The Effect of Climate on Turtle Clutch Size

By: Karolyn McGovern (300118923)

BIO 4009 – Research Project Supervisor: Dr. Gabriel Blouin-Demers

University of Ottawa

Department of Biology

April 26th, 2024

ABSTRACT

Past climate changes have influenced animal populations and dynamics. The current pace of climate change, however, may exceed the capacity of species to adapt or migrate. Ectotherms are highly sensitive to temperature variations, thus leading to changes in behaviour, physiology, and spatial distribution. In this study, I aimed to determine the effect of climate on the clutch size of turtles. I used the mean daily temperatures of spring and fall, as these are the periods of egg development. I obtained data on clutch size from three species in Ontario, Canada: the common snapping turtle (*Chelydra serpentina*), the painted turtle (*Chrysemys picta*), and the blanding's turtle (*Emydoidea blandingii*). I found no significant relationships between temperature and clutch size. I had limited power to detect the effects of annual variation in climate on clutch size due to a modest sample size (n = 7 years) and a lack of climate variation over the duration of my study. My findings highlight the complexity of climate-reproductive dynamics in turtles as well as the need for further research with larger datasets and extended temporal coverage.

Keywords: Ectotherms, Reproduction, Follicular development, Common snapping turtle, Painted turtle, Blanding's turtle

RÉSUMÉ

Les changements climatiques survenus dans le passé ont influencé les populations animales et leurs dynamiques. Toutefois, le rythme actuel du changement climatique pourrait dépasser la capacité d'adaptation ou de migration des espèces. Les ectothermes sont très sensibles aux variations de température, ce qui entraîne des changements dans leur comportement, leur physiologie et leur répartition spatiale. Dans cette étude, j'ai cherché à déterminer l'effet du climat sur la taille des couvées des tortues. J'ai utilisé les températures quotidiennes moyennes du printemps et de l'automne, car ce sont les périodes de développement des œufs. J'ai obtenu des données sur les tailles des couvées de trois espèces en Ontario, au Canada: la tortue serpentine (Chelydra serpentina), la tortue peinte (Chrysemys picta) et la tortue mouchetée (Emydoidea *blandingii*). Je n'ai trouvé aucune relation significative entre la température et la taille des pontes. J'avais une puissance limitée pour détecter les effets des variations annuelles de climat sur la taille des couvées en raison de la taille modeste de l'échantillon (n = 7 ans) et une variation climatique limitée pendant mon étude. Mes résultats soulignent la complexité de la dynamique du climat et de la reproduction chez les tortues ainsi que l'importance de poursuivre les recherches avec des jeux de données plus importants et une couverture temporelle étendue.

Mots clés: Ectotherme, reproduction, développement folliculaire, tortue serpentine, tortue peinte, tortue mouchetée

ACKNOWLEDGEMENTS

I would like to thank Dr. Blouin-Demers for granting me the opportunity to conduct research in a field that deeply passions me. Dr. Blouin-Demers's mentorship and guidance have enriched my academic experience and allowed me to achieve greater success. For this, I am extremely grateful. Thank you to Scales Nature Park for contributing the reproductive data. Additionally, I would like to recognize and thank the dedicated students of the Herpetology Lab, Laurence, Sofia, Cecilia, Andrea, and Michelle, for their invaluable support and camaraderie. Lastly, thank you to my close friend Heidi for igniting within me a newfound appreciation and passion for R coding.

TABLE OF CONTENTS

INTRODUCTION
METHODS
Reproductive Data11
Climate Data11
Statistical Analyses
RESULTS
DISCUSSION
Climate Data Variability
<i>Egg Size</i>
Multiple Clutches
Limitations14
REFERENCES

LIST OF TABLES

Table 1: Number of observations of mothers and their respective nest per year for each species. One year of data was removed from the data set for each species due to insufficient size (less than five observations). Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles. This was done with the aim of determining the effect of annual climate variations on clutch size.

