Temperature modulates the strength of density-dependent habitat selection in ectotherms: expanding and testing theory with red flour beetles and common gartersnakes

William David Halliday

Supervisor:
Gabriel Blouin-Demers

Committee Members:
Julie Morand-Ferron and Lenore Fahrig

Examiners:
Vincent Careau (Internal) and Philip McLoughlin (External)

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements
for the Doctorate in Philosophy degree in the
Ottawa-Carleton Institute of Biology

Department of Biology
Faculty of Science
University of Ottawa

Abstract

Density dependence is a common phenomenon in nature, and the intensity of density dependence is driven by competition over depletable resources. Habitat selection patterns are often density-dependent, and are driven by decreasing population mean fitness in a habitat as population density increases in that habitat. Yet not all resources are depletable, and non-depletable resources may sometimes be most important in dictating patterns of habitat selection. Ectotherms, for example, are defined by their dependence on environmental temperature to regulate body temperature, and temperature is often the most important resource for ectotherms. Is density dependence an important mechanism in ectotherms, especially when temperature is a limiting factor?

In this thesis, I examine density dependence of fitness and habitat selection by ectotherms using red flour beetles and common gartersnakes. In chapter one and three, I test whether density-dependent habitat selection occurs when habitats differ in both temperature and food availability with red flour beetles and common gartersnakes, respectively. In chapter two, I modify the isodar model of habitat selection to account for the effect of temperature on ectotherms, derive predictions from the modified model, and test these predictions with controlled experiments with red flour beetles selecting between habitats that differ in food quantity and temperature. Finally, in chapter four, I examine the effect of density on metrics of fitness and habitat selection with common gartersnakes.

Red flour beetles exhibited strong density dependence in both habitat selection and fitness at their optimal temperature, but density dependence weakened at lower temperatures. Common gartersnakes exhibited mostly density-independent habitat selection with a strong preference for warm field habitat over cool forest habitat, but exhibited some density dependence in habitat selection within field habitat. Overall, my thesis demonstrates that ectotherms have variable density-dependent responses, and that these responses are strongly modulated by temperature.

Résumé

La densité dépendance est un phénomène commun en nature, et l'intensité de la densité dépendance découle de la compétition pour les ressources épuisables. Les patrons de sélection d'habitat dépendent souvent de la densité de la population, et découlent de la baisse de l’aptitude moyenne dans un habitat lorsque la densité de la population augmente dans cet habitat. Pourtant, toutes les ressources ne sont épuisables, et les ressources non-épuisables sont parfois les plus importantes pour dicter la sélection d'habitat. Les ectothermes, par exemple, dépendent par définition de la température ambiante pour réguler leur température corporelle, et la température est souvent la ressource la plus importante pour ce groupe. Est-ce que la densité dépendance est un mécanisme important chez les ectothermes, surtout lorsque la température est un facteur limitant?

Dans cette thèse, j’examine la densité dépendance de l’aptitude et de la sélection d’habitat chez les triboliums rouges de la farine et chez les couleuvres rayées. Aux chapitres un et trois, je vérifie si la sélection d'habitat dépendante de la densité se produit lorsque les habitats diffèrent en température et en nourriture chez les triboliums rouges et chez les couleuvres rayées, respectivement. Au chapitre deux, je modifie le modèle isodar de la sélection d'habitat pour tenir compte de l'effet de la température chez les ectothermes, je dérive des prédictions du modèle modifié et je vérifie ces prédictions avec des expériences contrôlées où les triboliums rouges peuvent choisir des habitats qui diffèrent en température et en nourriture. Enfin, au chapitre quatre, je détermine comment l’aptitude et la sélection d'habitat varient en fonction de la densité de la population chez les couleuvres rayées.

Chez les triboliums rouges, la sélection de l'habitat et l’aptitude dépendent fortement de la densité à leur température optimale, mais cette dépendance faiblit de façon marquée à des températures plus basses. Les couleuvres rayées choisissent leur habitat de manière largement indépendante de la densité, et préfèrent généralement les champs, un habitat chaud, par rapport aux habitats forestiers plus froids. Par contre, elles présentent une sélection de l'habitat en fonction de la densité au sein des champs. Dans l'ensemble, ma thèse démontre que les ectothermes ont des réponses variables par rapport à la densité dépendance, et que ces réponses sont fortement modulées par la température.

Acknowledgements

This thesis would not have been possible without the help of many people. First, thank you to all of the people who have assisted me in the lab and the field: J. Châteauvert, P. Fassina, S. Karabatsos, F. Lanoix, Z. Maillet, T. Newman, K. Pilon, F. Retamel Diaz, M. Routh, and A. Tran. My supervisor, Gabriel, has been an inspiration throughout this entire process. He has been a wonderful sounding board for all of my research ideas, encouraged me to conduct research that excites me, and has helped to make this thesis significantly more coherent than it would have been without his input. Thank you to Julie Morand-Ferron and Lenore Fahrig, my committee members, who have helped to guide me to asking the right questions with my research. I thank my various sources of funding, without which this thesis would not have been possible: the University of Ottawa; the Government of Ontario for an Ontario Graduate Scholarship; the Ottawa Field-Naturalists' Club for a research grant; the Natural Sciences and Engineering Research Council (NSERC) of Canada for an Alexander Graham Bell Canada Graduate Scholarship; and finally, NSERC for Gabriel's Discovery Grant. Finally, I am especially grateful to my wife, Laura, who has assisted me with experiments, listened to my thoughts and ideas while planning, conducting, and writing about my research (despite not wanting to hear about snakes because they frighten her), and for dealing with the unconventional hours and time spent away during my research. This thesis would have been dramatically different without her love and support.

Table of Contents

ABSTRACT..II

RÉSUMÉ...IV

ACKNOWLEDGEMENTS...VI

TABLE OF CONTENTS..VII

LIST OF TABLES...XI

LIST OF FIGURES..XVI

LIST OF ABBREVIATIONS..XX

LIST OF APPENDICES..XXII

GENERAL INTRODUCTION...1

CHAPTER ONE...8

INTRODUCTION...9

METHODS..12

Thermal Preference...12

Habitat Selection..13

Relative Effects of Thermal Quality and of Food Abundance in Habitat Selection..............................14

Fitness Consequences of Habitat Selection..15

Results..16

Thermal Preference..16

Habitat Selection..16

Relative Effects of Thermal Quality and of Food Abundance in Habitat Selection 17

Fitness Consequences of Habitat Selection..17

Discussion..17

CHAPTER TWO..29

Introduction..30

Theory..31

Methods..35

Study Species..35

Experiments..35

Statistical Analyses..36

Results..37

Discussion..38

CHAPTER THREE..47

INTRODUCTION..48

METHODS..51

Observational Test of Density-Dependent Habitat Selection...............................51

Experimental Test of Density-Dependent Habitat Selection................................55

Fitness Consequences of Field and Forest Habitat Selection..............................57

RESULTS..59

Observational Test of Density-Dependent Habitat Selection...............................59

Experimental Test of Density-Dependent Habitat Selection................................60

Fitness Consequences of Field and Forest Habitat Selection..............................60

DISCUSSION..61

CHAPTER FOUR..70

INTRODUCTION..71

METHODS..74

Growth Rate and Reproductive Output..76

Foraging..77

Habitat Selection..77

RESULTS..78

Growth Rate and Reproductive Output..78

Foraging..78

Habitat Selection..79

DISCUSSION..79

CONCLUSION..92

APPENDIX ONE..97

APPENDIX TWO..101

LITERATURE CITED..108

List of Tables

Table 1-1. Isodars for red flour beetles (*Tribolium castaneum*) in three treatments that varied the quantity of food in habitats when habitats differed in thermal quality. Treatment = Equal (equal food between habitats), High (4 times more food in warm than in cool habitat), and Low (4 times more food in cool than in warm habitat). In the equation, Warm refers to the abundance of beetles in the warm habitat, and Cool is the number of beetles in the cool habitat. C.I. (Int) is the 95% confidence interval for the intercept and C.I. (Sl) is the 95% confidence interval for the slope.................................21

Table 1-2. Model selection (top section) and final model output (bottom section) describing habitat selection of red flour beetles (*Tribolium castaneum*) in habitats differing in thermal quality and food as food increases in one habitat. Proportion = # beetles in low food habitat / total # of beetles; Treatment = equal thermal quality and unequal thermal quality (intercept in bottom panel); Food = multiples of food in the high food habitat; Food^2 = square of Food; *k* = number of parameters in a model; AICc = bias-corrected Akaike's information criteria value; ΔAICc = difference between AICc of the model with the lowest AICc and each other model. The best model (bolded) has an AICc within 2 units of the lowest AICc, and is most parsimonious. *represents a competing, but less parsimonious model..22

Table 1-3. Model selection (top section) and parameters of the final model (bottom section) describing per capita fitness (square root-transformed) of red flour beetles (*Tribolium castaneum*) as temperature, food abundance, and population density are varied. Fitness = per capita # of eggs laid over four days; Temperature = temperature treatment (20 or 30°C); Food = food treatment (0.625 or 2.5 ml of flour); Density = number of beetles in a treatment; Density^2 = square of Density; *k* = number of parameters in a model; AICc = bias-corrected Akaike's information criteria value; ΔAICc = difference between AICc of the best model (bolded) and each other model. *represents a competing, but less parsimonious model..23

Table 2-1. Isodar equations, built via geometric mean regression, for habitat selection by red flour beetles (*Tribolium castaneum*) selecting between low and high food habitats across a range of densities at three temperatures. Y is the number of beetles in the high food habitat and X is the number of beetles in the low food habitat. Mixed Group refers to an equal sex ratio, and Female Groups refer to experiments with only females. CI represents the 95% confidence interval..41

Table 2-2. Model selection and final model output for linear mixed effects models examining per capita eggs laid (total eggs / population density) by red flour beetles (*Tribolium castaneum*) in high and low food habitats at different population densities and at three temperatures. *k* is the number of parameters in the model, AIC is the Akaike's criteria value, and ΔAIC is the difference between the AIC of the best model and the focal model...42

Table 2-3. Model selection and final model output for linear mixed effects models examining per capita eggs laid (eggs in a habitat / beetles in a habitat) by red flour beetles (*Tribolium castaneum*) in high and low food habitats at different population densities and at three temperatures, and as the number of beetles in a habitat varied. *k* is the number of parameters in the model, AIC is the Akaike's criteria value, and ΔAIC is the difference between the AIC of the best model and the focal model...43

Table 3-1. Isodars (calculated via geometric mean regression) for habitat selection by common gartersnakes (*Thamnophis sirtalis*) between field and forest in experimental enclosures that straddled each habitat. Each model refers to different food treatments, where Equal is equal food in each habitat, Field is high food in the field, and Forest is high food in the forest. CI refers to the 95% confidence interval around the intercept and slope..............66

Table 3-2. Reproductive output (number of offspring) and growth rates (mass and snout-vent length (SVL)) of female common gartersnakes (*Thamnophis sirtalis*) kept in enclosures in field and in forest. C.I. refers to the 95% confidence interval around the mean for each metric...67

Table 4-1. Model selection and final model output for linear mixed effects models examining the mass growth rate of common gartersnakes (*Thamnophis sirtalis*) living in experimental enclosures at different population densities in Pontiac County, Québec. Gartersnakes were in different reproductive classes, and were in the experiment for different lengths of time (week). *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc...83

Table 4-2. Model selection and final model output for linear mixed effects models examining the snout-vent length (SVL) growth rate of common gartersnakes (*Thamnophis sirtalis*) living in experimental enclosures at different population densities in Pontiac County, Québec. Gartersnakes were in different reproductive classes, and were in the experiment for different lengths of time (week). *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc. * represents competing models that are within two AICc units of the model with the lowest AICc. Final model output is based on model averaging between competing models..84

Table 4-3. Model selection and final model output for linear mixed effects models examining the snout-vent length (SVL) of newborn common gartersnakes from mothers that lived in experimental enclosures at different population densities in Pontiac County, Québec. Mothers varied in SVL and had different litter sizes. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc. * represents competing models that are within 2 AICc units of the model with the lowest AICc. Final model output is based on model averaging between competing models.....................................85

Table 4-4. Model selection and final model output for linear models examining the litter size of female common gartersnakes (*Thamnophis sirtalis*) that lived in experimental enclosures at different population densities in Pontiac County, Québec. Mothers varied in SVL. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc. * represents competing models that are within 2 AICc units of the model with the lowest AICc. Final model output is based on model averaging between competing models...86

Table 4-5. Model selection and final model output for linear mixed effects models examining the total number of worms in pitfall traps found inside and outside experimental enclosures housing different densities of common gartersnakes (*Thamnophis sirtalis*) in Pontiac County, Québec. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc. 87

Table 4-6. Model selection and final model output for linear mixed effects models examining the total number of slugs in pitfall traps found inside and outside experimental enclosures housing different densities of common gartersnakes (*Thamnophis sirtalis*) in Pontiac County, Québec. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc..........88

List of Figures

Figure I-1. Fitness density functions (A), and two isodars (B and C) derived from the fitness-density functions. In the legend, “Hab” is short for habitat....................................7

Figure 1-1. The temperatures selected by red flour beetles (*Tribolium castaneum*) in a thermal gradient ranging from 25 to 40°C. The box represents the interquartile range, the line within the box represents the median value, and the whiskers represent the maximum and minimum values...25

Figure 1-2. Isodars for habitat selection experiments with red flour beetles (*Tribolium castaneum*), where habitats were set at 20 and 30°C with food equal between habitats (A), food higher in the warm habitat (B), and food higher in the cool habitat (C). Density is the total number of beetles in a habitat. The solid line represents the isodar, calculated using geometric mean regression, and the dashed line represents equality between habitats. N = 10 replicates for each density treatment, which are represented as diagonal rows of points. Bold outlines represent overlapping data points......................................26

Figure 1-3. The proportion of red flour beetles (*Tribolium castaneum*) using the low food habitat (Proportion Occupying Low Food) as the amount of food in the high food habitat increased (Ratio of Food Abundance). Habitats either had equal thermal quality (30°C) or unequal thermal quality (20 and 30°C), and the low food habitat was always set at 30°C. Ratio of Food Abundance (x-axis) refers to how much more food the high food habitat had compared to the low food habitat. Linear regression best described the unequal thermal quality treatment (solid line) and the equal thermal quality treatment (dashed line). Each point represents the mean value for that treatment (n = 10), and the error bars represent the standard error of the mean..27

Figure 1-4. Per capita fecundity of red flour beetles (*Tribolium castaneum*) in treatments set at 30°C with 0.625 ml of flour (A) and 2.5 ml of flour (B), and set at 20°C with 0.625 ml of flour (C) and 2.5 ml of flour (D) as density increases from 10 to 50 beetles. Density is the total number of individuals in a treatment, and per capita fecundity is the number of eggs laid over four days divided by the density of beetles in the treatment. N = 10 replicates for each density treatment....................28

Figure 2-1. (A) Mean fitness-density functions, based on per capita egg laid, under high and low food treatments at three temperatures (20, 25, and 30°C) for red flour beetles (*Tribolium castaneum*) (adapted from Halliday et al. 2015A). (B) Theoretical isodars predicting habitat selection based on temperature....................44

Figure 2-2. Experimental isodars for red flour beetles (*Tribolium castaneum*) selecting between habitats with high food and low food when temperature was 20°C (A, D), 25°C (B, E), and 30°C (C, F). The dashed lines represent equal selection of each habitat, and the solid lines represent statistically significant isodars. Isodars were built using mixed groups of males and females (A-C) and with female-only groups (D-F)....................45

Figure 2-3. Overall per capita number of eggs (A-C) and habitat per capita number of eggs (D-F) laid by red flour beetles (*Tribolium castaneum*) in low food and high food habitats over 24 hours at different density treatments when temperature was 20°C (A, D), 25°C (B, E), and 30°C (C, F). Overall per capita eggs was calculated as the number of eggs in a habitat divided by the total number of beetles in the treatment. Habitat per capita eggs was calculated as the number of eggs in a habitat divided by the number of beetles in that same habitat....................46

Figure 3-1. Mean number of individuals of three species of snakes detected in field and forest at Queen's University Biological Station, Ontario, Canada: *Storeria dekayi* (Dekay's brownsnake; shortened to *S. dekayi*), and *Storeria occipitomaculata* (red-bellied snake; shortened to *S. occipito*), and *Thamnophis sirtalis* (common gartersnake; shortened to *T. sirtalis*). Each bar was calculated using the number of individuals of each snake species caught on each of five 50 × 50 m grid across six sampling periods (n = 30 for each bar). Each bar represents the mean value, and error bars represent the standard error around the mean. Note that no *S. dekayi* or *S. occipitomaculata* were detected in forest..................68

Figure 3-2. Isodars for an experimental test of density-dependent habitat selection with common gartersnakes (*Thamnophis sirtalis*) between field and forest in enclosures straddling both habitats, when food is equal between habitats (A), when food was higher in the field (B), and when food was higher in the forest (C). Filled symbols represent two overlapping points. Lines represent the predicted intercepts and slopes from a geometric mean regression..69

Figure 4-1. Design schematic for an experiment examining the effect of population density on fitness and habitat selection for common gartersnakes (*Thamnophis sirtalis*) in Pontiac County, Québec. Rectangles at the top of the figure represent the experimental enclosures, and the numbers within the rectangles represent the density of common gartersnakes within each enclosure. A and B represent the two sides of each enclosure. The rectangle at the bottom demonstrates the locations of cover objects and feeding stations within each enclosure..89

Figure 4-2. Mass (A) and snout-vent length (SVL; B) growth rate for common gartersnakes (*Thamnophis sirtalis*) in different reproductive classes living in experimental enclosures at different population densities in Pontiac County, Québec..90

Figure 4-3. An isodar representing the number of common gartersnakes (*Thamnophis sirtalis*) in the side of the enclosure with food supplementation versus the number of common gartersnakes in the side of the enclosure without food supplementation for snakes living in experimental enclosures in Pontiac County, Québec. The dashed line (equality) represents an isodar with an intercept of zero and a slope of one, which would demonstrate no preference between habitats..91

List of Abbreviations

CHAPTER ONE.

AICc	bias-corrected Akaike's information criteria
B_i	basic habitat suitability of habitat i
$f(d_i)$	function describing negative density dependence of fitness in habitat i
k	number of parameters in a model
S_i	suitability of habitat i

CHAPTER TWO.

AIC	Akaike's information criteria
b	isodar slope
C	isodar intercept
E_B/E_i	efficiency of extracting resources, consumption and conversion into descendants in habitat i relative to the best habitat B
IFD	ideal free distribution
k	number of parameters in a model
N_i	population density in habitat i
p	per capita demand on resources
R_i	quantity of resources in habitat i corrected by renewal rate
T_o	optimal temperature

T_{Ri}......................the effect of temperature on the intercept of a fitness-density function in habitat i

T_{Ui}...........................the effect of temperature on the slope of a fitness-density function in habitat i

Ui...................combination of the per capita demand on resources and the extraction efficiency in habitat i.

W_i..fitness in habitat i

CHAPTER THREE.

d_e...thermal quality, absolute deviations of T_e from T_{set}

IFD...ideal free distribution

MANOVA...multivariate analysis of variance

QUBS...Queen’s University Biological Station

SVL...snout-vent length

T_e...environmental temperature

T_{set}...preferred temperature

CHAPTER FOUR.

AICc...bias-corrected Akaike’s information criteria

k...number of parameters in a model

SVL...snout-vent length

List of Appendixes

Appendix 1. Thermal preferences of various snake species from published studies.

Appendix 2. List of additional publications completed during my PhD that are not thesis chapters.

General Introduction

Explaining patterns in the distribution and abundance of organisms is at the core of ecology, and theories of habitat selection are one attempt to explain these patterns (Fretwell and Lucas 1969; Rosenzweig 1981; Morris 2011). One of the more popular models of habitat selection is the ideal free distribution (Fretwell and Lucas 1969), which posits that individuals should be distributed between habitats such that the mean fitness of individuals is equal across habitats. As population density increases, fitness in a habitat decreases. Different habitats can have different fitness-density relationships, which can lead to patterns of habitat selection that change with population density. The latter pattern is formally known as density-dependent habitat selection. The ideal free distribution assumes that individuals have equal competitive abilities, have a perfect knowledge of the distribution of resources and competitors between habitats, and are free to move between all habitats. While these assumptions can be considered unrealistic (Kennedy and Gray 1993), the ideal free distribution and density-dependent habitat selection have been demonstrated in a plethora of organisms, including birds (Fretwell and Calver 1969; Shochat et al. 2002; Jensen and Cully 2005; Zimmerman et al. 2009), mammals (e.g., Morris 1988; Ovadia and Abramsky 1995; Lin and Batzli 2002; Tadesse & Kotler 2010), fish (e.g., Rodríguez 1995; Morita et al. 2004; Haugen et al. 2006; Knight et al. 2008), lizards (Calsbeek and Sinervo 2002), and invertebrates (e.g., Krasnov et al. 2003; Krasnov et al. 2004; Lerner et al. 2011), and the ideal free distribution continues to be used as the backbone for theoretical advances in habitat selection (Tregenza 1995; Krivan 1997, 2014; Cressman and Krivan 2006; Cantrell et al. 2010; Matthiopoulos et al. 2015).

Ectotherms comprise the vast majority of animals on Earth. By definition, ectotherms rely on external environmental temperatures to maintain their internal body temperature, which often leads to large variations in internal body temperature simply because environmental temperature varies through time and space. Body temperature has large impacts on many proximate measures of fitness, including metabolic rate (Gillooley et al. 2001; Dubois et al. 2009), energy acquisition and assimilation (Angilletta 2001), growth rate (Angilletta et al. 2004), reproductive success (Berger et al. 2008; Halliday and Blouin-Demers 2015A; Halliday et al. 2015A), and even population growth rates (Halliday et al. 2015A). For this reason, temperature is often considered the most important environmental driver for ectotherms, and especially so in their habitat preferences. For example, side-blotched lizards in the southwest United States have a strong preference for territories with more rocks, which provide a wide range of temperatures for thermoregulation (Calsbeek and Sinervo 2002). Milksnakes in eastern Ontario prefer open macrohabitats over shaded forested habitat because open habitats are thermally superior (Row and Blouin-Demers 2006). Finally, grasshoppers in the United Kingdom tend to be distributed between habitats based on their thermoregulatory needs (Willott 1997). These three examples illustrate the importance of temperature in habitat selection by ectotherms.

One important consideration for density-dependent habitat selection is that resources must be limiting and depletable for density dependence to occur. For example, food is a depletable resource, whereas environmental temperature may not be depletable unless individuals compete over basking sites, as happens in lizards (e.g., Calsbeek and Sinervo 2002). Because temperature is often the most important factor for habitat selection by ectotherms, and because temperature is typically not depletable, will density-dependent habitat selection occur in ectotherms? Because habitat suitability depends on the availability of multiple resources, it is possible that temperature varies simultaneously with depletable resources such as food, which then allows for some density dependence in habitat selection. Yet if temperature truly is limiting for some ectotherms, then density-dependent habitat selection may not occur at all, and individuals should only be found in the habitat that provides optimal temperatures.

