Do Blanding's turtles in poor quality habitats experience reduced immunocompetence and increased parasitaemia?

By

Mélanie Lacroix
5764158

Supervisor: Dr. Gabriel Blouin-Demers

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u Ottawa

L'Université canadienne
Canada's university

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Table of Contents

Abstract

Human activities play an important role in the loss of biodiversity and habitat loss is the greatest known threat to the survival of most species at risk, such as Blanding's turtles. I tested the hypothesis that individuals in poor habitats are in poor condition. I assessed habitat quality by the land use in the home range and I assessed condition by heterophil:lymphocyte ratios and levels of parasitaemia. I expected to see an increase in H:L ratios and parasite counts as the proportion of anthropogenic lands and agricultural fields in the home range increased. This study was conducted at five study sites in southwestern Québec. A total of 42 turtles were monitored by radio-telemetry and location data were used to determine the MCP of each turtle. Blood samples were drawn from each turtle and leukocyte counts were done. I found that immunological response varied negatively with the density of roads whereas parasitaemia increased as a function of proportion of wetlands.

Introduction

It is well established that human activity is the largest threat to species throughout the world, and habitat loss is the most common cause of species endangerment (Tilman *et al*., 1994). Urban developments across the globe are continuously expanding their limits, slowly covering more and more surface, altering and destroying habitats in the process (Dietz and Adger, 2003). These environmental changes can lead to important changes in population sizes, densities and even distribution (Kim and Byrne, 2006). Furthermore, additional habitat loss has a greater impact on remaining populations driving species to decline drastically (Fahrig, 2003; Tilman *et al.* 1994). We understand that biodiversity plays a crucial role in our lives by providing important ecological services such as decomposition, ecosystem balance, primary production, nutrient and CO_2 flux, etc. (Connery, 2009; Dobson *et al*., 2006; Thompson and Starzomski, 2007). It is therefore no surprise that the effects of human activities on biodiversity have become a growing concern worldwide.

Habitat is generally defined as a portion of land that can support the population of a given species providing necessities crucial for survival. Therefore, habitat loss implies that the environment can no longer support the needs of individuals, impeding their survival and reproductive success. While it is clear that complete habitat destruction has a great impact on biodiversity, we must recognize that these events are much less frequent than reductions in habitat quality. For instance, pollution affects a wide variety of ecosystems rendering habitats less suitable for populations of all kinds (McNeely, 1992). Past studies have focused their attention on this problem in terrestrial habitats (Giordani *et al*., 2002; Hafez and Elbestawy, 2009; Szaro *et al*., 2002) as well as aquatic ones (Fabricius and De'ath, 2004; Freedman and Beauchamp, 1998). Moreover, conversion of lands for agricultural, industrial or residential

purposes can cause loss of biodiversity (McNeely, 1992). Fragmentation is also often a result of these types of modifications, creating patches and increasing edge to area proportions (Fahrig, 2003; Tilman *et al*., 1994). This can impede species dispersal which can in turn have a significant impact on population dynamics (Fahrig, 1997, 2003; Franklin *et al.* 2002).

In this study, I will be using data on Blanding's turtles, *Emydoidea blandingii*, to determine the effect of habitat quality on fitness. Specifically, I will test the hypothesis that poor habitat quality compromises immunocompetence and increases parasite loads in Blanding's turtles. *Emydoidea blandingii* populations are classified as endangered in Nova Scotia (Canada) and threatened in the Great Lakes and St-Lawrence region (COSEWIC). To this day we know that the Blanding's turtle is especially affected by fragmentation of their home range, road mortality and predators because of their life cycle (low reproductive output and late maturity) and distribution (Roe *et al*., 2011; Browne and Hecnar, 2007). Thus, grasping how fitness varies with habitat quality can help guide our conservation efforts.

Habitat quality is measured by a wide variety of techniques. Many studies have used a direct approach for evaluating habitat quality by measuring different factors such as pH, temperature, forest cover, inclination, soil components, water characteristics, etc. (Johnson, 2007). Others compiled such measures to create an index that allows them to rank habitat patches by quality. Many scales exist to accommodate different measures available to the researcher; some examples include IBI (Index of Biotic Integrity), IEC (Index of Environmental Condition) or IEI (Index of Ecological Integrity) (Diaz *et al*., 2004). In this study, I defined habitat quality by the different components included in an individual's home range. In total six categories will be used to describe the environment of a given turtle: proportion of wetlands, agricultural lands, forests, open water, anthropogenic lands, and other (all types of lands that are not included in the

above mentioned categories). Habitat selection studies concerning Blanding's turtles have shown that this species selects wetlands surrounded by forest cover during their active season (Beaudry *et al.* 2009; Millar and Blouin-Demers, 2011; Rowe and Moll, 1991). I therefore identify agricultural, anthropogenic and other types of lands that have been altered as poor quality habitats for Blanding's turtles.