Table 2: Significance of temperature period in the six linear mixed-effects models. This was done to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

LIST OF FIGURES

Figure 1: Study site in Muskoka region, Ontario. There is an approximate buffer zone of 75 km from Muskoka (Esri Inc, 2024). The aim was to determine the effect of annual climate variations on clutch size. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

Figure 2: Blanding's turtle's regression of clutch size (log-transformed) against temperature for both the fall and spring. A linear regression is in blue, with a 95% confidence interval area in grey. For the fall regression, $R^2 \approx 0$, and for the spring regression, $R^2 = 0.0027$. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

Figure 3: Snapping turtle's regression of clutch size (log-transformed) against temperature for both the fall and spring. A linear regression is in blue, with a 95% confidence interval area in grey. For the fall regression, $R^2 = 0.004$, and for the spring regression, $R^2 = 0.00039$. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

Figure 4: Painted turtle's regression of clutch size against temperature for both the fall and spring. A linear regression is in blue, with a 95% confidence interval area in grey. For the fall regression, $R^2 = 0.0066$, and for the spring regression, $R^2 = 0.016$. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

APPENDIX

Figure 5: Histogram of residuals for each of the six linear mixed-effects models. Clutch size is a dependent variable, mean temperature and carapace length are predictor variables, and year is a random effect. BLTU corresponds to blanding's turtles, MPTU corresponds to painted turtles, and SNTU corresponds to snapping turtles. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023.

INTRODUCTION

The global climate is warming. Climate change refers to long-term shifts in temperature and weather patterns (United Nations, 2024). Following the industrial revolution of 1975, the rate of temperature increased by approximately 0.15 to 0.20°C per decade (NASA, 2023). Global temperatures are expected to rise anywhere from 1.5°C to 5.9°C by the end of the 21st century (Lindsey & Dahlman, 2020; Houghton, 2001). Climate history reveals that significant changes have occurred throughout the earth's past, and have altered animal populations and dynamics (Nogu'es-Bravo et al., 2018). Both short-term and long-term mechanisms exist within species to respond to these environmental variations. These include behavioral changes, such as phenological shifts to reproduce during more favorable periods (Iler et al., 2021), physiological changes, such as increasing thermal tolerances (Bradshaw and Holzapfel, 2010; Brook et al., 2008), and spatial shifts, such as altering range limits (Parmesan, 2006). However, the rate at which climate is being altered may be too rapid to allow for adaptation (Santidrián et al., 2023; Bradshaw & Holzapfel, 2010).

Ectotherms depend highly on their environment to regulate bodily functions (James et al., 2006). Temperature is a measure of average kinetic energy, the addition or absence of energy from an organism's systems will have profound physiological impacts (Atkinson, 1994). Turtles modulate their energy through two primary avenues: nutrition and behaviour (Brown et al., 1994). Modified behavioural strategies may be observed to regulate a turtle's body temperature. This includes prolonged basking, adjusting body position, timing of activity, and selection of thermally suitable locations (Stevenson, 1985). When body temperature is maintained within an optimal range, increased efficiency of both metabolic and physiological performance is observed (Huey & Kingslover, 1989; Stevenson, 1985). In turtles, both rate and efficiency of digestion are positively

correlated to body temperature (Knight et al., 1990). As such, environmental temperature is extremely important in regulating the physiology of turtles.

The follicular development of oviparous reptiles depends on temperature. In female turtles, oogenesis and vitellogenesis are the critical processes of gametogenesis (Hamann et al., 2003). Oogenesis refers to the formation of oocytes, which in turn produce eggs (Guraya, 2013; Hamann et al., 2003). Within oogenesis, occurs folliculogenesis, where ovarian follicles are formed (Guraya, 2013). Follicles play a vital role in the development of oocytes, stimulating growth and yolk synthesis by regulating important hormones and proteins (Guraya, 2013; Donini et al., 2021). Vitellogenin is a crucial protein precursor to the egg yolk; it is synthesized in the liver and transported to the ovaries as part of an important lipoprotein complex (Donini et al., 2018; Hamann et al., 2003). Energetic limitations will reduce the turtle's ability to produce crucial vitellogenin and lipids required by the oocytes (Donini et al., 2018). Temperature modulates the energy expenditure necessary for follicular development and inhibits the process (Ganzhorn & Licht, 1983). Inhibition is due to the termination of essential hormonal pathways (Ganzhorn & Licht, 1983). As various aspects of female reproduction are influenced by temperature, seasonality may be observed.

