Does landscape composition affect wetland occupancy by Blanding's turtles (*Emydoidea blandingii*) in the National Capital Region?

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ABSTRACT

Within a landscape, a species' occurrence is dictated by the availability of suitable habitat and resources needed for survival; however, occurrence is not only affected by the characteristics of occupied sites, but also by the characteristics of the surrounding landscape. The endangered Blanding's turtle (*Emydoidea blandingii*), a semi-aquatic freshwater turtle, occupies a wide range of wetlands and landscapes in southeastern Canada and the northeastern United States. While the effects of habitat characteristics on wetland occupancy by Blanding's turtles have been documented, here I explore whether the probability of wetland occupancy by Blanding's turtles is affected by the surrounding landscape. I used visual surveys, environmental DNA, and atlas data to document the presence of Blanding's turtles. I then used boosted regression tree modelling to determine how landscape composition explains wetland occupancy. Forest cover around the surveyed wetlands was the strongest positive driver of turtle occupancy while anthropogenic land cover was the strongest negative driver of turtle occupancy. Generally, human disturbances in a landscape lowered the probability of occupancy. Overall, I determined that wetland occupancy by Blanding's turtles is affected by landscape composition and that, therefore, wetland occupancy can successfully be predicted from the composition of the surrounding landscape.

RÉSUMÉ

Dans un paysage, la présence d’une espèce est dictée par la disponibilité d’habitat convenable et des ressources nécessaires pour sa survie. Par contre, la présence d’une espèce n’est pas seulement affectée par les caractéristiques des sites occupés, mais aussi par les caractéristiques du paysage environnant. La tortue mouchetée (*Emydoidea blandingii*), une tortue semi-aquatique d’eau douce, occupe une vaste gamme de milieux humides du sud-est du Canada et du nord-est des États-Unis. Bien que les effets des caractéristiques de l'habitat sur l’occupation des milieux humides chez la tortue mouchetée aient déjà été documentés, ici j'examine si la probabilité d'occupation des milieux humides chez la tortue mouchetée est affectée par le paysage environnant. J’ai utilisé la modélisation par arbres de régression augmentés pour déterminer comment la composition du paysage peut expliquer l’occupation des milieux humides chez la tortue mouchetée. Parmi les milieux humides échantillonnés, le couvert forestier des terres adjacentes était le facteur principal affectant l’occupation positivement, tandis que le couvert anthropogénique était le facteur principal affectant l’occupation négativement. Généralement, les dérangements anthropogéniques dans un paysage abaissent la probabilité d’occupation. En somme, j’ai déterminé que l’occupation des terres humides chez la tortue mouchetée est affectée par la composition du paysage et que l’on peut prédire avec succès l’occupation par la tortue mouchetée en se servant de la composition des paysages environnants.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Worldwide, species are currently facing many challenges to their continued survival due to the impacts of human activities. Habitat loss and degradation, climate change, and other unsustainable practices threaten biodiversity and have led to the extinction of many species (Sisk et al., 1994; Thomas et al., 2004; Tilman et al., 2017). Currently, an estimated 1 million species worldwide are at risk of extinction due to anthropogenic threats, and even populations of some common and widespread species are in decline (IPBES, 2019; Rosenberg et al., 2019). For example, avifauna in North America has lost nearly 3 billion individuals since 1970, a loss of approximately half of North America's present bird population (Rosenberg et al., 2019). The measurable and catastrophic impacts of humans on the Earth's climate, habitats, and biodiversity have led many to advocate a new epoch: the Anthropocene (Crutzen, 2006; Dirzo et al., 2014; Pievani, 2014). As such, the preservation of habitat and biodiversity is instrumental in maintaining healthy ecosystems for the benefit of humans, in the form of ecosystem services, and for the benefit of all other species (Christie et al., 2012; Lefcheck et al., 2015).

Reptiles are no exception in the current biodiversity crisis: over a fifth of reptile species worldwide are currently threatened with extinction (IUCN, 2020). Threats to reptiles are diverse and include agricultural practices, urban development, and collection for food, pets, and medicine (da Nóbrega Alves et al., 2008; IUCN, 2020; Klemens and Thorbjarnarson, 1995). Out of 43 species of reptiles found in Canada, there are currently 26 listed as at-risk under the Species at Risk Act (SARA, 2020; Seburn and Seburn, 2000), a federal law governing the protection of species in Canada. Of these 26 species of reptiles listed as at-risk, 16 are listed as 'threatened' or 'endangered', designations that offer legal protection from habitat loss or physical harm (SARA, 2002).

Included in the legal protections offered to threatened and endangered species in Canada is the designation of critical habitat: habitat deemed essential for the persistence of a species (SARA, 2002). Identifying critical habitat 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). Currently in Canada, nine species of reptile have officially designated critical habitat (SARA, 2020) and, for many species, this designated critical habitat only encompasses a small portion of their known range and utilized habitat. 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). My thesis focuses on the latter: the effects of the landscape on the probability of occurrence of a species at risk, the Blanding's turtle (*Emydoidea blandingii*).

1 INTRODUCTION

Landscape composition affects the abundance, occurrence, and behaviour of the species that inhabit that landscape (Guerry and Hunter, 2002; Knutson et al., 1999; Tremblay et al., 1998). Within a landscape, a species' occurrence and abundance are dictated by the availability of suitable habitat and of resources needed for survival and reproduction (Johnson, 1980). Knowing that the likelihood of a species occupying a given area in a landscape is dependent on the presence of that species' preferred habitat characteristics, those habitat characteristics can be used to predict the occupancy of a species within a given area of a landscape. For example, by modelling habitat characteristics of sites occupied by Coho salmon (*Oncorhynchus kisutch*), Anlauf-Dunn et al. (2014) were able to estimate the probability of occupancy of *O. kisutch* across their study area.

A species' occupancy is not only affected by characteristics of the sites they occupy, but also by characteristics of the landscape they inhabit. Mazerolle et al. (2005) found that the probability of pond occupancy by green frogs (*Lithobates clamitans*) in New Brunswick, Canada was significantly correlated with landscape features such as wetland cover and forest cover at various scales. 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 of *L. clamitans* (Martof 1953) can influence site occupancy. Similarly, probability of site occupancy by Eastern newt (*Notophthalmus viridescens*) 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 demonstrate how threats like habitat fragmentation and habitat loss at the landscape scale can act negatively on a local population (Burkey, 1995; Cushman, 2006; Fahrig, 2003).

In freshwater turtles, habitat fragmentation and habitat loss, along with more specific threats such as road mortality and collection for the pet or food trades, are the most significant drivers of population declines (Gibbon et al., 2000; Steen and Gibbs, 2004; Turtle Conservation Fund, 2002). The Blanding's turtle (*Emydoidea blandingii*), 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 (a generation is estimated at approximately 40 years) owing largely to habitat loss and road mortality (COSEWIC, 2016). Although Blanding's turtles inhabit wetlands such as swamps, ponds, and marshes (Edge et al., 2010; Ross and Anderson, 1990), they also use upland habitat for nesting and inter-wetland travel (Edge et al., 2010; Markle and Chow-Fraser, 2014; Millar and Blouin-Demers, 2011). Eleven studies have included the tracking of Blanding's turtles with radio-telemetry and have documented the mean home range area to be between 1 and 95 ha (Fortin at al., 2012; Millar and Blouin-Demers, 2011); home ranges as large as 255 ha and as long as 3.2 km have been documented (Fortin et al., 2012). Although both male and female turtles demonstrate similar home range areas, gravid females during the nesting period exhibit the longest movements and have been documented travelling more than 6 km (Edge et al., 2010; Millar and Blouin-Demers, 2011). Since the Blanding's turtle is a vagile species and road mortality and habitat loss are putative drivers of its population decline, landscape features such as road density and human development may be good predictors of its presence. Landscape features are good at estimating site occupancy for the eastern musk turtle (*Sternotherus odoratus*) in Georgian Bay, where the probability of site occupancy was negatively correlated with the density of surrounding roads, docks, and cottages (Markle et al., 2018). Similarly, northern map turtles (*Graptemys geographica*) prefer natural shoreline over developed shoreline at a macrohabitat scale (Carrière and Blouin-Demers, 2010). Literature on Blanding's turtle site occupancy is largely focused on microhabitat selection and on habitat suitability modelling at the provincial scale (Edge et al., 2010; Markle and Chow-Fraser, 2014; Millar and Blouin-Demers, 2012; Ross and Anderson, 1990). Here, I investigate whether we can estimate the probability of wetland occupancy by Blanding's turtles based on landscape composition.

To determine whether landscape composition affects the probability of wetland occupancy by Blanding's turtles, I determined wetland occupancy using visual surveys, environmental DNA (eDNA), and sightings from the Ontario Reptile and Amphibian Atlas (Ontario Nature, 2018). Species detection with 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; Raemy and Ursenbacher, 2018; Thomsen et al., 2012). For instance, eDNA was used successfully to detect an invasive species of carp in the Lake Michigan watershed before its detection by traditional survey methods (Jerde et al., 2011). eDNA was superior to visual surveys, but inferior to trap surveys, for the detection of the European pond turtle (*Emis orbicularis*) (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, I complemented and validated eDNA data with visual surveys and occurrence records from the Ontario Reptile and Amphibian Atlas.

1.1 Modelling

I used boosted regression tree (BRT) modelling to test whether the probability of wetland occupancy by Blanding's turtles can be predicted from landscape composition. BRTs are a relatively new method used to model ecological interactions and have been used with success in assessing landscape effects on organisms (Elith et al., 2008; Ruso et al., 2019). BRT models are a machine learning method that works by building regression trees of a set complexity and scaling their contribution for inclusion in a final model (Elith et al., 2008). A distinct advantage of BRT modelling for my study is that, unlike other modelling methods, the final model predictions are little affected by outliers and collinearity amongst predictor variables (Elith et al., 2008; Main et al., 2015), which is almost inevitable in landscape studies because increased cover of a given habitat necessarily means less cover of the other habitats. The main disadvantages of BRT models is that they are susceptible to model overfit and can show noise in fitted functions (Elith et al., 2008; Naghibi et al., 2016); however, neither are necessarily critical issues (Elith et al., 2008).

1.2 Hypothesis and Predictions

I hypothesize that the probability of wetland occupancy by Blanding's turtles (*Emydoidea blandingii*) can be estimated from the composition of the landscape surrounding a wetland. I predict that an increase in human activity, such as roads and urban areas, will decrease the probability of Blanding's turtle occupancy. Apart from the direct effect of roadways increasing wildlife mortality and being a threat to Blanding's turtles (COSEWIC, 2016; Trombulak and Frissell, 2000), roads also degrade habitat connectivity by acting as barriers to movement (Attum et al., 2008; Proulx et al., 2014). Roadways and increased human presence can also lead to an increase in activities that threaten Blanding's turtles, such as collection for the pet trade (COSEWIC, 2016; Trombulak and Frissell, 2000). I also predict that, since the preferred habitats of Blanding's turtles are wetlands (Edge et al., 2010), increased wetland cover in the surrounding landscape will increase the probability of occupancy. Since it is difficult to know a priori at which scales landscape components will affect the probability of occupancy, I test each variable at multiple scales to identify the scale of maximum effect. For example, Mazerolle et al. (2005) found that a decrease in the probability of occupancy of *Lithobates clamitans* was related to an increase in forest cover within 250 m of the focal wetlands, but the opposite was true when measuring forest cover within 1000 m, demonstrating the difficulty of estimating landscape effects at predetermined scales.

1.3 Significance

Properly documenting how a landscape can influence the occurrence of Blanding's turtles is important for the conservation of the species because it can inform habitat protection. By assessing which landscape features can be used to estimate Blanding's turtle occupancy, it will be possible to better identify where to invest conservation resources at the landscape scale. This relationship between landscape and occupancy can be applied to, among other applications, legally required critical habitat mapping. In addition, documenting how a landscape can influence the occurrence of Blanding's turtles will provide valuable knowledge of the relationship between landscape components and the species that inhabit that landscape.

Blanding's turtles are a good species for this study for several reasons. (1) Although Blanding's turtles inhabit a wide variety of wetlands (Edge et al., 2010), they are not ubiquitous across the study area like more common turtle species, such as the painted turtle (*Chrysemys* *picta*), allowing for a wider range of occupied and unoccupied wetlands to be included in the study. (2) The decline of Blanding's turtles in the study area has, in part, been linked to road mortality and habitat loss (COSEWIC, 2016); threats which can be attributed to road density and land cover classifications which can be spatially modelled (Falcucci et al., 2007; Gibbs and Shriver, 2002). Finally, (3) since there is interest in conserving the remaining populations of Blanding's turtles (Environment Canada, 2016), having the ability to estimate its presence based on landscape features will provide another tool for conservation biologists working on this species.

2 METHODS

2.1 Study sites

I conducted fieldwork from May to August 2018 and 2019 within the limits of the City of Ottawa, Ontario, Canada (Figure 2). All the wetlands I studied were within the ~2,800 km^2 area of the City of Ottawa, a low-lying and predominantly flat region of the mixedwood plains ecozone whose geology is described primarily as glacial deposits and marine deposits atop Paleozoic sedimentary formations (Harrison, 1979; Richard, 1982). The study area was comprised of 48% agriculture, 18% wetlands, 16% forests, 15% anthropogenic lands (which include urban developments, roadways, waste facilities, and quarries), and 4% open water. Road density of all road types across the study area was 2.3 km/km^2 on average.

I studied 155 wetlands: I surveyed 117 wetlands for Blanding's turtles by visual surveys or environmental DNA sampling (70 wetlands overlapped between the two techniques) and the remaining wetlands were included based on Blanding's turtle sightings obtained from the Ontario Reptile and Amphibian Atlas. Wetlands were spread out across the study area to ensure sufficient occupied sites for use as positive controls for environmental DNA surveying as well as to stratify adjacent lands of surveyed sites with the landscape composition of the study area. Land cover within a 2 km buffer of the included wetlands was 38% agriculture, 20% wetlands, 20% forests, 18% anthropogenic lands, and 3% open water while road density was 2.6 km/km^2.

2.2 Blanding’s turtle occupancy

To determine whether wetlands are occupied by Blanding’s turtles, I surveyed for Blanding’s turtles by means of visual surveys and environmental DNA sampling. Survey efforts were supplemented by Blanding’s turtle sightings from the Ontario Reptile and Amphibian Atlas.

2.2.1 Environmental DNA

During the summer of 2019, I took 445 water samples from 89 wetlands to survey for Blanding’s turtles with environmental DNA (eDNA). I sampled wetlands by gathering five 1 L samples of water per wetland in sterilized polypropylene bottles. I collected one sample in the wetland inflow, if present, and the remaining samples were spread out around the wetland with at least one sample located in the outflow. I placed the samples on ice in a cooler and transported them to the laboratory where I filtered the samples through glass microfiber filter paper (Watman GC/F) using a vacuum pump for suction. I placed the filter papers in microtubes which were frozen at -20 °C for short term storage and -80 °C for long term storage. I sterilized equipment between wetlands by soaking in a 0.5% solution of sodium hypochlorite for 10 minutes then rinsing. Sample bottles were also flushed with water from the target wetland before sampling. I took an additional ten samples using distilled water, ten samples using City of Ottawa tap water, and five samples from an outdoor water source that was certain to not contain Blanding’s turtles. These 25 negative control samples were gathered and processed using equipment sterilized after being used for sites known to contain Blanding’s turtles.

DNA extraction, PCR, and Blanding’s turtle primer development was completed by the laboratory of Dr. Yann Surget-Groba at the Université du Québec en Outaouais (Appendix 1). They extracted the DNA from the samples using a QIAgen DNeasy kit. The samples were diluted by a factor of 10 and amplified using qPCR. They amplified a minimum of three replicates per sample.