In this thesis, I investigate density-dependent habitat selection by ectotherms. Since temperature is such an important driver of habitat suitability for ectotherms, I examine how temperature affects density dependence of both fitness and habitat selection. I first test the general hypothesis that density-dependent habitat selection occurs in ectotherms when habitat suitability is a function of thermal quality. I then test the general hypothesis that the strength of density-dependent habitat selection in ectotherms is modulated by temperature. I use two study systems to test these hypotheses: red flour beetles (*Tribolium castaneum*) in the laboratory, and common gartersnakes (*Thamnophis sirtalis*) in the field. The laboratory experiments with flour beetles offered more convenience and control for manipulation and experimentation, while the observational and experimental approaches in the field with gartersnakes offered more ecological realism, and also allowed to test for density dependence in a taxon believed to select habitats independently of density (Harvey and Weatherhead 2006).

In chapters one and three, I test whether density-dependent habitat selection can occur when habitat suitability is a function of temperature. I use red flour beetles in chapter one and common gartersnakes in chapter three. In chapter one, I test the hypothesis that both thermal quality and food availability explain habitat selection patterns of red flour beetles because thermal quality affects their ability to extract resources. In chapter three, I test the hypothesis that habitat selection by gartersnakes is not a function of conspecific density because the fitness of snakes is more tightly linked to thermal quality, a non-depletable resource, than to the availability of depletable resources. Mean fitness may be so low in cool habitats that it is never beneficial for individuals to use this habitat. In this case, habitat selection should be density-independent because individuals should always use the warm habitat, regardless of density. I compare temperatures in each habitat to the temperatures selected by each species in a thermal gradient. I then measure fitness in each habitat, and set up experiments examining density-dependent habitat selection between these two habitats.

In chapter two, I build upon density-dependent habitat selection theory to add the effect of temperature. In chapter one, I demonstrate that the strength of negative density dependence of fitness decreases as temperature deviates from the optimal temperature in red flour beetles. I use these results to adapt habitat selection models for ectotherms, and derive and test predictions for density-dependent habitat selection between habitats differing in the quantity of available food as temperature changes in red flour beetles.

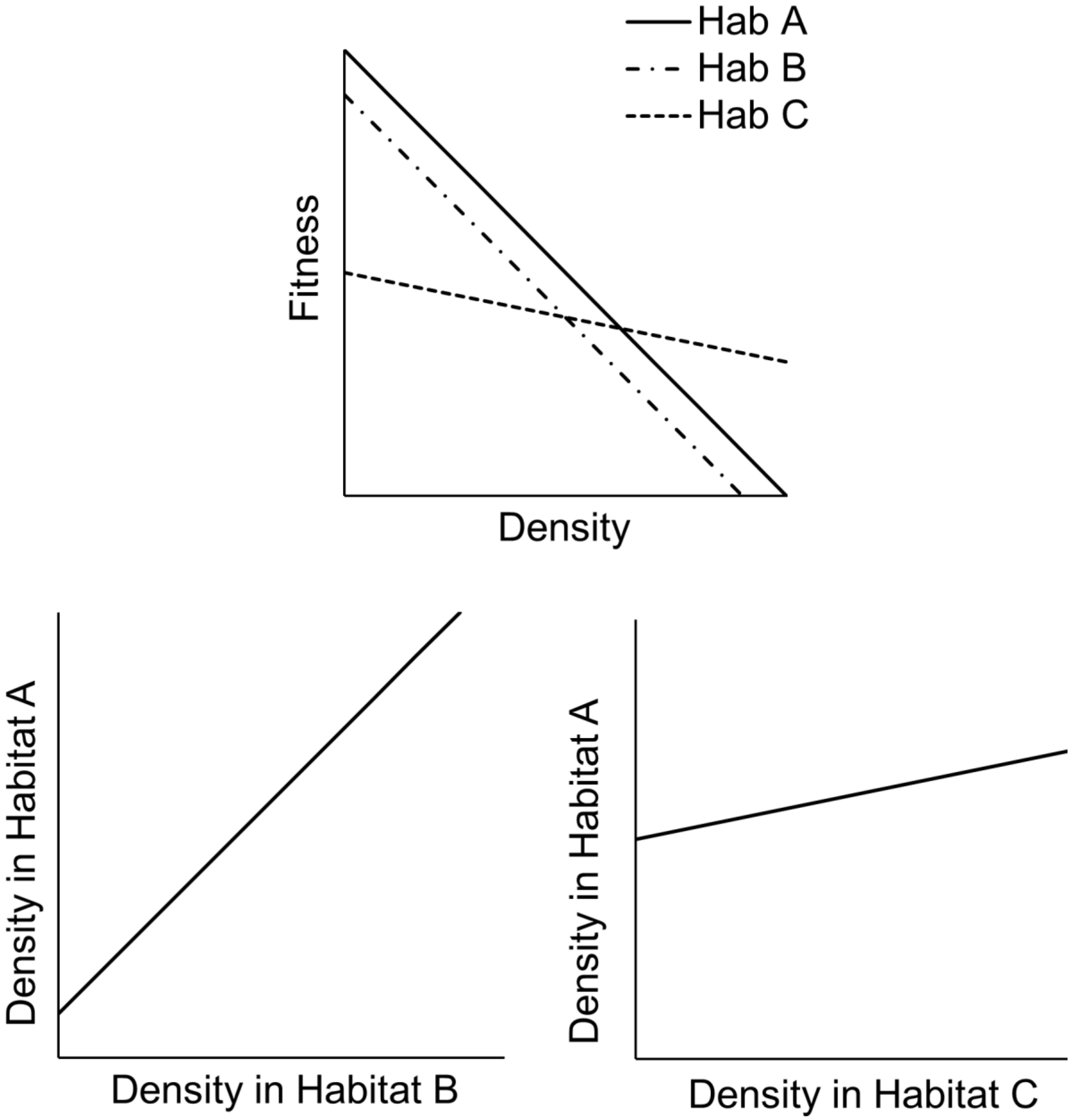
In my fourth chapter, I examine fitness and habitat selection by common gartersnakes between patches differing in food in field habitat while simultaneously manipulating population density. I empirically test if snakes demonstrate density dependence in fitness and habitat selection, and test the specific hypothesis that gartersnakes select habitats independently of density because their low energetic requirements should make their fitness independent of density at ecologically relevant densities. Overall, my thesis examines density dependence in ectotherms, and specifically examines how temperature affects density dependence in habitat selection and fitness.

Isodars

Isodars are a common method for analyzing density-dependent habitat selection (Morris 1988). Using the ideal free distribution as a starting point, isodars predict the density of individuals in habitat A given their density in habitat B. Isodars start from the assumption that fitness in a given habitat decreases as density in that habitat increases (Figure I-1A). These fitness-density functions can then be used to predict the distribution of individuals between two habitats given the assumption that individuals are attempting to maximize their fitness via their habitat choice (Figure I-1B,C). The isodar is a line of equal fitness ("iso" represents equal, "dar" represents Darwinian fitness) when examining the density of individuals in two habitats. Isodars have two important characteristics: the intercept and the slope. The intercept provides information on the fitness differences between habitats, and demonstrates the habitat preference of a species at low density. An intercept greater than zero implies preference for the habitat on the y-axis at low density, an intercept less than zero implies a preference for the habitat on the x-axis at low density, and an intercept not different from zero implies equal preference for both habitats at low density. The slope provides information on how habitat preference changes as density increases. A slope of one implies that an equal number of individuals select each habitat as density increases (the absolute difference in the number of individuals between habitats at low density being maintained at all densities) and that habitat preference does not change with density, a slope greater than one implies that more individuals select the habitat on the y-axis as density increases (and thus that preference for the habitat on the y-axis increases as density increases), and a slope less than one implies that more individuals select the habitat on the x-axis as density increases (and thus that preference for the habitat on the x-axis increases as density increases). A slope not different from zero or infinity demonstrates density-independent habitat selection. Similarly, an isodar that is not statistically significant demonstrates either density-independent habitat selection or random habitat selection. An isodar with an intercept of zero and a slope of one demonstrates equal preference for the two habitats, or the inability of individuals to differentiate between the two habitats. An isodar with an intercept of zero and a slope of one is the null model of habitat selection when examining density-dependent habitat selection because it represents no significant habitat preference.

Isodars can be calculated from population density data with geometric mean regressions (also known as reduced major axis regression) of the density of individuals in one habitat by the density of individuals in the second habitat. Geometric mean regression is used because density in one habitat is dependent upon density in the other habitat, and because there is measurement error in both axes. While ordinary least squares regression tends to bias intercept values to be higher and slopes to be lower, geometric mean regression tends to equalize the effect of one variable on the other and estimate a slope and intercept that are closer to their true values (Bradbury and Vehrencamp 2015). Isodars that are curvilinear demonstrate an ideal despotic distribution, where dominant individuals exclude subordinates from high quality habitat and have higher fitness than subordinates (Fretwell and Lucas 1969; Morris 1988). These curvilinear isodars can be logarithmically transformed for the isodar analysis.

Figure I-1. Fitness-density functions (A), and two isodars (B and C) derived from the fitness-density functions. In the legend, “Hab” is short for habitat.



Chapter One

Red flour beetles balance thermoregulation and food acquisition via density-dependent habitat selection

This chapter formed the basis for the following publication:

Halliday WD, Blouin-Demers G (2014) Red flour beetles balance thermoregulation and food acquisition via density-dependent habitat selection. Journal of Zoology 294: 198-205

Introduction

Theories of habitat selection assume that individuals select habitats in a manner that allows each individual to obtain the same expectation of fitness (Fretwell and Lucas 1969; Rosenzweig 1981; Rosenzweig and Abramsky 1986; Morris 1988). This starting point is known as the ideal free distribution (Fretwell and Lucas 1969). Since all animals must obtain food, food is often considered to be one of the most important factors in habitat selection. In a simple scenario, individuals should distribute themselves between habitats in proportion to food availability in those habitats (see review in Kennedy and Gray 1993). The underlying mechanism for this habitat selection pattern is negative density dependence (Fretwell and Lucas 1969; Rosenzweig 1981; Morris 1988): as population density increases, individual fitness decreases because per capita food availability decreases.

Although most theories of habitat selection go beyond food availability and often include factors such as interference competition (the ideal despotic distribution: Fretwell and Lucas 1969; Fretwell 1972; Morris 1988), interspecific competition (Rosenzweig and Abramsky 1986; Morris 1988), and predation risk (Moody et al. 1996; Grand and Dill 1999), theories of habitat selection typically do not account for factors such as moisture or temperature (Huey 1991), which do not decline with density. This general lack of consideration for environmental density-independent factors is likely a result of most theories of habitat selection being originally applied to endotherms such as birds (Fretwell 1969; Fretwell and Calver 1969) and mammals (Rosenzweig and Abramsky 1986; Morris 1988), and only later applied to ectotherms (e.g. Rodríguez 1995; Haugen et al. 2006; Knight et al. 2008). Although theories of habitat selection can still be applied to ectotherms, they should incorporate environmental temperature, a density-independent factor, as an important driver of habitat selection because temperature profoundly affects performance of ectotherms (Huey 1991; Blouin-Demers and Weatherhead 2001; Buckley et al. 2012). Furthermore, a theory of habitat selection that incorporates temperature would also be important for endotherms because endotherms are also affected by temperature (e.g., Schwab and Pitt 1991; Humphries et al. 2002), albeit less dramatically than in ectotherms.

Habitat suitability for ectotherms depends on environmental temperature (Huey 1991; Blouin-Demers and Weatherhead 2001, 2002, 2008; Row and Blouin-Demers 2006) because temperature has a marked effect on performance (Blouin-Demers and Weatherhead 2008) and, ultimately, on fitness (Gilchrist 1995). Thus, thermal quality (how far, on average, environmental temperature is from the optimal temperature for performance) of a habitat is a key driver of habitat selection in ectotherms. It has even been suggested that some ectotherms, such as snakes, select habitats independently of density because temperature is more important than food availability to fitness (Harvey and Weatherhead 2010), thus some ectotherms may trade-off temperature for food in habitat selection.

According to Fretwell and Lucas (1969), the suitability (S) of habitat i can be described by

$$S_i = B_i - f(d_i)$$

where B_i is the basic suitability of a habitat and $f(d_i)$ is some function describing the negative effects of density on suitability. Note that if habitat suitability is independent of depletable resources such as food, then $f(d_i) = 0$ and habitat selection will be density-independent. The ideal free distribution starts with this equation, and suggests that individuals will choose a habitat with the highest suitability, which leads to each individual obtaining the same fitness. Using the ideal free distribution as a starting point, Morris (1988) created isodars that predict the density of individuals in habitat A given their density in habitat B. Isodars use geometric mean regressions of the density of individuals in habitat A by the density of individuals in habitat B to estimate the density at which individuals will begin using habitat B (intercept), and the rate at which individuals will select between habitats as density increases (slope). Because differences in available resources between habitats are a function of conspecific density, of the quantity of depletable resources, and of the ability of animals to extract those resources (which could be a function of thermal quality, for instance), isodars may be used successfully to predict habitat selection by ectotherms. Isodars are also able to detect effects of interference competition where dominant individuals exclude subordinates from preferred habitats (Morris 1988; Knight et al. 2008); isodars demonstrating interference competition are curvilinear (Morris 1988).

In this study, I use red flour beetles (*Tribolium castaneum*) to test the hypothesis that both thermal quality and food availability explain habitat selection patterns of ectotherms because thermal quality affects the ability of ectotherms to extract resources (i.e. obtain, digest, and assimilate resources). Specifically, I test the prediction that red flour beetles select habitats based on food availability, which is a function of conspecific density, but that density-dependent habitat selection is strongly modulated by thermal quality, a density-independent factor. I determined the thermal preference of red flour beetles by allowing beetles to select their preferred body temperature in a thermal gradient. I created high thermal quality (within the preferred temperature range) and low thermal quality (below the preferred temperature range) habitats, and then created equal food and unequal food treatments between thermal habitats. I allowed beetles to select habitats under three treatment combinations at varying densities. Finally, I determined the fitness consequences of habitat selection by measuring oviposition rates in four food and temperature treatment combinations to determine if negative density dependence is modified by the thermal quality of a habitat.

Methods

I conducted all experiments with a colony of red flour beetles (*Tribolium castaneum*) originally obtained from Carolina Biological Supply Company (Burlington, North Carolina, USA). The starting colony consisted of 200 individuals, and I let the colony grow to approximately 5000 individuals. I raised beetles in large cultures containing 95% all-purpose wheat flour and 5% brewer's yeast (all future mention of flour refers to this mixture). I maintained the cultures at 25°C and 70% humidity. I identified the sex of beetles at the pupal stage (Good 1936) and separated males and females for experiments.

I conducted four experiments to determine if density-dependent habitat selection by red flour beetles is modified by thermal quality: 1) I determined the thermal preference of flour beetles; 2) I allowed beetles to select between one habitat set at their preferred temperature and a second habitat set at 10°C below their preferred temperature while varying food abundance and density; 3) I determined how much food was required to cause beetles to switch habitat preference to a lower thermal quality habitat; and 4) I determined the fitness effects of habitat selection by examining oviposition rate under different temperature, food, and density combinations, which allowed me to examine the assumptions of density-dependent habitat selection. Each of these four experiments is detailed below.

Thermal Preference

I determined the thermal preference of red flour beetles by placing beetles in a thermal gradient ranging from 25 to 40°C. I created the thermal gradient by placing a metal box (30 × 30 cm) with five runways (5 cm wide) in an environmental chamber set at 25°C and placing heating pads under one end of the gradient. I generated a thermal map of the gradient by measuring temperature every 1 cm. I placed 10 beetles in each lane, allowed them to acclimate to the gradient for one hour, and then took pictures of the beetles in the gradient every 5 minutes for one hour using a digital camera. I assigned a temperature to each beetle in each picture based on its location on the thermal map. I used 100 male and 100 female beetles in this experiment, for a total of 10 replicates of 10 individuals for each sex. I estimated the thermal preference of all beetles as the interquartile range of all selected temperatures (Huey 1991).

Habitat Selection

I created three habitat selection treatments. All treatments had a habitat with high thermal quality (30°C, within the preferred temperature for red flour beetles – see Results; henceforth referred to as “warm”) and a habitat with low thermal quality (20°C; henceforth referred to as “cool”). I created habitats that varied in thermal quality by placing a clear plastic container ($l \times w \times h = 31 \times 17 \times 10$ cm) with 1 cm of sand as substrate in a thermal chamber set to 20°C. I then placed one end of the plastic container on heat tape set to 30°C. To create variation in food availability, I placed flour on two glass slides (75 × 25 mm) 20 cm apart in the plastic container, one in each thermal habitat. The first treatment had equal food in the warm and cool habitats (2.5 ml of flour), the second treatment had high food in the warm habitat (2.5 ml of flour in warm, 0.625 ml of flour in cool), and the third treatment had high food in the cool habitat (2.5 ml of flour in cool, 0.625 ml of flour in warm). I placed five densities of female flour beetles in the middle of each container (10, 20, 30, 40, and 50 beetles) with 10 replicates of each density for each treatment. I used female beetles in this experiment because male red flour beetles emit aggregation pheromones (Suzuki 1980) that could potentially change a beetle’s perception of habitat suitability. I counted the number of beetles in each habitat (defined as the total number of beetles found in each container half) after 24 hours. I also counted the number of beetles in each habitat after 48 hours, but I found no difference in distribution between 24 and 48 hours and, therefore, I only present the data for 24 hours for simplicity.

I used isodar analysis (Morris 1988) to test for density-dependent habitat selection in each treatment. I analysed the abundance of beetles in the warm habitat as a function of the abundance of beetles in the cool habitat for each treatment using geometric mean regressions in R (package: lmodel2; function: lmodel2; Legendre 2014). I tested for differences between isodars for each treatment by comparing the 95% confidence interval around the intercept and slope for each treatment isodar.

Relative Effects of Thermal Quality and of Food Abundance in Habitat Selection

I used the same habitats as in the previous experiment, but this time I used both unequal thermal quality (20°C and 30°C) and equal thermal quality (30°C on both sides) treatments. I placed 0.625 ml of flour in the warm habitat (30°C). I then created eight treatments with increasing food in the cool habitat (equal increments from 0.625 ml to 5.0 ml). I placed 10 female red flour beetles in the centre of each container, and then counted the number of beetles in each habitat after 24 hours. I replicated each treatment combination (eight food treatments in each of the two thermal quality treatments) 10 times.

I analysed the proportion of individuals in the low food habitat using analysis of covariance in R (package: stats; function: lm; R Core Team 2015). I used thermal quality treatment, amount of food in the second habitat, the square of the amount of food in the second habitat, and all two-way interactions as independent variables. I used bias-corrected Akaike's information criteria (package: qpcR; function: AICc; Spiess 2014) to select the best model. I considered the model with the lowest AICc to be the "best" model as long as the difference in AICc (ΔAICc) between models was > 2; when ΔAICc < 2, I chose the most parsimonious (fewest parameters) model (Akaike 1973; Bozdogan 1987). I also confirmed that statistical assumptions (i.e. normality, homogeneity of variance) were met.

Fitness Consequences of Habitat Selection

I tested the fitness consequences of habitat selection by measuring the fecundity (oviposition rate) of flour beetles. I placed five densities of beetles (10, 20, 30, 40, and 50) in 10 replicates of four habitat treatments: 30°C with 2.5 ml of flour, 30°C with 0.625 ml of flour, 20°C with 2.5 ml of flour, and 20°C with 0.625 ml of flour. Prior to the experiment, flour was sifted through a 250 µm sieve so that flour particles could not be confused with beetle eggs. I placed the flour in a plastic petri dish (10 cm diameter) and then added beetles non-selectively obtained from a culture of ~5000 beetles. I assumed a 1:1 sex ratio in the culture based on pilot studies, where the sex ratio of pupae in three different cultures was 51.1 ± 0.01% female (three samples per culture per week over 12 weeks). Although the probability of obtaining an unequal sex ratio was higher for the low density than for the high density treatments, the probability was equal across a given density treatment, and the differences should have averaged out. I did not use beetles previously identified to sex as pupae due to the large number of beetles required for this experiment (1500 per sex). I placed each petri dish in an incubator set to 20 or 30°C. After 4 days, I removed each petri dish, sifted all eggs from the flour using a 250 µm sieve, and counted all eggs. I calculated per capita fecundity as the number of eggs laid divided by the density of individuals in the treatment.

I analysed per capita fecundity (square root-transformed) using multiple linear regression in R (package: stats; function: lm), with temperature (20 or 30°C), food (0.625 or 2.5 ml), density (10, 20, 30, 40, or 50), density^2, and all two and three-way interactions (excluding the three-way interaction with density and density^2) as independent variables. Again, I used bias-corrected Akaike's information criteria to select the best model.

Results

Thermal Preference

Consistent with King and Dawson (1973), the thermal preference of red flour beetles was 28.8 to 33.8°C, with a mean selected temperature of 31.8°C and a median selected temperature of 29.9°C (Figure 1-1). I used the median temperature (rounded to 30°C) as the high thermal quality habitat in the following experiments. Using the median selected temperature is a common method for determining the preferred temperature of a species (Hertz et al. 1993).

Habitat Selection

Red flour beetles preferred the warm habitat over the cool habitat across all densities when there was equal food in each habitat ($n = 50$, $R^2 = 0.44$, $p < 0.0001$, Figure 1-2A) and when there was more food in the warm habitat ($n = 50$, $R^2 = 0.26$, $p < 0.001$, Figure 1-2B). In the third treatment (high food in cool habitat), beetles preferred the warm habitat over the cool habitat at low population density, but started to prefer the cool habitat over the warm habitat as density increased ($n = 50$, $R^2 = 0.11$, $p = 0.02$, Figure 1-2C). The 95% confidence intervals around the intercepts for all three isodars overlapped, but the slopes for the equal and high food in warm habitat treatments were higher than the slope for the high food in cool habitat treatment (Table 1-1).

Relative Effects of Thermal Quality and of Food Abundance in Habitat Selection

The proportion of beetles in the low food habitat decreased as the amount of food increased in the high food habitat in both thermal quality treatments ($df = 156$; $R^2 = 0.38$, $p < 0.0001$, Figure 1-3, Table 1-2). The proportion of beetles in the low food habitat was lower in the equal thermal quality treatment than in the unequal thermal quality treatment ($t_{1,156} = 3.91$, $p = 0.0001$) and the relationship between the proportion of beetles in the low food habitat and the abundance of food in the high food habitat was different between thermal quality treatments (Food × Treatment: $t_{1,156} = 2.61$, $p = 0.01$), where the slope was steeper for the unequal thermal quality treatment than for the equal thermal quality treatment (Figure 1-3). Two closely competing, but less parsimonious models (Table 1-2) included the non-significant effect of food^2, and were therefore not considered the best models.

Fitness Consequences of Habitat Selection

Per capita fecundity was higher at 30°C (high thermal quality) than at 20°C (low thermal quality; temperature: $t_{1,193} = 23.60$, $p < 0.0001$), and per capita fecundity decreased as food availability decreased (food: $t_{1,193} = 9.84$, $p < 0.0001$; full model: $R^2 = 0.80$, $p < 0.0001$, Figure 1-4, Table 1-3). Negative density dependence was strong and curvilinear in the 30°C treatments, but was linear and nearly absent in the 20°C treatment (density × temperature: $t_{1,193} = 4.99$, $p < 0.0001$; density^2 × temperature: $t_{1,193} = 3.04$, $p < 0.01$).