In northern turtles, egg production may be divided into four essential phases: recrudescent, preparatory, egg-laying, and quiescent periods (Hedrick et al., 2018; Rollinson et al., 2012; Sarkar et al., 1996). Recrudescent refers to the period prior to brumation. During this phase, follicular development will begin. In the northern temperate species, this period occurs over August, September, and October (Hedrick et al., 2018; Rollinson et al., 2012). After brumation, the preparatory phase occurs, where the remainder of follicular development is completed (Hedrick et al., 2018; Rollinson et al., 2012).

al., 2018; Rollinson et al., 2012). In the northern temperate species, this period occurs over May and June (Hedrick et al., 2018).

The phases of follicular development occur at different rates in different species (Hedrick et al., 2018; Rollinson et al., 2012). Common snapping turtles (*C. serpentina*) complete approximately 80% of follicular development in the fall, and the remaining 20% in the spring. Painted turtles (*C. picta*) complete approximately 50% of follicular development in the fall and the remaining 50% in the spring (Rollinson et al., 2012; Congdon et al., 1982). There is no data regarding the specific dominance of follicular development periods of balnding's turtles (*E. blandingii*). Several factors may account for the variation of follicular development periods between species, including turtle size, preferred habitat, and reproductive strategy (Guraya, 2013).

Turtles may modulate egg size, clutch size, or number of clutches in response to increased or decreased metabolic energy. Optimal egg size theory states that variation in reproductive output should be realized primarily in terms of clutch size, and secondarily in terms of egg size (Congdon & Gibbons, 1987; Rollinson et al., 2012). Due to the limited availability of vitellogenin, a tradeoff between clutch size and optimal egg size may be observed (Rollinson et al., 2012). Additionally, turtles may produce one or multiple clutches in the same season, depending on the species (Gibbons, 1982; Rollinson et al., 2012). The production of a second or third seasonal clutch may be seen as a response to resource availability (Gibbons, 1982). Clutch frequency is deemed one of the most critical parameters of reproductive output in iteroparous turtles (Gibbons, 1982). Snapping and painted turtles may lay multiple clutches seasonally, whereas blanding's turtles typically only lay one clutch per season (Congdon et al., 1983; Samson, 2004; Standing et al., 1999)

This study aims to observe the impact of annual climatic variations on the clutch size of turtles. I used data from three species (snapping, painted, and blanding's turtles) in Ontario. I hypothesized that annual variations in mean temperature will dictate clutch size, as temperature should dictate the ability of turtles to mobilize resources for reproduction. I predicted an increase in clutch size with increasing temperatures.

METHODS

Reproductive Data

I obtained data from Scales Nature Park (SNP). SNP provided data from 2016 until 2023 for three species (snapping, painted, and blanding's turtles) in southwestern Ontario (Figure 1). I had data on carapace length, mother ID, and clutch size. Due to the small number of clutches in certain years (less than five), one year of data was omitted for each species for analyses.

Climate Data

I obtained air temperature (C°) data from the Muskoka Ontario station (44.974722°, -79.303333°) of Environment and Climate Change Canada (ECCC, 2024). The Muskoka weather station was a relatively central point in my study area. I calculated the daily mean temperature for the spring (May and June) and fall (August, September, October) by averaging the daily means of the months within each seasonal period. For climate data to represent conditions the mother experienced before laying the clutch, the fall period is from the calendar year before the clutch was laid, and the spring period is from the same year the clutch was laid.