2.2.2 Visual surveys

I conducted visual surveys for Blanding's turtles from late April to mid-June when Blanding's turtles are most likely to be basking and thus easiest to detect (Millar and Blouin-Demers, 2011). During this time, I visited 98 wetlands with a spotting scope and binoculars to search for Blanding's turtles. Wetlands were visited from mid-morning to late afternoon on days without precipitation and I spent approximately one hour per wetland per visit. I visited wetlands an average of three times (a minimum of one and a maximum of 10) and no longer visited wetlands once I had confirmed Blanding's turtles to be present.

2.2.3 Ontario Reptile and Amphibian Atlas

I used Blanding's turtle sightings from the Ontario Reptile and Amphibian Atlas to provide additional wetlands with confirmed presence. I retained sightings within the City of Ottawa which could be attributed to a specific wetland (i.e. within or on the edge of a wetland). I further filtered sightings to only include observations from the past ten years. The Ontario Reptile and Amphibian Atlas provided 840 Blanding's turtle observations within the study area, 522 of which were from the years 2008 to 2018.

2.3 Landscape composition

I acquired land cover data from the Ontario Land Cover Classification V2 (OMNRF, 2014) in raster form with 15 meter resolution and 28 land cover classes. I merged land cover classes into the following 6 categories: 1- agriculture, 2- anthropogenic (buildings, roadways, gravel pits and quarries, and other human disturbed sites), 3- forest, 4- wetlands, 5- open water, and 6- other (all other land cover types which did not fall into the previous five categories, such as alvar and bedrock). I acquired road information from Open Street Maps (2019) and included motorways, primary, secondary, tertiary, and residential roads. I delineated wetlands at a scale of 1:5000 using aerial photographs taken in the spring of 2014 (National Capital Commission, 2014) and ground truthed for accuracy. I determined wetland age (in years) from historical aerial photographs (City of Ottawa 1958, 1976, 1991, 1999, 2008; National Capital Commission 1965, 2001, 2014; University of Toronto 1954) as the mean of the age of the air photo in which the wetland first appeared and the age of the next oldest air photo. The earliest air photos covering the study site were taken in 1954, so I deemed wetlands already present in 1954 to be 65 years old.

I created buffers around wetlands in 100 meter increments from 100 to 4000 m (Fortin et al., 2012). After wetlands were buffered by each radius, I tabulated land cover (as a percentage of the buffer area excluding the focal wetland) and road density (km/km^2) within each incremental buffer. I then calculated point biserial correlation between each variable and Blanding's turtle occupancy at all buffer scales to determine the variables' scale of maximum effect. I retained each variable at its scale of maximum effect for model building. I completed all geospatial analyses using ArcGIS 10.4.1 (ESRI, 2016) and Python 2.7.10 (Python Software Foundation, 2015).

2.4 Modelling

I used boosted regression tree (BRT) modelling to determine whether landscape composition affects the probability of wetland occupancy by Blanding's turtles. Using the ‘dismo’ (Hijmans et al., 2017) and ‘gbm’ (Greenwell et al., 2019) packages in R 3.5.2 (R Development Core Team, 2018), I built six models using the eDNA data, the visual survey data, the Ontario Reptile and Amphibian Atlas data, and all combinations of data sources (with the exception of solely Ontario Reptile and Amphibian atlas data which is a presence only dataset). When combining data sources and conflicting occupancy data existed, presences overrode absences regardless of survey method. All six models included eight explanatory variables: open water cover, wetland cover, forest cover, anthropogenic land cover, agricultural land cover, road density (km/km^2), wetland age (years), and wetland area (hectares). The buffer size within which each land cover class and road density was tabulated varied between models and was determined based on the scale of maximum effect (Table 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, I set the tree complexity to five. Next, I built models with decreasing learning rates from 0.01 to 0.001 to determine the optimal value. Similarly, I 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, I tested tree complexity with values of two, three, and four, which are considered suitable for small 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 (AUC), which are more reliable in evaluating model performance than self-statistics such as residuals (Elith et al., 2008; Elith and Leathwick, 2017). Percent deviance explained, calculated as (total deviance - CV deviance)/total deviance (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). The number of trees was also examined, because models are ideally fit with at least 1000 trees (Elith et al., 2008).

Regardless of model performances, I retained the model built using data from all three data sources, and hence the most complete dataset, as the most comprehensive model to determine the effects of landscape composition on Blanding's turtle occupancy. To test for model overfit, which indicates a model may not make accurate predictions of occupancy on wetlands not included in model building, I picked a random subset of 100 wetlands from the original 155 which I used as training data to build a new model and the remaining 55 wetlands were retained as validation data. I repeated this process 100 times with a new random subset of 100 wetlands for each model. I used the subset models to estimate wetland occupancy by Blanding's turtles for the 100 training wetlands and the 55 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, I used the comprehensive model to make estimations of wetland occupancy for all 155 wetlands to compare between wetlands where Blanding's turtles are present and absent.

3 RESULTS

3.1 Blanding's turtle occupancy

Environmental DNA sampling, visual surveys, and the Ontario Reptile and Amphibian Atlas resulted in six datasets, each of which I used for modelling Blanding's turtle occupancy. These datasets are as follows, with their abbreviation in brackets: environmental DNA (eDNA) consisting of 89 wetlands, visual surveys (Visual) consisting of 98 wetlands, eDNA and visual surveys (Visual + eDNA) consisting of 117 wetlands, eDNA and Ontario Reptile and Amphibian Atlas (eDNA + ORAA) consisting of 131 wetlands, visual surveys and Ontario Reptile and Amphibian Atlas (visual + ORAA) consisting of 143 wetlands, and eDNA, visual surveys, and Ontario Reptile and Amphibian Atlas (Comprehensive) consisting of 155 wetlands.

3.1.1 Environmental DNA

The eDNA results were unreliable, since three of the 25 negative controls (four of 75 replicates) tested positive for Blanding's turtle DNA due to contamination either during the sampling, filtering, or DNA extraction. For this reason, I deemed sites occupied based on strict criteria to avoid possible false-positives. I filtered samples by quantification cycle (Cq) values and number of positive replicates. Based on the Cq values from positive control replicates, I determined that values between 8 and 16 represent values that are unlikely to be contamination (Figure 3). Additionally, sites where only one of the 15 replicates was positive I eliminated as possible contamination. After eliminating possible contamination (29 wetlands were eliminated), 26 of the 89 sites tested positive for Blanding's turtle DNA.

I took eDNA samples from 20 wetlands where Blanding's turtles had been visually confirmed to provide positive controls, as well as three additional wetlands that had been visually confirmed by other researchers within a week prior to sampling. With few exceptions, I took samples from positive control wetlands within one day of Blanding's turtle observations. Eleven of the positive control wetlands tested positive for Blanding's turtle DNA while 12 of the 23 wetlands resulted in false-negatives.

3.1.2 Visual surveys

I confirmed the presence of Blanding's turtles at 24 of the 98 wetlands with visual surveys. Of the 24 wetlands occupied by Blanding's turtles, I confirmed presence on the first visit for 19 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.

3.1.3 Ontario Reptile and Amphibian Atlas

I used Blanding's turtle sightings from the Ontario Reptile and Amphibian Atlas to confirm occupancy at an additional 47 wetlands. Of these wetlands, I had surveyed nine 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 88 out of the 155 wetlands included in the study.

3.2 Landscape composition

The scale of maximum effect for each landscape composition variable, determined as the buffer size with the highest correlation between the landscape variable and wetland occupancy, varied for each of the six datasets. Between the six datasets, the scale of maximum effect for open water cover varied from 700 to 4000 m, wetland cover from 100 to 3500 m, forest cover from 200 to 4000 m, anthropogenic land cover from 300 to 4000 m, agricultural land cover from 200 to 4000 m, and road density from 300 to 4000 m (Table 1, Figure 4a-f). Within the retained buffer sizes, the proportions of the various land covers and road density were comparable to those of the study area (Table 2).

3.3 Modelling

Using the ‘dismo’ (Hijmans et al., 2017) and ‘gbm’ (Greenwell et al., 2019) packages in R 3.5.2 (R Development Core Team, 2018), I fit BRT models to the data using a tree complexity of five, with the exception of the model using only eDNA data which required a tree complexity of three to successfully build due to its smaller sample size and number of positive sites. I determined that a learning rate of 0.001 and a bag fraction of 0.6 resulted in the best performing models. Model performances are summarized in Table 3.

The model built using visual survey data and Ontario Reptile and Amphibian Atlas (Visual + ORAA) data is the best performing model. This model explains 28.8% of the deviance in Blanding’s turtle occupancy and had a cross-validation area under the receiver operating curve (CV AUC) of 0.847 (Figure 5). The next best performing models were the comprehensive model (built using all data sources; 17.8% deviance explained and CV AUC of 0.795) and the model using the eDNA and ORAA data (16.4% deviance explained and CV AUC of 0.780). The worst performing model uses only the eDNA data and explains 0.9% of the deviance in occupancy and has a CV AUC of 0.599. Models which use the eDNA data are generally the poorest performing models, the worst of which uses only eDNA data.

Although model performance varied greatly, there were similarities in the relative influence (a rank of the importance of a variable in predicting Blanding’s turtle occupancy in relation to the other variables) and marginal effects of variables across all models. Forest cover and wetland age were the most consistent variables; in all models forest cover showed a strong positive relationship with Blanding's turtle occupancy and it ranked in the top three most important variables with a relative influence (RI) ranging from 11.8% to 34.8% (Figure 6 and Figure 7a-f) and wetland age, although not ranked as highly as forest cover, also showed a positive relationship across all the models (2.5% to 16.5% RI). Wetland area (9.3% to 21.2% RI), wetland cover (7.1% to 24.4% RI), and open water cover (6.6% to 20.2% RI), although not consistent across all models, generally showed a positive relationship with occupancy. Anthropogenic land cover (3.7% to 14.8% RI) and road density (3.5% to 11.1% RI) were generally showed a negative relationship with occupancy. Agricultural land cover (5.7% to 18.0% RI) was much less clear in its effects on wetland occupancy and varied between showing a positive and a negative relationship with occupancy.

The comprehensive model, built using data from the visual surveys, eDNA sampling, and Ontario Reptile and Amphibian Atlas, ranks wetland cover as the most important variable (21.0% RI) followed by forest cover (19.5% RI), and wetland area (18.1% RI) (Figure 6). Marginal effects show increased wetland cover, forest cover, wetland area, and wetland age to have a positive relationship with occupancy while anthropogenic and agricultural land covers have a negative relationship with occupancy (Figure 7b). Overall trends of occupancy based on increased water cover and road density are difficult to assess. The comprehensive model, when used to estimate occupancy for all 155 wetlands, estimates wetlands where Blanding's turtles are present to have a significantly higher ($p < 0.001$) probability of occupancy than wetlands where Blanding's turtles are absent (Figure 8; mean 74.5%, SE 1.6% for occupied wetlands; mean 33.4%, SE 2.3% for unoccupied wetlands). The subset models, built using 100 randomly selected wetlands, performed worse than the comprehensive model (mean deviance explained: 10.8%; mean CV AUC: 0.730); however, Welch's t-test determined there was a significant difference ($p < 0.001$) between the occupied training wetlands and the unoccupied training wetlands, between the occupied testing wetlands and the unoccupied testing wetlands, between the occupied training wetlands and the occupied testing wetlands, and between the unoccupied training wetlands and the unoccupied testing wetlands (Figure 9).

4 DISCUSSION

4.1 Landscape effects on occupancy

I tested the hypothesis that wetland occupancy by Blanding's turtles is affected by landscape composition around the wetland. Overall, I found that landscape composition is a factor in whether a wetland is occupied by Blanding's turtles, with the highest performing BRT model explaining over a quarter of the deviance in Blanding's turtle occupancy. Interestingly, wetlands located in less disturbed landscapes with a higher proportion of natural land cover types, such as wetland cover and forest 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 not as likely to be occupied by Blanding's turtles. This is consistent with existing literature on the relationship between Blanding's turtles and landscape features.

While BRT models have power in estimating Blanding's turtle occupancy based on the surrounding landscape, the exact importance of each landscape variable is difficult to determine due to high collinearity amongst predictor variables. If two variables included in building a BRT model are perfectly correlated, one of the two variables will be assigned a relative importance of 0% and show no effect on the model's predictions. If, hypothetically, the first of the two perfectly correlated variables is an important driver of occupancy while the second variable has no effect, the model may associate the effects of the first variable with the second variable. For this reason, it is important to examine collinearity when making assumptions about the importance and effects of a variable. The proportion of forest cover, for example, had a very high and significant correlation with anthropogenic land cover (Table 4). Therefore, it is difficult to determine the exact dynamics of the relationship between forest cover, anthropogenic land cover, and Blanding's turtle occupancy. In this case, the importance of forest cover (ranked as high as 34.8% relative importance) may be over or underestimated in favour of anthropogenic land cover (ranked as high as 14.8% relative importance).

Road mortality and illegal collection are among the leading causes of Blanding's turtle population decline (COSEWIC, 2016), so landscape features like roadways and urban areas that facilitate these threats should negatively affect Blanding's turtle populations in nearby wetlands. This is consistent with the findings of my study which suggest 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, the preferred habitat type of Blanding's turtles (Edge et al., 2010; Millar and Blouin-Demers, 2011), is found to increase the probability of occupancy. In addition to being the preferred habitat, additional wetlands in close proximity may increase Blanding's turtle immigration which reduces the likelihood of local extinction. However, it would be difficult to disentangle to what extent local wetlands increase the probability of occupancy due to immigration versus the increased habitat quality. Forest cover also increases 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 and, as a result, may increase probability of occupancy simply by merit of not being a heavily influenced by humans. Its importance may also be conflated with anthropogenic land cover due to their high correlation (Table 4).

Agricultural land cover did not have a strong effect on the probability of wetland occupancy by Blanding's turtles. The negative impact of agricultural land on Blanding's turtle populations found in a previous study (Mui et al., 2016) was not evident from my models. Of note is that the landscape cover data I used does not distinguish between agricultural lands that are currently in use and lands that have been fallow for as many as 50 years. While it is difficult to get an exact proportion of agricultural lands that are fallow versus active, my estimate based on air photos is that about 10% of the agricultural lands across the study area are fallow. My estimate of fallow lands increases to over 50% in conservation and wilderness areas such as the Greenbelt and Marlborough Forest where many of the surveyed wetlands are located. The lack of distinction between fallow and active agriculture lands may have contributed to the weak effects of agriculture in the BRT models because fallow lands may provide nesting opportunities for Blanding's turtles, but without threats such as pesticides and agricultural machinery (Mui et al., 2016).

Wetland age was consistent in effect and importance across models. Older wetlands had a higher likelihood of being occupied by Blanding's turtles, although the effect was not as strong as that of other variables (2.5% to 16.5% relative influence). Although Blanding's turtles are relatively mobile compared to other freshwater turtles, populations in close geographic proximity can have low gene flow 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. My 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 no difference in the probability of occupancy for wetlands younger than approximately 40 years. 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 observed between 40 and 50 years after construction (Dupuis-Desormeaux et al. 2018). Colonization should occur more rapidly in my study area because the closest known Blanding's turtle population to Tommy Thompson Park is 15 km away (Dupuis-Desormeaux et al. 2018) while the mean distance between unoccupied wetlands and the nearest occupied wetland in my study area is 3.8 km (minimum 0.1 km and maximum 15.6 km).