Discussion

Theories of density-dependent habitat selection were originally adapted for endothermic animals and focus on the effect of the quantity of depletable resources, such as food, yet many ectothermic animals may be limited more by environmental temperature than by food (e.g., Huey

1991; Buckley et al. 2012). My habitat selection experiments with red flour beetles demonstrate that ectotherms do select habitats based on conspecific density, but the effects of thermal quality and of food abundance on habitat selection interact. Beetles in the equal food and in the high food in warm habitat treatments both preferred the warm habitat across all densities, despite the differences in food between these treatments. The distribution of beetles in the high food in cool habitat treatment provided clear evidence of the interaction between thermal quality and food abundance in the habitat selection of ectotherms: beetles demonstrated a preference for the warm habitat at low density and an increasing preference for the cool habitat as density increased. This suggests that under low competition (low density), beetles select their preferred temperature rather than more food in the cool habitat, but as competition increases individuals are forced to use temperatures outside their preferred range to obtain sufficient food. In this way, individuals are potentially maximizing fitness by trading off heat and food in their habitat preference. It also appears that beetles were competing for food through exploitative competition rather than through interference competition, as demonstrated by my linear (rather than curvilinear) isodars (Morris 1988).

My experiment, designed to assess the relative effects of food abundance and thermal quality on habitat selection, demonstrated that beetles in the equal thermal quality treatment followed the predictions of habitat matching, whereas beetles in the unequal thermal quality treatment followed a distribution that depended partly on food, but was strongly modified by thermal quality. Beetles switched habitat preference for the cool habitat when the cool habitat had 4 times more food than the warm habitat, and beetles continued to show increased preference for the cool habitat as food increased in that habitat. These results are strong indications that habitat selection is based on both food and thermal quality in red flour beetles.

While the spatial distribution of animals is the focus of work on habitat selection, variation in population growth across habitats is an important mechanism driving variation in density. My oviposition results demonstrate a clear fitness difference between warm and cool habitats. In warm habitats, negative density dependence was strong and a decrease in food abundance caused a sharp decrease in oviposition (although not exactly in proportion to food abundance). In cool habitats, however, negative density dependence was weak, and the effect of food abundance on oviposition was also weak. This suggests that when thermal quality is low, the ability to process resources, not the ability to acquire resources, is the rate limiting factor for fitness in ectotherms. Transposed to my habitat selection experiments, these results indicate that beetles selecting the cool habitat would only have achieved equal fitness to beetles selecting the warm habitat when food was low in the warm habitat and when beetles were at the highest densities. This raises a very interesting question: why did beetles select the cool habitat at all when it would have yielded lower fitness? One possible explanation is that beetles moved to the cool habitat to forage, but then moved back to the warm habitat to process their food. Since my habitats were relatively close together, this behavioural response was possible. Future studies could test this hypothesis by marking the beetles and monitoring their movements to determine if beetles move between habitats more often when habitats differ in thermal quality than when thermal quality is equal. Alternatively, oviposition rates may not be an ultimate measure of fitness and a better measure of fitness, such as the number of descendants reaching reproductive age, may have indicated perfect habitat matching. Future work should examine the ultimate fitness consequences of habitat selection in flour beetles. Finally, another alternative hypothesis to explain this surprising pattern of beetle distribution is that beetles are also selecting habitat based on the size of the habitat, and not just on food abundance. Flour is the food source for flour beetles, but it is also the substrate in which they conduct many of their activities. While my fitness experiment related oviposition rates to the availability of heat and food, it was not designed to assess the effect of habitat size because beetles were forced to live within one habitat in a finite space. My habitat selection experiment, on the other hand, allowed individuals to select between habitats that differed in the amount of food, but also in the size of the food patch because food abundance and patch size are confounded in my design. Although the habitats were the same size (one half of the container), the food patches within the habitats differed in volume. It is thus possible that space was limiting in the low food patch. Beetles could therefore be distributing themselves based on the number of individuals that can fit in the food patch rather than based on the amount of food in that habitat. Future work could assess this hypothesis by mixing wheat flour with a lower quality food, such as corn flour, and allowing beetles to select between patches of equal size, but with different food quality (King and Dawson 1973).

Thermal quality is clearly an important aspect of habitat selection by ectotherms, and theories of habitat selection should reflect this importance. In fact, under some circumstances, thermal quality is the rate-limiting factor for fitness of ectotherms. All ectotherms require food, but they also all require a high thermal quality habitat to process food. While I expect thermal quality to be most important in predicting habitat selection patterns in ectotherms given their reliance on environmental temperature to regulate their body temperature, thermal quality actually also influences habitat selection by endotherms. Indeed, some large mammalian herbivores must select habitats to avoid heat stress (Schwab and Pitt 1991) while some small hibernating mammals must select hibernacula that are not too cold (Humphries et al. 2002). Therefore, including thermal quality as a factor that modifies habitat suitability would allow for a more accurate prediction of the distribution of animals between habitats.

Table 1-1. Isodars for red flour beetles (*Tribolium castaneum*) in three treatments that varied the quantity of food in habitats when habitats differed in thermal quality. Treatment = Equal (equal food between habitats), High (4 times more food in warm than in cool habitat), and Low (4 times more food in cool than in warm habitat). In the equation, Warm refers to the abundance of beetles in the warm habitat, and Cool is the number of beetles in the cool habitat. C.I. (Int) is the 95% confidence interval for the intercept and C.I. (Sl) is the 95% confidence interval for the slope.

Treatment	Equation	C.I. (Int.)	C.I. (Sl.)	R^2	p
Equal	Warm = 3.25 + 2.26 × Cool	-1.22 – 6.86	1.82 – 2.81	0.44	<0.001
High	Warm = 5.73 + 1.95 × Cool	1.22 – 9.25	1.52 – 2.50	0.26	<0.001
Low	Warm = 3.46 + 0.73 × Cool	-0.01 – 6.10	0.55 – 0.95	0.11	0.02

Table 1-2. Model selection (top section) and final model output (bottom section) describing habitat selection of red flour beetles (*Tribolium castaneum*) in habitats differing in thermal quality and food as food increases in one habitat. Proportion = # beetles in low food habitat / total # of beetles; Treatment = equal thermal quality and unequal thermal quality (intercept in bottom panel); Food = multiples of food in the high food habitat; Food^2 = square of Food; *k* = number of parameters in a model; AICc = bias-corrected Akaike's information criteria value; ΔAICc = difference between AICc of the model with the lowest AICc and each other model. The best model (bolded) has an AICc within 2 units of the lowest AICc, and is most parsimonious. *represents a competing, but less parsimonious model.

Model	*k*	AICc	ΔAICc
Proportion ~ Food + Treatment + Food : Treatment	**5**	**-75.96**	**1.22**
Proportion ~ Food + Treatment + Food^2 + Food : Treatment	6	-77.18	0.00*
Proportion ~ Food + Treatment + Food^2 + Food : Treatment + Treatment : Food^2	7	-76.14	1.04*
Proportion ~ Food + Treatment + Food^2	5	-72.36	4.82
Proportion ~ Food + Treatment	4	-70.88	6.30

Parameter	Estimate	S.E.	*t*	*p*
Intercept	0.75	0.05	16.17	< 0.01
Food	-0.07	0.009	8.00	< 0.01
Treatment (equal)	-0.26	0.07	3.91	< 0.01
Food : Treatment (equal)	0.03	0.01	2.61	0.01

Table 1-3. Model selection (top section) and final model output (bottom section) describing per capita fitness (square root-transformed) of red flour beetles (*Tribolium castaneum*) as temperature, food abundance, and population density are varied. Fitness = per capita # of eggs laid over four days; Temperature = temperature treatment (20 or 30°C); Food = food treatment (0.625 or 2.5 ml of flour); Density = number of beetles in a treatment; Density^2 = square of Density; *k* = number of parameters in a model; AICc = bias-corrected Akaike's information criteria value; ΔAICc = difference between AICc of the best model (bolded) and each other model. *represents a competing, but less parsimonious model.

Model	k	AICc	ΔAICc
Fitness = Temperature + Food + Density + Density^2 Temperature : Density + Temperature : Density^2	**8**	**146.44**	**0**
Fitness = Temperature + Food + Density + Temperature : Food Density^2 Temperature : Density + Temperature : Density^2	9	147.54	1.10*
Fitness = Temperature + Food + Density + Temperature : Food Density^2 Temperature : Density + Temperature : Density^2 + Food : Density + Food : Density^2	11	150.70	4.26
Fitness = Temperature + Food + Density + Temperature : Food Density^2 Temperature : Density + Temperature : Density^2 + Food : Density + Food : Density^2 + Temperature : Food : Density + Temperature : Food : Density^2	13	154.42	7.98

Parameter	Estimate	S.E.	*t*	*p*

Intercept	-1.39	0.13	10.75	< 0.01
Temperature	0.11	0.005	23.60	< 0.01
Food	0.25	0.03	9.84	< 0.01
Density	6.11	1.73	3.52	<0.01
Density^2	7.34	1.74	4.23	< 0.01
Temperature : Density	-0.34	0.07	4.99	< 0.01
Temperature : Density^2	-0.21	0.07	3.04	< 0.01

Figure 1-1. The temperatures selected by red flour beetles (*Tribolium castaneum*) in a thermal gradient ranging from 25 to 40°C. The box represents the interquartile range, the line within the box represents the median value, and the whiskers represent the maximum and minimum values.

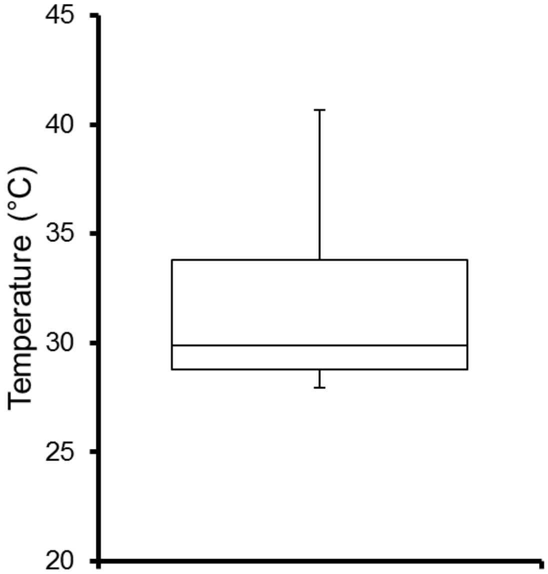


Figure 1-2. Isodars for habitat selection experiments with red flour beetles (*Tribolium castaneum*), where habitats were set at 20 and 30°C with food equal between habitats (A), food higher in the warm habitat (B), and food higher in the cool habitat (C). Density is the total number of beetles in a habitat. The solid line represents the isodar, calculated using geometric mean regression, and the dashed line represents equality between habitats. N = 10 replicates for each density treatment, which are represented as diagonal rows of points. Bold outlines represent overlapping data points.

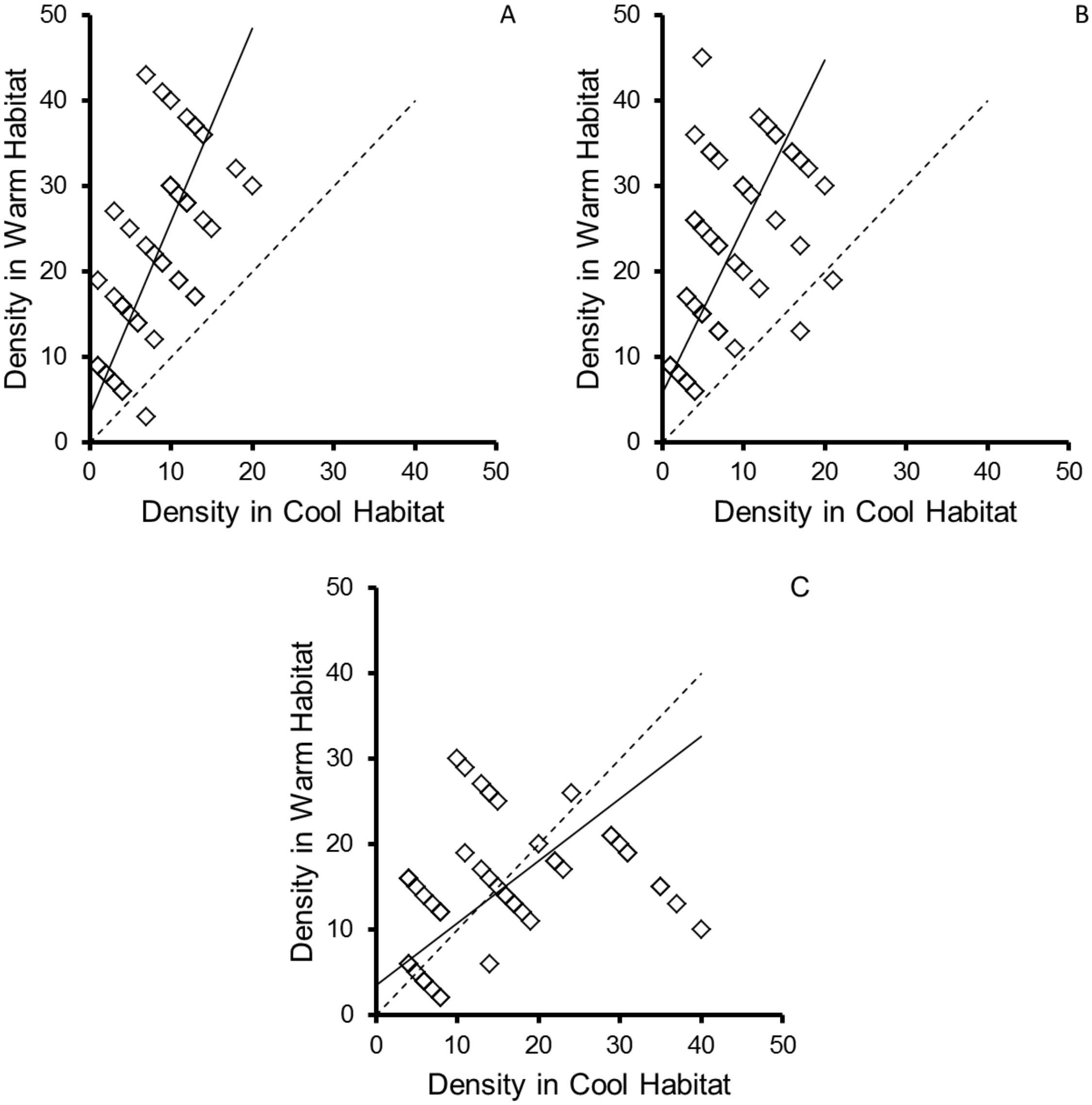


Figure 1-3. The proportion of red flour beetles (*Tribolium castaneum*) using the low food habitat (Proportion Occupying Low Food) as the amount of food in the high food habitat increased (Ratio of Food Abundance). Habitats either had equal thermal quality (30°C) or unequal thermal quality (20 and 30°C), and the low food habitat was always set at 30°C. Ratio of Food Abundance (x-axis) refers to how much more food the high food habitat had compared to the low food habitat. Linear regression best described the unequal thermal quality treatment (solid line) and the equal thermal quality treatment (dashed line). Each point represents the mean value for that treatment (n = 10), and the error bars represent the standard error of the mean.

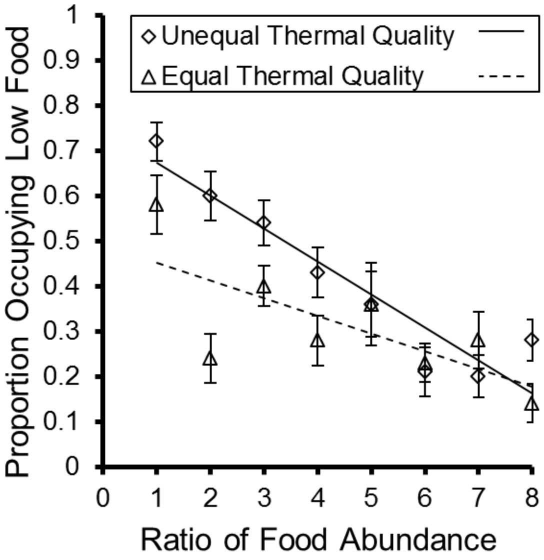
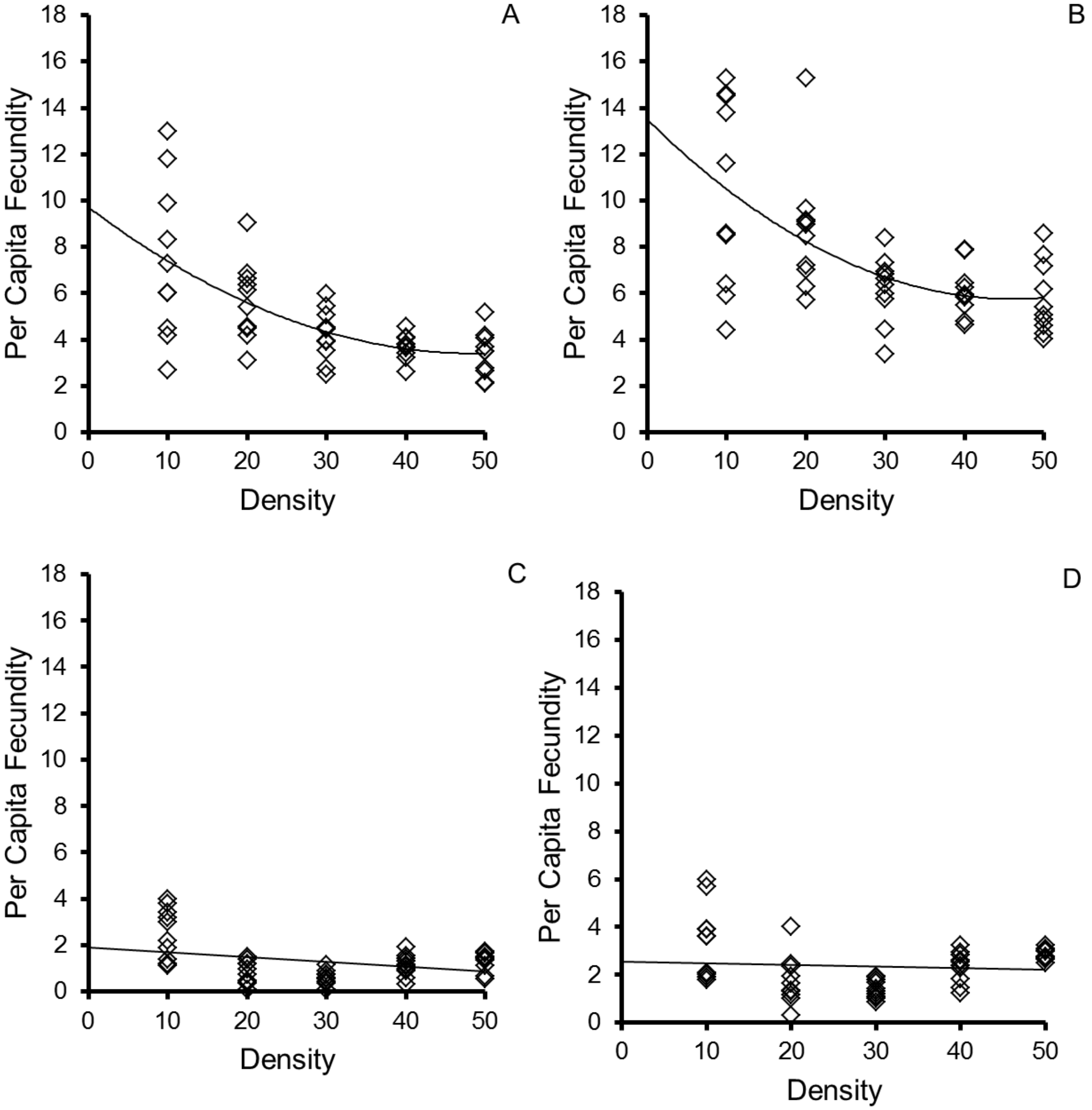


Figure 1-4. Per capita fecundity of red flour beetles (*Tribolium castaneum*) in treatments set at 30°C with 0.625 ml of flour (A) and 2.5 ml of flour (B), and set at 20°C with 0.625 ml of flour (C) and 2.5 ml of flour (D) as density increases from 10 to 50 beetles. Density is the total number of individuals in a treatment, and per capita fecundity is the number of eggs laid over four days divided by the density of beetles in the treatment. N = 10 replicates for each density treatment.



Chapter Two

Temperature modifies density-dependent habitat selection for ectotherms: theory and a test with red flour beetles

Introduction

The distribution and abundance of organisms between habitats is a fundamental aspect of ecology (Fretwell and Lucas 1969; Rosenzweig 1981; Morris 2003, 2011; Buckley et al. 2012). Habitat selection theory attempts to explain patterns in animal distribution and abundance by relating habitat suitability to fitness (Fretwell and Lucas 1969; Morris 2011). In the simplest sense, each habitat represents a set of resources that differ from the sets in other habitats, and the set of resources dictates the fitness achieved in each habitat (Fretwell and Lucas 1969). Animals maximize fitness by choosing to live in the habitat offering the greatest fitness rewards, and the mean fitness in each habitat decreases as population density increases due to increased competition. Under the ideal free distribution (IFD; Fretwell and Lucas 1969), individuals in a population maximize fitness via their density-dependent distribution between habitats such that mean fitness is the same in the various habitats, but decreases as density increases. The IFD has three main assumptions: 1) individuals have equal competitive abilities, 2) individuals have ideal knowledge of the distribution and suitability of habitats and of the distribution of competitors between those habitats, and 3) individuals are free to move between habitats without travel costs (Fretwell and Lucas 1969). Despite the unrealistic assumptions of the IFD (Kennedy and Gray 1993; Åström 1994 and Milinski 1994), density-dependent habitat selection has been demonstrated in a variety of organisms, from endothermic vertebrates such as birds (e.g., Fretwell 1969; Fretwell and Calver 1969; Jensen and Cully 2005; Zimmerman et al. 2009) and mammals (e.g., Rosenzweig and Abramsky 1986; Morris 1988; Morris et al. 2012), to ectothermic vertebrates such as lizards (Calsbeek and Sinervo 2002) and fish (e.g., Rodríguez 1995; Haugen et al. 2006; Knight et al. 2008; Falcy 2015), to invertebrates (Krasnov et al. 2003; Krasnov et al. 2004; Lerner et al. 2011; Halliday and Blouin-Demers 2014).