Statistical Analyses

I completed my statistical analyses in R, version 4.3.3, using Rstudio (RStudio Team, 2024). My analysis used clutch size as a dependent variable, mean temperature and carapace length as predictor variables, and year as a random effect. I produced six linear mixed-effects models, two for each of the three species and one for each temperature period, spring and fall. I assessed the correlation between predictor variables to ensure that there were no multicollinearity issues (r = -0.004). Additionally, a strong positive correlation exists between clutch size and carapace length (r = 0.82). Thus, when carapace size increases, clutch size will also increase. I log-transformed clutch size for snapping and blanding's turtles to ensure normal distributions. To test the normality of each model, I plotted a histogram of the residuals to identify the distribution (Figure 5). Normality could be assumed for all six models. To further test if the temperature period had a significant effect on each model, I conducted a significant of predictor test, where an ANOVA model is fitted with a reduced model (excluding temperature) and a full model (including temperature). Finally, a single-factor ANOVA model was fitted for both fall and spring to determine if there was a significant difference between annual temperatures.

RESULTS

Throughout the 8-year study period (n corrected to 7), 484 independent mothers and their respective nest were observed (179 blanding's, 211 snapping, and 94 painted). An average of 23 \pm 12.5 (range 6-47) nests were laid per species per year (Table 1).

Temperature had no significant effects on clutch size (Table 2). The significance of the predictor test confirmed this observation. Additionally, there is a lack of significant annual differences in both fall and spring, with the fall period ($F_{(7, 23)} = 0.12$, p = 0.99) and the spring period ($F_{(7, 15)} = 0.20$, p = 0.97). Plotting the regressions of clutch size against temperature for both

fall and spring demonstrates an insignificant relationship. This is seen in the R^2 values of the linear regressions ranging from 0 to 0.016 (Figure 2, Figure 3, and Figure 4).

DISCUSSION

I found no relationship between clutch size and temperature in fall or spring for three turtle species in Ontario. Factors such as climate data variability, egg size, multiple clutches, and limitations greatly influenced the observed results.

Climate Data Variability

Air temperature displayed no trend. Annual mean temperature only differs by $\pm 1.17^{\circ}$ C throughout the fall periods and $\pm 1.09^{\circ}$ C throughout the spring periods. The stable temperatures likely did not impact the availability of vitellogenin, lipids, and physical parameters essential to the synthesis of new oocytes and yolks. Thus, no effect could be observed. It is essential to recognize that I used the means of each month's daily mean temperature. My datum was very averaged and may not have fully accounted for the variation that may have occurred. In the case that my datum did not account for these minor temperature variations, although present, behavioural strategies could have been implemented to account for the minute temperature microclimatic variations (Bentley et al., 2020; Stevenson, 1985). Variation in clutch size would not be observed as mothers actively regulated their body temperatures.

Egg Size

Although no trend could be observed between clutch size and temperature, it is possible that egg size may have been modified. A longer period of follicular development allows eggs to grow larger rather than adding eggs to the clutch (Ganzhorn & Licht, 1983; Congdon & Tinkle, 1982; Iverson,1992; Rollinson et al., 2012). This is seen across the geographic ranges where the egg size of painted turtles correlates with the duration of high summer temperatures (Ganzhorn & Licht, 1983). Thus, a trade-off may be observed between clutch size and optimal egg size (Rollinson et al., 2012). In my study, minor environmental variations may have caused the eggs to slightly decrease or increase in size rather than adding or removing an egg from the clutch. This would have occurred in cases of slightly extended spring or fall periods.

Multiple Clutches

As the reproductive data only contained one nest per mother per year, the maximal reproductive success of seasonal iteroparous species, painted and snapping, could not be fully considered. This is because, in some ectothermic reptiles, increased annual temperatures result in increased clutch frequency (Meiri et al., 2013). Thus, the additional energetic availability should entail the production of a second or third seasonal clutch (Gibbons, 1982). However, the second or third clutch produced is typically smaller than the first clutch (Rollinson et al., 2012; Gibbons, 1982). If my reproductive data only contained information on one of these clutches, it would fail to fully represent reproductive ability.