Fitness, which is described as an individual's capacity to survive and pass on genes to the next generation, is at times difficult to measure directly in a field setting (Groom *et al*., 2006; Johnson, 2007). Therefore, I will be evaluating physiological factors relevant to health instead of measuring fitness directly. For instance, immune system response measures have been employed in previous studies to assess the physiological status of turtles in the presence of disease. An increased immune system is a reflection of poor health and therefore lowered fitness (Auld *et al.,* 2012; Tavares-Dias *et al*., 2009). White blood cell analyses are widely used as indicators of immunocompetence. Hence, I will be interpreting the immunological investment of individuals at a point in time by examining leukocytes found in the peripheral blood stream. More specifically, I will be observing the presence of heterophils and lymphocytes as these particular leukocytes vary in number as disease or environmental stressors are present (Thrall *et al.*, 2004). I will therefore be calculating a heterophil:lymphocyte (H:L) ratio to evaluate the relative abundance of both types of cells in individual turtles. I predict that H:L ratios will increase with a decrease in habitat quality. This method was used for the first time as an indicator of stress in poultry by Gross and Siegel (1983). Since then, this measure has been used to determine the effect of various factors on physical conditions of birds (Campo and Davila, 2002; Jones *et al*., 1988; Müller *et al*., 2011; Scope *et al*., 2002), amphibians (Davis and Durso, 2009; Davis and Maerz, 2008, 2009; Shutler *et al.,* 2009; Shutler and Marcogliese, 2011), fish (Houston *et al.,*

1996; Zinkl *et al.,* 1991), mammals (Anderson *et al.,* 1999; Rossdale *et al.,* 1982; Walsh *et al*. 2005), and reptiles (Case *et al.,* 2005; Davis *et al.,* 2011; Schwanz *et al.,* 2011; Sperry *et al.,* 2009).

We will also be evaluating the physiological state of Blanding's turtles by determining the level of parasitaemia in the blood stream. Haemoparasites such as hemogregarina, trypanosomes and *Plasmodium* are common species found in reptiles.(Thrall *et al.*, 2004; Sperry *et al.,* 2009; Siddal and Desser, 2001). In high concentration, they can cause disease such as hemolytic anemia thus affecting the individual's health. (Sperry *et al.,* 2009; Thrall, 2004). Hence, level of parasitaemia can also be an indicator of an individual's fitness. I expect to see an increased level of parasitaemia with a decrease in habitat quality.

Methods

Site selection

The area of study was in southwestern Québec (Canada), more precisely from Gatineau Park (Collines-de-l'Outaouais county) to the West point of Clarendon (Pontiac county). A total of five study sites (60-130 km^2) were selected in this area. This allowed to achieve a gradient in the physical and natural characteristics. Clarendon, Bristol and Shawville sites present in the Ottawa Valley are composed of mixed forest cover, a low incline and wetlands. Eardley-Masham is characterized by a mixed forest cover, high elevation, and low density of wetlands. Lastly, West of Gatineau Park is majorly composed of wetlands surrounded by forest, agricultural fields, and active mines. In addition, Clarendon includes abandoned fields and a series of trails destined for all terrain vehicles. Bristol presents an increased level of human activity with mines, woodmills, crops and a higher road density than other sites. Shawville is located entirely on private terrains where agriculture is intense. There are also more urban and industrial developments paired with a medium density of roads. Finally, Eardley-Masham and West of Gatineau Park are part of the conservation grounds in Gatineau Park. There is therefore a low density of roads found at these sites.

Turtle capture and telemetry

All turtles were captured by hand, hoopnets or crabpots in the beginning of spring 2010. An identification number was assigned to each turtle (notches on the marginal scutes of the carapace). To determine the sex of individuals, length of the plastron was measured and concavity of this structure was assessed. A radio-transmitter (model #AI-2F, 33g, 36 months, Holohil Systems, Ontario, Canada) was attached to the posterior end of the carapace with two screws. The transmitter and screws did not surpass 5% body mass of individuals. A total of 42

individuals were tracked (21 females and 21 males) throughout the summer (May to September 2010). Turtle position was determined using a receiver (Lotek Wireless, # SRX 400A, Ontario, Canada; Communication Specialists, # R-1000, California, USA) coupled with an antenna (Advanced Telemetry Systems, Minnesota, USA). The location of turtles was determined every 2-4 days from May to August and once per week in September. All coordinates were recorded using a GPS (Garmin # GPSMap 60CSx, Kansas, USA). Some locations were determined by triangulation using Locatte III software (Pacer Computing, Nova Scotia, Canada).