Environmental temperature is a major component of habitat suitability for ectotherms due to the functional relationship between fitness and temperature (Huey 1991; Blouin-Demers and Weatherhead 2001, 2008; Row and Blouin-Demers 2006; Lelièvre et al. 2011; Weatherhead et al. 2012; Halliday and Blouin-Demers 2014, 2015A; Halliday et al. 2015A, 2015B). For example, in ectotherms several important processes are maximized within a narrow range of body temperatures, including locomotion (Stevenson et al. 1985; Blouin-Demers and Weatherhead 2008; Halliday and Blouin-Demers 2015A), growth rate (Angilletta et al. 2004), energy acquisition (Bergman 1987), energy assimilation (Stevenson et al. 1985; Angilletta 2001), and reproductive output (Berger et al. 2008; Halliday and Blouin-Demers 2014, 2015A; Halliday et al. 2015A, 2015B). Moreover, temperature can modulate the density dependence of fitness (Halliday and Blouin-Demers 2014; Halliday et al. 2015A), where negative density dependence is strongest at high temperature, and weakens as temperature decreases. Since the negative density dependence of fitness is a crucial aspect of density-dependent habitat selection (Fretwell and Lucas 1969; Rosenzweig 1981; Morris 1988, 2011), can changes in temperature modify the density dependence of habitat selection in ecotherms?

In this study, I first adapt density-dependent habitat selection theory to obtain temperature-dependent predictions. I then test these predictions using a controlled habitat selection experiment with red flour beetles (*Tribolium castaneum*).

Theory

From Morris (1988), fitness (W) in a habitat (i) can be modelled as:

$$W_i = \gamma \left[R_i - N_i p \frac{E_B}{E_i} \right] \tag{1}$$

where γ is a scaling constant, R_i is the amount of resources in a habitat corrected by its renewal rate, N_i is the population density in a habitat, E_B/Ei is the efficiency of extracting resources, consumption and conversion into descendants in habitat *i* relative to the best habitat *B*, and *p* is the per capita demand on resources. Equation 1 can be simplified to a density-dependent fitness line (Morris 1988), known as the fitness-density function, where per capita fitness decreases as density increases:

$$W_i = R_i - N_i U_i \tag{2}$$

In this simplified equation, *U* is a combination of the per capita demand on resources and the extraction efficiency in habitat *i*. For ectotherms, we can add temperature dependence of resource acquisition and resource assimilation (T) to equation 2:

$$W_i = T_{Ri} R_i - N_i T_{Ui} U_i \tag{3}$$

where *T* affects both the slope (T_{Ui}) and the intercept (T_{Ri}) of the fitness-density function. At the optimal temperature for the species (T_o), T_{Ui} equals 1, and as temperature deviates from T_o, T_{Ui} approaches 0. At temperatures much higher and much lower than T_o, $T_{Ui} = 0$ because organisms can no longer extract resources and convert them to fitness. T_{Ri} affects the intercept in the same way that T_{Ui} affects the slope, where at T_o for the species T_{Ri} equals 1, and approaches 0 as temperature deviates from T_o. As temperature deviates from T_o, fitness decreases and negative density dependence of fitness weakens (Halliday et al. 2015).

Under an ideal free distribution, individuals are distributed between habitats to equalize fitness (Morris 1988):

$$W_A = W_B \tag{4}$$

where W_A is fitness in habitat A, and W_B is fitness in habitat B. We can then substitute the right side of the fitness-density function (equation 3) for W in each habitat

$$T_{RA}R_A - N_A T_{UA}U_A = T_{RB}R_B - N_B T_{UB}U_B \tag{5}$$

and solve for N_A as previously done by Morris (1990):

$$N_A = \frac{T_{RB}R_B - T_{RA}R_A}{T_{UA}U_A} + \frac{T_{UB}U_B}{T_{UA}U_A} N_B \tag{6}$$

Equation 6 can then be simplified to give the habitat isodar (Morris 1988):

$$N_A = C + bN_B \tag{7}$$

where C, the isodar intercept, is the difference in total resources between the two habitats divided by extraction efficiency and per capita resource demand in habitat A

$$C = \frac{T_{RB}R_B - T_{RA}R_A}{T_{UA}U_A} \tag{8}$$

and b, the isodar slope, is the ratio of extraction efficiency and per capita resource demand between the two habitats

$$b = \frac{T_{UB}U_B}{T_{UA}U_A} \tag{9}$$

As with the temperature-dependent fitness-density function (equation 3), the temperature-dependent isodar (equation 6) will have the highest intercept and the steepest slope at T_o for the species, and both the intercept and the slope will decrease as temperature deviates from T_o until eventually habitat selection is either equalized (individuals show equal preference for each habitat) or habitat selection is density-independent.

To illustrate this theory, I used data from Halliday et al. (2015A) (Figure 2-1A). Briefly, fitness was the per capita number of eggs laid over four days by red flour beetles at five density treatments (10, 20, 30, 40, and 50 beetles), two food treatments (0.625 and 2.5 ml of wheat flour), and three temperature treatments (20, 25, and 30°C). In red flour beetles, T_o = 30°C according to multiple measures of fitness (Halliday and Blouin-Demers 2014, 2015A; Halliday et al. 2015A). The fitness data clearly demonstrate that the fitness-density function is strongest at T_o and weakens as temperature decreases. I then built predictive isodars using these fitness data (Figure 2-1B), where each isodar is based on the assumption that beetles select habitats to maximize individual fitness (i.e., according to the Ideal Free Distribution). I predicted that the slope of the isodar should be the steepest at T_o (strongest preference for high food), and should approach equality (slope of 1) as temperature deviated from T_o. I predicted that the slope of the isodar at 20°C should be 1 because the fitness of beetles is equal between habitats at that temperature (Halliday and Blouin-Demers 2014; Halliday et al. 2015A), and an isodar should have an intercept of zero and a slope of one when individuals do not differentiate between habitats. Alternatively, habitat selection at 20°C could also be density-independent because the relationship between fitness and density was not statistically significant at 20°C (Halliday et al. 2015A).

I used red flour beetles to conduct controlled habitat selection experiments across a range of population densities and three temperatures to test these predictions. I also confirmed the assumption that fitness decreases as density increases, and the pattern that the fitness difference between habitats weakens as temperature deviates from T_o.

Methods

Study Species

I obtained a colony of 200 red flour beetles (*Tribolium castaneum*) from Carolina Biological Supply Company (Burlington, North Carolina, USA), and kept beetles with ad libitum flour (95% all purpose flour and 5% yeast) at 30°C and 70% relative humidity, under a 12:12 hour light:dark cycle. The beetles lived under these conditions for 18 months prior to my experiments and colony size increased to over 5000 individuals. For experiments requiring only females, I sexed beetles at the pupa stage (Good 1936) and kept female pupa separately.

Experiments

I set up experimental habitats in clear plastic containers (31 × 17 × 10 cm) with 1 cm of sand as a substrate. I created two food patches in each container (one at each end, with 20 cm between them) by attaching two glass slides together with tape (combined dimensions: 75 × 50 mm): a low food patch (0.625 ml of flour) and a high food patch (2.5 ml of flour). I placed each container in an environmental chamber for 24 hours with a 12:12 hour light:dark cycle at a constant temperature. I placed beetles in the middle of each container, and after 24 hours I counted the number of beetles on each side of the container. I then sifted the flour through a 250 μm sieve and counted the number of eggs that had been laid in each food patch. I used 10 replicates of five density treatments (10, 20, 30, 40, and 50 beetles) and three temperature treatments (20, 25, and 30°C), with either mixed groups (~1:1 sex ratio) or female-only groups in a fully factorial design (n = 300 containers). I used mixed groups to obtain fitness estimates (number of eggs laid) in each habitat, and I used female-only groups to avoid noise in the habitat selection data associated with male aggregation pheromones (Suzuki 1980; Halliday and Blouin-

Demers, *unpublished data*). I randomly selected all beetles for each treatment and assumed a 1:1 sex ratio in the mixed group of beetles based on the observed 1:1 sex ratio of pupa in my cultures (Halliday and Blouin-Demers *unpublished data*).

Statistical Analyses

I analysed habitat selection data with isodars (Morris 1988). I built isodars for each temperature treatment for both mixed groups and female-only groups using geometric mean regression in R (package: lmodel2; function: lmodel2; Legendre 2014) with the number of beetles in the high food habitat as Y and the number of beetles in the low food habitat as X. I compared isodars using the confidence intervals around their intercept and slope, and with the fit of each model.

I calculated the per capita number of eggs laid in a habitat two ways. First, I calculated the per capita number of eggs laid by dividing the number of eggs in a habitat by the number of beetles in the treatment (overall per capita eggs). Second, I calculated the per capita number of eggs laid by dividing the number of eggs in a habitat by the number of beetles counted in that habitat (habitat per capita eggs). I identified the factors governing overall per capita eggs using linear mixed effects models in R (package: nlme; function: lme; Pinheiro et al. 2015) with density treatment, temperature treatment, habitat type, and all two- and three-way interactions as fixed effects, and with replicate as a random effect. I identified the factors governing habitat per capita eggs with a similar model, but with the number of beetles in a habitat rather than the density treatment as a fixed effect. I compared models using Akaike's information criterion (package: stats; function: AIC; R Core Team 2015) and considered the best model the one with the lowest AIC value.

Results

Beetles demonstrated the strongest preference for the high food patch at 30°C in both mixed and female-only groups (Table 2-1, Figure 2-2). This preference for the high food patch disappeared at 25°C and at 20°C for mixed groups, and weakened at 25°C and at 20°C for female-only groups. Models for female-only groups had higher R^2 values than mixed groups at all temperature treatments.

Overall per capita eggs had the strongest negative density dependence at 30°C (Table 2-2, Figure 2-3), and density dependence was stronger in high food patches than in low food patches (high food patch: per capita eggs = 1.42 - 0.03 × density; low food patch: per capita eggs = 0.46 – 0.01 × density). The negative density dependence of egg production was weaker at 25°C, and almost absent in the low food patches (high food patch: per capita eggs = 0.54 – 0.007 × density; low food patch: per capita eggs = 0.14 - 0.001 × density). Negative density dependence of egg production disappeared at 20°C in both habitats (both habitats: per capita eggs = 0.02 + 0.001 × density).

Habitat per capita eggs had similar negative density dependence in high food patches and in low food patches (Table 2-3, Figure 2-3). Negative density dependence was strongest at 30°C (high food patch: per capita eggs = 2.65 – 0.07 × density; low food patch: per capita eggs = 1.45 - 0.07 × density), weakened at 25°C (high food patch: per capita eggs = 0.71 – 0.01 × density; low food patch: per capita eggs = 0.51 - 0.01 × density), and was absent at 20°C.

Discussion

In agreement with my prediction, density-dependent habitat selection weakened as temperature decreased from T_o, for both the slope of the relationship and for the fit of the model. This indicates that the weakening of density dependence as temperature deviates from T_o does indeed appear to affect habitat selection by beetles. Because the number of eggs laid is only one of a number of possible proximate measures of fitness, future work could examine other fitness-density functions and their ability at predicting habitat selection. For example, rates of energy acquisition and assimilation vary with temperature (Angilletta 2001), and may also decrease as density increases due to increased competition for food. These fitness metrics may therefore also be good predictors of habitat selection given the relationship between habitat suitability and energy acquisition (Morris 2014).

Habitat selection at 20°C was density-independent, in agreement with the prediction of Morris (1988) that density-independent fitness functions should lead to density-independent habitat selection. It is unsurprising that density dependence disappears at 20°C in red flour beetles because, although they lay eggs, none of these eggs successfully develop (Halliday et al. 2015A). Therefore, the non-significant fitness-density function at 20°C is indicative of zero fitness at that temperature. Beetles at 25°C had fitness that was intermediate to that at 20°C and 30°C, and the strength of density dependence was similarly intermediate to that at 20°C and 30°C. Accordingly and as predicted, this intermediate fitness-density function led to an isodar that was again intermediate to the isodars at 20°C and 30°C.

The presence of males weakened patterns of density-dependent habitat selection, as I expected. This is likely due to the aggregation pheromones that are released by males (Suzuki

1980). Individuals may therefore select habitat based on the location of pheromones rather than the factors that I manipulated (Halliday and Blouin-Demers, *unpublished data*). Even with the noise introduced by the aggregation pheromones secreted by males, beetles matched my general prediction, but the trend was much stronger when I only used females.

I found relatively weak patterns of habitat selection in all of my treatments (highest R^2 = 0.12). The strength of the relationships decreased as temperature deviated from T_o, as I predicted. It is plausible that creating habitats with a more marked difference in food quantity would have led to a more striking preference for the high food habitat, and a stronger relationship between density and habitat preference. For example, in a previous study where I manipulated habitat quality by creating habitats with different temperatures (Halliday and Blouin-Demers 2014), the isodars had much stronger fits (highest R^2 = 0.44), likely because differences in temperature had a much larger impact on habitat suitability than the differences in food that I used in this study.

My results have striking implications for our understanding of habitat selection by ectotherms. Since density dependence is strongly affected by temperature in ectotherms, field studies examining habitat selection could have confounding effects of temperature that could mask or negate the effect of density. It becomes increasingly difficult to detect density dependence as temperature departs from T_o. For example, a study conducted when environmental temperatures are well below T_o, such as the 20°C treatment in my study, could lead to the conclusion that a species does not select habitats in a density-dependent fashion, or that the species has equal preference for two habitats that actually have very different food availabilities. Moreover, in temperate ecosystems with short summers, density dependence may only be important during the few warm weeks when environmental temperatures approach T_o. It is therefore imperative to consider explicitly the role that temperature plays on density dependence when working with ectotherms.

Table 2-1. Isodar equations, built via geometric mean regression, for habitat selection by red flour beetles (*Tribolium castaneum*) selecting between low and high food habitats across a range of densities at three temperatures. Y is the number of beetles in the high food habitat and X is the number of beetles in the low food habitat. Mixed Group refers to an equal sex ratio, and Female Groups refer to experiments with only females. CI represents the 95% confidence interval.

Treatment	Isodar Equation	Intercept CI	Slope CI	R^2	p
		Mixed Groups			
20°C	Y = 30.57 – 1.04 X	27.31 to 34.90	-1.38 to -0.79	0.01	0.43
25°C	Y = 32.44 – 1.24 X	29.30 to 36.62	-1.65 to -0.93	< 0.01	0.99
30°C	Y = 5.78 + 1.70 X	0.92 to 9.46	1.29 to 2.46	0.07	0.06
		Female Groups			
20°C	Y = 0.78 + 1.43 X	-4.72 to 4.95	1.09 to 1.89	0.06	0.08
25°C	Y = 2.31 + 1.52 X	-2.92 to 6.29	1.16 to 2.00	0.10	0.03
30°C	Y = -1.34 + 1.92 X	-7.69 to 3.51	1.47 to 2.52	0.12	0.01

Table 2-2. Model selection and final model output for linear mixed effects models examining per capita eggs laid (total eggs / population density) by red flour beetles (*Tribolium castaneum*) in high and low food habitats at different population densities and at three temperatures. *k* is the number of parameters in the model, AIC is the Akaike's criteria value, and ΔAIC is the difference between the AIC of the best model and the focal model.

Model	*k*	AIC	ΔAIC
Per Capita Eggs = Density + Temperature + Habitat + all two- and three-way interactions	10	32.61	0.00
Per Capita Eggs = Density + Temperature + Habitat + all two-way interactions	9	36.72	4.11

Parameter	Estimate	S.E.	*t*	*p*
Intercept	-2.94	0.26	11.34	< 0.01
Density	0.06	0.008	7.83	< 0.01
Temperature	0.14	0.01	14.03	< 0.01
Habitat (Low)	2.13	0.37	5.81	< 0.01
Density : Temperature	-0.003	0.0003	9.25	< 0.01
Density : Habitat (Low)	-0.04	0.01	3.80	< 0.01
Temperature : Habitat (Low)	-0.10	0.01	7.07	< 0.01
Density : Temperature : Habitat (Low)	0.002	0.0004	4.51	< 0.01

Table 2-3. Model selection and final model output for linear mixed effects models examining per capita eggs laid (eggs in a habitat / beetles in a habitat) by red flour beetles (*Tribolium castaneum*) in high and low food habitats at different population densities and at three temperatures, and as the number of beetles in a habitat varied. *k* is the number of parameters in the model, AIC is the Akaike’s criteria value, and ΔAIC is the difference between the AIC of the best model and the focal model.

Model	*k*	AIC	ΔAIC
Per Capita Eggs = Beetles + Temperature + Habitat + Habitat : Temperature + Beetles : Temperature	8	766.69	0.00
Per Capita Eggs = Beetles + Temperature + Habitat + all two-way interactions	9	776.12	9.43
Per Capita Eggs = Beetles + Temperature + Habitat + all two- and three-way interactions	10	785.87	19.18

Parameter	Estimate	S.E.	*t*	*p*
Intercept	-4.65	0.68	6.88	< 0.01
Beetles	0.13	0.03	4.62	< 0.01
Temperature	0.23	0.03	8.48	< 0.01
Habitat (Low)	2.61	0.62	4.22	< 0.01
Beetles : Temperature	-0.006	0.002	5.60	< 0.01
Temperature : Habitat (Low)	-0.12	0.02	4.88	< 0.01

Figure 2-1. (A) Mean fitness-density functions, based on per capita egg laid, under high and low food treatments at three temperatures (20, 25, and 30°C) for red flour beetles (*Tribolium castaneum*) (adapted from Halliday et al. 2015A). (B) Theoretical isodars predicting habitat selection based on temperature.

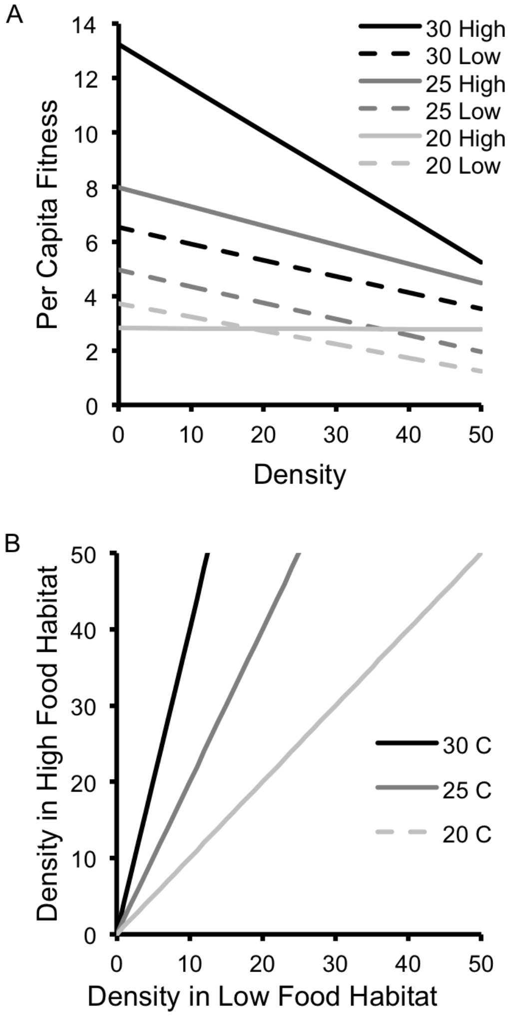


Figure 2-2. Experimental isodars for red flour beetles (*Tribolium castaneum*) selecting between habitats with high food and low food when temperature was 20°C (A, D), 25°C (B, E), and 30°C (C, F). The dashed lines represent equal selection of each habitat, and the solid lines represent statistically significant isodars. Isodars were built using mixed groups of males and females (A-C) and with female-only groups (D-F).

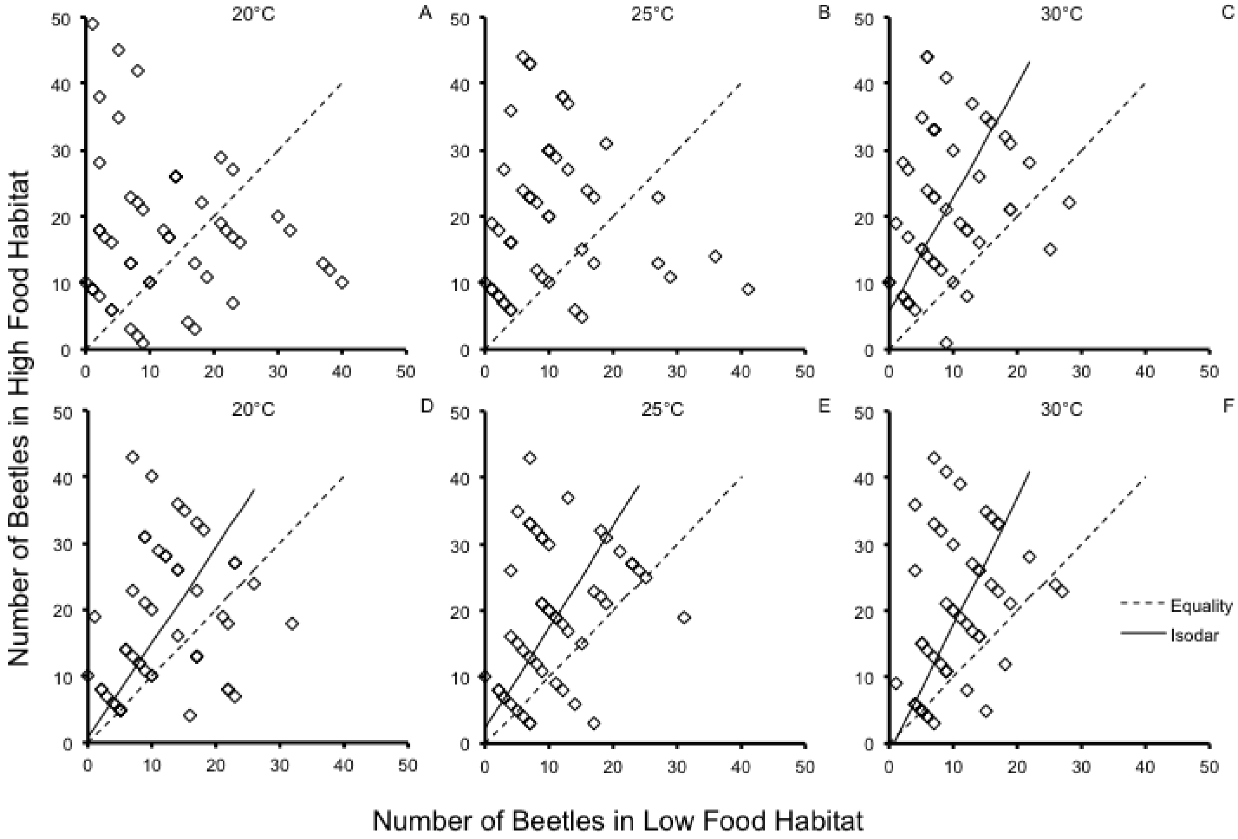
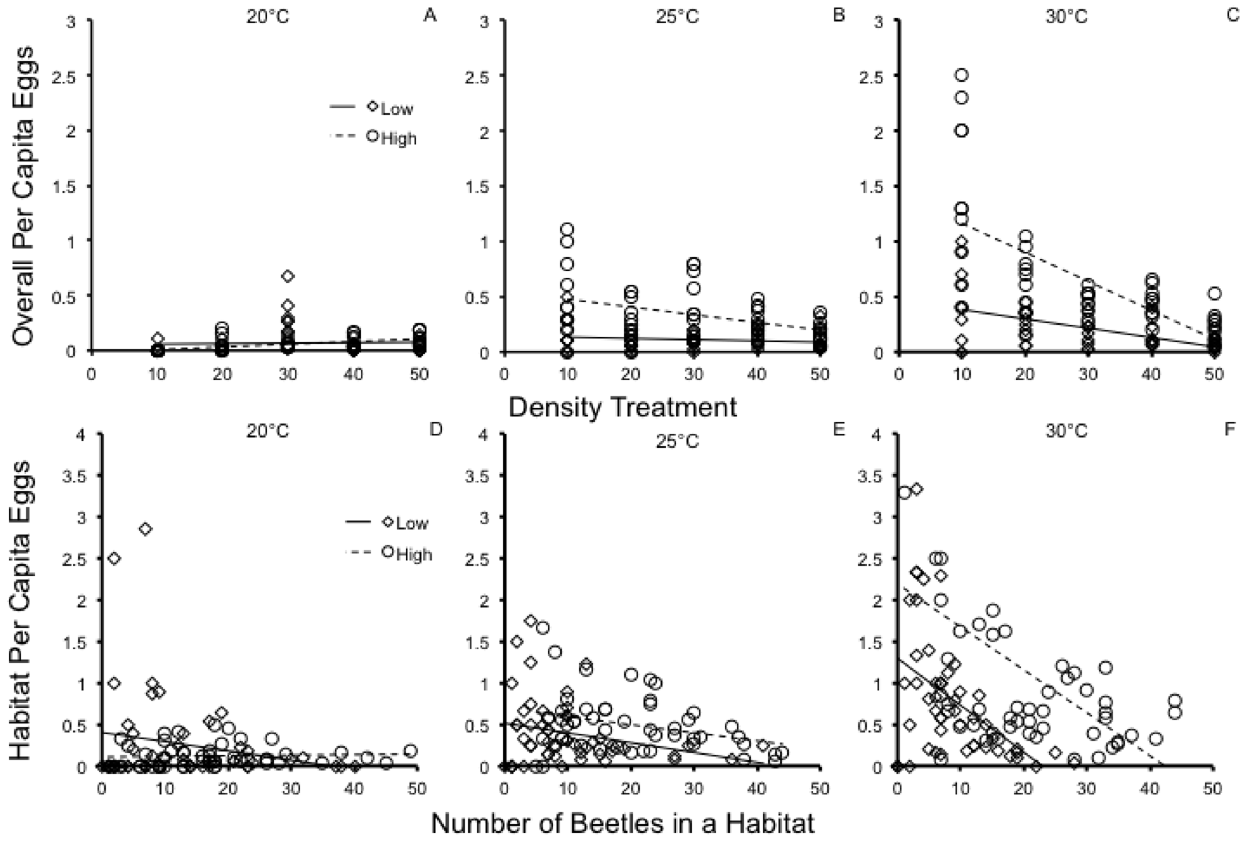


Figure 2-3. Overall per capita number of eggs (A-C) and habitat per capita number of eggs (D-F) laid by red flour beetles (*Tribolium castaneum*) in low food and high food habitats over 24 hours at different density treatments when temperature was 20°C (A, D), 25°C (B, E), and 30°C (C, F). Overall per capita eggs was calculated as the number of eggs in a habitat divided by the total number of beetles in the treatment. Habitat per capita eggs was calculated as the number of eggs in a habitat divided by the number of beetles in that same habitat.