Wetland size is the only explanatory variable I tested which does not significantly correlate with any other variable and, as a result, its relative importance in predicting Blanding's turtle occupancy is likely not conflated by collinearity. 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).

4.2 Comprehensive model

Although it is difficult to know which of the six models gives the most accurate representation of the effects of landscape composition on Blanding's turtle occupancy, I retained the model using data from all three sources as the comprehensive model because it includes the largest number of both occupied and unoccupied wetlands. The comprehensive model indicated that landscape composition explains nearly one fifth of the deviance in Blanding's turtle occupancy (Figure 5) and is consistent with the overall findings that more human disturbed landscapes decrease the probability of occupancy. The results also suggest landscape to be a much stronger driver of wetland occupancy by Blanding's turtles than a similar study in the Pontiac region of Québec, but both studies show similar effects for the included land cover types (Fortin and Blouin-Demers, 2012).

The comprehensive model's cross-validation AUC score of 0.795, which is lower than the training AUC of 0.970, suggests the model is overfit to the data. However, overfitting of BRT models is not necessarily an issue (Elith et al., 2008). To test the overfit and evaluate the predictive performance of the model, I built 100 models using random subsets of 100 of the 155 wetlands used to build the Comprehensive model. I used these models to predict the probability of occupancy for all 155 wetlands. Welch's t-test determined that there was a significant difference in predicted occupancy between the training wetlands and the validation wetlands (Figure 9). This suggests the resulting models are indeed overfit to the data as indicated by the cross-validation AUC; however, regardless of overfit, there was a significant difference between the estimated probabilities of occupancy for occupied versus unoccupied validation wetlands suggesting the models still had power to predict wetland occupancy by Blanding's turtles for external data.

The comprehensive model, when used to predict the probability of wetland occupancy by Blanding's turtles for all 155 wetlands (Figure 8), provides some insight into possible survey errors. Four wetlands have a low probability of occupancy (25% to 45%; mean predicted probability of occupancy for occupied sites is 74.5%, SE 1.6%), but are found to have Blanding's turtles present. Blanding's turtles were detected in three of these wetlands based on eDNA, but I did not find Blanding's turtles at those three 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 not possible to verify. The fourth occupied site, which had the lowest predicted probability of occupancy, is a seasonal pond in downtown Ottawa that is drained each fall. It is thought 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. My subsequent visual surveys did not confirm the presence of Blanding's turtles to be present despite the pond's ease of surveying. Sites with a high predicted probability of occupancy in which I did not find Blanding's turtles may be false-negative survey results, a common issue with rare and elusive species (Miller et al., 2011; Zhou and Griffiths, 2007). Nine wetlands stand out as having a high predicted probability of occupancy (61% to 78%; mean predicted probability of occupancy for unoccupied sites is 33.4%, SE 2.3%), but were not occupied by Blanding's turtles All nine wetlands are located in areas that have well established Blanding's turtle populations and are in close proximity to other wetlands where Blanding's turtles are known to occur (mean 1.6 km, minimum 0.2 km, and maximum 5.5 km).

The comprehensive model, with its ability to successfully estimate wetland occupancy by Blanding's turtles, can also be used to estimate Blanding's turtle occupancy across a landscape. Predicting occupancy across a landscape can be particularly useful for mapping exercises, such as determining critical habitat. Predictive mapping across a landscape, however, rather than estimating the probability of occupancy at a known wetland, does have the drawback of not having the ability to factor in wetland age or wetland area. As an exercise in predictive mapping, I rebuilt the comprehensive model without the wetland age or area variables and applied the estimations to a 1 km grid across the study area by assuming the centroids of the grid cells were the wetlands (Figure 10). While there was a loss of model performance resulting from the exclusion of wetland age and area (CV deviance explained is 14.2%, down from 17.8%), the resulting predictive map was, based on my turtle observations and historic turtle observations, generally a very good representation of where Blanding's turtles are known to be found across the study area.

4.3 Survey method comparison

4.3.1 Occupancy results comparison

The eDNA samples and the visual surveys were both able to detect 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, nine 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 nine 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. While 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). If we assume that none of the sites where Blanding's turtles were detected by eDNA are false-positives, then eDNA was slightly more effective than visual surveys at detecting Blanding's turtles, which is consistent with one previous study of an elusive freshwater turtle (Raemy and Ursenbacher 2018). Neither method is perfect and a combination of the data from both methods yields the most comprehensive picture of where turtles occur. Data from visual surveys can be easily improved with additional site visits while improvements to eDNA data are more difficult to achieve.

4.3.2 Model comparison

The BRT models that included Blanding's turtle occupancy data from multiple sources (i.e., some combination of visual surveys, eDNA sampling, and the Ontario Reptile and Amphibian Atlas) were in agreement with existing literature on how landscape features should influence turtle populations. The results from the models using only eDNA data or only visual survey data were not as easily interpreted. A likely explanation is that these survey methods alone did not give an adequate representation of Blanding's turtle occupancy throughout the study area. The models including eDNA data performed poorly and were the most difficult to interpret, likely because these models included several false-negatives. Interestingly, the data from visual surveys generally performed better than the eDNA data even though the visual surveys were not more effective at detecting Blanding's turtles than the eDNA surveys. This may be because false-positives are much more likely with eDNA than with visual surveys. Due to results achieved using eDNA data and the high possibility of both false-positive and false-negative results, I recommend eDNA be used only in conjunction with other survey methods.

5 CONCLUSION

My thesis demonstrates that wetland occupancy by Blanding's turtles can be successfully estimated by landscape composition. While there are certainly many untested factors influencing whether a wetland is occupied by Blanding's turtles, I show the importance of the surrounding landscape in determining occupancy. The finding that human influenced landscapes are generally less suitable for Blanding's turtles is not surprising given what we know about the impacts of humans on biodiversity; however, the findings do provide valuable knowledge into the complicated relationships between landscape components and the species that inhabit those landscapes. The findings also provide many practical applications for the conservation of Blanding's turtles, including critical habitat mapping and habitat restoration.

The ability to properly define critical habitat of at-risk species is thought to be one of the most difficult aspects of species conservation, and determining critical habitat requires knowledge of a species' interactions with the biotic and abiotic world at multiple scales (Rosenfeld and Hatfield, 2006). My study provides information at the landscape scale that can be directly applied to delineating critical habitat for Blanding's turtles.

6 TABLES

Table 1: Summary of the scales of maximum effect (meters) of six landscape variables on Blanding's turtle occupancy, as determined by each of the six datasets. The scale of maximum effect is determined as the buffer size (ranging from 100 m to 4000 m in 100 m increments) surrounding a wetland in which the landscape value has the highest correlation with wetland occupancy (point biserial correlation). Visual = data from the visual surveys; eDNA = data from the eDNA sampling; ORAA = data from the Ontario Reptile and Amphibian Atlas; Comprehensive = data from Visual, eDNA, and ORAA. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

Landscape variable	Visual + ORAA	Comprehensive	eDNA + ORAA	Visual	Visual + eDNA	eDNA
Water	3700	3900	4000	700	700	4000
Wetland	3000	3300	3500	100	200	1700
Forest	4000	4000	4000	4000	200	300
Anthropogenic	4000	4000	4000	4000	300	300
Agriculture	2300	2300	2900	4000	4000	200
Road	4000	4000	4000	4000	300	300

Table 2: Land cover proportions (%) and road density (km/km^2) of the study area and the buffered areas used in building the six BRT models. Models are identified by their source data and buffer sizes are in meters. Visual = data from the visual surveys; eDNA = data from the eDNA sampling; ORAA = data from the Ontario Reptile and Amphibian Atlas; Comprehensive = data from Visual, eDNA, and ORAA. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

	Water (%)	**Wetland (%)**	**Forest (%)**	**Anthropogenic (%)**	**Agriculture (%)**	**Other (%)**	**Road (km/km^2)**
Study area	3.5	17.8	15.8	15.0	47.8	0.0	2.3
Comprehensive - 2300 m	3.6	21.4	19.1	17.6	38.2	0.1	2.6
Comprehensive - 3300 m	4.0	19.6	18.0	17.8	40.6	0.1	2.6
Comprehensive - 3900 m	4.3	19.0	17.7	17.5	41.5	0.1	2.6
Comprehensive - 4000 m	4.4	18.9	17.7	17.4	41.6	0.1	2.6
Visual + ORAA - 2300 m	3.8	21.7	19.1	17.6	37.7	0.1	2.6
Visual + ORAA - 3000 m	4.1	20.2	18.1	17.9	39.6	0.1	2.6
Visual + ORAA - 3700 m	4.4	19.4	17.6	17.9	40.6	0.1	2.6
Visual + ORAA - 4000 m	4.6	19.2	17.4	17.8	40.9	0.1	2.6
eDNA + ORAA - 2900 m	3.6	20.6	18.7	17.4	39.7	0.1	2.6
eDNA + ORAA - 3500 m	3.8	19.7	18.3	17.3	40.9	0.1	2.6

eDNA + ORAA - 4000 m	4.0	19.1	18.0	16.9	41.9	0.1	2.5
Visual - 100 m	1.2	38.5	24.3	7.9	28.1	0.0	1.2
Visual - 700 m	2.5	22.1	23.7	18.7	33.0	0.0	2.9
Visual - 4000 m	3.7	15.5	15.1	23.9	41.9	0.0	3.4
Visual + eDNA - 200 m	1.1	35.8	26.4	9.6	27.0	0.0	1.5
Visual + eDNA - 300 m	1.3	32.6	26.5	11.4	28.1	0.0	1.8
Visual + eDNA - 700 m	2.0	24.9	24.3	16.8	31.9	0.0	2.6
Visual + eDNA - 4000 m	3.4	17.6	16.7	20.5	41.8	0.1	3.0
eDNA - 200 m	1.0	35.9	27.6	10.5	25.0	0.0	1.6
eDNA - 300 m	1.2	33.1	27.9	12.0	25.9	0.0	1.9
eDNA - 1700 m	3.0	20.2	20.1	21.5	35.2	0.1	3.1
eDNA - 4000 m	2.9	18.3	17.4	18.8	42.6	0.1	2.7

Table 3: Summary of the performances of the six BRT models used to predict Blanding's turtle occupancy. Models are identified by their source data and are built with a tree complexity of 5, a learning rate of 0.001, and a bag fraction of 0.6 with the exception of the eDNA model which is built with a tree complexity of 3. CV = cross-validation; AUC = area under the receiver operating curve; SE = standard error. Visual = data from the visual surveys; eDNA = data from the eDNA sampling; ORAA = data from the Ontario Reptile and Amphibian Atlas; Comprehensive = data from Visual, eDNA, and ORAA.

Model	**# of trees**	**Total deviance**	**Residual deviance**	**CV deviance (SE)**	**Training AUC**	**CV AUC (SE)**
Visual + ORAA	2800	1.378	0.587	0.981 (0.079)	0.974	0.847 (0.032)
Comprehensive	3200	1.368	0.651	1.124 (0.077)	0.970	0.795 (0.034)
eDNA + ORAA	1900	1.365	0.788	1.141 (0.074)	0.945	0.780 (0.035)
Visual	2050	1.113	0.620	0.936 (0.091)	0.953	0.786 (0.064)
Visual + eDNA	550	1.295	1.133	1.282 (0.022)	0.890	0.577 (0.044)
eDNA	450	1.208	1.103	1.197 (0.033)	0.861	0.599 (0.059)

Table 4a: Correlations (Pearson's correlation coefficient) between the explanatory variables of the BRT model built using the visual survey data and the Ontario Reptile and Amphibian Atlas data (Visual + ORAA). Asterisk (*) represents significant correlation ($p < 0.05$). Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

Water	-0.32*	-0.25*	0.08	-0.10	0.06	0.11	-0.06
	Wetland	0.60*	-0.51*	-0.43*	-0.51*	0.33*	0.11
		Forest	-0.71*	-0.29*	-0.72*	0.47*	0.01
			Anthropogenic	-0.26*	0.98*	-0.48*	-0.09
				Agriculture	-0.23*	-0.21*	0.10
					Road	-0.45*	-0.08
						Age	0.09
							Area

Table 4b: Correlations (Pearson's correlation coefficient) between the explanatory variables of the BRT model built using the visual survey data, the eDNA data, and the Ontario Reptile and Amphibian Atlas data (Comprehensive). Asterisk (*) represents significant correlation ($p < 0.05$). Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

Water	-0.34*	-0.27*	0.00	0.00	0.08	0.12	-0.06
	Wetland	0.62*	-0.53*	-0.43*	-0.52*	0.29*	0.11
		Forest	-0.72*	-0.32*	-0.72*	0.44*	0.02
			Anthropogenic	-0.22*	0.98*	-0.47*	-0.09
				Agriculture	-0.21*	-0.19*	0.09
					Road	-0.44*	-0.08
						Age	0.10
							Area

Table 4c: Correlations (Pearson's correlation coefficient) between the explanatory variables of the BRT model built using the environmental DNA data and the Ontario Reptile and Amphibian Atlas data (eDNA + ORAA). Asterisk (*) represents significant correlation ($p < 0.05$). Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

Water	-0.35*	-0.29*	0.06	-0.08	0.04	0.13	-0.06
	Wetland	0.66*	-0.58*	-0.43*	-0.58*	0.30*	0.11
		Forest	-0.74*	-0.30*	-0.73*	0.44*	0.03
			Anthropogenic	-0.20*	0.98*	-0.49*	-0.11
				Agriculture	-0.19*	-0.15	0.09
					Road	-0.45*	-0.10
						Age	0.11
							Area

Table 4d: Correlations (Pearson's correlation coefficient) between the explanatory variables of the BRT model built using the visual survey data (Visual). Asterisk (*) represents significant correlation ($p < 0.05$). Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

Water	-0.23*	-0.07	0.09	-0.15	0.09	0.17	-0.06
	Wetland	-0.09	0.07	-0.02	0.10	0.26*	0.09
		Forest	-0.68*	0.03	-0.69*	0.49*	-0.06
			Anthropogenic	-0.58	0.98*	-0.46*	-0.01
				Agriculture	-0.54*	0.04	0.09
					Road	-0.43*	-0.01
						Age	0.06
							Area

Table 4e: Correlations (Pearson's correlation coefficient) between the explanatory variables of the BRT model built using the visual survey data and the environmental DNA data (Visual + eDNA). Asterisk (*) represents significant correlation ($p < 0.05$). Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

Water	-0.24*	-0.11	0.18	-0.14	0.11	0.15	-0.05
	Wetland	-0.02	-0.38*	0.03	-0.34*	0.30*	0.09
		Forest	-0.43*	-0.13	-0.41*	0.34*	-0.05
			Anthropogenic	-0.34*	0.86*	-0.41*	-0.05
				Agriculture	-0.30*	0.05	0.07
					Road	-0.43*	-0.06
						Age	0.07
							Area

Table 4f: Correlations (Pearson’s correlation coefficient) between the explanatory variables of the BRT model built using the environmental DNA data (eDNA). Asterisk (*) represents significant correlation ($p < 0.05$). Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

Water	-0.31*	-0.21	0.17	0.20	-0.03	0.16	-0.05
	Wetland	0.40*	-0.51	-0.42*	-0.49*	0.50*	0.17
		Forest	-0.49*	-0.50*	-0.48*	0.40*	-0.09
			Anthropogenic	-0.11	0.83*	-0.47*	-0.06
				Agriculture	0.01	-0.28	-0.07
					Road	-0.55*	-0.07
						Age	0.10
							Area

7 FIGURES

Figure 1: A Blanding's turtle (*Emydoidea blandingii*) nesting on the shoulder of a road in eastern Ontario, Canada.