Limitations

The greatest limiting factor of my study was the sample size. With only seven years of reproductive data, I was only able to analyze the variation of temperature (fall and spring) on seven years of reproductive output. A reproductive database ranging from 20 to 30 years would have been beneficial to a more robust statistical analysis. I could make one large model for all three species and use individual months of each reproductive period as predictor variables. From this, several smaller models could be used to determine the best fit. Additionally, a reproductive

database of a longer timescale would potentially allow a longer timescale to observe reproductive adaptation and a longer timescale to observe climate change.

Further research — Although my study did not observe an effect of temperature on clutch size, this was largely due to a lack of variability in temperature data. Additional research should continue to explore the effect of climate on turtle reproductive output. I recommend using several sites for greater climate variations and to compare populations. Using clutch size and egg mass would be beneficial, as without egg mass, it is difficult to determine if there was no relationship between clutches and temperature (Congdon & Gibbons, 1987; Rollinson et al., 2012).

REFERENCES

- Atkinson, D. (1994). Temperature and Organism Size—A Biological Law for Ectotherms? Advances in Ecological Research, 25, 1–58. https://doi.org/10.1016/S0065-2504(08)60212-3
- Bentley, B. P., Kearney, M. R., Whiting, S. D., & Mitchell, N. J. (2020). Microclimate modelling of beach sand temperatures reveals high spatial and temporal variation at sea turtle rookeries. *Journal of Thermal Biology*, 88, 102522–12. https://doi.org/10.1016/j.jtherbio.2020.102522
- Bradshaw, W. E., & Holzapfel, C. M. (2010). Light, time, and the physiology of biotic response to rapid climate change in animals. *Annual review of physiology*, 72, 147-166.
- Brook, B. W., Sodhi, N. S., & Bradshaw, C. J. A. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, 23(8), 453–460.
- Brown, G. P., Bishop, C. A., & Brooks, R. J. (1994). Growth rate, reproductive output, and temperature selection of snapping turtles in habitats of different productivities. *Journal of Herpetology*, pp. 405–410.
- Congdon, J. D., & Gibbons, J. W. (1987). Morphological constraint on egg size: a challenge to optimal egg size theory? *Proceedings of the National Academy of Sciences*, 84(12), 4145– 4147.
- Congdon, J. D., Dunham, A. E., & van Loben Sels, R. C. (1994). Demographics of common snapping turtles (Chelydra serpentina): implications for conservation and management of long-lived organisms. *American Zoologist*, 34(3), 397–408.

- Congdon, J. D., Tinkle, D. W., Breitenbach, G. L., & van Loben Sels, R. C. (1983). Nesting ecology and hatching success in the turtle Emydoidea blandingi. *Herpetologica*, 417-429.
- Congdon, J. D., & Tinkle, D. W. (1982). Reproductive energetics of the painted turtle (Chrysemys picta). *Herpetologica*, 228-237.
- Donini, J., Lechowicz, C., & Valverde, R. (2018). Comparisons of summer and winter patterns in ovarian development, plasma vitellogenin, and sex steroids in female Diamondback
 Terrapins (malaclemys terrapin) in southern Florida. *Chelonian Conservation and Biology*, 17(2), 227. https://doi.org/10.2744/ccb-1310.1
- Environment and Climate Change Canada. (2024). Historical Climate Data. Retrieved from https://climate.weather.gc.ca/index_e.html
- Esri Inc. (2024). ArcGIS Pro (Version 3.2). *Esri Inc*. Retrieved from https://www.esri.com/enus/arcgis/products/arcgis-pro/overview
- Ganzhorn, D., & Licht, P. (1983). Regulation of seasonal gonadal cycles by temperature in the painted turtle, Chrysemys picta. *Copeia*, 347-358.
- Gibbons, J. W. (1982). Reproductive patterns in freshwater turtles. Herpetologica, 222-227.
- Guraya, S. S. (2013). Ovarian follicles in reptiles and birds (Vol. 24). *Springer Science & Business Media*.
- Hamann, M., Limpus, C. J., Owens, D. W., Lutz, P. L., Musick, J. A., & Wyneken, J. (2003). Reproductive cycles of males and females. In P. L. Lutz, J. A. Musick, & J. Wyneken (Eds.), *The Biology of Sea Turtles* (pp. 135-161).