Chapter Three

Differential fitness in field and forest habitat explains density-independent habitat selection by gartersnakes

This chapter formed the basis for the following publication:

Halliday WD, Blouin-Demers G (in press) Differential fitness in field and forest habitat explains density-independent habitat selection by gartersnakes. Oecologia.

Introduction

The ideal free distribution (IFD; Fretwell and Lucas 1969) is the predominant framework for habitat selection theory (Rosenzweig 1981; Morris 2003). The IFD predicts that organisms will distribute themselves between habitats in proportion to the suitability of the various habitats such that each individual obtains the same fitness (Fretwell and Lucas 1969). Habitat suitability was defined as “the average potential contribution from that habitat to the gene pool of succeeding generations” (Fretwell and Lucas 1969), thus explicitly linking habitat suitability to mean potential fitness. Habitat suitability is determined by several factors such as food abundance, interference competition (the ideal despotic distribution: Fretwell and Lucas 1969; Fretwell 1972; Morris 1988), interspecific competition (Rosenzweig and Abramsky 1986; Morris 1988), and predation risk (Moody et al. 1996; Grand and Dill 1999), the influence of most of which is density-dependent. Importantly, therefore, the IFD assumes that habitat suitability always decreases with density (Fretwell and Lucas 1969). Finally, the IFD assumes that individuals are ‘ideal’ in the sense that they select the habitat that will maximize their fitness and ‘free’ in the sense that they can enter any habitat on an equal basis with all their conspecifics (Fretwell and Lucas 1969). The IFD has been tested extensively in birds (e.g., Shochat et al. 2002; Jensen and Cully 2005; Zimmerman et al. 2009), mammals (e.g., Morris 1988; Ovadia and Abramsky 1995; Lin and Batzli 2002; Tadesse & Kotler 2010), fish (e.g., Rodríguez 1995; Morita et al. 2004; Haugen et al. 2006; Knight et al. 2008), and invertebrates (e.g., Krasnov et al. 2003; Krasnov et al. 2004; Lerner et al. 2011), and has also been used to examine optimal foraging behaviour (see Kennedy and Gray 1993 for a review). Despite the unrealistic ‘free’ and ‘ideal’ assumptions of the IFD (see review in Kennedy and Gray 1993; but see also Åström 1994 and Milinski 1994), the IFD remains an important and powerful theoretical framework to predict the habitat selection of organisms.

One important assumption of the IFD that has received less attention is whether habitat suitability always decreases as population density increases. In animals that are limited by depletable resources such as food, the assumption of density dependence is reasonable. Yet, food or other depletable resources are not always the most important limiting factor (Hutchinson 1959; Huey 1991; Buckley et al. 2012), especially for ectotherms that must regulate their body temperatures by selecting habitats with appropriate temperatures to perform maximally (Huey 1991; Calsbeek and Sinervo 2002; Buckley et al. 2012). Since, under most circumstances, temperature is not a depletable resource, thus violating the density dependence assumption of the IFD, can the IFD still predict the distribution of organisms that are limited by temperature in addition to food? If individuals distribute themselves between habitats based on temperature alone, we should expect density-independent habitat selection unless access to preferred temperatures was limited by competition (e.g., Calsbeek and Sinervo 2002). If habitat suitability is dictated by the interacting effects of temperature and food abundance, however, then individuals should distribute themselves among habitats according to the combined effects of temperature and food abundance (Halliday and Blouin-Demers 2014).

Snakes are probably limited more by temperature than by food; snakes can go for weeks without eating due to their relatively slow metabolism (e.g., Shine 1986) and the typically large meals they ingest. Although, to the best of my knowledge, no study has tested if snakes select habitats independently of density, it is often assumed to be the case (e.g., Harvey and Weatherhead 2006). Habitat selection studies on snakes frequently show that thermal quality is very important in dictating patterns of habitat selection (e.g., Blouin-Demers and Weatherhead

2001; Row and Blouin-Demers 2006; Lelièvre et al. 2011; Weatherhead et al. 2012), and thermal quality can be more important than the availability of food in dictating patterns of habitat selection (Blouin-Demers and Weatherhead 2001). Snakes are therefore logical organisms for testing whether the IFD can successfully predict the distribution of animals when habitat suitability is largely a function of thermal quality, and thus probably largely density-independent contrary to a key assumption of the IFD.

In this study, I tested the hypothesis that habitat selection by snakes is not a function of conspecific density because the fitness of snakes is more tightly linked to thermal quality, a non-depletable resource, than to the availability of depletable resources. More specifically, I tested the predictions that snake habitat selection patterns are density-independent and that variation in snake density across habitats is better explained by differences in thermal quality than by differences in food abundance. I tested this hypothesis with an observational study and with a manipulative study. For my observational study, I measured the abundance of small northern snakes, specifically common gartersnakes (*Thamnophis sirtalis*), red-bellied snakes (*Storeria occipitomaculata*), and Dekay's brownsnakes (*Storeria dekayi*), in field and forest, two habitats with very different thermal qualities. These habitats are dominant land covers in my study area, which should be encountered commonly by snakes. I consider both land covers to be habitats for these species because, although previous studies have demonstrated that gartersnakes show a strong preference for open habitats (Carpenter 1952; Burger et al. 2004), forests are also used (see Results). I examined the habitat suitability of field and forest based on thermal quality (in relation to the preferred body temperature of most snakes) and based on prey abundance. For my manipulative study, I used enclosures that encompassed both field and forest and manipulated common gartersnake density and food abundance. I quantified habitat selection in response to variation in conspecific density and in food abundance. Finally, I examined the fitness consequences of habitat selection to confirm that the habitat selection choices of snakes were adaptive. I measured reproductive output and growth rate of female common gartersnakes living in enclosures in field and in forest under two food regimes.

Methods

Observational Test of Density-Dependent Habitat Selection

I conducted an observational study of snake habitat selection at Queen’s University Biological Station (QUBS; 44°33’N, 76°21’W) in eastern Ontario, Canada. Although QUBS is home to nine species of snakes, I only obtained sufficient capture data for common gartersnakes (*Thamnophis sirtalis*), Dekay’s brownsnakes (*Storeria dekayi*), and red-bellied snakes (*Storeria occipitomaculata*). All three species have relatively generalized diets, but *T. sirtalis* eats invertebrates and small amphibians, whereas *S. dekayi* and *S. occipitomaculata* only eat invertebrates.

I set up five 50 × 100 m study plots that each encompassed 50% field and 50% forest. All of the fields were cut once per year, and were thereby maintained as a mixed grass and forb community. Forests were mixed hardwood, mainly sugar maple (*Acer saccharum*), ironwood (*Ostrya virginiana*), and American beech (*Fagus grandifolia*). I placed sixteen 60 × 60 cm plywood cover boards on each grid (four rows of four boards spaced every 25 m) to act as snake refuges (Halliday and Blouin-Demers 2015B), with half of the boards placed in field and half placed in forest. I surveyed field and forest habitats twice a day (09h00 and 14h00) for three days every two weeks from 5 May 2013 to 16 July 2013. These three-day periods represent one sampling period, for a total of six sampling periods throughout the study. During each survey, I walked across the plots at a constant pace and checked under every cover board. I hand-captured each snake that I encountered and gave each individual a unique mark by branding its ventral scales using a medical cautery unit (Bovie Aaron Low-Temp Reusable Cautery Unit, Clearwater, Florida; technique and rationale for branding described in Winne et al. 2006). I then released each individual at its point of capture.

I estimated the abundance of each snake species during each three-day sampling period by counting the number of individual snakes caught on each plot during the sampling period. I only counted snakes found under cover boards (snakes captured under cover boards represented 94% of the 132 snakes captured in field and 2 of the 3 snakes captured in forest) because my ability to detect snakes in field outside of the cover boards may have decreased as the season progressed due to vegetation growth.

I estimated the abundance of amphibian prey in each habitat of each plot by counting the number of amphibians encountered during each sampling period while surveying for snakes. I identified each amphibian to species. I mostly encountered northern leopard frogs (*Lithobates pipiens*) in field and American toads (*Anaxyrus americanus*) in forest, but I also encountered wood frogs (*Lithobates sylvaticus*), spring peepers (*Pseudacris crucifer*), blue-spotted salamanders (*Ambystoma laterale*), and eastern newts (*Notophthalmus viridescens*) occasionally.

I monitored invertebrate prey density in both habitats every two weeks from 21 May 2013 to 16 July 2013. I built 1 m drift fences using 10 cm wide tin material, and dug 15 cm deep pitfall traps at both ends of each drift fence with a plastic cup in each pit. I poured ~200 ml of soapy water in each pitfall trap. I built three traps in each habitat in each plot, and emptied the traps every two weeks. I identified the invertebrates from each habitat of each plot to groups

(Annelida, Arachnida, Coleoptera, Diplopoda, Lepidoptera, Mollusca, Orthoptera, and other) and weighed the wet mass of all invertebrates in each group. Although it is unlikely that snakes eat all of these invertebrates, they likely all consume a subset of these prey items. For example, both red-bellied snakes and Dekay's brownsnakes are known to eat molluscs and earthworms (Rossman and Myer 1990), and common gartersnakes are successfully fed earthworms in captivity (see fitness experiments).

I monitored the temperature that could be achieved by snakes in each habitat (environmental temperature, T_e) using copper snake models. Copper models were made of 30 cm long copper pipe (1.9 cm in diameter) with a cap soldered on one end, filled with water, and sealed on the other end with a rubber stopper. The models were approximately the same size as the most common species in my study, the common gartersnake. I placed an iButton (Maxim Integrated, San José, California) inside the model, which measured temperature every 30 minutes. I placed one copper model under a cover board and one outside of a cover board in all five forest plots and in four of the five field plots.

To assess thermal quality of the two habitats, I determined the thermal preference of common gartersnakes, the most abundant snake in my samples, by placing individual snakes (n = 49) in a thermal gradient ranging from 15 to 40°C. The thermal gradient was a wooden box (l × w × h = 170 × 74 × 35 cm) with two lanes (30 cm wide), heated at one end by heat pads and cooled at the other end by coiled tubes of cold well water. After an acclimation period of one hour, I recorded the skin temperature of each snake at their head, mid body, and tail using an infrared thermometer (Raytek Corp, Santa Cruz, CA) every hour for six hours. I then averaged these three measurements for each hour to obtain six measures of mean body temperature for each snake. Snakes used in this experiment were not part of the habitat selection experiment, but were captured in fields and wetlands within 5 km of the study plots. All snakes fasted in captivity for at least 24 hours before being placed in the thermal gradient. I calculated the thermal preference (T_{set}) of all individuals as the 25th to 75th percentiles of the selected body temperatures (Hertz et al. 1993). The T_{set} of common gartersnakes (n = 49) ranged from 25.5 ± 0.4°C to 27.4 ± 0.3°C.

I assessed the thermal quality of habitats by calculating the absolute deviations of T_e from T_{set} (d_e; Hertz et al. 1993), where a lower d_e indicates a higher thermal quality. I used T_{set} of common gartersnakes as a proxy for T_{set} of Dekay's brownsnakes and red-bellied snakes because common gartersnakes made up ~55% of the individuals caught in my study (71 common gartersnakes, 34 red-bellied snakes, 24 Dekay's brownsnakes), and the thermal preference of common gartersnakes is very similar to the mean thermal preference of other snakes in a thermal gradient (average T_{set} of other snakes = 26.8 ± 0.9°C to 30.7 ± 0.8°C; see Appendix 1 for data and details).

I analysed habitat selection data of each species using a linear mixed effects model in R (package: nlme; function: lme; Pinheiro et al. 2015). I used the abundance of each species as the response variable, habitat type as the fixed effect, and plot nested within sampling date as random effects.

I quantified the differences between field and forest habitat in terms of thermal quality and prey abundance using a MANOVA (package: stats; function: aov; R Core Team 2015), where the response variables were thermal quality (d_e), invertebrate biomass, and amphibian abundance, and the independent variable was habitat with sample period as a repeated measure. I only used d_e from daylight hours (08h00 to 20h00) because snakes in this region are mostly diurnal (e.g., Weatherhead et al. 2012). I repeated this analysis with a subset of the invertebrate data that only included the biomass of molluscs and earthworms because these two groups have been confirmed in the diets of the three species in my study (e.g., Rossman and Myer 1990).

Experimental Test of Density-Dependent Habitat Selection

I experimentally tested whether habitat selection between field and forest by common gartersnakes is density-dependent. I built experimental enclosures in Pontiac County, Québec, Canada, on a property owned by the Nature Conservancy of Canada. Enclosures were 8 × 16 m with 1.3 m walls, with the bottoms of the walls partially buried into the ground to prevent snakes from escaping. Frames of the enclosures were built with lumber and the walls were made with polyethylene vapour barrier. Half of each enclosure was in field (8 × 8 m) and the other half was in forest. The forest was mostly comprised of trembling aspen (*Populus tremuloides*) with minimal understory growth. The field was typical old field habitat with common grasses and flowering plants such as goldernrod (*Solidago* sp.), milkweed (*Asclepias* sp.), purple loosestrife (*Lythrum salicaria*), and vetch (*Vicia cracca*).

I placed three 60 × 60 cm wooden cover boards and one feeding station in each habitat within each enclosure. Feeding stations consisted of a 10 × 30 × 7 cm plastic container inserted into the ground, filled to ¾ with soil, and covered by a 30 × 30 cm wood cover with a 5 cm gap between the top of the container and the wood cover. I used these feeding stations to add large earthworms to a habitat to supplement the natural food. I moistened the soil with water whenever it became dry.

I created three food treatments and three density treatments in the enclosures in a fully factorial design, with two replicates of each food by density treatment. Each replicate lasted 7 days. The food treatments consisted of 10 earthworms in the field, 10 earthworms in the forest, or 5 earthworms in each habitat. These worms were added on day 1 of the replicate, and then counted and replenished on days 2, 4, 6, and 7 of each replicate. I collected common gartersnakes from different source populations around Ottawa, Ontario, and Pontiac, Québec to populate my enclosures (snout-vent length range = 271 to 617 mm, approximately equal sex ratio). I added snakes to the enclosures at three densities (3, 6, or 9 snakes per enclosure) ensuring an approximately even distribution of body sizes and aiming for an equal sex ratio. These numbers translate to 234, 468, and 702 snakes per hectare. The highest density of snakes that I found during the observational study was 24 snakes per hectare. Therefore, these experimental densities represent unnaturally high densities that should allow me to detect an effect of competition if it were present. I released snakes in the edge habitat in each enclosure (interface between field and forest) on day 1 of the replicate, and then counted the number of snakes in each habitat on days 2, 4, 6, and 7. I entered the enclosure as quietly as possible and quickly moved to the edge habitat in the enclosure. I caught all snakes in the edge habitat, and then systematically checked each habitat and looked under all covers in each habitat and caught every snake that I encountered. As soon as a snake was caught, I measured its body temperature using an infrared thermometer.

I analysed the experimental habitat selection data by building isodars (Morris 1988) for each food treatment using geometric mean regression in R (package: lmodel2; function: lmodel2; Legendre 2014), with the number of snakes in the field as the dependent variable and the number of snakes in the forest as the independent variable. I calculated the number of snakes in each habitat on each day of data collection; I assigned snakes found in the edge to either field or forest for this analysis depending on their body temperature, where snakes with temperatures closer to those experienced by snakes in the field were assigned to the field, and *vice versa*. I then calculated the mean distribution of snakes between field and forest within each replicate of each treatment (mean distribution across four days of data collection). Each isodar was therefore built using six data points (three density treatments, two replicates per treatment). I compared isodars for each treatment with the 95% confidence intervals around the intercepts and slopes. This analysis served to test the density dependence prediction of the IFD. Statistically significant isodars indicate density dependence, intercepts significantly different than zero indicate a strong preference for a habitat, and a slope steeper than one combined with an intercept higher than zero indicates strong habitat preference across all densities (Morris 1988).

Fitness Consequences of Field and Forest Habitat Selection

I examined the fitness consequences of living in field and forest by placing female common gartersnakes in six small enclosures in each habitat. I built small enclosures (2.67 × 2.67 x 1.3 m) in the same way I built the habitat selection enclosures. To populate my enclosures, I captured female common gartersnakes between late April and early June 2014 in a field and a wetland within 10 km of my enclosures. I placed 10 females in the field enclosures and 10 females in the forest enclosures with up to two snakes per enclosure. I ensured that each female had the opportunity to mate before starting the experiment by placing each female in a large container for thirty minutes with three adult male common gartersnakes from the same population. I assigned females in each habitat to one of two feeding treatments. I assigned females to habitat and food treatments randomly, but taking body size into account to obtain an approximately even distribution of body sizes within each treatment. Females assigned to the high food treatment were fed one large earthworm four times per week, while females in the low food treatment were fed one large earthworm two times per week. I fed snakes by isolating each snake within their enclosure and dropping an earthworm in front of their head. This normally elicited a feeding response where the snake either struck the earthworm immediately, or began flicking its tongue at the worm. I also measured the snout-vent length and mass of each female once per week throughout the experiment. I continued this experiment until the beginning of September to allow for all births to occur. A few snakes escaped or were eaten during my experiment, making the final sample size in each habitat six (one low food and five high food females in field, three females in each treatment in forest).

I examined the growth rate of female common gartersnakes for snout-vent length (SVL) and mass. First, I regressed the SVL and mass for each individual by time (the number of weeks that the snake was in the experiment), calculated the slope for each measure, and used these slopes as the growth rate for each individual for each measure. Using the slopes of the regressions allowed me to include females that escaped the enclosures or were eaten before the end of the experiment. I then used MANOVA in R (package: stats: function: manova) to examine differences in growth rate between habitat and food treatments. I used growth rate for SVL and mass as dependent variables, and habitat, food treatment, and their interaction as independent variables.

I also examined the reproductive output of females by habitat and food treatment with an analysis of variance in R (package: stats; function: aov). I used the number of offspring per female as the dependent variable, with habitat, food treatment, and their interaction as independent variables.

Results

Observational test of density-dependent habitat selection

All three species of snakes exhibited a strong and significant preference for field. Almost all common gartersnakes were captured in field (mean abundance in field = 1.83 ± 0.29, mean abundance in forest = 0.07 ± 0.07; $t_{1,29} = 6.31$; $p < 0.0001$; Figure 3-1) whereas all red-bellied snakes and all Dekay's brownsnakes were captured in field (mean Dekay's brownsnake abundance in field = 0.53 ± 0.11; $t_{1,29} = 4.98$; $p < 0.0001$; mean red-bellied snake abundance in field = 0.80 ± 0.18; $t_{1,29} = 4.55$; $p < 0.0001$).

Diurnal thermal quality was higher in field than in forest (mean diurnal d_e in field = 5.0 ± 0.2°C; forest = 6.2 ± 0.5°C; $F_{1,31} = 7.12$, $p = 0.01$). Invertebrate biomass was higher in field than in forest (mean biomass in field = 85.1 ± 9.0 g; forest = 43.7 ± 6.3 g; $F_{1,31} = 19.13$, $p < 0.001$). When I restricted the analysis of invertebrate prey to earthworms and molluscs (known prey items of all three snake species), the difference in biomass between field and forest disappeared ($F_{1,31} = 1.83$, $p = 0.19$). Amphibian abundance was not different between field and forest (mean abundance in field = 5.9 ± 2.3; forest = 2.5 ± 2.0; $F_{1,31} = 0.84$, $p = 0.37$). According to the habitat matching prediction of the IFD, snakes should have been twice as abundant in field than in forest based on total invertebrate biomass and equally abundant in field and forest based on their main invertebrate prey (worms and molluscs) or based on amphibian abundance. Contrary to the habitat matching prediction, all red-bellied snakes and all Dekay's brownsnakes were caught in field, and the majority of common gartersnakes were caught in field (field : forest ratio = 26:1).