Home range

All precise positions and positions found by triangulation were imported in ArcGIS 10 software (ArcView, Environmental Systems Research Institute, California, USA). All data were resampled randomly by Hawth`s tools (Hawthorne Beyer, Spatial Ecology) to have 34-35 positions per animal for the active season observed (May to September). To determine habitat composition of a given individual's home range, we used the minimum convex polygon (MCP) (Row *et al.,* 2006).

Habitat composition

The proportions of habitat types were determined using ArcGIS 10 software using land use layers. These were obtained from Base de données topographiques du Québec (BDTQ), Système d'information écoforestière (SIEF), Base de données des cultures assurées (BCDA), and Ducks Unlimited Canada (DUC). A scale of 1 : 20 000 was used for all layers except BCDA which was 1 : 50 000. Layers were in the form of vectors. In total, six categories were used to characterize habitat composition:

1) Wetlands that include wetlands and wooded wetlands (DUC, BDTQ),

2) Agriculture that include agriculture fields and agroforestry (BCDA, SIEF)

3) Forest (BDTQ)

4) Open water (BDTQ)

5) Anthropogenic composed of either gravel pits or highly or slightly modified areas (BDTQ, SIEF)

6) Other composed of any other type of land not included in the above categories

We also included in this study the density of roads (km/km^2). All measures were examined at eight spatial scales: MCP and MCP with buffer zones surrounding it (250, 500, 1000, 1500, 2000 and 4000 m).

Blood samples

One blood sample was collected from each individual in late June by standard caudal puncture (Bulté *et al.* 2006). A heparinized syringe of 1 cc (Hepalean, haparin sodium injection for intravenous or subcutaneous use, 1000 U.S.P. units/ml) was used to avoid coagulation of the blood. In total, 0.2 ml of blood was collected per individual. Blood samples were then transferred to slides (Fisherfinest, 25 x 75 x 1 mm, frosted) and Wright-Giemsa stained (Wright-Giemsa sure stain) for a total of one to four replicates per individual.

H :L ratios and parasitaemia

All blood slides were analyzed under a 400x objective using a standard light microscope (Olympus, Model: CX41RF). An estimate of the absolute number of heterophils and lymphocytes was calculated per 4000 erythrocytes as suggested by Davis *et al.* (2011). Leukocytes (heterophils and lymphocytes) were identified according to Thrall *et al.* (2004).

Heterophil:lymphocyte ratios were calculated with the relative numbers of both cell types. Only fields of view with even layers of erythrocytes were analyzed (Davis *et al*., 2011). Fields of view composed of 15 or less erythrocytes were not considered (Davis *et al.,* 2008). Blood parasites (intra-erythrocytic and free forms) were counted per 10,000 erythrocytes (Manwell, 1977). Genus of haemoparasites was identified using Thrall *et al.* (2004).

Statistical analyses

Statistical analyses were conducted in R version 2.13.2. A stepwise regression model using Akaike Information Criterion (AIC) was used to analyze data for initial MCP and each of the seven buffer areas surrounding MCP (250, 500, 1000, 1500, 2000 and 4000 m). The model with the lowest AIC was retained as the final model. The dependent variables used in data analyses were heterophil:lymphocyte ratios (Ratio) and total parasite count (Para). The independent variables were the proportions (%) of agricultural lands (Agri), open water (Water), anthropogenic lands (Ant), wetlands (Wet), forests (Forest), all other types of lands (Other) and total density of roads (km/km^2) (TR). Before proceeding to stepwise regression, correlation analyses were conducted between independent variables for MCP and MCP with buffer zones of 250, 500, 1000, 1500, 2000, 3000 and 4000 m. High correlation was defined by r > 0.6 (Cohen, 1988). Hence, Forest variable was removed from all data sets and TR (total road density) was removed from analyses involving MCP 500 and MCP 1000 to avoid multicolinearity. The latter was verified by the Variance Inflation Factor (VIF). Variables were considered independent when VIF coefficient was lower than 5 allowing greater statistical power to detect the individual effect of each independent variable (Stine, 1995). The following model assumptions were examined: normality (Shapiro-Wilk normality test), homoscedasticity (studentized Breush-Pagan test), serial autocorrelation (Durbin-Watson test), and linearity (Reset test). All data was square

root transformed to better fit normality (sragri, srwater, srant, srwet, srforest, srother and srtr). Results were considered significant when p<0.05. Stepwise regression models were obtained for six spatial scales (MCP, MCP 250, MCP 500, MCP 1000, MCP 1500 and MCP 2000). The remaining MCP with 3000 and 4000 m buffer zones were not analysed due to a high correlation between all independent variables.