- Hedrick, A. R., Klondaris, H. M., Corichi, L. C., Dreslik, M. J., & Iverson, J. B. (2018). The effects of climate on annual variation in reproductive output in Snapping Turtles (Chelydra serpentina). *Canadian Journal of Zoology*, 96(3), 221-228.
- Houghton, J. (2001). The Science of Global Warming. *Interdisciplinary Science Reviews*, 26(4), 247–257. https://doi.org/10.1179/isr.2001.26.4.247
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in ecology & evolution*, 4(5), 131-135.
- Iler, A. M., CaraDonna, P. J., Forrest, J. R. K., & Post, E. (2021). Demographic consequences of phenological shifts in response to climate change. Annual Review of Ecology, *Evolution*, and Systematics, 52, 221–245.
- Iverson, J. B. (1992). Correlates of reproductive output in turtles (Order Testudines). *Herpetological Monographs*, 25-42.
- James, M. C., Davenport, J., & Hays, G. C. (2006). Expanded thermal niche for a diving vertebrate: a leatherback turtle diving into near-freezing water. *Journal of Experimental Marine Biology and Ecology*, 335(2), 221–226.
- Knight, T. W., Layfield, J. A., & Brooks, R. J. (1990). Nutritional status and mean selected temperature of hatchling snapping turtles (Chelydra serpentina): is there a thermophilic response to feeding?. *Copeia*, pp. 1067–1072.
- Lindsey, R., & Dahlman, L. (2020). Climate change: Global temperature. Climate. gov, 16.
- Mazaris, A., Kramer-Schadt, S., Tzanopoulos, J., Johst, K., Matsinos, G., & Pantis, J. (2009). Assessing the relative importance of conservation measures applied on sea turtles:

comparison of measures focusing on nesting success and hatching recruitment success. *Amphibia-Reptilia*, 30(2), 221-231.

- Meiri, S., Bauer, A. M., Chirio, L., Colli, G. R., Das, I., Doan, T. M., ... & Van Damme, R. (2013). Are lizards feeling the heat? A tale of ecology and evolution under two temperatures. *Global Ecology and Biogeography*, 22(7), 834-845.
- NASA. (2023). World of change: Global temperatures. *NASA*. Retrieved from https://earthobservatory.nasa.gov/world-of-change/global-temperatures
- Nogués-Bravo, D., Rodríguez-Sánchez, F., Orsini, L., Boer, E., Jansson, R., Morlon, H., & Fordham, D. A. (2018). Cracking the code of biodiversity responses to past climate change. *Trends in Ecology & Evolution*, 33(10), 765–776.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637–669.
- Rollinson, N., Farmer, R. G., & Brooks, R. J. (2012). Widespread reproductive variation in North American turtles: temperature, egg size and optimality. *Zoology*, 115(3), 160-169.
- Santidrián Tomillo, P., Pujol, F., Félix, G., Núñez-Reyes, V., Saba, V., Tomás, J., & Marco, A. (2023). Colonization of new nesting areas could provide climate refuge to loggerhead turtles under climate change. *Biological Conservation*, 284, 110146-. https://doi.org/10.1016/j.biocon.2023.110146
- Stevenson, R. D. (1985). The Relative Importance of Behavioral and Physiological Adjustments Controlling Body Temperature in Terrestrial Ectotherms. *The American Naturalist*, 126(3), 362–386. https://doi.org/10.1086/284423

United Nations. (2024). What is climate change?. United Nations. https://www.un.org/en/climatechange/what-is-climate-change

•

Table 1: Number of observations of mothers and their respective nest per year for each species. One year of data was removed from the data set for each species due to insufficient size (less than five observations). Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles. This was done with the aim of determining the effect of annual climate variations on clutch size.