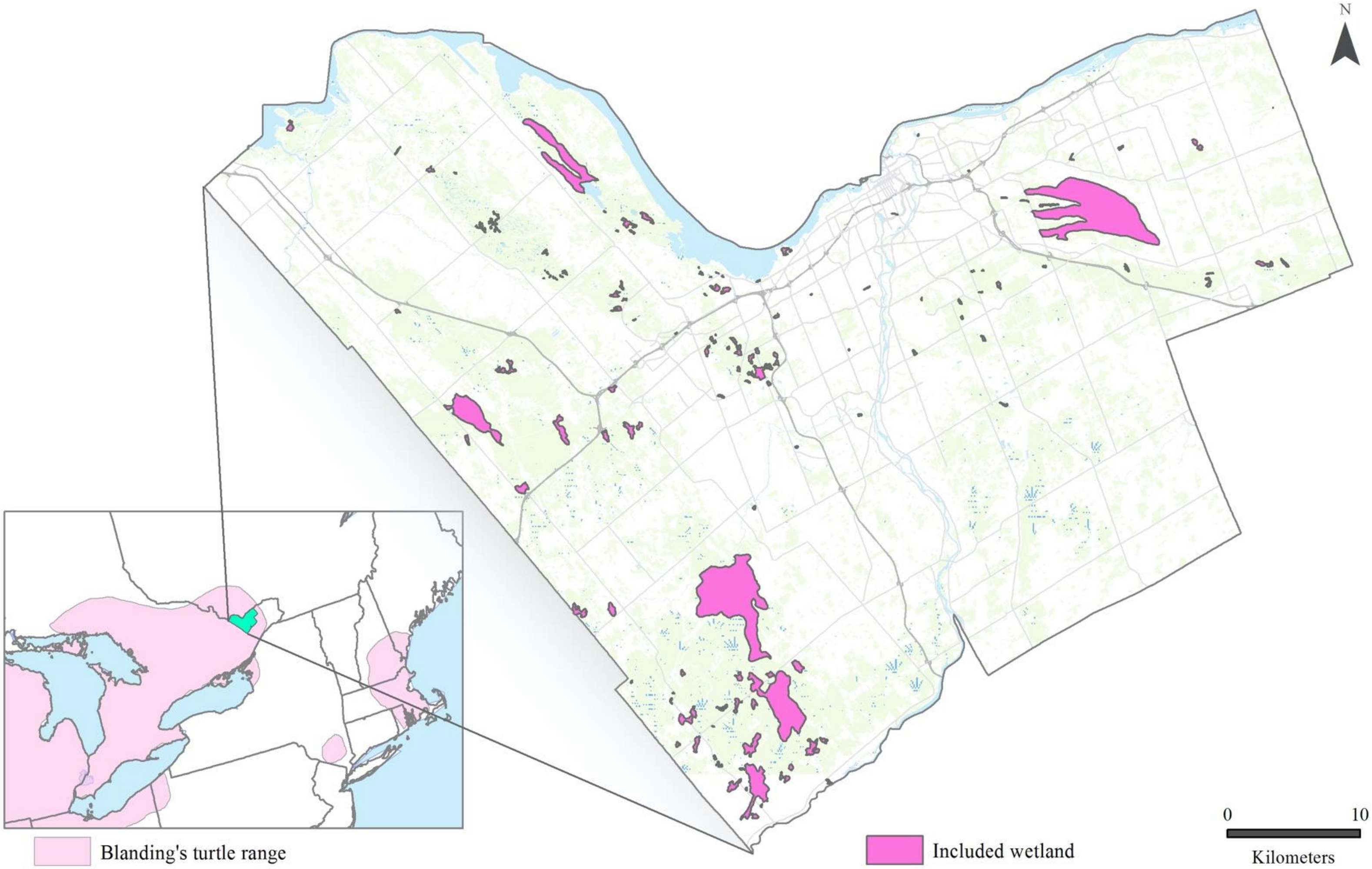

Figure 2: Map of the study area where the effect of landscape composition on the probability of wetland (n = 155) occupancy by Blanding's turtles was studied in eastern Ontario, Canada.

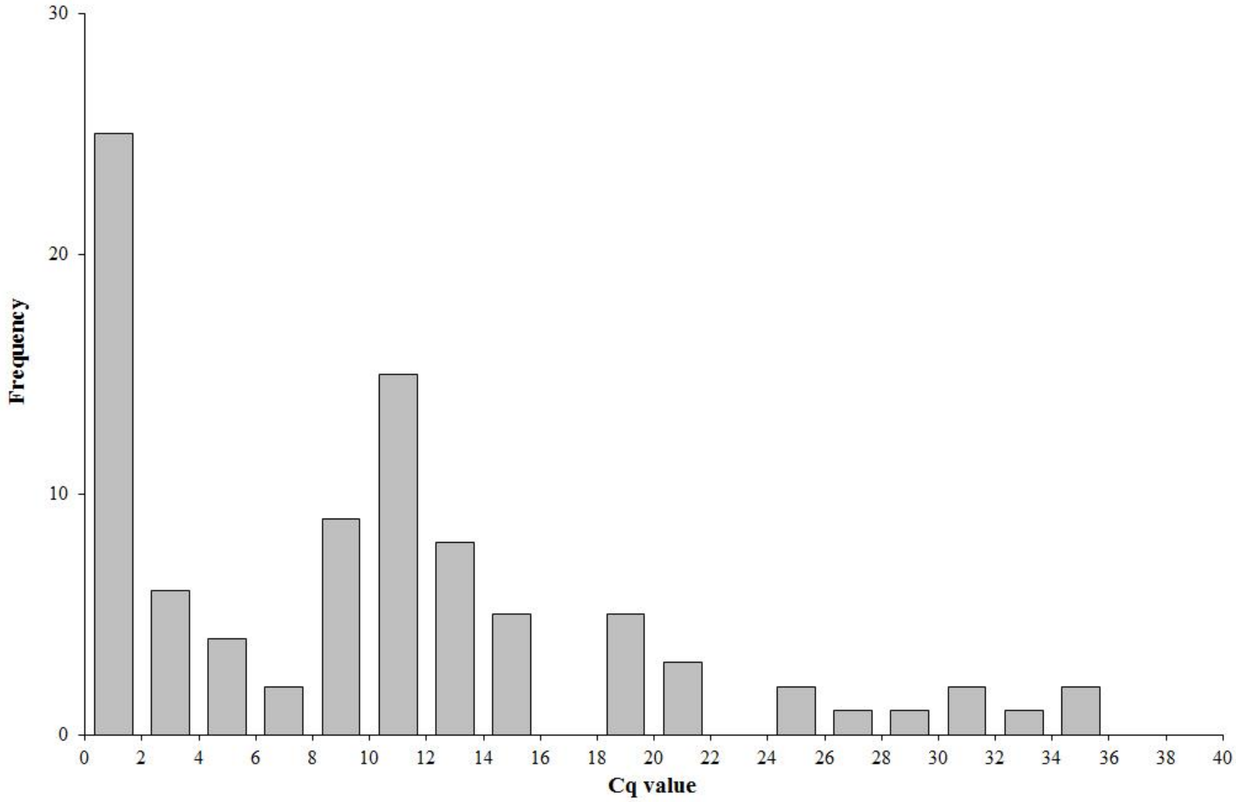

Figure 3: The frequency of quantification cycle values (Cq values) for the 92 of 345 positive control replicates that registered a Cq value.

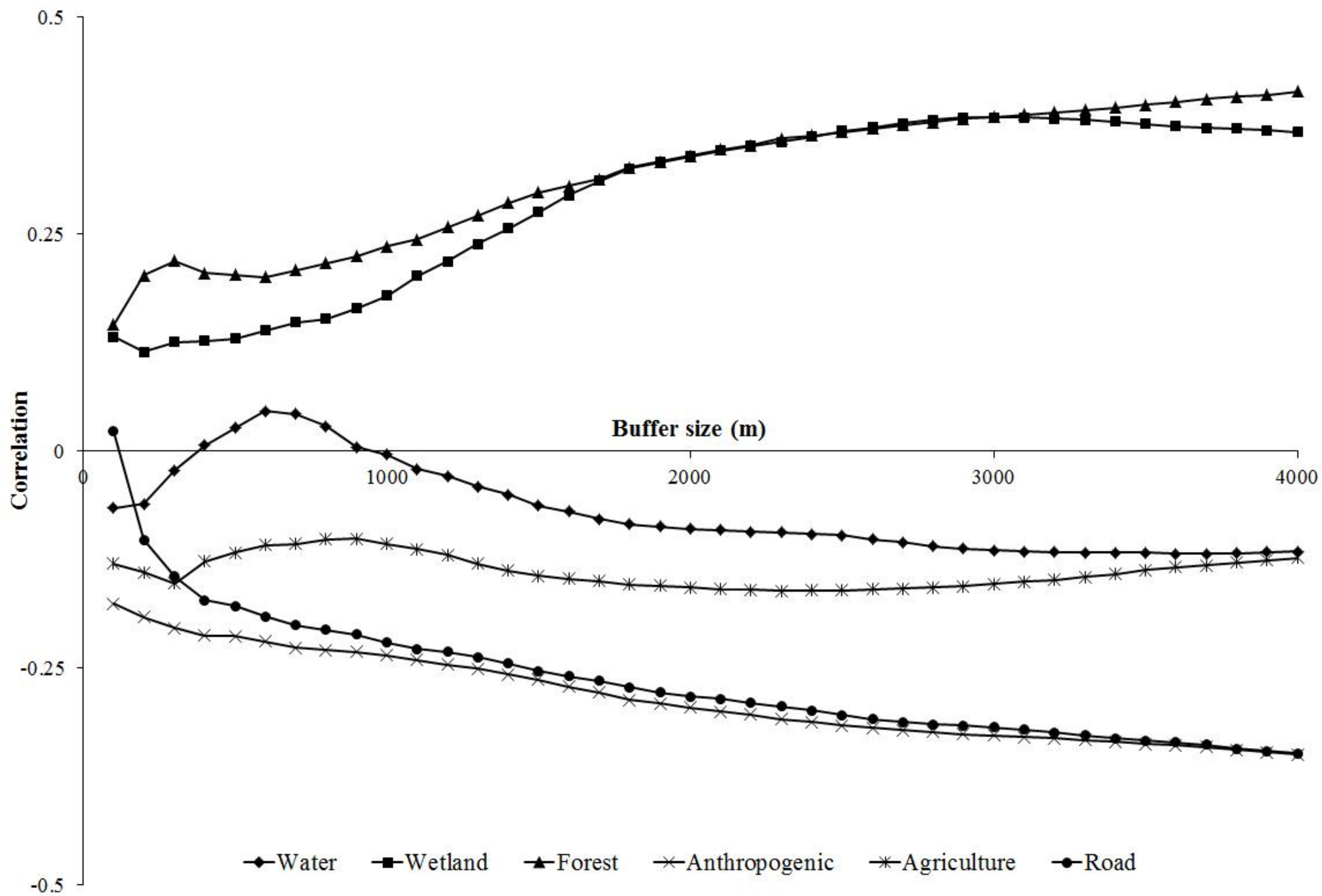

Figure 4a: Point biserial correlations between wetland occupancy by Blanding's turtles in eastern Ontario, Canada, and six landscape variables measured in buffers of increasing size (meters), calculated from 'Visual + ORAA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

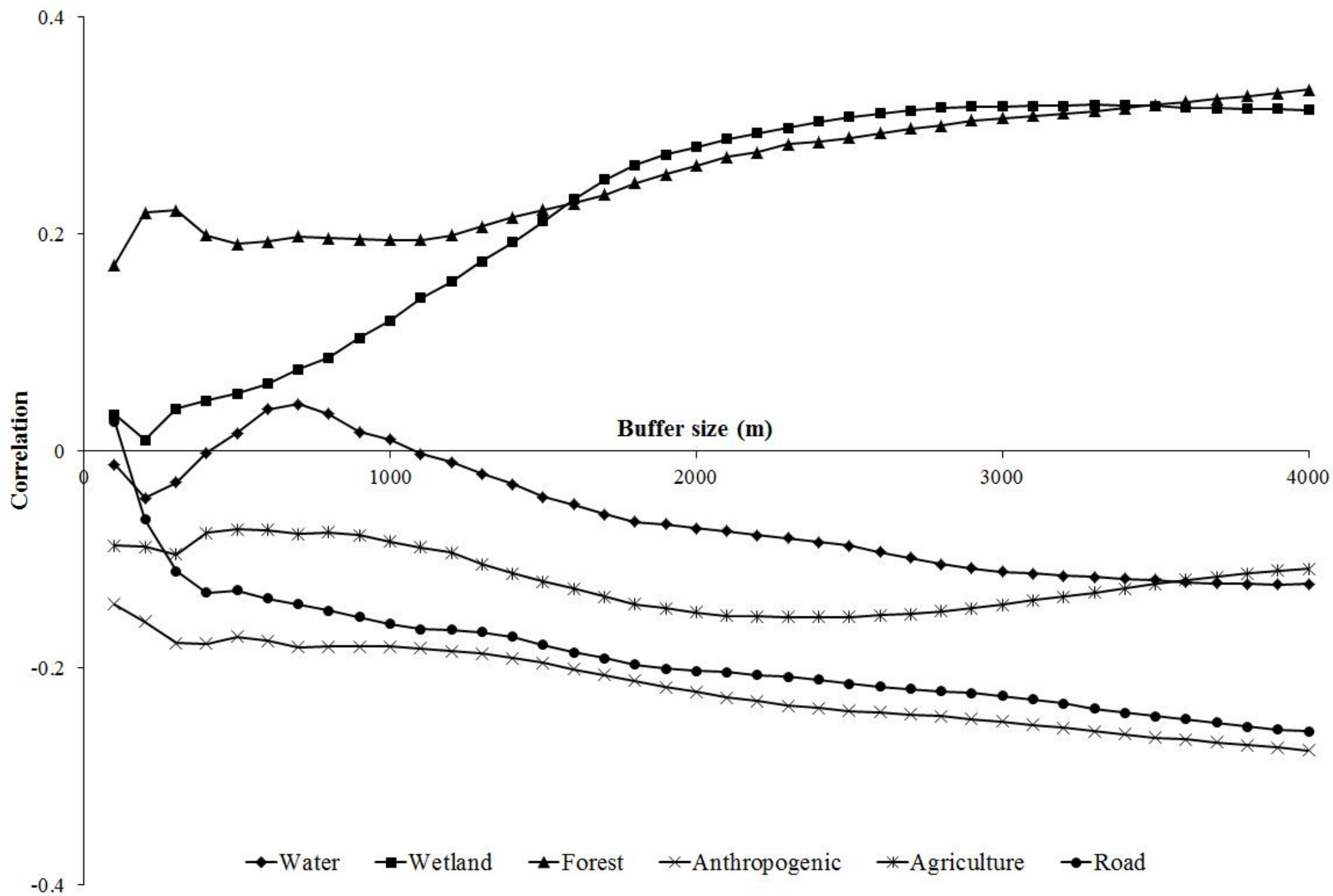

Figure 4b: Point biserial correlations between wetland occupancy by Blanding's turtles in eastern Ontario, Canada, and six landscape variables measured in buffers of increasing size (meters), calculated from 'Comprehensive' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