Experimental test of density-dependent habitat selection

Common gartersnakes always preferred field over forest, regardless of the food treatment or of density (Figure 3-2, Table 3-1). The preference for field was strong across all densities when food was higher in the field than in the forest (isodar equation: Snakes in Field = 4.77 + 5.72 × Snakes in Forest) or when food was equal between field and forest (isodar equation: Snakes in Field = 4.17 + 4.20 × Snakes in Forest). The preference for field was weakest when food was higher in the forest than in the field (isodar equation: Snakes in Field = 3.68 + 1.99 × Snakes in Forest) and when density was high (Figure 3-2, Table 3-1). Habitat selection was partly density-dependent: snakes in all food treatments used forest at the highest population density, but not at the lowest population density (Figure 3-2). However, the isodars for the high food in field and high food in forest treatments were non-significant ($p = 0.08$ and 0.33, respectively), which indicates density independence, or low statistical power. The isodar for the equal food treatment was significant ($p = 0.04$), which indicates density dependence.

Fitness consequences of field and forest habitat selection

None of the six female common gartersnakes in forest gave birth, whereas three females out of six in field gave birth (Table 3-2). The growth rate (based on mass or SVL) of female common gartersnakes was higher in field than in forest (Table 3-2). Females in the field gained mass and increased in SVL, whereas snakes in the forest increased in SVL, but lost mass (Table 3-2). As predicted, therefore, fitness was higher in the habitat with the higher thermal quality. Contrary to my prediction, however, food abundance had no effect on fitness (reproduction: $F_{1,14} = 0.10$, $p = 0.76$; mass: $F_{1,14} = 0.08$, $p = 0.78$; SVL: $F_{1,14} = 0.07$, $p = 0.80$).

Discussion

I found some support for my hypothesis that habitat selection by snakes is not a function of conspecific density, but rather a function of thermal quality. Although my isodar analysis did provide some evidence of density-dependent habitat selection, this evidence was weak. I also found that the habitat with the highest thermal quality provided the greatest fitness rewards, no matter the food abundance. I found no evidence of density-dependent habitat selection in my observational test: all red-bellied snakes and all Dekay's brownsnakes were found in field, and the vast majority of common gartersnakes were found in field. Perhaps the natural density of snakes in my study area was too low to create competition for food; density dependence may only occur when densities are so high that food becomes limiting. In my manipulative experiment, in which snake densities were much higher than those in my observational study (maximum of 24 common gartersnakes/ha compared to over 200 common gartersnakes/ha), I found that gartersnakes continued to prefer field over forest, regardless of food abundance. I found weak evidence for density dependence in only one of three treatments. Overall, my results provide weak evidence for density-dependent habitat selection by snakes when habitats differ in thermal quality, but clear evidence that the habitat with the highest thermal quality is the preferred habitat. While my experiments cannot conclusively demonstrate that thermal quality is the factor causing these patterns due to other uncontrolled factors such as habitat structure and predation risk, my results do match with fitness results based on thermal physiology (Halliday, unpublished data). Assuming that thermal quality is driving habitat preference for gartersnakes, it is likely that large differences in thermal quality between habitats can lead to largely density-independent habitat selection at naturally low snake population densities. Although I only found weak evidence for density-dependent habitat selection between field and forest, it is possible that snakes exhibit density-dependent habitat selection between other habitat pairs. For instance, density dependence could be stronger between habitat pairs of equivalent thermal quality, such as old fields and wetlands. Wetlands can have higher densities of gartersnakes than fields (Halliday, *unpublished data*). Future studies should examine the density dependence of habitat selection between multiple habitat pairs. Future work should also confirm that thermal quality drives density-dependent habitat selection by snakes by controlling for the factors that were overlooked in this study and by manipulating thermal quality of one habitat. One option would be to build enclosures entirely in one macrohabitat (such as old field) and then shade one half of the enclosures so that the only difference between the two sides of the enclosures is thermal quality.

I found unambiguous support for my prediction that snakes should prefer the habitat with the highest thermal quality (field). I also confirmed that choosing the habitat with the highest thermal quality translated to increased fitness, both in terms of reproductive output and of growth rate. This leads to the obvious question: why would snakes ever use forest? Free-ranging snakes may use forest while moving between patches of their preferred field habitat. Snakes may also use forest for foraging and then return to their preferred field habitat for thermoregulation. Indeed, in my habitat selection experiment worms were consumed in the forest although I rarely found snakes in the forest. In my manipulative experiment, common gartersnakes were only found in forest at high densities, and more often when more food was provided in the forest. It is also possible that forest represent a sink habitat (Pulliam 1988) since snakes in the forest did not grow and did not give birth. It remains unclear whether population density affects fitness in snakes because I did not test for the effect of density on fitness, only for its effect on proximate measures of fitness. Snake species that are exceptionally abundant in nature should be used to test for the presence of density dependence.

My study focused on snakes because I wanted to determine whether density-dependent habitat selection could occur for a species that was likely more limited by temperature than by food. Repeating this study with a different ectotherm could yield very different results, simply because other taxa may have stronger responses to food availability, different levels of competition, or higher natural densities. Gartersnakes did not appear to compete for access to basking sites because I often found them coiled together. I have observed gartersnakes apparently competing over food items, however, where two snakes would attempt to eat the same earthworm. Therefore, I would not expect territoriality over thermal habitats in gartersnakes, but possibly over foraging patches. In a similar study on red flour beetles, I demonstrated strong density-dependent habitat selection between habitats differing in thermal quality, and the pattern of density-dependent habitat selection changed as the abundance of food in each habitat changed (Halliday and Blouin-Demers 2014). Flour beetles clearly respond strongly to both temperature and food availability. A study on lizards demonstrated strong territoriality over basking batches, where dominant individuals defended the patches with the highest thermal quality, forcing subordinates to use patches with lower thermal quality (Calsbeek and Sinervo 2002). Increased competition for resources that are limiting can increase the strength of density dependence.

Thermal quality was a strong predictor of snake habitat selection in my study, but I worked in an area that is thermally challenging for snakes. At my study site, snakes are only active six to seven months of the year and, based on the temperatures of my snake models, preferred body temperatures were only available, on average, for 1.2 hours per day in the field during my observational study. It is therefore possible that snakes living at more temperate or at tropical latitudes are not as limited by temperature. For example, Weatherhead et al. (2012) demonstrated that ratsnakes (*Pantherophis* sp.) changed their habitat preferences and activity times at different latitudes. Gray ratsnakes (*Pantherophis spiloides*) in Ontario (at my observational study site) preferred forest edges and were diurnal, eastern ratsnakes (*Pantherophis alleghaniensis*) in Illinois preferred forests and were diurnal, and western ratsnakes (*Pantherophis obsoletus*) in Texas preferred forest, but were nocturnal (Weatherhead et al. 2012). Ratsnakes used the thermally superior edge habitat in more northern latitudes, and switched to forest further south. These findings suggest that the strong preference for field in my study could change for gartersnakes at more southern latitudes where factors other than thermal quality may be more important.

Previous studies have demonstrated that environmental temperature is a very strong predictor of habitat selection in snakes (e.g., Blouin-Demers and Weatherhead 2001; Burger et al. 2004; Carfagno and Weatherhead 2006; Row and Blouin-Demers 2006), yet that food can also be important (e.g. Reinert 1984; Robertson and Weatherhead 1992; Diller and Wallace 1996). To the best of my knowledge, however, my study is the first to examine explicitly density-dependent habitat selection in snakes. Using both observational and experimental approaches, I found evidence that habitat selection can be density-dependent at unnaturally high snake densities, but habitat selection was density-independent at natural snake densities. Future studies could more fully examine the fitness effects of habitat selection, for instance by obtaining data on survival in addition to growth and reproductive output. Understanding how competition (density dependence) affects the habitat selection of snakes is crucial since density dependence is an assumption of several important ecological models (e.g. Lotka-Volterra equation (Volterra 1926); Ricker equation (Ricker 1954); ideal free distribution (Fretwell and Lucas 1969)). If density dependence does not apply to snakes, then do these ecological models apply to snake populations, and to ectotherms more generally? This is a fruitful avenue for future research.

Table 3-1. Isodars (calculated via geometric mean regression) for habitat selection by common gartersnakes (*Thamnophis sirtalis*) between field and forest in experimental enclosures that straddled each habitat. Each model refers to different food treatments, where Equal is equal food in each habitat, Field is high food in the field, and Forest is high food in the forest. CI refers to the 95% confidence interval around the intercept and slope.

Treatment	Intercept	Intercept CI	Slope	Slope CI	R^2	p
Equal	4.17	2.50 to 4.92	4.20	1.87 to 9.42	0.58	0.04
Field	4.77	2.98 to 5.49	5.72	2.27 to 14.37	0.42	0.08
Forest	3.68	0.31 to 4.79	1.99	0.65 to 6.04	0.05	0.33

Table 3-2. Reproductive output (number of offspring) and growth rates (mass and snout-vent length (SVL)) of female common gartersnakes (*Thamnophis sirtalis*) kept in enclosures in field and in forest. C.I. refers to the 95% confidence interval around the mean for each metric.

	Field		Forest			
	Mean	C.I.	Mean	C.I.	*F*	*p*
Number of Offspring	4	0 to 8	0	NA	6.07	0.03
Mass (g/week)	1.3	0.8 to 1.8	-0.2	-0.6 to 0.2	21.9	< 0.001
SVL (mm/week)	2.3	1.4 to 3.2	0.9	0.3 to 1.5	6.21	0.03

Figure 3-1. Mean number of individuals of three species of snakes detected in field and forest at Queen’s University Biological Station, Ontario, Canada: *Storeria dekayi* (Dekay’s brownsnake; shortened to *S. dekayi*), and *Storeria occipitomaculata* (red-bellied snake; shortened to *S. occipito*), and *Thamnophis sirtalis* (common gartersnake; shortened to *T. sirtalis*). Each bar was calculated using the number of individuals of each snake species caught on each of five 50 × 50 m grid across six sampling periods (n = 30 for each bar). Each bar represents the mean value, and error bars represent the standard error around the mean. Note that no *S. dekayi* or *S. occipitomaculata* were detected in forest.

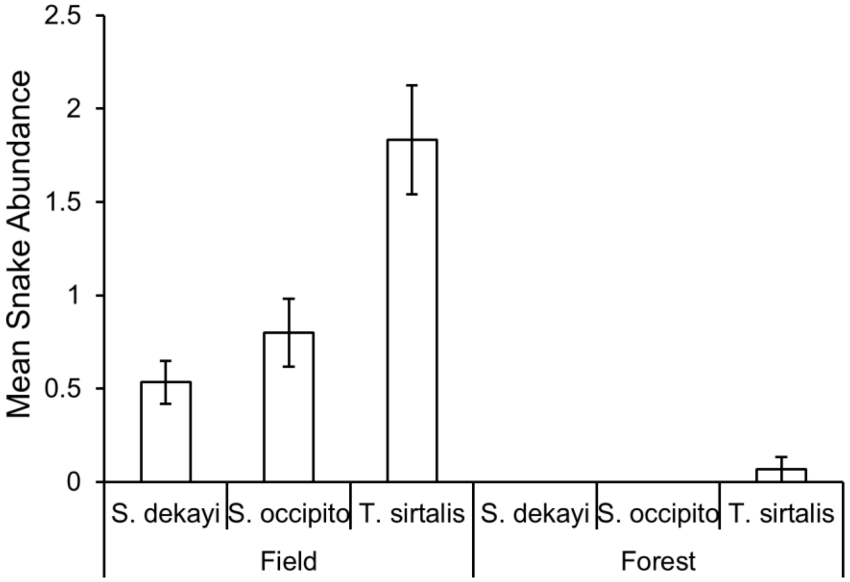
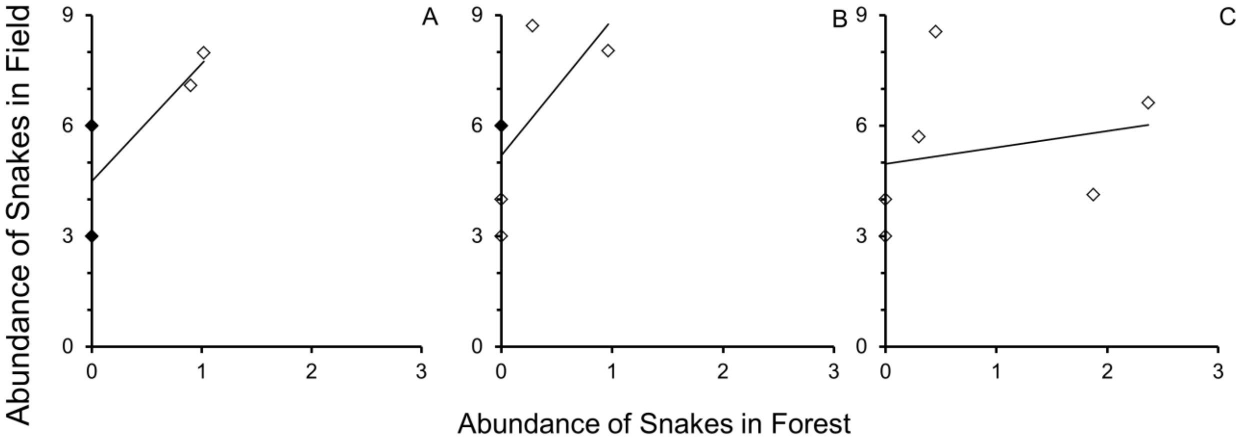


Figure 3-2. Isodars for an experimental test of density-dependent habitat selection with common gartersnakes (*Thamnophis sirtalis*) between field and forest in enclosures straddling both habitats, when food is equal between habitats (A), when food was higher in the field (B), and when food was higher in the forest (C). Filled symbols represent two overlapping points. Lines represent the predicted intercepts and slopes from a geometric mean regression.



Chapter Four

Common gartersnakes show density dependence in habitat selection despite no density dependence in fitness

Introduction

The pioneering essay of Malthus (1798) in which he argued that populations grow geometrically whereas food supplies increase arithmetically, thus predicting that populations will eventually outgrow their food supplies, established the principle of density dependence of population growth. The classic experiments by Gause (1934) in which he demonstrated density-dependent population growth and density-dependent competitive exclusion between *Paramecium* species empirically demonstrated the importance of density dependence. We now know that processes that vary with population density are prevalent. Population density affects the growth rate (e.g., Björnsson 1994) and reproductive output (e.g., Ferrer and Donazar 1996; Halliday et al. 2015) of individuals, growth rates of populations (e.g., Lin and Batzli 2001), habitat selection patterns (e.g., Morris 1989; Halliday and Blouin-Demers 2014), and even community organization through the effects of density on interspecific competition (Morris 1988; Harms et al. 2000; Morris et al. 2012). Population density affects all types of organisms; for instance, density dependence has been documented in bacteria (e.g., Stevens and Holbert 1995), in single-celled algae (Moses et al. 2013), in plants (Goldberg et al. 2001), in insects (Halliday et al. 2015), and in most vertebrate classes (mammals: Morris et al. 2012; birds: Rodenhouse et al. 2003; fish: Hixon and Carr 1997; lizards: Massot et al. 1992).

Density-dependent habitat selection is a special case of density dependence and it arises because of the negative density dependence of fitness. If individuals are selected to maximize fitness via habitat choice, then patterns of habitat selection should change with density. At low density, individuals should live in the highest quality habitat. Habitat quality is determined by the factors that influence fitness, such as food availability and safety from predators. As density increases in a habitat, mean per capita fitness decreases because at least some of the resources in the habitat are depletable, such as food, and per capita resource availability decreases as density increases. The highest quality habitat eventually will offer the same fitness rewards as lower quality habitats because of the high population density in the highest quality habitat. At this point, it becomes beneficial for some individuals to live in the lower quality habitats at low density because the same mean per capita fitness can be achieved. Therefore, individual habitat selection choices should change as population density changes because of the negative density dependence of fitness. Under an ideal free distribution (Fretwell and Lucas 1969), individuals use the habitat offering the highest fitness rewards, and individuals are distributed between habitats such that the mean fitness is the same in all habitats. The ideal free distribution has three main assumptions: 1) individuals have perfect knowledge of the quality of all available habitats and of the distribution of competitors between those habitats; 2) all competitors are equal; and 3) individuals are free to move between habitats and there is no travel cost associated with moving between habitats (Fretwell and Lucas 1969). Although the ideal free distribution was proposed nearly half a century ago, and although its assumptions are unrealistic (reviewed by Kennedy and Gray 1993), the ideal free distribution is still commonly used as the foundation for theoretical advances (e.g., Krivan 2014; Cressman and Tran 2015; Matthiopoulos et al. 2015).

Temperature is one of the most important environmental drivers in ecology (e.g., Buckley et al. 2012; Sunday et al. 2012; Araújo et al. 2013), yet its modulating effect on density dependence has only recently been established (Halliday and Blouin-Demers 2014; Halliday et al. 2015A). The vast majority of animals are ectotherms and, by definition, do not maintain their internal body temperature via metabolism. Instead, most ectotherms use behavioural thermoregulation to regulate their body temperature. Because physiological processes depend on temperature, the performance and fitness of ectotherms is tightly linked to their body temperature

(Bennett 1980; Huey and Kingsolver 1989; Angilletta et al. 2002; Halliday and Blouin-Demers 2015A). For example, red flour beetles can survive between 5 and 40ºC, but they can only successfully reproduce between 23 and 35ºC (Halliday et al. 2015A). Within this range of temperatures over which reproduction is possible, red flour beetles produce the most eggs at 30ºC (Halliday and Blouin-Demers 2014, 2015A; Halliday et al. 2015A). If ectotherms are unable to maintain their body temperature within the optimal range for performance, then density dependence weakens due to the reduced ability of individuals to assimilate energy (Halliday et al. 2015A).

Snakes are ectotherms that are considered to exhibit weak density dependence (Nowak et al. 2008). Snakes may even select habitats independently of density (Harvey and Weatherhead 2006) although, to the best of my knowledge, this has not been formally established. Because negative density dependence of fitness is caused by increased competition for depletable resources (often food) as density increases, Nowak et al. (2008) postulated that functional and numerical responses by snakes should be much weaker than for endothermic predators because of the low energetic requirements of snakes, and because of the generally much longer time between meals for snakes. For example, female long-tailed snakes (*Philodryas chamissonis*) in Chile require approximately one 30 g rodent per week throughout the 24-week reproductive season, which corresponds to approximately 16% of their body mass every week (Bozinovic and Rosenmann 1988). These energetic requirements are 4 to 5% that of a similar sized endothermic predator (Bozinovic and Rosenmann 1988). Given that snakes have low energetic requirements and apparently do not compete for food, changes in population density may not affect their rates of energy acquisition, and ultimately their fitness. If density does not affect fitness in snakes, then density should similarly have no effect on habitat selection.

In this study, I examine density dependence in fitness and habitat selection in common gartersnakes (*Thamnophis sirtalis*). I test the hypothesis that gartersnakes select habitats independently of density because their low energetic requirements should make their fitness independent of density at ecologically relevant densities. I used experimental enclosures in which I manipulated gartersnake density and food abundance. I monitored growth and female reproductive output as measures of fitness, and documented habitat selection.

Methods

I collected all gartersnakes for this experiment in late May and early June 2015 at a wetland in Pontiac County, Québec. I used active searches and cover boards to capture snakes (Halliday and Blouin-Demers 2015B). I marked each snake with ventral scale branding (Winne et al. 2006) and transported them to my experimental enclosures. Following the experiment, I released all snakes back at their capture site. My methods were approved by the University of Ottawa Animal Care Committee (protocol BL-278), which follows the guidelines of the Canadian Council on Animal Care. All snakes were used under the authority of Québec Permis Scientifique 20150307001SF.

I built nine experimental enclosures in an old field in Pontiac County, Québec. The dominant vegetation in this old field included grasses (*Poa* spp.), goldenrod (*Solidago* sp.), milkweed (*Asclepias* sp.), and vetch (*Vicia* sp.). I built frames with lumber and walls with polyethylene sheeting; I buried the bottom 10 cm of each wall in the ground. Each enclosure was 1.3 m tall, 2.7 m wide, and 10.7 m long. I surrounded the enclosures with an electric fence and placed nylon mesh (2 cm spacing) over the enclosures to keep predators out. I created one foraging patch at each end of each enclosure by digging a 30 x 10 x 7 cm plastic container into the ground, filling half the container with soil, and adding a 60 x 60 cm plywood cover. I placed six earthworms in one foraging patch and none in the other. I alternated the patch receiving food every two weeks for eight weeks. I also added one 60 x 60 cm piece of tin to each end of the enclosure as cover.

I created three density treatments with three replicates of each by adding 2, 4 or 6 common gartersnakes to each enclosure (Figure 1). I systematically placed individuals in each enclosure according to their snout-vent length (SVL) so that enclosures had similar distributions of SVL and sex-ratios. Once per week, I measured the SVL and mass of each snake to monitor growth. Thrice per week, I counted the number of snakes in each half of the enclosure and then replenished the worms. I replaced snakes that disappeared during the experiment with individuals of approximately the same size.

Near the end of the experiment, gravid females gave birth. I collected all offspring from the enclosures and measured the mass and SVL of each offspring. Common gartersnakes mate as they exit their hibernaculum in the spring, and this typically occurs in late April and early May at my study site. All females therefore had the opportunity to mate prior to be being collected for my experiment. Gartersnakes tend to reproduce biennially (Blem 1982; Whittier and Crews 1990), therefore I expected half the females to be gravid during my experiment.

I monitored the natural abundance of worms and slugs in each enclosure, as well as at four locations just outside of the enclosures. At each sampling location, I dug holes in the ground, placed plastic cups (7 cm diameter, 15 cm height) in the holes, and half filled each cup with soil. Once per week, I counted and removed all slugs and worms from each cup.

Growth Rate and Reproductive Output

I calculated the growth rate for mass and for SVL for each snake in each enclosure by regressing mass and SVL by time. I used the slopes of these regressions for each individual as their growth rates. For gravid females, I calculated mass growth rate up until the week before they gave birth. I tested for density dependence of growth with linear mixed effects models in R (package: nlme; function: lme; Pinheiro et al. 2015). I used SVL or mass growth rate as the dependent variable, density, reproductive status (male, gravid female, non-gravid female), initial mass or SVL, and number of weeks that the snake was in the experiment as fixed effects, and enclosure as a random effect. I included reproductive status, initial size, and weeks into the experiment as control variables. I only had a single non-gravid female in the lowest density treatment, and its mass growth rate was an outlier. I therefore removed this outlier and re-analyzed the data. I used bias-corrected Akaike's information criteria (AICc; package: qpcR; function: AICc; Spiess 2015) for model selection, and considered the model with the lowest AICc to be the best model. I considered models to be competing if they were within 2 AICc units of the best model (Bozdogan 1987).

I tested for density dependence of reproductive output with linear regression (package: stats; function: lm; R Core Team 2015). I used the number of offspring per female as the dependent variable, and density treatment and initial SVL of the female as independent variables. I also examined the SVL of offspring using linear mixed effect models, with density, SVL of the mother, and litter size as fixed effects, and female ID as a random effect.