Species	2023	2022	2021	2020	2019	2018	2017	2016
E. blandingii	16	44	26	43	24	14	12	
C. serpentina		21	21	28	47	41	33	20
C. picta marginata		13	10	10	28	13	14	6

Table 2: Significance of temperature period in the six linear mixed-effects models. This was done with the aim of determining the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

Linear mixed-effects model		Estimate	Std. Error	t value	Pr(> t)	\mathbb{R}^2
E. blandingii	Fall	0.088	0.19	0.46	0.65	0.19
	Spring	-0.038	0.20	-0.19	0.85	0.19
C. serpentina	Fall	0.25	0.34	0.75	0.49	0.25
	Spring	0.24	0.35	0.66	0.54	0.25
C. picta	Fall	0.18	0.22	0.53	0.41	0.0061
	Spring	-0.24	0.21	-1.17	0.24	0.016

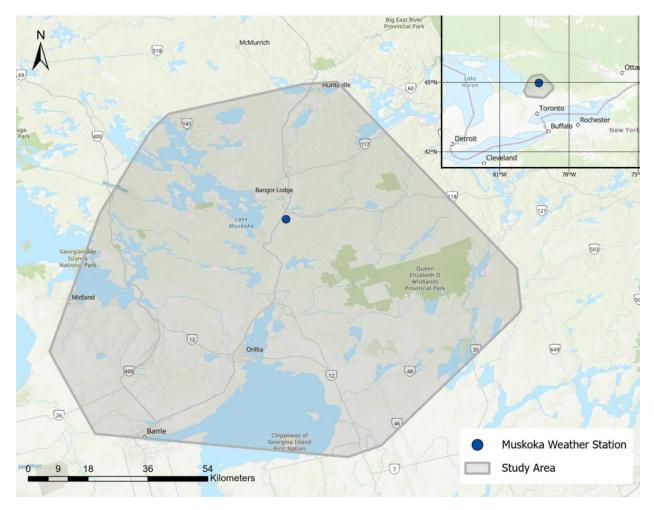


Figure 1: Study site in Muskoka region, Ontario. There is an approximate buffer zone of 75 km from Muskoka (Esri Inc, 2024). The aim was to determine the effect of annual climate variations on clutch size. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

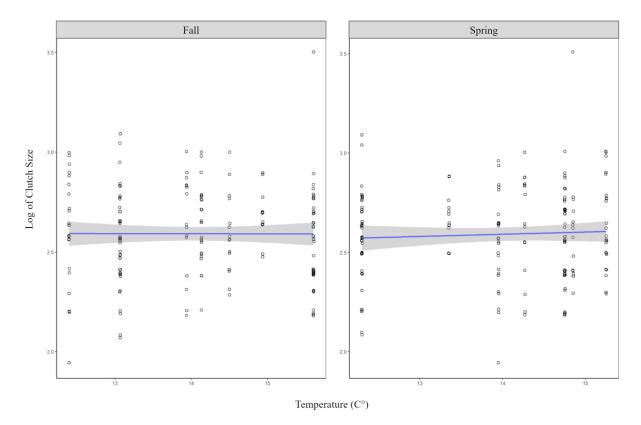


Figure 2: Blanding's turtle's regression of clutch size (log-transformed) against temperature for both the fall and spring. A linear regression is in blue, with a 95% confidence interval area in grey. For the fall regression, $R^2 \approx 0$, and for the spring regression, $R^2 = 0.0027$. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