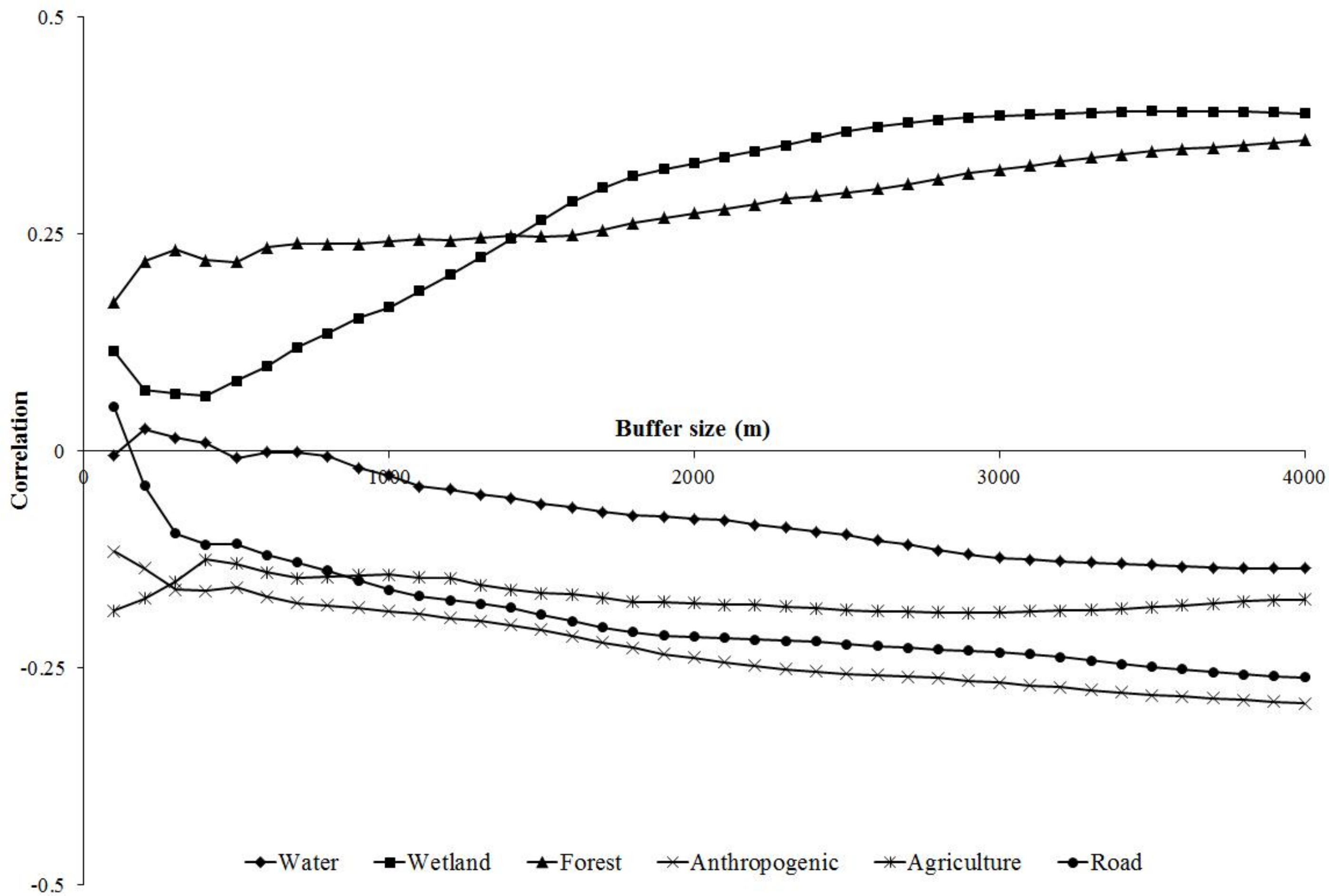

Figure 4c: Point biserial correlations between wetland occupancy by Blanding's turtles in eastern Ontario, Canada, and six landscape variables measured in buffers of increasing size (meters), calculated from 'eDNA + ORAA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

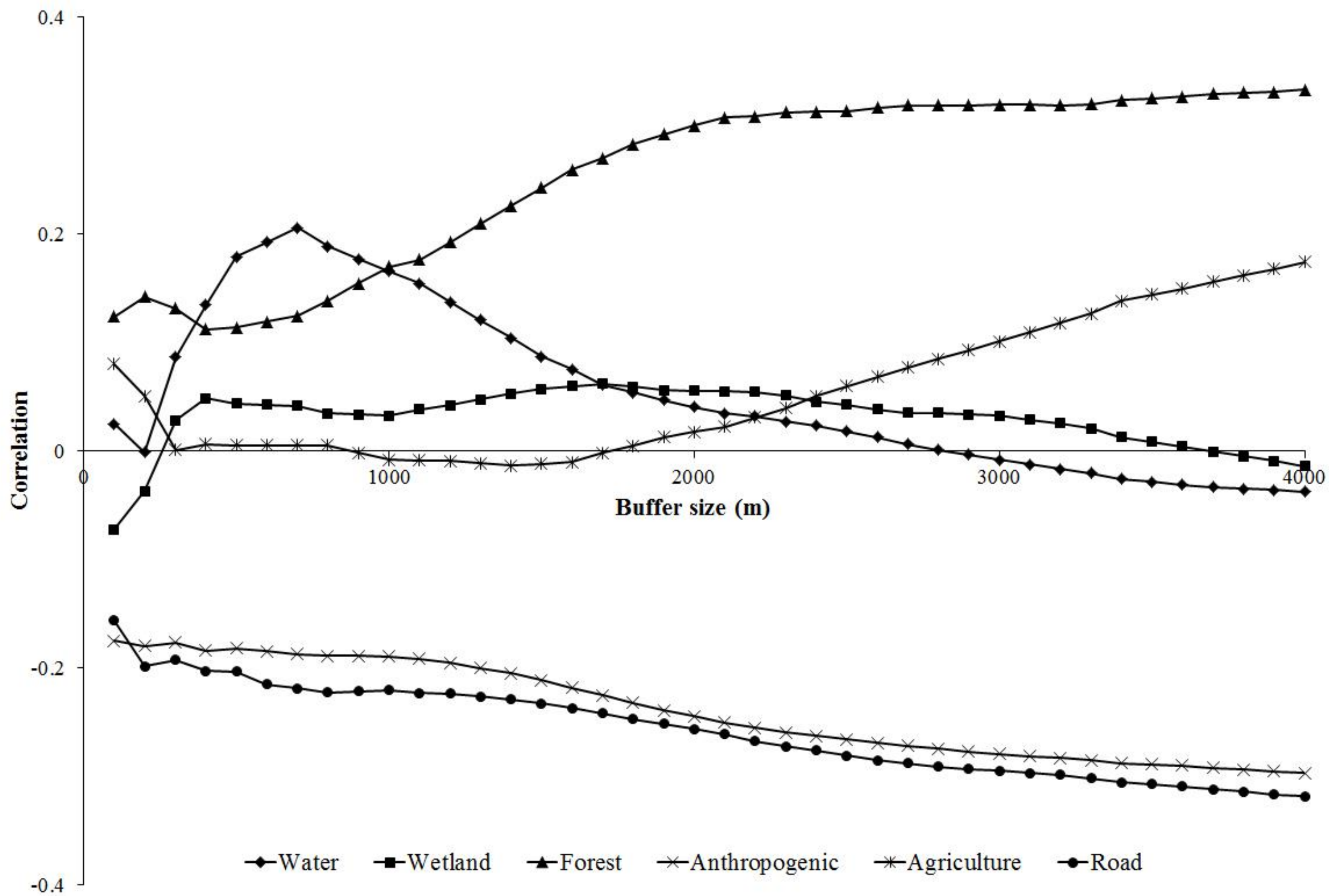

Figure 4d: Point biserial correlations between wetland occupancy by Blanding's turtles in eastern Ontario, Canada, and six landscape variables measured in buffers of increasing size (meters), calculated from 'Visual' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

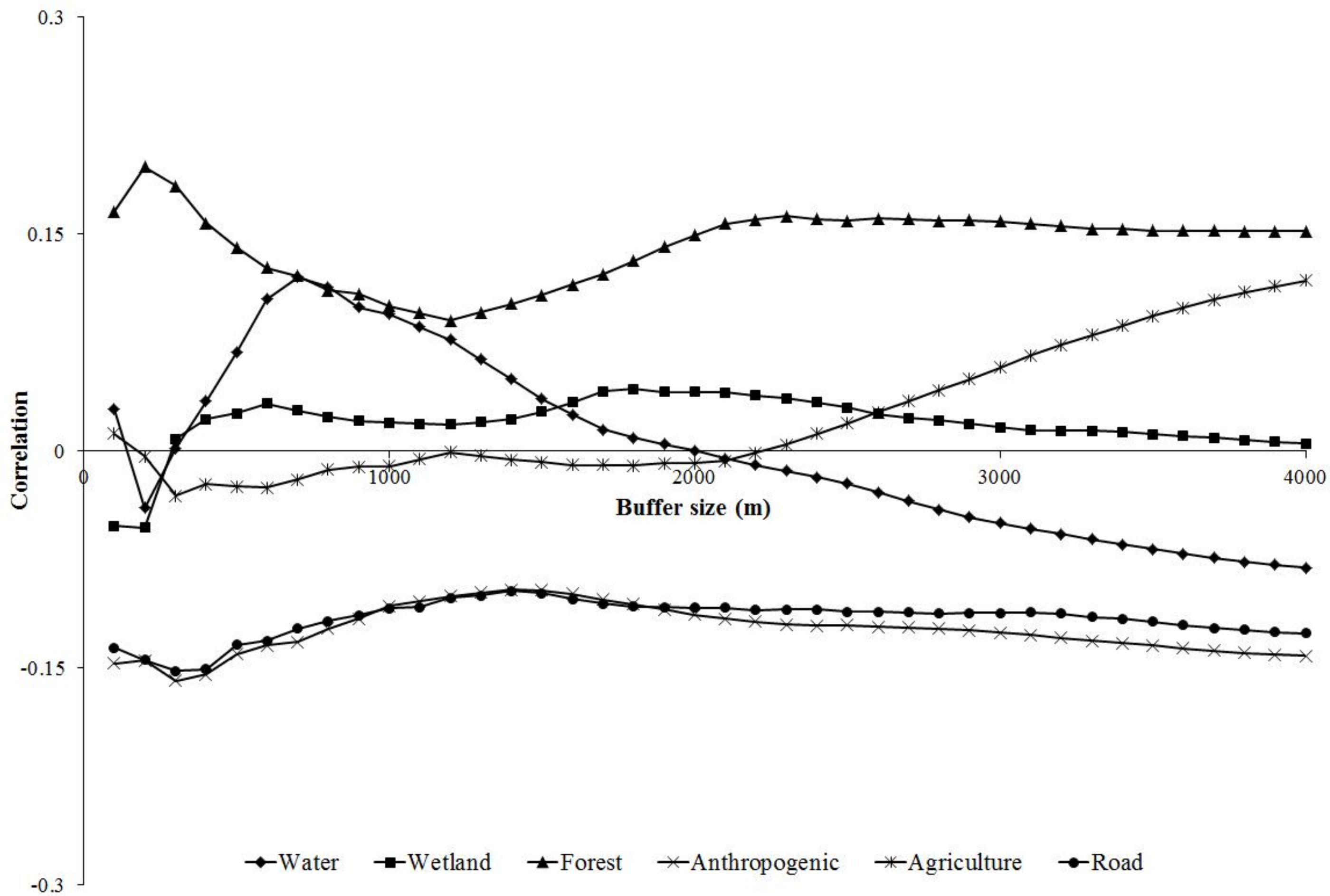

Figure 4e: Point biserial correlations between wetland occupancy by Blanding's turtles in eastern Ontario, Canada, and six landscape variables measured in buffers of increasing size (meters), calculated from 'Visual + eDNA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

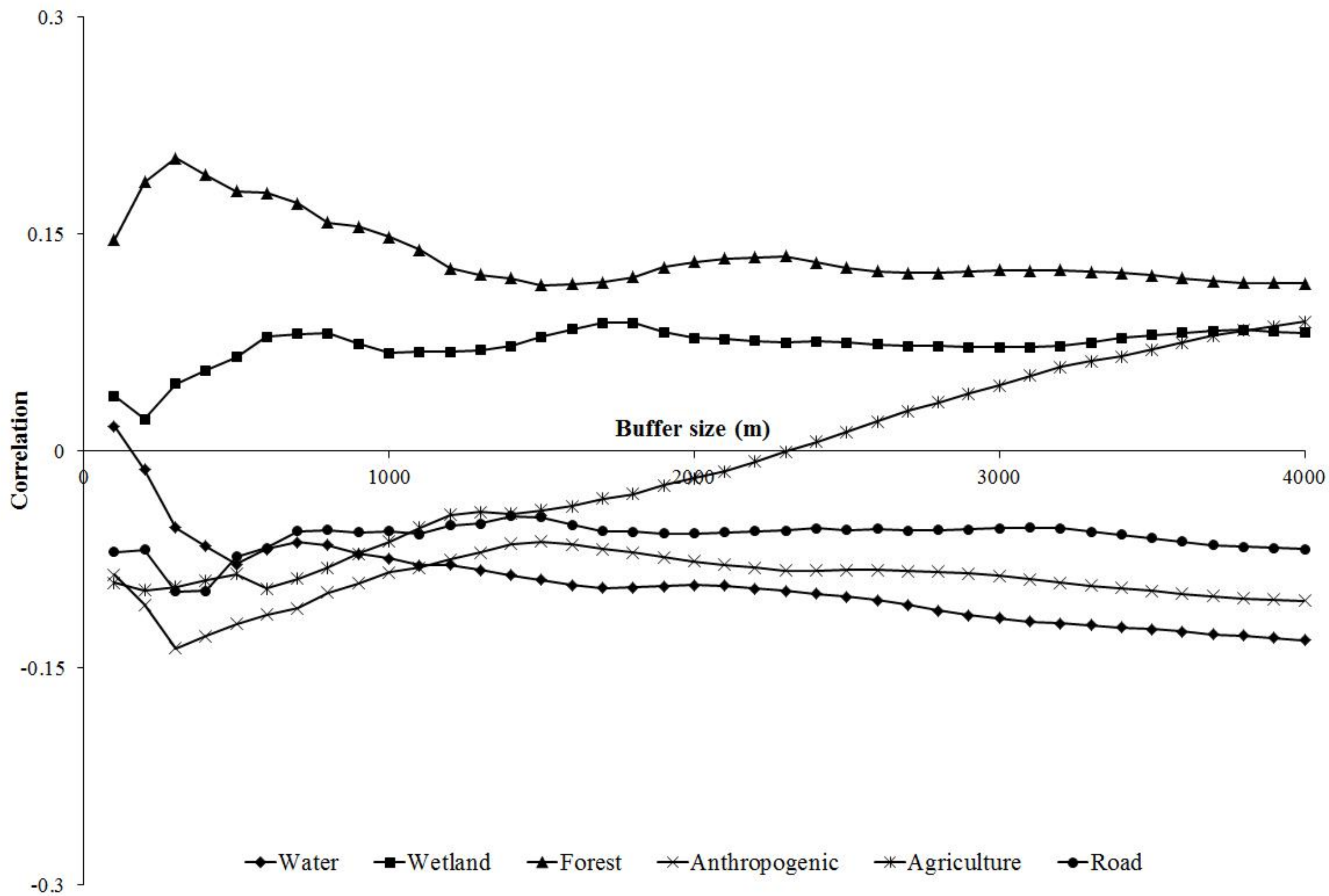

Figure 4f: Point biserial correlations between wetland occupancy by Blanding's turtles in eastern Ontario, Canada, and six landscape variables measured in buffers of increasing size (meters), calculated from 'eDNA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2).