Results

Stepwise regression model for MCP with 250 meter buffer zone is statistically significant (DF=38, F=3.135, p=0.037) and explains 13,51% of variance of H:L ratio (Adjusted R-squared = 0.14). The resulting model contains three independent variables: proportion of agricultural lands, proportion of other types of lands, and total road density. In addition, this model demonstrated that total road density has a statistically significant effect on H:L ratio (p=0.019).

Stepwise regression for MCP with 250 m buffer zone produced a statistically significant final model composed of the proportion of wetlands and proportion of other types of lands (DF=39, F=3.256, p=0.049). The adjusted R-squared value is 0.099.

Discussion

My hypothesis concerning habitat quality and its effect on immunocompetence and levels of parasitaemia in Blanding's turtles was evaluated using heterophil:lymphocyte ratios and hemoparasite count. H:L ratios are linked to elevated levels of glucocorticoids in the peripheral blood system (Davis *et al.* 2008). These hormones are released in the blood stream when individuals are faced with biological stressors such as exposure to agricultural pesticides (Shutler and Marcogliese, 2011), to water treatment plant runoffs (Polo-Cavia *et al.* 2009), to parasites (Shutler *et al.* 2009; Sperry *et al*. 2009), and other types of human activities (Davis *et al.* 2008,

2011; Scope *et al.,* 2002). These factors typically cause leukocyte counts to vary in all vertebrate species (Davis *et al.* 2008). As such, heterophil (in reptiles and birds) or neutrophil (in mammals, fish or amphibians) levels tend to increase and lymphocytes are likely to decrease when an individual's metabolism is strained (Thrall *et al*., 2004). Thus, in stressful environments or situations, animals are prone to heterophilia (or neutrophilia) and lymphopenia (Davis *et al*. 2008; Davis and Maerz, 2008; Shutler and Marcogliese, 2011; Thrall *et al.,* 2004). Studies have suggested that elevated levels of heterophils indicate stress, disease or illness universally among vertebrates (Davis *et al*. 2008). Auld *et al.* (2012) propose that an increase in immunological activity does not necessarily reflect an increase immunity to illness or an increase in fitness. On the contrary, an increased immune response would likely represent an increased expense in energy or immunopathology indicating a reduced fitness. Therefore, H:L ratios are a useful tool to evaluate the physical condition of individuals.

I found a significant decrease in H:L ratio as a function of the total road density (km/km^2) within the home range (MCP with a buffer of 250 meters) of Blanding's turtles (figure 1). This result seems to contradict most literature where H:L ratios tend to increase with environmental stressors. I expected H:L ratios to increase as habitat quality decreased. As such, presence of roads does not seem to affect the physical health of turtles to the extent of reducing their fitness. We must not, however, mistakenly conclude that roads do not have an impact on *Emydoidea blandingii*'s as road mortality plays an important role in semi-terrestrial freshwater turtle populations decline (Beaudry *et al.,* 2008; Gibbs and Shriver, 2002).

Haemoparasites were found in 39 of the 42 turtles assessed. Slide analysis revealed that intracellular parasites present were of the *Hemogregarina* genus. I observed a significant increase in parasitaemia as the proportion of wetlands and other types of lands increased in the

home range (MCP with 250 m buffer zone) (figure 2). These haemoparasites are transmitted by leeches found in wetlands and possibly in lands categorized as "other" (Telford *et al.*, 2001; Siddall and Desser, 1993). It has been suggested that these leeches may have similar habitats to freshwater turtles and therefore, may also be affected negatively by degraded habitats (Dodd, 1988). As such, our results are consistent with Siddall and Sherwin (1992) where Blanding's turtles exposed to leeches throughout summer months had higher levels of parasitaemia. According to Thrall *et al.* (2004) parasites are commonly found in reptiles and may potentially cause diseases such as hemolytic anemia. While it is clear that parasites may have a negative impact on the general health of an individual, mean parasite count was relatively low varying from 0 to 32 per 10 000 erythrocytes. Therefore, parasite load was likely to be too small to cause any real physiological repercussion (Bouma and Smallridge, 2007).