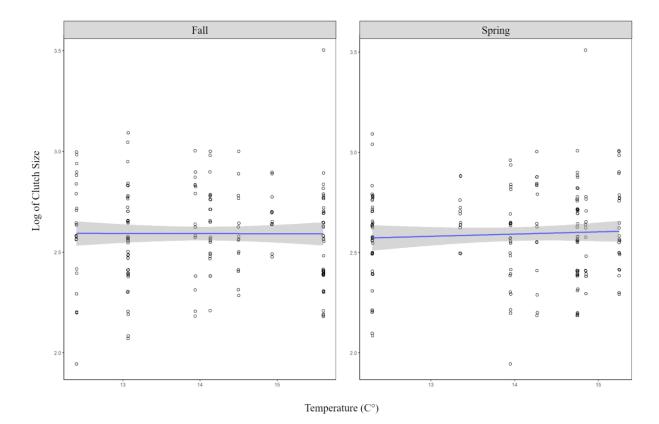


Figure 3: Snapping turtle's regression of clutch size (log-transformed) against temperature for both the fall. A linear regression is in blue, with a 95% confidence interval area in grey. For the fall regression, $R^2 = 0.004$, and for the spring regression, $R^2 = 0.00039$. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

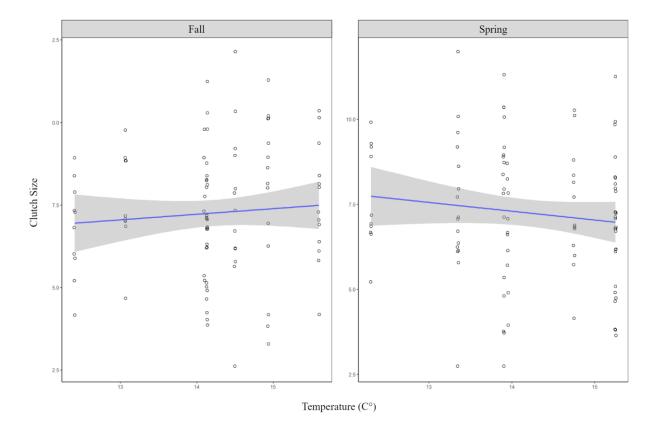


Figure 4: Painted turtle's regression of clutch size against temperature for both the fall and spring. A linear regression is in blue, with a 95% confidence interval area in grey. For the fall regression, $R^2 = 0.0066$, and for the spring regression, $R^2 = 0.016$. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023 and observed blanding's, snapping, and painted turtles.

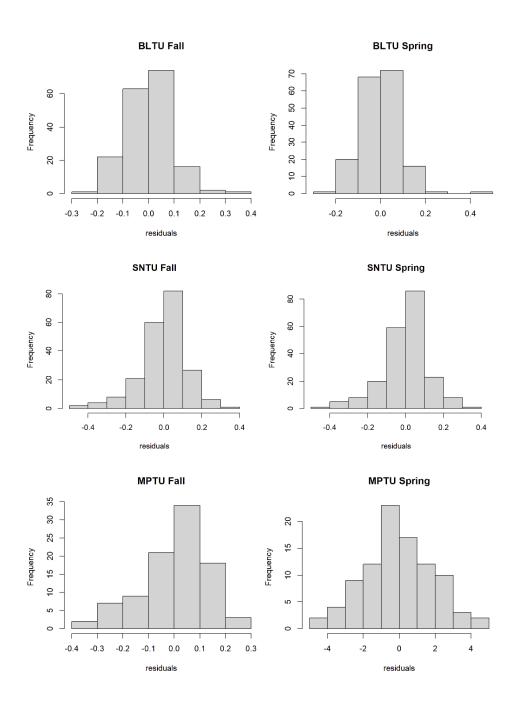


Figure 5: Histogram of residuals for each of the six linear mixed-effects models. Clutch size is a dependent variable, mean temperature and carapace length are predictor variables, and year is a random effect. BLTU corresponds to blanding's turtles, MPTU corresponds to painted turtles, and SNTU corresponds to snapping turtles. This study aims to determine the effect of annual climate variations on clutch size. Observations were made in southwestern Ontario, within a 75 km buffer zone from Muskoka, Ontario. The study encompassed an 8-year period from 2016 to 2023.