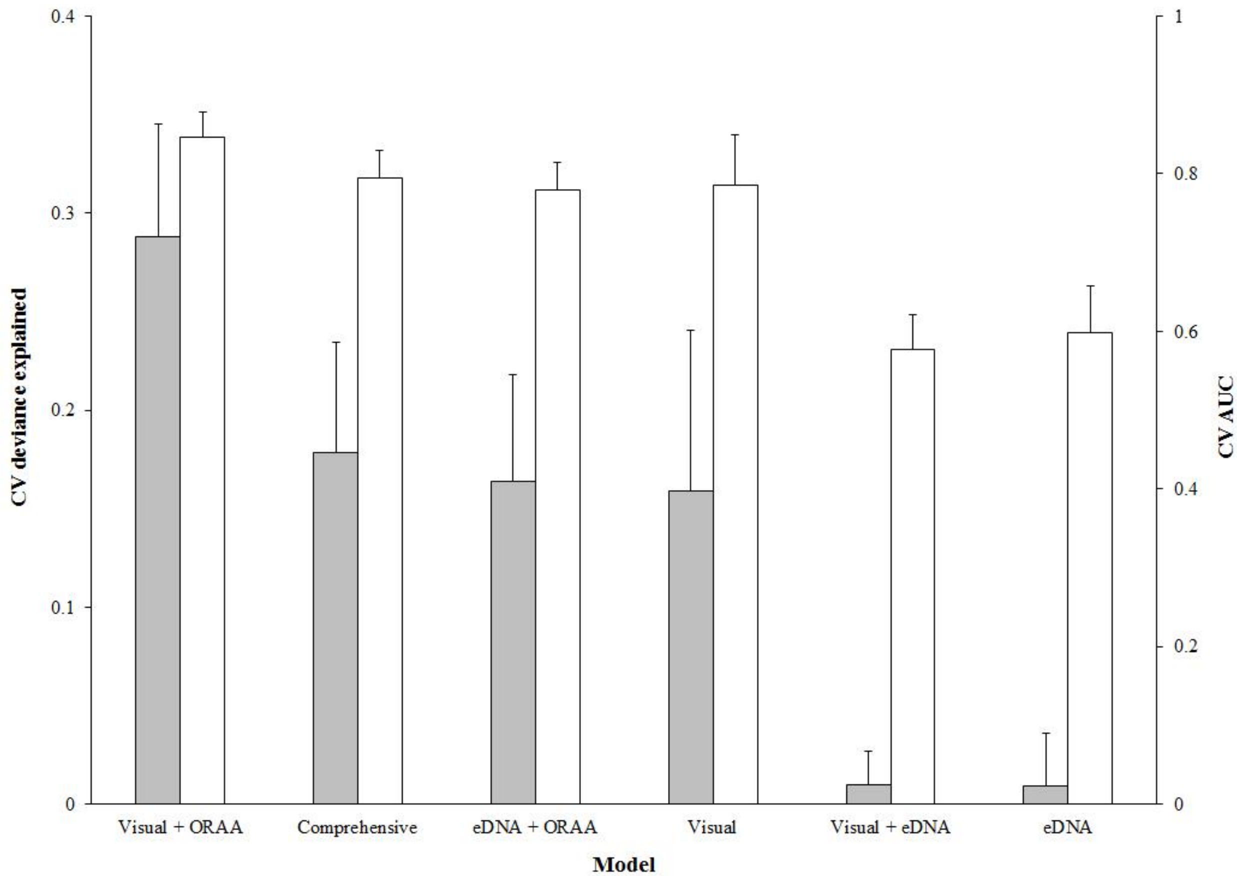

Figure 5: Percent deviance explained (based on cross-validation (CV) deviance; grey bars) and cross-validation area under the receiver operating curve (CV AUC; hollow bars) for the six BRT models used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada. Models are identified by their source data: Visual = data from the visual surveys; eDNA = data from the eDNA sampling; ORAA = data from the Ontario Reptile and Amphibian Atlas; Comprehensive = data from Visual, eDNA, and ORAA. Error bars represent standard error.

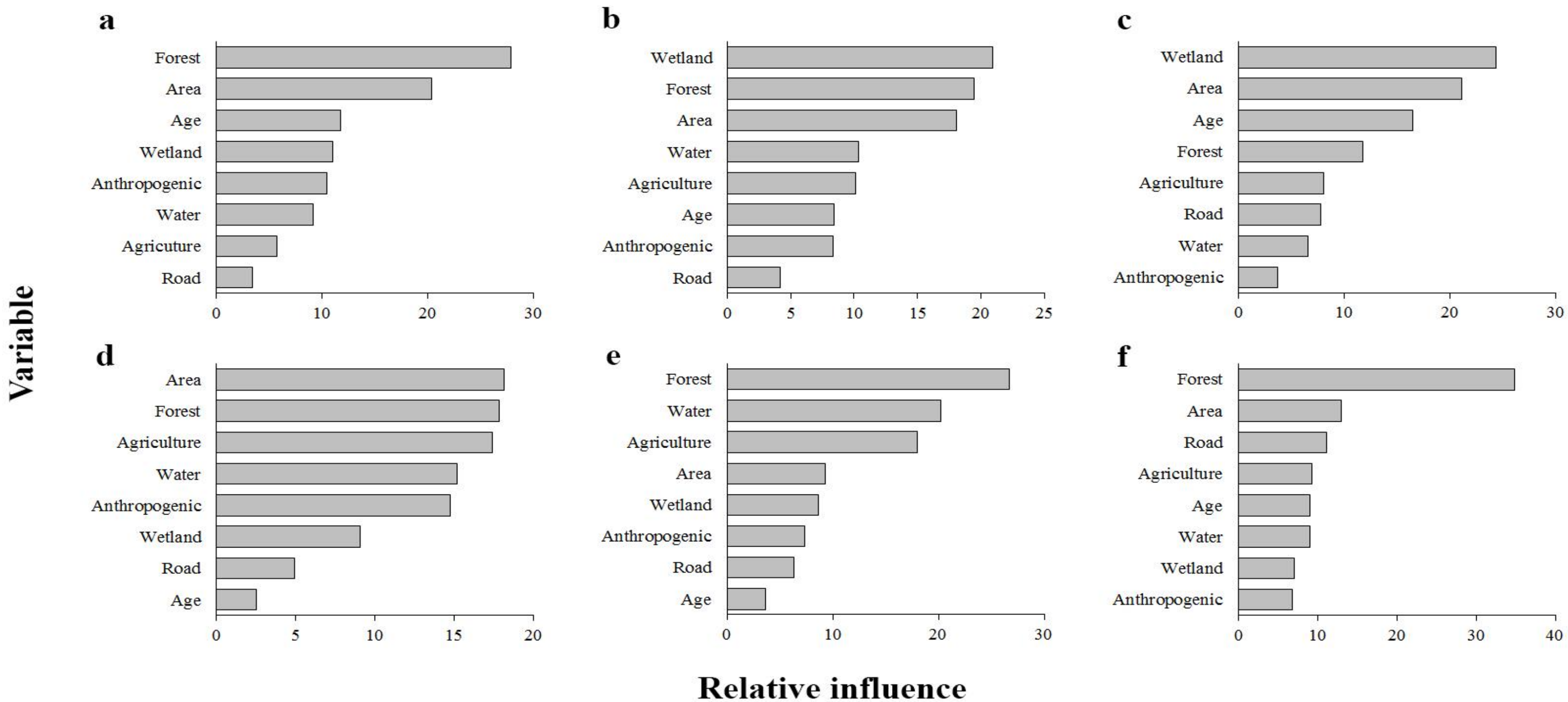

Figure 6: Relative influence (%) of the explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the six BRT models. 'a' = Visual + ORAA model; 'b' = Comprehensive model; 'c' = eDNA + ORAA model; 'd' = Visual model; 'e' = Visual + eDNA model; 'f' = eDNA model. Visual = data from the visual surveys; eDNA = data from the eDNA sampling; ORAA = data from the Ontario Reptile and Amphibian Atlas; Comprehensive = data from Visual, eDNA, and ORAA. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

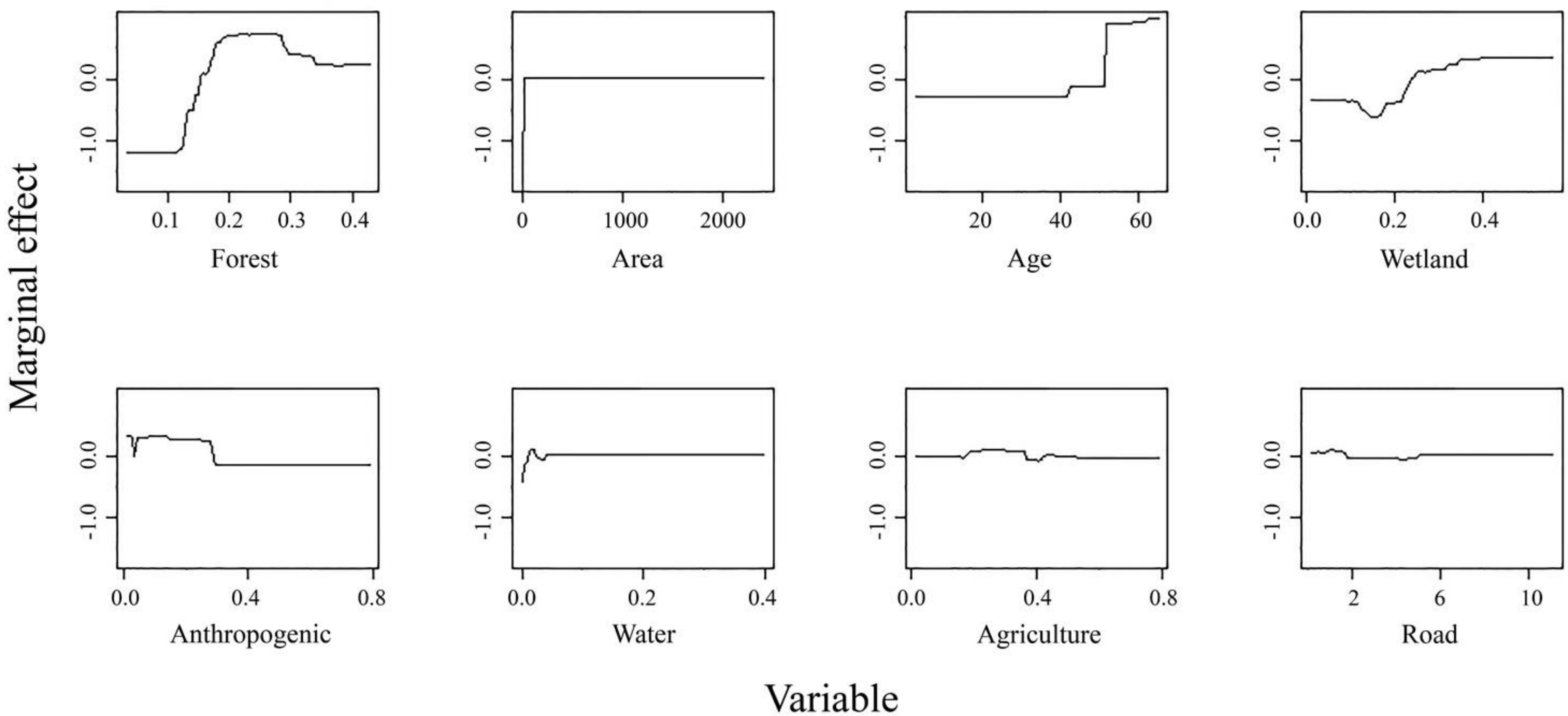

Figure 7a: The marginal effects of the eight explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the BRT model built using the 'Visual + ORAA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

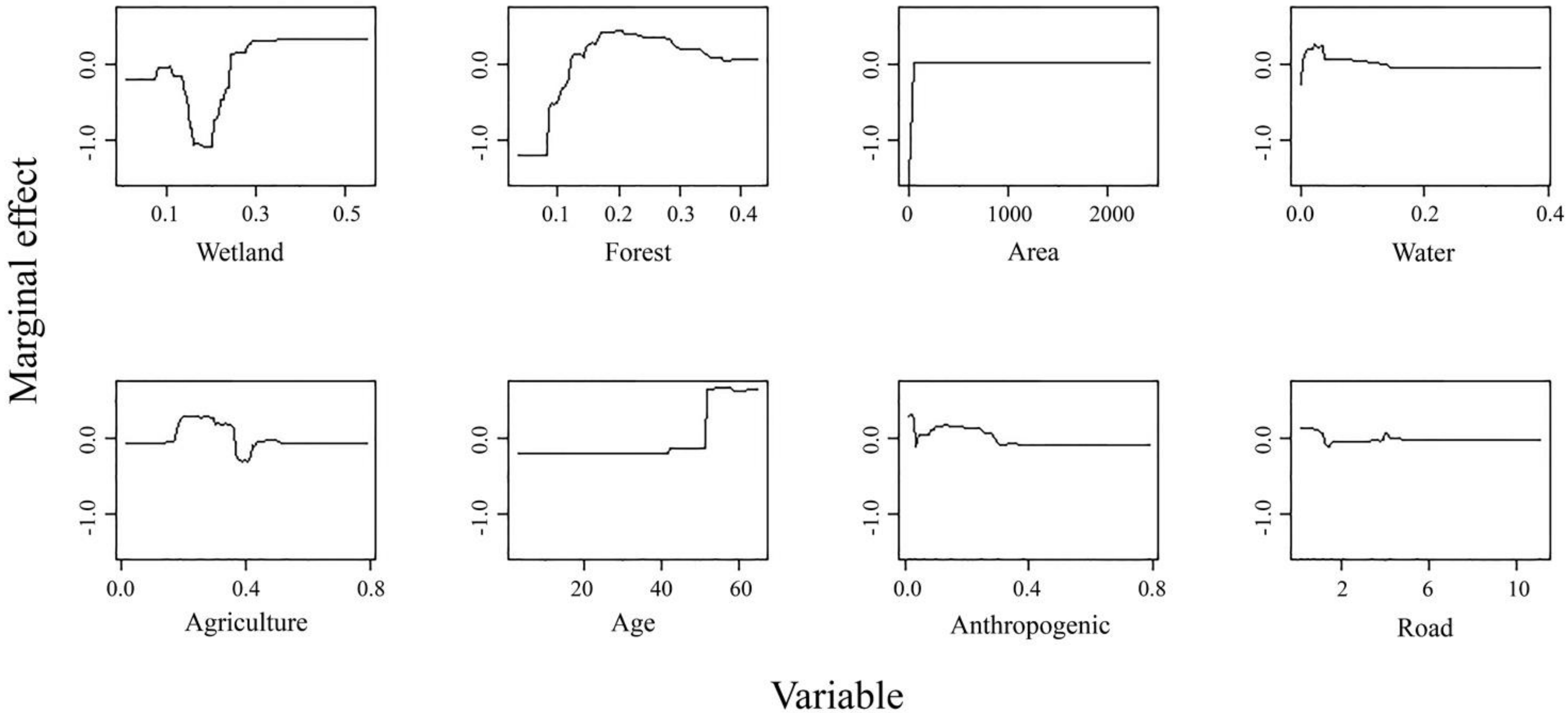

Figure 7b: The marginal effects of the eight explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the BRT model built using the 'Comprehensive' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