Another factor that must be considered is the relationship between heterophil:lymphocyte ratios and haemoparasites. Auld *et al*. (2012) suggested that immunological activity may be due to resistance to parasites or to infections. Although these trends were observed in other studies, I do not believe this is the case here as there was no significant correlation between H:L ratios and parasitaemia levels. In addition, as previously mentioned, the low parasite load observed was unlikely to cause an important physiological response.

In conclusion, I found that immunological response varied negatively as a function of the density of roads present in the home range of Blanding's turtles. This was inconsistent with previous findings where heterophil:lymphocyte ratios increased as habitat quality decreased (Shutler and Marcogliese, 2011; Polo-Cavia et al. 2009; Shutler *et al.* 2009). In addition, parasitaemia increased as a function of proportion of wetlands and lands falling in the "other" category of our study. These results are similar to the study conducted by Siddall and Sherwin

(1992) where parasite load increased in *Emydoidea blandingii* with exposure to leeches. Hence, as I hypothesized, habitat quality had an effect on immunocompetence and levels of parasitaemia. However, my prediction that percentage of parasites and H:L ratios would increase with larger proportions of anthropogenic lands and agricultural fields was not confirmed. Moreover, as we were able to perceive an effect of habitat quality in the relatively pristine setting of this study in or on the outskirts of Gatineau Park, there is a possibility that a more important effect may be detected in habitats that present a greater degradation. Future studies should consider measuring these fitness measures in highly disturbed environments.

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Appendix

Table 1: Stepwise regression model for heterophils:lymphocytes ratios square root transformed. Table includes results for six spatial scales (degrees of freedom, Ajusted R-squared, F-statistic and p-value of total model) and square root transformed variables in final model (srwet: square root transformed percentage of wetlands; srant: square root transformed percentage of anthropogenic lands; srother: square root transformed percentage of other lands; sragri: square root transformed percentage of agricultural lands; srtr: square root transformed total road density (km/km^2)). Statistically significant p-values ($p < 0.05$) are bolded. (n=42)

Spatial Scale	Variables	DF	Ajusted R-squared	F-statistic	p-value
MCP	srwet, srant	39	0.06595	2.447	0.0997
MCP 250	sragri, srother, srtr	38	0.13510	3.135	**0.0366**
MCP 500	srwet, srant	39	0.05425	2.176	0.1271
MCP 1000	srwet, srant	39	0.04728	2.017	0.1467
MCP 1500	srother, srant	39	0.06200	2.355	0.1083
MCP 2000	srother, srant	39	0.08836	2.987	0.0621

Table 2: Stepwise regression model for levels of parasitaemia (square root transformed). Table includes results for six spatial scales (degrees of freedom, ajusted R-squared, F-statistic and p-value of total model) and square root transformed variables in final model (srwet: square root transformed percentage of wetlands; srother: square root transformed percentage of other type of lands; srant: square root transformed percentage of anthropogenic lands). Statistically significant p-values ($p < 0.05$) are bolded. (n=42)

Spatial Scale	Variables	DF	Ajusted R-squared	F-statistic	p-value
MCP	srwet, srother	39	0.03301	1.7	0.196
MCP 250	srwet, srother	39	0.09912	3.256	**0.04926**
MCP 500	srwet, srother	39	0.05697	2.238	0.1201
MCP 1000	srant	40	0.02695	2.135	0.1517
MCP 1500	srant	40	0.02556	2.075	0.1575
MCP 2000	srant	40	0.03214	2.362	0.1322

Figure 1: srratio: square root transformed heterophil:lymphocyte ratio; sragri: square root transformed percentage of agricultural lands; srother : square root transformed percentage of other types of lands; srtr : square root transformed total road density (km/km^2). Panel A: Component and residual plot illustrating variation of srratio as a function of sragri corrected for the effect of srother and srtr included in the model. (n=42) Panel B: Component and residual plot illustrating variation of srratio as a function of srother corrected for the effect of sragri and srtr included in the model. (n=42) Panel C: Component and residual plot illustrating variation of srratio as a function of srtr corrected for the effect of sragri and srother included in the model. (n=42)

Figure 2: srpara: square root transformed parasite count; srwet: square root transformed percentage of wetlands; srother : square root transformed percentage of other types of lands. <u>Panel A:</u> Component and residual plot illustrating variation of srpara as a function of srwet corrected for the effect of srother included in the model. (n=42). <u>Panel B:</u> Component and residual plot illustrating variation of srpara as a function of srother corrected for the effect of srwet included in the model. (n=42).