Foraging

Snakes had access to the worms I provided in foraging stations and to the food naturally available in the enclosures. Thus, I tested for the effect of snake density on natural food abundance (worms and slugs) with linear mixed effects models. I conducted two analyses with different dependent variables: total number of worms or total number slugs in the three pitfall traps. For each analysis, I used snake density (2, 4, or 6 snakes/enclosure), days since last collection of the potential prey (which varied early in the experiment), and weeks into the experiment as fixed effects, and the ID of the trap station as a random effect. I assigned a snake density of zero to pitfall traps outside of the enclosures. Although there were certainly some snakes outside of the enclosures, their density would be much lower compared to densities in my enclosures. Density in my enclosures ranged from 700 to 2100 gartersnakes/ha, whereas the highest natural density that I have measured in the same habitat was 24 gartersnakes/ha (Chapter One).

Habitat Selection

I used isodar analysis (Morris 1988) to compare the abundance of snakes on one side of the enclosure versus snakes on the other side of the enclosure in relation to the location of food provisioning. I built isodars using geometric mean regression in R (package: lmodel2; function: lmodel2; Legendre 2014), with the number of snakes on the side of the enclosure with food supplementation as y and the number of snakes on the side of the enclosure without food supplementation as x. To build the isodar, I took the mean distribution of snakes in each enclosure during each two-week period of the experiment (n = 36). I compared the isodar intercept to zero and slope to one with their 95% confidence intervals. An isodar with an intercept significantly different from zero demonstrates preference for a habitat, and a slope that is significantly different from one demonstrates that habitat preference changes with population density (Morris 1988). Statistically significant isodars are considered evidence of density dependence in habitat choice, and the R^2 represents the strength of density dependence (Morris 1988).

Results

Growth Rate and Reproductive Output

When I included the outlier in my analysis, non-gravid females lost mass as density increased ($t_{30} = 2.87$, $p < 0.01$), but this effect disappeared when I removed the outlier ($t_{30} = 0.14$, $p = 0.89$; Figure 4-2A, Table 4-1). Growth in length was unaffected by density, regardless of reproductive status ($t_{30} = 0.77$, $p = 0.46$; Figure 4-2B, Table 4-2). Based on the analysis without the outlier, reproductive females gained more mass (4.0 ± 0.7 g/week, $t_{31} = 5.89$, $p < 0.01$) than males (0.2 ± 0.8 g/week, $t_{31} = 4.92$, $p < 0.01$), and non-gravid females lost mass (-0.3 ± 0.8 g/week; $t_{31} = 5.53$, $p < 0.01$; Figure 4-2, Table 4-1).

Females living at high density did not give birth to smaller offspring ($t_{75} = 1.50$, $p = 0.19$, Table 4-3) and did not have smaller litters ($t_{75} = 1.71$, $p = 0.14$, Table 4-4) than females living at lower densities.

Foraging

Pitfall traps caught fewer worms as snake density increased ($t_{102} = 2.61$, $p = 0.02$; Table 4-5). On the other hand, the abundance of slugs was unaffected by snake density ($t_{102} = 0.21$, $p = 0.84$; Table 4-6).

Habitat Selection

Gartersnakes had an equal preference for both sides of the enclosure, and this equal preference was density-dependent. The isodar examining habitat selection between the two sides of the enclosures in relation to the location of food provisioning was statistically significant (p = 0.02, R^2 = 0.15; Figure 4-3), but the intercept was not different from zero (intercept = 0.18, 95% CI = -0.54 to 0.71) and the slope was not different from one (slope = 1.03, 95% CI = 0.75 to 1.42).

Discussion

Density dependence is prevalent in most animal taxa, and my experiment suggests that it also occurs in snakes. I found no evidence for density dependence of growth rate and reproductive output in common gartersnakes but, surprisingly, I still found evidence of density dependence in habitat selection. Researchers have suggested that density dependence is not important for snakes, either because snakes naturally live at population densities that are too low to detect density dependence (Harvey and Weatherhead 2006) or because their energetic requirements are too low for competition to be important (Nowak et al. 2008). The highest density of gartersnakes that I have measured in the field was 24 snakes/ha (Chapter One), but to maximize my chances of detecting density dependence I used gartersnake densities between 700 and 2100 snakes/ha in my experiment. It appears likely that snakes rarely reach natural densities high enough for density dependence to be important, but my experiment provides evidence that under some circumstances density dependence can be important for snakes, at least in habitat selection. Many temperate snakes congregate at communal hibernacula. For example, red-sided gartersnakes (*Thamnophis sirtalis parietalis)* hibernacula in Manitoba, Canada, can contain over

20,000 individuals (Shine et al. 2001). If snakes are feeding before dispersing from their hibernacula, then such densities may be high enough for density dependence to occur.

Gartersnakes demonstrated equal preference for both sides of my enclosures at all densities, regardless of the location of food supplementation. Snakes often ate all worms at feeding stations, it is therefore possible that I did not provide enough worms to successfully bias the distribution of snakes to one side of the enclosures. It is also possible that my food supplementation was relatively small compared to the naturally available worms in my enclosures. The fact that I captured fewer worms in my pitfall traps when snake density was high does indicate that snakes were depleting their naturally available prey population. Thus, it is possible that snakes in my enclosures were competing for the naturally available worms, that I presume were equally distributed in my enclosures given the homogeneous habitat, but that competition for my supplemental worms was short-lived because they were rapidly exhausted. If that inference is correct, it would explain why I found evidence of density dependence in habitat selection (a statistically significant isodar), but no preference for the side of the enclosure containing supplemental worms (an intercept not statistically different from 0). Future studies could use higher levels of food supplementation to assess if habitat preference is based on the distribution of food, but food saturation could equally lead to the inability to detect density dependence. A natural experiment in a system with patchily distributed food could be used to examine habitat preference based on the location of food, but natural snake densities would have to be high enough for competition to occur. Therefore, testing for density dependence in snakes will remain a challenge.

I detected no density dependence in my metrics of fitness. Again, it is possible that my food supplementation was relatively small compared to the naturally available worms in my enclosures, and that the food supply may have been high enough to supress competition. Given the functional relationship between energy acquisition (foraging) and fitness (Morris 2014), it appears likely that snakes in high-density enclosures still managed to eat enough worms to have fitness equal to that of snakes in low-density enclosures. If that were the case, the density-dependent habitat selection I observed was not driven by density dependence of foraging rate. Density dependence can also occur in other aspects of fitness that are not related to energetics. For example, predation risk can increase as density increases because predators are attracted to habitats with a high density of prey (e.g., Rangeley and Kramer 1998), or because prey are more conspicuous when aggregated. Thus, snakes in my enclosures could have been attempting to reduce predation risk by being distributed equally in the enclosure because a clumped distribution could have increased predation risk. Conversely, it is possible that high snake density did cause competition for food in my experiment, but that my measures of fitness were not sensitive enough to detect the fitness consequences of such competition. Although my experiment lasted for eight weeks during the peak of growth for temperate snakes, it is possible that a longer experiment would have been required to detect density dependence in growth or reproduction, especially if reproduction and growth at least partly depend on accumulated energy reserves (Bonnet et al. 1998). Finally, given the high variance inherent to growth rate measurements (Blouin-Demers 2003), maybe an increased sample size would have been required to detect negative density dependence in growth.

Although some studies have suggested that density dependence is not important for temperate snakes due to naturally low population densities (Harvey and Weatherhead 2006), it is possible that density dependence is important for snakes closer to the tropics. For example, Weatherhead et al. (2012) demonstrated that habitat selection patterns of ratsnakes changed across their geographic range as the thermal quality of the environment changed. Porter and Tracy (1974) suggested that the weekly energetic requirements of gartersnakes increase for snakes farther south in their range. Snakes closer to the tropics are able to maintain optimal body temperatures for longer than temperate snakes. For this reason, snakes in the tropics may have higher energetic demands, which would make them more likely to compete over food than temperate snakes. In fact, Nowak et al. (2008) suggested that tropical snakes that eat frequently would be good models to look for density dependence. Future work could examine density dependence in wide ranging species to determine if density dependence is more prevalent in southern versus northern populations.

In summary, common gartersnakes showed evidence of density-dependent habitat selection, but no evidence of negative density dependence in growth rate or reproductive output. Although I used gartersnake population densities much higher than those typically observed in nature, my experiment revealed, for the first time, that density dependence can occur in snakes. The next steps are to determine if and when density dependence occurs in free ranging snakes, which is especially important given that density dependence is an important assumption in basic models of population growth and regulation (e.g,. Lotka-Volterra equation (Volterra 1926); Ricker equation (Ricker 1954)).

Table 4-1. Model selection and final model output for linear mixed effects models examining the mass growth rate of common gartersnakes (*Thamnophis sirtalis*) living in experimental enclosures at different population densities in Pontiac County, Québec. Gartersnakes were in different reproductive classes, and were in the experiment for different lengths of time (week). *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc.

Model	*k*	AICc	ΔAICc
Mass GR = Reproduction	6	188.36	0.00
Mass GR = Reproduction + Week	7	191.18	2.82
Mass GR = Reproduction + Week + Density	8	194.12	5.76
Mass GR = Reproduction + Week + Density + Starting Mass	9	203.71	15.35
Mass GR = Reproduction + Week + Density + Starting Mass + Reproduction : Density	11	207.44	19.08

Parameter	Estimate	S.E.	*t*	*p*
Intercept	3.95	0.67	5.89	< 0.01
Reproduction (Non-gravid Female)	-4.32	0.78	5.53	< 0.01
Reproduction (Male)	-3.81	0.77	4.92	< 0.01

Table 4-2. Model selection and final model output for linear mixed effects models examining the snout-vent length (SVL) growth rate of common gartersnakes (*Thamnophis sirtalis*) living in experimental enclosures at different population densities in Pontiac County, Québec. Gartersnakes were in different reproductive classes, and were in the experiment for different lengths of time (week). *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. * represents competing models that are within two AICc units of the model with the lowest AICc. Final model output (lower panel) is based on model averaging between competing models.

Model	*k*	AICc	ΔAICc
SVL GR = 1	4	203.67	0.00
SVL GR = Reproduction	6	203.91	0.24*
SVL GR = Density + Reproduction + Starting Mass + Weeks	9	216.70	13.03
SVL GR = Density + Reproduction + Starting Mass + Weeks + Density : Reproduction	11	219.12	15.45

Parameter	Estimate	S.E.	*t*	*p*
Intercept	3.66	0.36	10.67	< 0.01
Reproduction (Non-gravid Female)	1.05	0.93	1.13	0.26
Reproduction (Male)	0.54	0.95	0.57	0.57

Table 4-3. Model selection and final model output for linear mixed effects models examining the snout-vent length (SVL) of newborn common gartersnakes from mothers that lived in experimental enclosures at different population densities in Pontiac County, Québec. Mothers varied in SVL and had different litter sizes. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. * represents competing models that are within 2 AICc units of the model with the lowest AICc. Final model output (lower panel) is based on model averaging between competing models.

Model	*k*	AICc	ΔAICc
SVL = Density	4	530.68	0.00
SVL = Density + Mother SVL + Litter Size	6	532.53	1.85*
SVL = 1	3	536.36	5.68

Parameter	Estimate	S.E.	*t*	*p*
Intercept	48.83	34.77	2.41	0.02
Density	9.43	6.63	1.50	0.19
Mother SVL	0.11	0.21	0.54	0.62
Litter Size	0.72	0.89	0.81	0.48

Table 4-4. Model selection and final model output for linear models examining the litter size of female common gartersnakes (*Thamnophis sirtalis*) that lived in experimental enclosures at different population densities in Pontiac County, Québec. Mothers varied in SVL. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. * represents competing models that are within 2 AICc units of the model with the lowest AICc. Final model output (lower panel) is based on model averaging between competing models.

Model	*k*	AICc	ΔAICc
Litter Size = Mother SVL	3	70.63	0.00
Litter Size = 1	2	71.09	0.46*
Litter Size = Mother SVL + Density	4	71.85	1.22*

Parameter	Estimate	S.E.	*t*	*p*
Intercept	-41.16	3.44	3.10	0.01
Mother SVL	0.12	0.06	1.95	0.09
Density	3.43	2.00	1.71	0.14

Table 4-5. Model selection and final model output for linear mixed effects models examining the total number of worms in pitfall traps found inside and outside experimental enclosures housing different densities of common gartersnakes (*Thamnophis sirtalis*) in Pontiac County, Québec. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc.

Model	*k*	AICc	ΔAICc
Total Worms = Density + Week + Days Since Last Check	6	444.16	0.00
Total Worms = Density	4	479.28	35.12
Total Worms = Density + Week	5	485.00	40.84

Parameter	Estimate	S.E.	*t*	*p*
Intercept	1.52	0.46	3.35	< 0.01
Density	-0.17	0.07	2.61	0.02
Week	0.14	0.09	1.50	0.14
Days Since Last Check	-0.11	0.10	1.08	0.28

Table 4-6. Model selection and final model output for linear mixed effects models examining the total number of slugs in pitfall traps found inside and outside experimental enclosures housing different densities of common gartersnakes (*Thamnophis sirtalis*) in Pontiac County, Québec. *k* is the number of parameters in the model, AICc is the bias-corrected Akaike's information criterion vale, and ΔAICc is the difference in AICc between the model and the model with the lowest AICc. Final model output (lower panel) is for the model with the lowest AICc.

Model	*k*	AICc	ΔAICc
Total Slugs = Density + Week + Days Since Last Check	6	480.18	0.00
Total Slugs = Week	4	510.38	30.20
Total Slugs = Density	4	538.47	58.29

Parameter	Estimate	S.E.	*t*	*p*
Intercept	-0.80	0.53	1.52	0.13
Density	0.01	0.07	0.21	0.84
Week	0.29	0.11	2.55	0.01
Days Since Last Check	0.15	0.12	1.19	0.24

Figure 4-1. Design schematic for an experiment examining the effect of population density on fitness and habitat selection for common gartersnakes (*Thamnophis sirtalis*) in Pontiac County, Québec. Rectangles at the top of the figure represent the experimental enclosures, and the numbers within the rectangles represent the density of common gartersnakes within each enclosure. A and B represent the two sides of each enclosure. The rectangle at the bottom demonstrates the locations of cover objects and feeding stations within each enclosure.

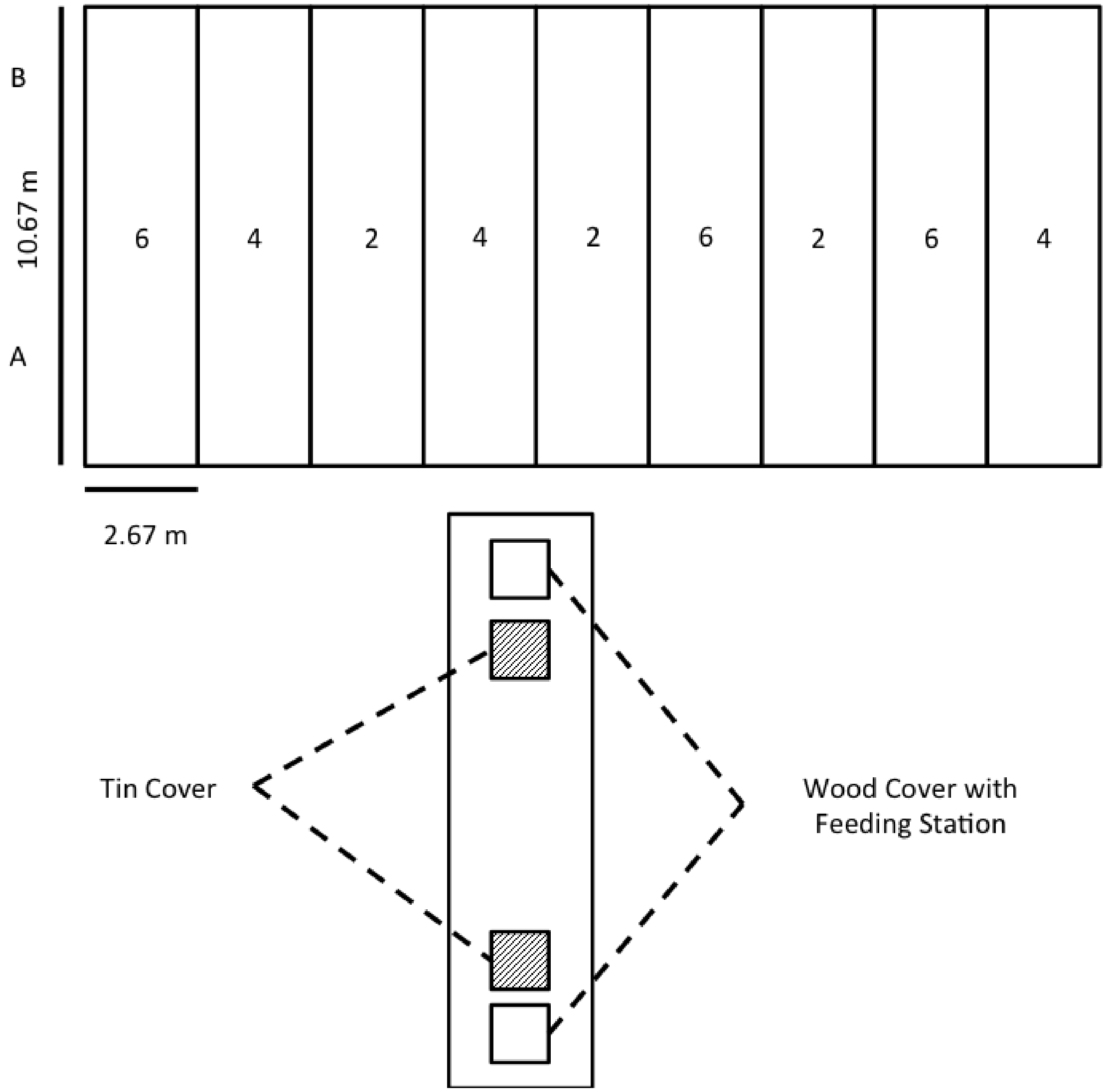


Figure 4-2. Mass (A) and snout-vent length (SVL; B) growth rate for common gartersnakes (*Thamnophis sirtalis*) in different reproductive classes living in experimental enclosures at different population densities in Pontiac County, Québec. FG = gravid females, FN = non-gravid females, and M = males.

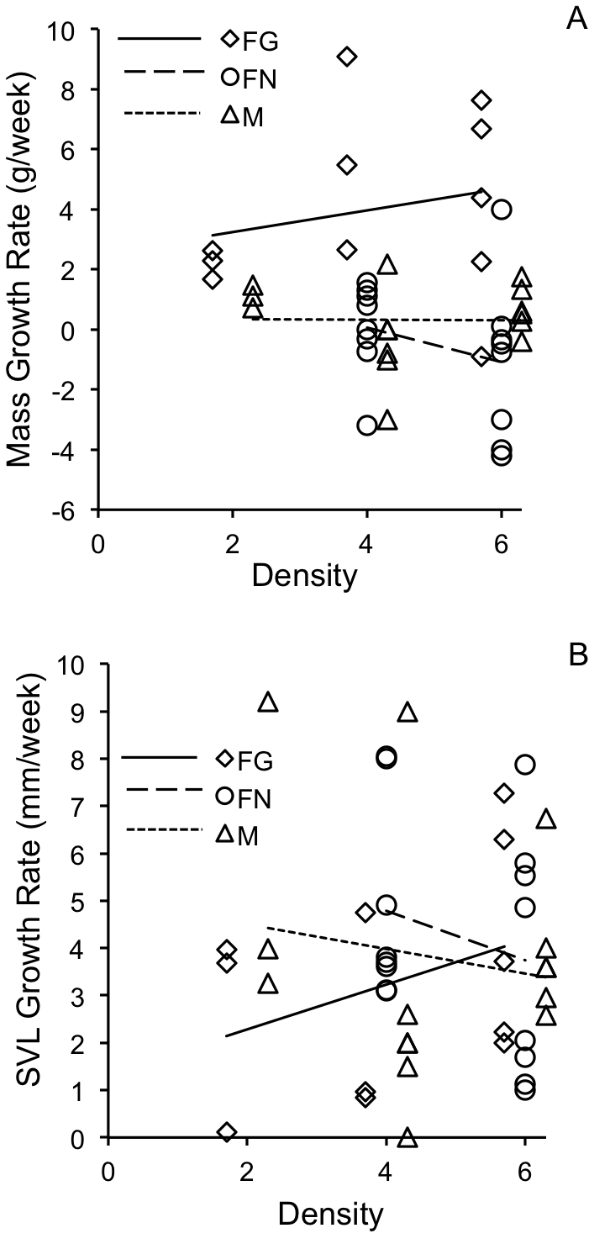
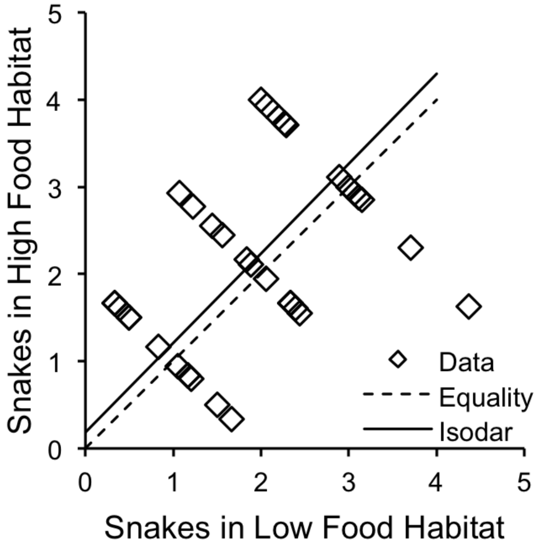


Figure 4-3. An isodar representing the number of common gartersnakes (*Thamnophis sirtalis*) in the side of the enclosure with food supplementation versus the number of common gartersnakes in the side of the enclosure without food supplementation for snakes living in experimental enclosures in Pontiac County, Québec. The dashed line (equality) represents an isodar with an intercept of zero and a slope of one, which would demonstrate no preference between habitats.



Conclusion

I have demonstrated that density dependence is strongly modulated by environmental temperature in ectotherms (Halliday et al. 2015A), and this has important implications for density-dependent habitat selection. In chapter one, I demonstrated that fitness-density functions for red flour beetles vary with temperature: the functions are strongest at the optimal temperature of the species and weaken as temperature departs from this optimal temperature. These differences in the strength of density dependence then lead to interesting patterns of density-dependent habitat selection between habitats differing in temperature and in food, where beetles always prefer the high temperature habitat except when food is highest in the cool habitat. In chapter two, I modified the isodar model of density-dependent habitat selection to include the effect of temperature for ectotherms, and demonstrated that, as expected given the effect of temperature on the density dependence of fitness, the strength of isodars weakened as temperature deviated from the optimal temperature.

In chapter three, I used a mix of observational and experimental studies with common gartersnakes to demonstrate that fitness and habitat quality are higher in field than in forest habitat, which led to density-independent habitat selection with a strong preference for field habitat. In fact, fitness was so low in forest that it probably represents a sink habitat. In chapter four, I tested more directly whether gartersnakes exhibit density-dependent habitat selection based on food abundance, given the lack of compelling evidence in chapter three. I found evidence of density-dependent habitat selection but, somewhat surprisingly, no evidence of density dependence in growth rate or reproductive output.