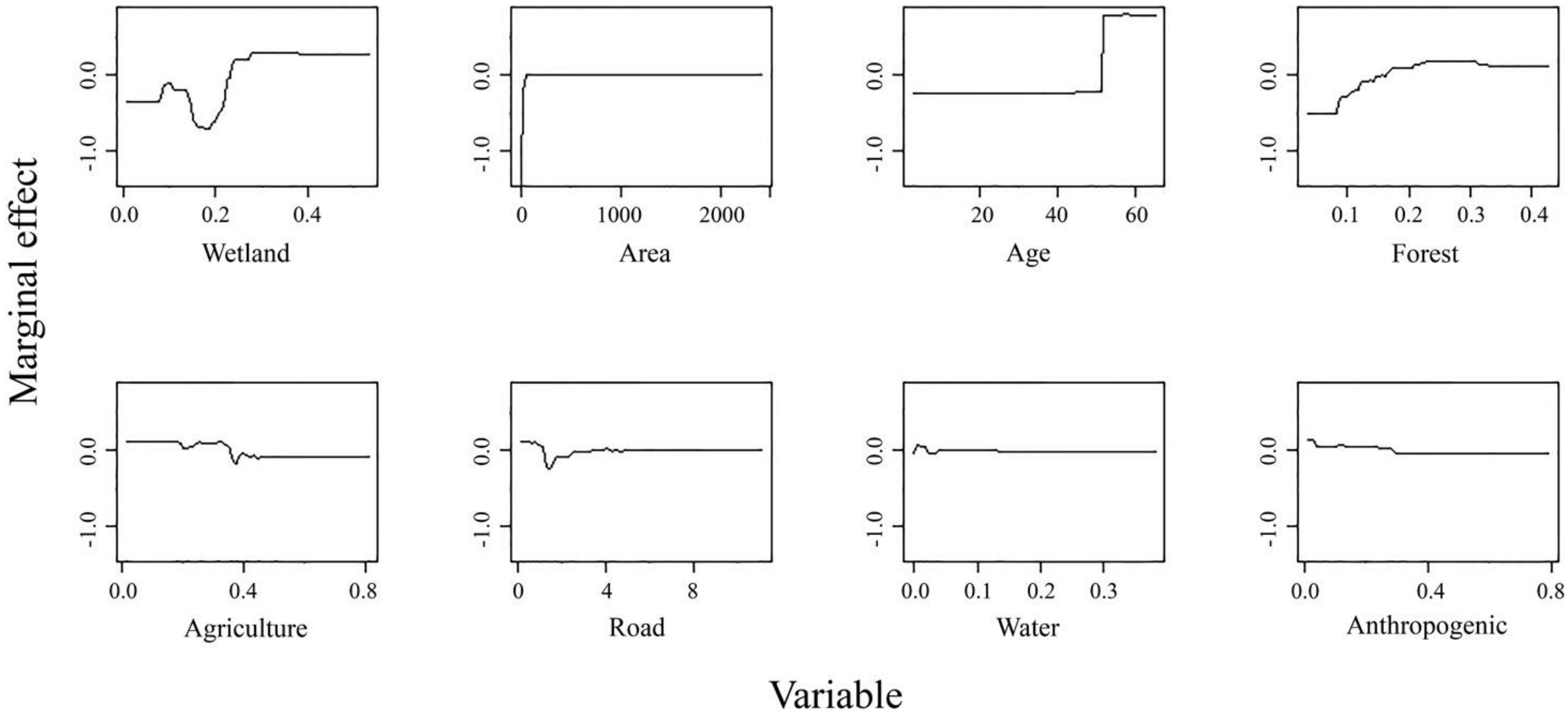

Figure 7c: The marginal effects of the eight explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the BRT model built using the 'eDNA + ORAA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

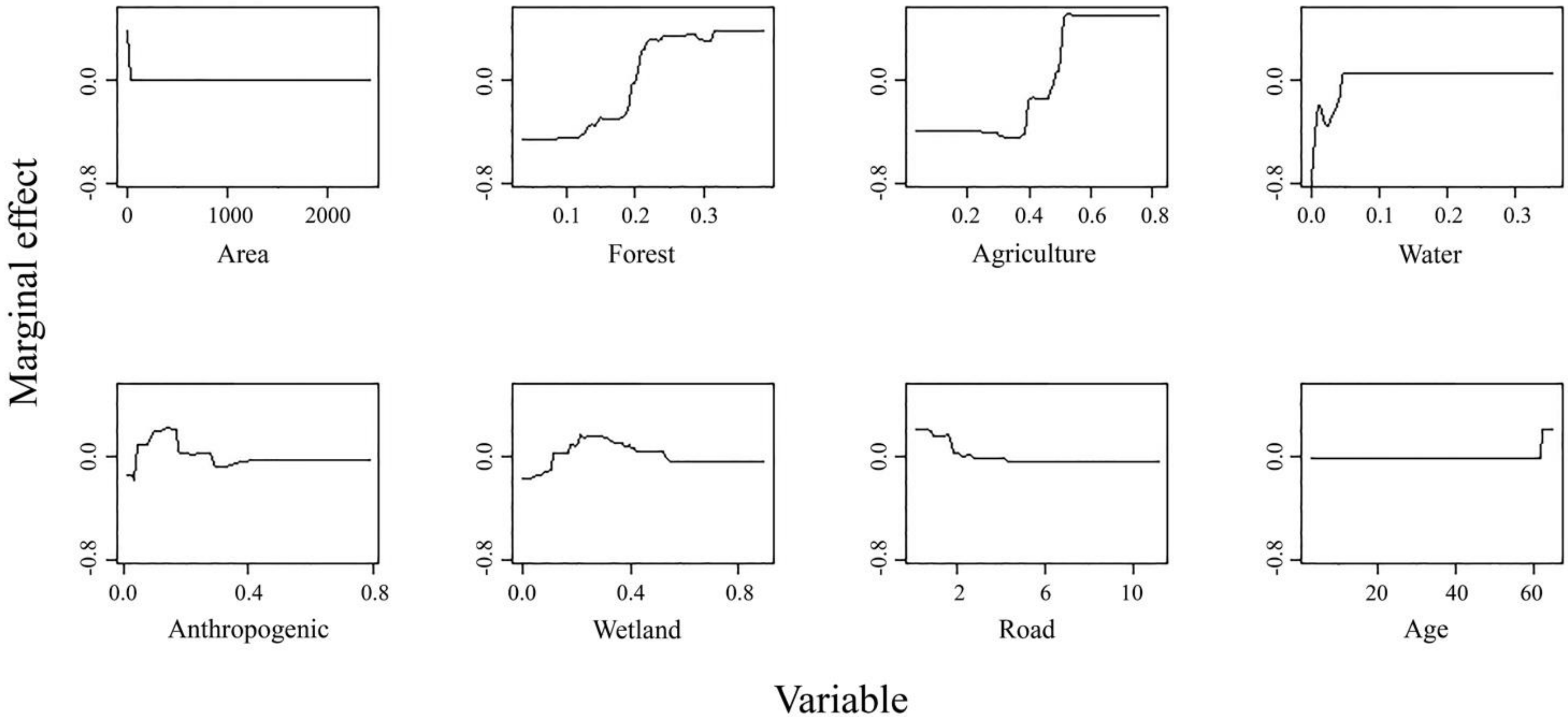

Figure 7d: The marginal effects of the eight explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the BRT model built using the 'Visual' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

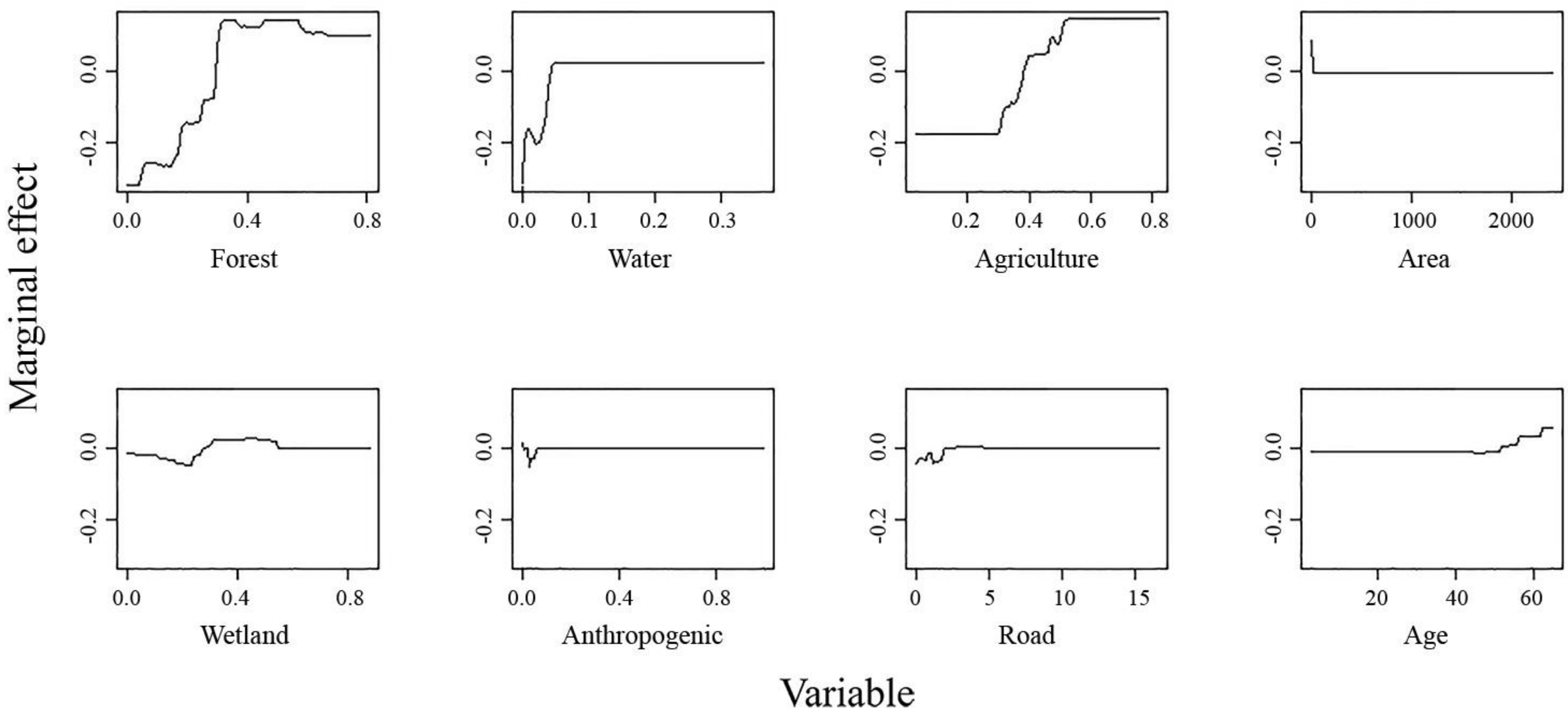

Figure 7e: The marginal effects of the eight explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the BRT model built using the 'Visual + eDNA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

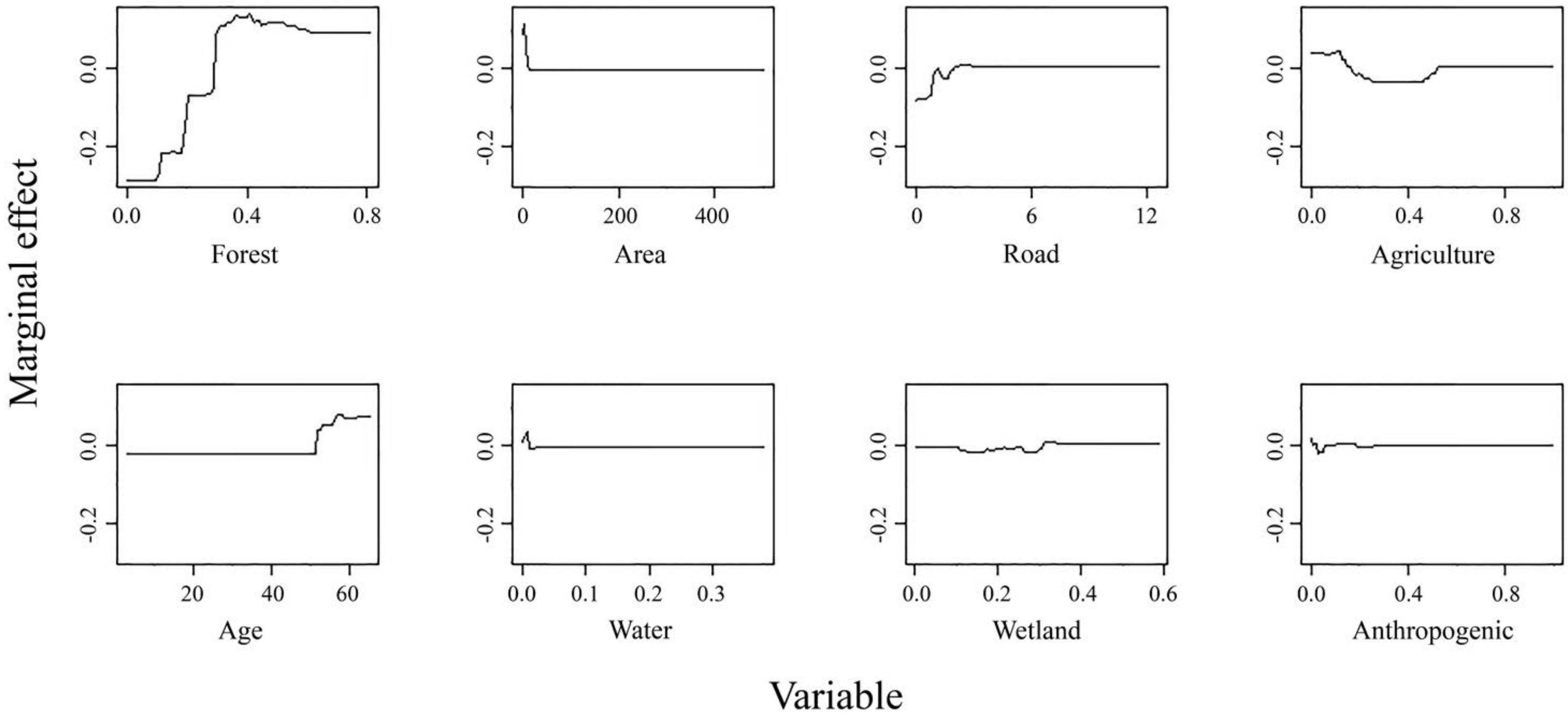

Figure 7f: The marginal effects of the eight explanatory variables used to predict wetland occupancy by Blanding's turtles in eastern Ontario, Canada, as determined by the BRT model built using the 'eDNA' data. Water = open water proportion; Wetland = wetland proportion; Forest = forest proportion, Anthropogenic = anthropogenic land proportion; Agriculture = agricultural land proportion; Road = road density (km/km^2); Age = wetland age (years); Area = wetland area (hectares).

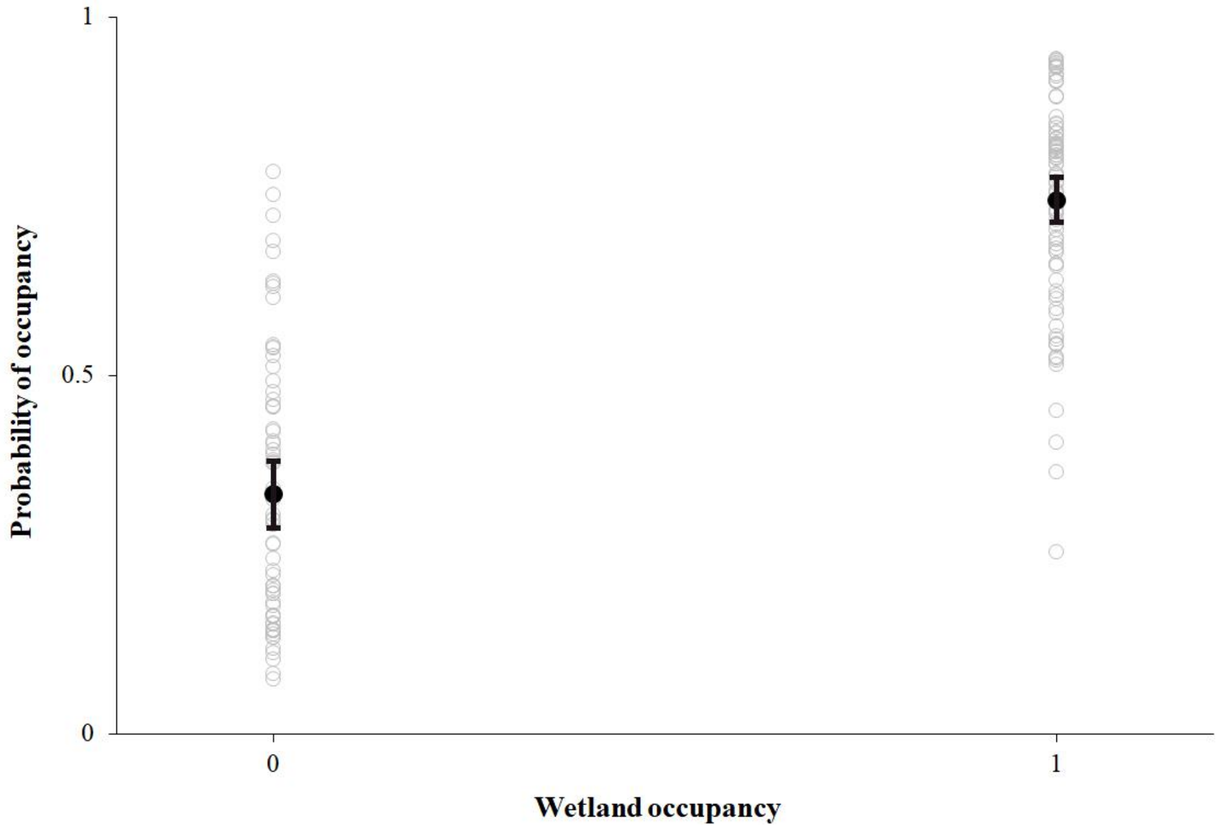

Figure 8: Probability of wetland occupancy by Blanding's turtles for all 155 wetlands, as predicted by the BRT model built using data from the visual surveys, the eDNA data, and the Ontario Reptile and Amphibian Atlas (Comprehensive). Wetlands are sorted by Blanding's turtle occupancy (0 = unoccupied; 1 = occupied). Filled circles represent the mean values (0.33 for unoccupied wetlands; 0.74 for occupied wetlands) with 95% confidence intervals.

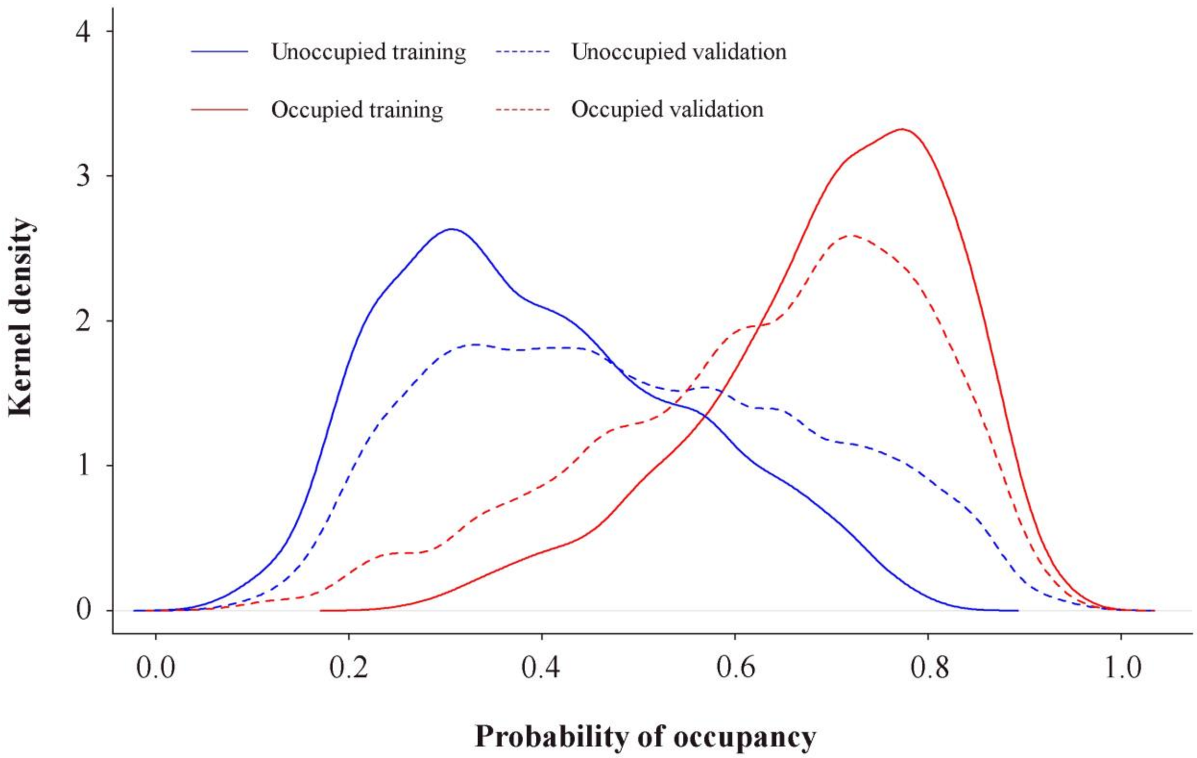

Figure 9: Kernel density plots for the probability of wetland occupancy by Blanding's turtles as predicted by 100 BRT models, each built using a different random subset of 100 wetlands from the 155 wetlands of the Comprehensive dataset (visual surveys, eDNA data, and the Ontario Reptile and Amphibian Atlas). Wetlands are sorted by Blanding's turtle occupancy (blue = unoccupied; red = occupied) and data source (solid = training wetlands; dashed = validation wetlands). All density curves have a bandwidth of 0.025 and n = 4329 for unoccupied training, n = 5671 for occupied training, n = 2371 for unoccupied validation, and n = 3129 for occupied validation.

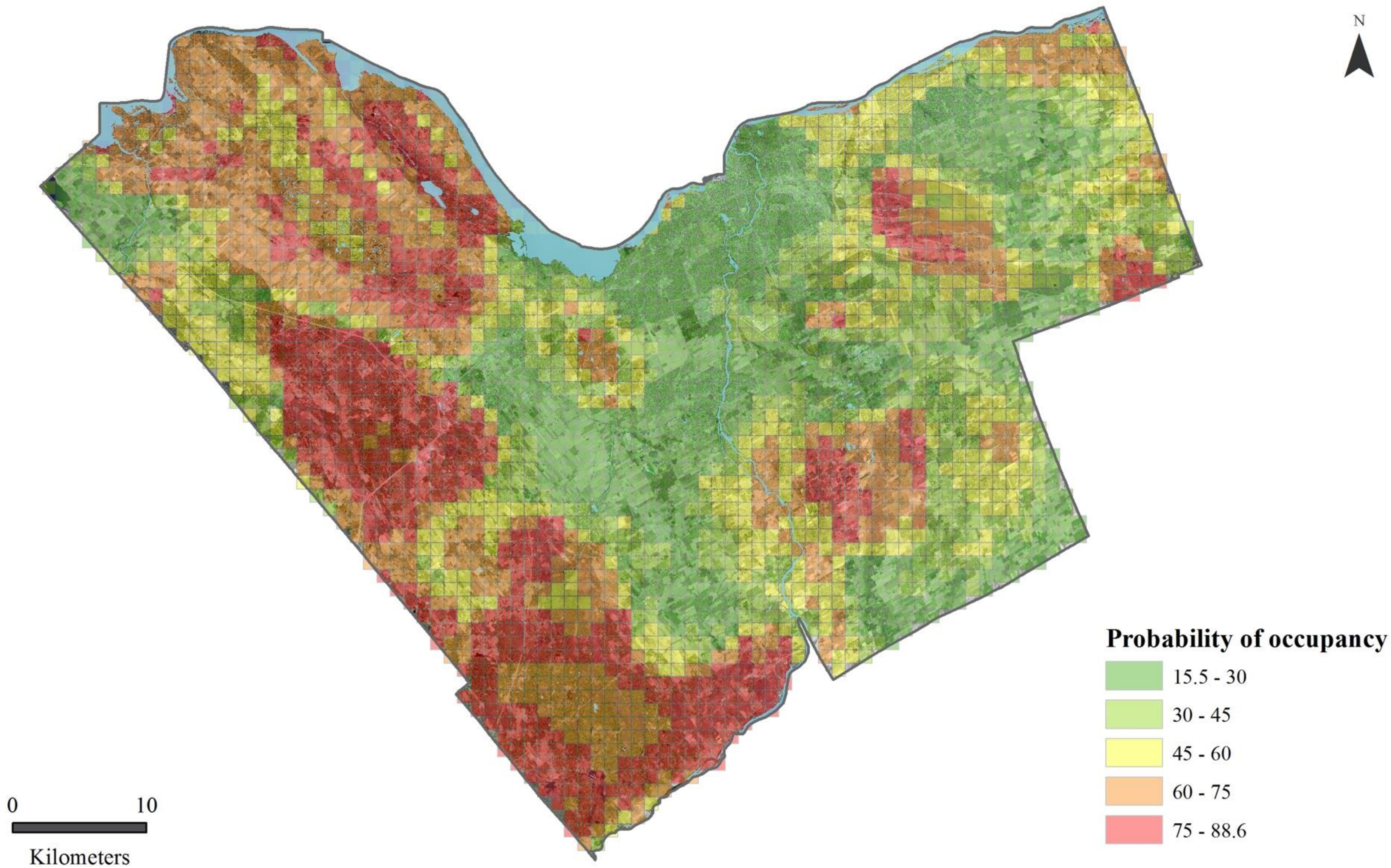

Figure 10: Probability of wetland occupancy by Blanding's turtles (%) predicted to 1 km grid cells across the study area. Predictions were made using a BRT model built using the 'Comprehensive' data and wetland proportion, forest proportion, anthropogenic land proportion, agricultural land proportion, and road density as explanatory variables.

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APPENDIX

Appendix 1:

DNA extraction and amplification protocol

2020-02-25 L.D.

Liste des étapes effectuées en laboratoire permettant de détecter la présence ou l'absence de spécimens de tortue mouchetée et de rainette faux-grillon dans les plans d'eau échantillonnés – Projet CCN 2018 et 2019 – Vincent Fyson

1) Extraction de l'acide nucléique contenu dans les filtres. – se référer à :
    - « Extraction d'ADN - FILTRE à sec - version courte »
    - « Protocole d'extractions d'ADNe de filtres » (version très détaillée)

    À noter que les filtres de 2018 ont été incubés durant la nuit (environ 15h) alors que les filtres de 2019 ont été incubé pour une période de 2h tel qu'indiqué aux protocoles mis en références.

2) Dilution 10X des ADNe extraits
3) Réactions qPCR – se référer à :
    - Tableaux 1 pour connaître les paramètres de température utilisés
    - Tableau 2-1 et 2-2 pour connaître les volumes de chacun des réactifs

Tableau 1– Paramètres de température utilisés pour les réactions qPCR – volume total : 20ul

Étapes	Température	Durée
1- Dénaturation initiale	95°C	2 minutes
2- Dénaturation	95°C	15 secondes
3- Hybridation	60°C	30 seconde – lecture
39 répétitions des étapes de dénaturation et d'hybridation		

Tableau 2-1 -Volume des composantes d'une réaction qPCR-sonde d'ADNe

Composantes	**Concentration initiale**	**Concentration finale**	**1 réaction (ul)**
SsoAdvanced Universal Probes Supermix	1 X	2 X	10
Amorce F	10 uM	0,25 uM	0,5
Amorce R	10 uM	0,25 uM	0,5
Sonde	10 uM	0,25 uM	0,5
ddH2O	s/o	s/o	4,5
ADNe 1/10	s/o	s/o	4
Volume final	s/o	s/o	20

Tableau 2-2-Volume des composantes d'une réaction qPCR d'ADNe pour les échantillons suivants :19.2500.E1, 19.2501.E1, 19.2546.E1, 19.2595.E1, 19.2628.E1, 19.2629.E1, 19.2660.E1, 19.2661.E1, 19.2692.E1, 19.2693.E1, 19.2759.E1, 19.2817.E1, 19.2818.E1, 19.2849.E1, 19.2850.E1, 19.2881.E1, 19.2882.E1, 19.2945.E1, 19.2946.E1, 19.212.E1, 19.213.E1, 19.232-1.E1, 19.232-2.E1, 19.257-1.E1, 19.257-2.E1, 19.281.E1, 19.282.E1, 19.283.E1

Composantes	**Concentration initiale**	**Concentration finale**	**1 réaction (ul)**
SsoAdvanced Universal Probes Supermix	1 X	2 X	10
Amorce F	10 uM	0,25 uM	0,5
Amorce R	10 uM	0,25 uM	0,5
Sonde	10 uM	0,25 uM	0,5
ddH2O	s/o	s/o	6,5
ADNe 1/10	s/o	s/o	2
Volume final	s/o	s/o	20

Transmis par Yann Surget-Groba le 2018-08-20

Peaufiné le 2018-10-31 et le 202-02-25 par L.D.

Note : ce protocole est la version courte des manipulations. Voir le protocole rédigé le 2019-11-18 GLC pour la version la plus détaillée. / Légères modifications apportés au protocole du 180820 YSG / 181031 LD pour lister les étapes utile que pour l'extraction de filtre à sec. L.D. 200225

Extraction d'ADN - FILTRE à sec - version courte.

Modifications et ajouts au protocole DNeasy Blood & Tissue kit

1) Incuber le filtre avec 540µl de tampon ATL et 20µl de protéinase K à 56°C avec agitation pendant 2h.

2) Transférer le filtre, dans un colonne QiaShredder et centrifuger à 20000g pendant 5'

3) Transférer le liquide dans le tube de 2ml qui contient le reste du mélange ATL-prot. K. (Jeter le filtre et la colonne QiaShredder.)

4) Ajouter 1200µl du mélange AL - éthanol (600ul AL et 600uL EtOH 100%) à l'échantillon et bien mélanger au vortex

effectuer une centrifugation rapide des tubes au besoin

5) Transférer 600µl de l'échantillon dans une colonne DNeasy, centrifuger 1' à 6000g, jeter le liquide et remettre le tube sur la colonne. Répéter autant de fois que nécessaire pour passer tout l'échantillon. *Note : Quatre transferts sont nécessaires*

6) Déposer la colonne DNeasy dans un nouveau « collection tube »

7) Ajouter 500 ul du tampon AW1 à la colonne DNeasy, puis centrifuger 1' à 6000g. Jeter tube + liquide

8) Déposer la colonne DNeasy dans un nouveau « collection tube »

9) Ajouter 500 ul du tampon AW2 à la colonne DNeasy, puis centrifuger 3' à 20 000g. Jeter tube + liquide

8) Déposer la colonne DNeasy dans un tube 1,5mL

9) Ajouter 100 ul du tampon AE à la colonne DNeasy, puis centrifuger 1' ;a 6000. Recueillir dans un tube de 1,5 ml. Refaire

!Attention! Les tampons AL, AW1 contiennent de la guanidine. Récupérer dans un contenant pour permettre une élimination sécuritaire!