My four chapters collectively demonstrate that ectotherms have different responses to temperature and population density. In gartersnakes, I found weak evidence of density-dependent habitat selection and no evidence of density dependence of fitness, whereas red flour beetles were strongly affected by density for both habitat selection and fitness. I speculate that the different responses to density are a function of the natural history of each species; gartersnakes have very low energetic requirements and flour beetles have high energetic requirements. Gartersnakes eat large food items infrequently and reproduce once per year or once every other year, whereas flour beetles eat and reproduce constantly. Flour beetles should therefore be much more sensitive to variations in energy availability than gartersnakes, and this should lead to stronger density dependence.

While I have focussed on ectotherms in this thesis, my results likely also apply to endotherms. While endotherms maintain stable body temperatures through metabolic means while environmental temperatures are within their thermal neutral zone (Angilletta et al. 2010), there is still an energetic cost associated with living within cooler habitats within the thermal neutral zone, and there are definitely costs associated with living in a habitat outside of the thermal neutral zone. Given the increased energetic costs associated with endothermy outside the thermal neutral zone, this may affect density dependence as temperature deviates from the optimal temperature for performance. For example, moose (*Alces alces*) are very susceptible to heat stress and when open habitats are warmer than their preferred temperature, moose move to habitats with more shade (van Beest et al. 2012). More work is needed to assess how density dependence in endotherms is affected by changes in temperature, and specifically to determine if relatively small changes in environmental temperature can affect density dependence in similar ways than it does in ectotherms.

My thesis also has wider implications than those discussed in each chapter. For example, the decreasing negative density dependence of fitness (Chapters 1 and 2) could lead to lower demographic parameters near the northern edge of the range for species whose distributions are limited by temperature. Shaded habitats could similarly be avoided by these species near the northern edge of their range, but could be used in warmer parts of their range. My results with common gartersnakes (Chapters 3 and 4) at the northern extreme of their range could be compared to those of studies conducted further south in the species' range. Similarly, there could also be temporal trends in the importance of density dependence in temperate climates. Density dependence should be weakest in colder shoulder seasons, and strongest in the warmer summer season.

Throughout this thesis, I use different proxies for fitness, including oviposition rates of beetles and growth rates and reproductive outputs of gartersnakes. I argue that all of these metrics are at least correlated with more ultimate measures of fitness, such as lifetime reproductive success. For beetles, trends in oviposition rates do reflect trends in the number of successful offspring produced (Halliday et al. 2015). For snakes, larger females can produce more offspring (Shine 1994), which means that the sooner that a female is large, the sooner she can reap the fitness benefits of this large body size. This link between growth rate and lifetime reproductive success is clear. Individual reproductive output is also clearly linked to lifetime reproductive success.

Since isodar theory is based on the ideal free distribution, it is worth discussing how both red flour beetles and common gartersnakes match the assumptions of the ideal free distribution. The three main assumptions of the ideal free distribution are ideal knowledge, freedom of movement, and equality of competitors. I observed flour beetles moving back and forth between habitats frequently (Halliday, unpublished data), which can be considered sampling behaviour. In this way, flour beetles would have had a near ideal knowledge of the availability of resources and of the density of competitors. Within the experimental containers that I set up, beetles had to travel over ~ 20 cm of flat sand to move between flour patches. This is a relatively short distance, and should not have imposed high travel costs. Moreover, I also compared different distances between flour patches and saw similar distributions of beetles between patches regardless of the distance (Halliday, unpublished data). I have no evidence of gartersnakes frequently moving between habitats, but many individuals did spend time on the edge of the forest and could have easily sampled the thermal quality of both habitats. My enclosures were also quite small relative to the distance that snakes would normally move in a day, and therefore did not impose high travel costs. The equality of competitors is an assumption that can be explicitly tested using isodars, where a curvilinear isodar represents an ideal despotic distribution (unequal competitors). None of my isodars were curvilinear, therefore I assume that both species were made up of relatively equal competitors. Overall, I am confident that both species met the assumptions of the ideal free distribution under my experimental conditions.

In summary, the most striking pattern from my thesis is that density dependence can be strongly modulated by environmental temperature for ectotherms, even within the ecologically relevant range of temperatures, and that density dependence can disappear if temperatures deviate from the optimal temperature. This result is novel, and is very important for theory, conservation, and management because the majority of animals are ectotherms, and likely also exhibit density dependence that is modulated by temperature. Therefore, models for ectotherms that include some form of density dependence should control for the effect of temperature on density dependence, which is not typically done for endotherms from which most of the density-dependent models are derived. Future research could build on my results by examining how temperature affects more ultimate measures of fitness, such as population growth and longevity, which are important for many ecological models, and expand on ecological models that include density-dependent parameters by incorporating the modulating effect of temperature.

Appendix One

Thermal preferences of various snake species from published studies

I compiled a list of studies that calculated the thermal preference of snakes. Most of these studies placed snakes in a thermal gradient and calculated the thermal preference of snakes as the 25th and 75th percentiles of all temperatures selected, although a few older studies did not state how they determined thermal preference. When multiple studies examined the same species, I took the mean lower and upper bounds of all studies as the value of T_{set} for that species. Based on these data, I calculated the global mean T_{set} (± S.E.) of snakes as 26.2 ± 0.7°C - 32.7 ± 0.8°C. I also recalculated mean T_{set} only using the studies that calculated T_{set} based on the 25th and 75th percentiles as 26.8 ± 0.9°C - 30.7 ± 0.8°C.

Scientific Name	Common Name	Lower Bound	Upper Bound	Reference (s)	Comments
Acanthophis antarticus	Common Death Adder	27.2	38.5	Lillywhite 1980	Did not use quartiles
Agkistrodon piscivorus	Cottonmouth	19.25	27.05	Crane and Greene 2008	Average female
Austrelaps superbus	Lowland Copperhead	26.6	37.1	Lillywhite 1980	Did not use quartiles
Boiga irregularis	Brown Treesnake	28.2	31.4	Anderson et al. 2005	Plateau pattern on figure
Bothrops insularis	Golden Lancehead	24.9	26.3	Bovo et al. 2012	Summer average
Coluber constrictor	Blue Racer	22.4	37.4	Kitchell 1969	Did not use quartiles
Elaphe obsoleta	Black Rat Snake	26.5	29.8	Blouin-Demers and Weatherhead 2001	

Elaphe quadrivirgata	Japanese Striped Snake	28	31.6	Tanaka 2007	
Eunectes notaeus	Yellow Anaconda	28	34	McConnachie et al. 2011	Terrestrial thigmothermal mosaic
Heterodon platyrhinos	Eastern Hog-nose snake	23.4	37	Kitchell 1969	Did not use quartiles
Hierophis viridiflavus	European Whipsnake	27.5	31	Lelièvre et al. 2010	
Hoplocephalus bungaroides	Broad-headed Snake	28.1	31.1	Webb and Shine 1998	
Hoplocephalus stephensii	Stephen's Banded Snake	27.8	29.3	Fitzgerald et al. 2003	
Lampropeltis triangulum	Milk snake	29	31	Row and Blouin-Demers 2006	
Natrix sipedon	Common Water Snake	20.8	34.7	Kitchell 1969	Did not use quartiles
Nerodia sipedon	Northern Water Snake	24.7	29.8	Brown and Weatherhead 2000	
Notechis scutatus	Western Tiger Snake	24.6	32.6	Lillywhite 1980; Ladyman and Bradshaw 2003	Average of two authors
Pseudechis porphyriacus	Red-bellied Black Snake	28.1	36.3	Lillywhite 1980	Did not use quartiles

Pseudonaja nuchalis	Western Brown Snake	31.8	36.9	Lillywhite 1980	Did not use quartiles
Pseudonaja textilis	Eastern Brown Snake	29.05	35.25	Lillywhite 1980; Whitaker and Shine 2002	Average of two authors
Sistrurus catenatus	Massasauga Rattlesnake	30	33.6	Harvey and Weatherhead 2010	
Thamnophis sirtalis	Eastern Garter Snake	20	35	Kitchell 1969	Did not use quartiles
Trimeresurus stejnegeri	Chinese Green Tree Vipers	20.3	24.3	Tsai & Tu 2005	Preprandial
Unechis flagellum	Little Whip Snake	24	36.2	Lillywhite 1980	Did not use quartiles
Vipera aspis	Asp viper	32.69	34.66	Lourdais et al. 2013	Gravid females
Vipera berus	Common European Adder	32.17	34.22	Herczeg et al. 2007; Lourdais et al. 2013	Average of two authors
Zamenis longissimus	Aesculapian Snake	21.5	25.5	Lelièvre et al. 2011	

Appendix Two

Abstracts of other publications completed during PhD

Halliday WD, Paterson JE, Patterson LD, Cooke SJ, Blouin-Demers G (2014) Testosterone, body size, and sexual signals predict parasite load in Yarrow’s spiny lizards (*Sceloporus jarrovii*). Canadian Journal of Zoology 92: 1075-1082.

Parasite load significantly impacts host health and fitness, and may vary substantially among individuals within a population. The immunocompetence handicap hypothesis posits that sexual signals are honest indicators of male quality because they are maintained by testosterone, an immunosuppressant that yields higher parasite loads. Additionally, testosterone may influence parasite load by increasing activity levels. We examined these two hypotheses in a wild population of Yarrow’s spiny lizards (*Sceloporus jarrovii* Cope, 1875) in Arizona. We 1) compared fecal testosterone levels to ectoparasite and haemoparasite loads, 2) tested if sexual signals (total coloured area, aggression, and head size), locomotor activity, and body size correlated with testosterone levels, and 3) compared sexual signals, locomotor activity, and body size to parasite load. Ectoparasite loads increased with total coloured area, and tended to increase with testosterone, but this latter relationship was only nearly significant. Parasite loads increased with body size. Thus, we found some support for the immunocompetence handicap hypothesis, and none for the activity hypothesis. Our results are consistent with an alternative hypothesis that larger individuals have more parasites because they have more surface area and/or have had longer to accumulate parasites. Future studies should examine the relative contributions of testosterone and glucocorticoids in driving variation in parasite loads.

Maillet Z, Halliday WD, Blouin-Demers G (2015) Exploratory and defensive behaviors change with sex and body size in eastern garter snakes (*Thamnophis sirtalis*). Journal of Ethology 33: 47-54.

Phenotypic traits are important to consider when examining behaviour because they can help explain behavioural variation. Behaviours such as exploration, boldness, and defense may vary because different intensities of a behaviour may be advantageous for males versus females, or at different body sizes. We tested the hypothesis that exploration, boldness, and defensive behaviours are related to body size, sex, and reproductive status in eastern garter snakes, *Thamnophis sirtalis*. We also tested whether the measured behaviours were consistent through time (i.e., whether eastern garter snakes could have personalities) and whether the measured behaviours were related to one another (i.e., whether behavioural syndromes could be present in eastern garter snakes). Males and non-gravid females were more likely to flee than gravid females when faced with an attack. Males and non-gravid females were also more active after the attack than before the attack, whereas gravid females were more active before the attack. Furthermore, longer females explored less than smaller females, with a more pronounced effect in gravid females. In contrast, longer males explored more than smaller males. Although we did not detect behavioural syndromes, within individuals the measured behaviours were repeatable through time, suggesting that eastern garter snakes could have personalities.

Halliday WD, Thomas AS, Blouin-Demers G (2015) High temperature intensifies negative density dependence of fitness in red flour beetles. Ecology and Evolution 5: 1061-1067.

Competition for food, space, or other depletable resources has strong impacts on the fitness of organisms, and can lead to a pattern known as negative density dependence, where fitness decreases with population density. Yet many resources that have strong impacts on fitness are non-depletable (e.g., moisture or temperature). How do these non-depletable resources interact with depletable resources to modify negative density dependence? We tested the hypothesis that negative density dependence is modulated by temperature in red flour beetles, and tested the prediction that the strength of negative density dependence should decrease as temperature decreases. We measured the number of eggs laid, offspring development time, and the number of offspring that reached maturity at three temperatures and two food treatment combinations as we simultaneously manipulated adult population density. We demonstrated that low temperatures weaken negative density dependence in the number of eggs laid; this pattern was most evident when food is abundant. Density had no effect on development time, but low temperatures increased development time. The percent of eggs that emerged as adults decreased with both density and temperature, and increased with food. Temperature, an abiotic driver, can thus modulate density-dependent processes in ectotherms. Therefore, models of population growth for ectotherms should incorporate the effects of temperature.

Halliday WD, Blouin-Demers G (2015) Efficacy of coverboards for sampling small northern snakes. Herpetology Notes 8: 309-314.

Using coverboards to monitor herpetofauna is common practice, yet few studies have formally tested the efficacy of using coverboards. We tested whether using coverboards on survey plots increased the number of small snakes detected in eastern Ontario, Canada. We set up twenty 2500 m^2 plots in field and forest habitat, ten with plywood coverboards and ten without coverboards. We sampled these twenty plots systematically for small snakes, and compared the number of snakes detected on plots with coverboards to the number detected on plots without coverboards. The number of snakes detected was always higher on plots with coverboards than on plots without coverboards, to the extent that we only detected the smallest snake species on plots with coverboards. We then examined whether *Storeria occipitomaculata* in western Québec, Canada prefer plywood or tin coverboards. We set up pairs of plywood and tin coverboards along transects, and monitored the use of these coverboards throughout the active season. Red-bellied Snakes preferred tin over plywood coverboards. We confirmed that coverboards are indeed a useful tool for monitoring small snakes, and that some snakes show preferences for specific types of coverboards. We therefore suggest that researchers use an array of different types of coverboards when attempting to monitor small snake communities, or determine which coverboards are preferred by their target species in a pilot study.

Halliday WD, Blouin-Demers G (2015) A stringent test of the thermal coadaptation hypothesis in flour beetles. Journal of Thermal Biology 52: 108-116.

Whole-organism performance depends on body temperature and ectotherms have variable body temperatures. The thermal coadaptation hypothesis posits that thermal reaction norms have coevolved with thermal preference such that organisms attain optimal performance under a narrow range of body temperatures commonly experienced in the wild. Since thermal reaction norms are often similar, researchers interested in the effects of temperature on fitness often use one easily measured thermal reaction norm, such as locomotor performance, and assume it is a good proxy for fitness when testing the thermal coadaptation hypothesis. The extent to which this assumption holds, however, is often untested. In this study, we provide a stringent test of the thermal coadaptation hypothesis in red and in confused flour beetles by comparing the thermal reaction norm for reproductive output to the preferred body temperature range. We also test the assumption that locomotor performance can serve as a proxy for the thermal reaction norm for reproductive output, a more ultimate index of fitness. In both species, we measured the number of eggs laid, righting time, and sprint speed at eight temperatures, as well as the thermal preference in a thermal gradient. The number of eggs laid increased with female sprint speed and with male righting time, and all three performances had similar thermal reaction norms, with 80% of the maximum achieved between 23 and 37°C. Red flour beetles had preferred body temperatures that matched the optimal temperature for performance; confused flour beetles had lower preferred body temperature than the optimal temperature for performance. We found support for the assumption that locomotor performance can serve as a proxy for reproductive output in flour beetles, but we only found evidence for thermal coadaptation in one of the two species.

Halliday WD, Gilmour KM, Blouin-Demers G (2015) Faecal corticosterone metabolite concentrations are not a good predictor of habitat suitability for common gartersnakes. Conservation Physiology 3: doi: 10.1093/conphys/cov047.

Measuring habitat suitability is important in conservation and in wildlife management. Measuring the abundance or presence/absence of a species in various habitats is not sufficient to measure habitat suitability because these metrics can be poor predictors of population success. Therefore, having some measure of population success is essential in assessing habitat suitability, but estimating population success is difficult. Identifying suitable proxies for population success could thus be beneficial. We examined whether faecal corticosterone metabolite (fCM) levels could be used as a proxy for habitat suitability in Common Gartersnakes, *Thamnophis sirtalis*. We conducted a validation study and confirmed that fCM levels indeed reflect circulating corticosterone levels. We estimated abundance, reproductive output, and growth rate of gartersnakes in field and in forest habitat, and we also measured fCM levels of gartersnakes from these same habitats. Common Gartersnakes were more abundant, had higher reproductive outputs, and higher growth rates in field habitat than in forest habitat, but fCM levels did not differ between the same two habitats. Our results suggest either that fCM levels are not a useful metric of habitat suitability in Common Gartersnakes, or that the difference in suitability between the two habitats was too small to induce changes in fCM levels. Incorporating fitness metrics in estimates of habitat suitability is important, but these metrics of fitness have to be sensitive enough to vary between habitats.

Literature Cited

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In *Second International Symposium on Information Theory*. (Petrov NB, Czaki BF eds), pp 267-281. Academiai Kiado, Budapest.

Anderson NL, Hetherington TE, Coupe B, Perry G, Williams JB, Lehman J (2005) Thermoregulation in a nocturnal, tropical, arboreal snake. Journal of Herpetology 39: 82-90.

Angilletta MJ (2001) Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). Ecology 82: 3044-3056.

Angilletta MF, Cooper BS, Schuler MS, Boyles JG (2010) The evolution of thermal physiology in endotherms. Frontiers in Bioscience E2: 861-881.

Angilletta MJ, Niewiarowski PH, Navas CA (2002) The evolution of thermal physiology in ectotherms. Journal of Thermal Biology 27: 249-268.

Angilletta MJ, Steury TD, Sears MW (2004) Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integrative and Comparative Biology 44: 498-509.

Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat freezes niche evolution. Ecology Letters 16: 1206-1219.

Åström M (1994) Travel cost and the ideal free distribution. Oikos 69:516-519.

Bennett AF (1980) The thermal dependence of behavioral performance in small lizards. Animal Behaviour 28: 752-762.

Berger D, Walters R, Gotthard K (2008) What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. Functional Ecology 22: 523-529.

Bergman E (1987) Temperature-dependent differences in foraging ability of two percids, *Perca fluviatilis* and *Gymnocephalus cernuus*. Environmental Biology of Fishes 19: 45-53.

Birch LC (1957) The role of weather in determining the distribution and abundance of animals. Cold Spring Harbor Symposium on Quantitative Biology 22: 203-218.

Björnsson B (1994) Effects of stocking density on growth rate of halibut (*Hippoglossus hippoglossus* L.) reared in large circular tanks for three years. Aquaculture 123: 259-270.

Blem CR (1982) Biennial reproduction in snakes: an alternative hypothesis. Copeia 1982: 961-963.

Blouin-Demers G (2003) Precision and accuracy of body-size measurements in a constricting, large-bodied snake (*Elaphe obsoleta*). Herpetological Review 34: 320-323.

Blouin-Demers G, Weatherhead PJ (2001) Habitat use by black rat snakes (*Elaphe obsoleta*) in fragmented forests. Ecology 82: 3025-3043.

Blouin-Demers G, Weatherhead PJ (2002) Habitat-specific behavioural thermoregulation by black rat snakes (*Elaphe o. obsoleta*). Oikos 97: 59-68.

Blouin-Demers G, Weatherhead PJ (2008) Habitat use is linked to components of fitness through the temperature-dependence of performance in ratsnakes (*Elaphe obsoleta*). Israel Journal of Ecology and Evolution 54: 361-372.

Bonnet X, Bradshaw D, Shine R (1998) Capital versus income breeding: an ectothermic perspective. Oikos 83: 333-342.

Bovo RP, Marques OA, Andrade DV (2012) When basking is not an option: thermoregulation of a viperid snake endemic to a small island in the South Atlantic of Brazil. Copeia 2012: 408-418.

Bozdogan H (1987) Model selection and Akaike’s Information Criterion (AIC): the general theory and its analytical extensions. Psychometrika 52: 345-370.

Bozinovic F, Rosenmann M (1988) Energetics and food requirements of the female snake Phillodryas chamissonis during the breeding season. Oecologia 75: 282-284.

Brook BW, Bradshaw CJA (2006) Strength of evidence for density dependence in abundance time series of 1198 species. Ecology 87: 1445-1451.

Brown GP, Weatherhead PJ (2000) Thermal ecology and sexual size dimorphism in northern water snakes, *Nerodia sipedon*. Ecological Monographs 70**:** 311-330.

Buckley LB, Hurlbert AH, Jetz W (2012) Broad-scale ecological implications of ectothermy and endothermy in changing environments. Global Ecology and Biogeography 21: 873-885.

Burger J, Jeitner C, Jensen H, Fitzgerald M, Carlucci S, Shukla S, Burke S, Ramos R, Gochfeld M (2004) Habitat use in basking northern water (*Nerodia sipedon*) and eastern garter (*Thamnophis sirtalis*) snakes in urban New Jersey. Urban Ecosystems 7: 17-27.

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, Berlin, Germany.

Calsbeek R, Sinervo B (2002) An experimental test of the ideal despotic distribution. Journal of Animal Ecology 71: 513-523.

Campbell JF, Runnion C (2003) Patch exploitation by female red flour beetles, *Tribolium castaneum*. Journal of Insect Science 3: 20.

Cantrell RS, Cosner C, Lou Y (2010) Evolution of dispersal and the ideal free distribution. Mathematical Biosciences and Engineering 7: 17-36.

Carfagno GLF, Weatherhead PJ (2006) Intraspecific and interspecific variation in use of forest-edge habitat by snakes. Canadian Journal of Zoology 84: 1440-1452.

Chapman RN (1924) Nutritional studies on the Confused Flour Beetle, *Tribolium confusum* Duval. Journal of General Physiology 6: 565-585.

Crane AL, Greene BD (2008) The effect of reproductive condition on thermoregulation in female *Agkistrodon piscivorus* near the northwestern range limit. Herpetologica 64: 156-167.

Cressman R, Krivan V (2006) Migration dynamics and the ideal free distribution. The American Naturalist 168: 384-397

Cressman R, Tran T (2015) The ideal free distribution and evolutionary stability in habitat selection games with linear fitness and Allee effect. In: Cojocaru M, Kotsireas IS, Makarov R, Melnik R, Shodiev H (eds) Interdisciplinary Topics in Applied Mathematics, Modeling and Computational Science. Springer International Publishing, pp. 457-463.

Diller LV, Wallace R (1996) Comparative ecology of two snakes species (*Crotalus viridis* and *Pituophis melanoleucus*) in southwestern Idaho. Herpetologica 52: 343-360.

Dubois Y, Blouin-Demers G, Thomas D (2008) Temperature selection in wood turtles (*Glyptemys insculpta*) and its implications for energetics. Écoscience 15: 398-406.

Edwards GP, Preu NDE, Crealy IV, Shakeshaft BJ (2002) Habitat selection by feral cats and dingoes in a semi-arid woodland environment in central Australia. Australian Ecology 27: 26-31.

Falcy MR (2015) Density-dependent habitat selection of spawning Chinook salmon: broad-scale evidence and implications. Journal of Animal Ecology 84: 545-553.

Ferrer M, Donazar JA (1996) Density-dependent fecundity by habitat heterogeneity in an increasing population of Spanish imperial eagles. Ecology 77: 69-74

Fitzgerald M, Shine R, Lemckert F (2003) A reluctant heliotherm: thermal ecology of the arboreal snake *Hoplocephalus stephensii* (Elapidae) in dense forest. Journal of Thermal Biology 2003: 515-524.

Fretwell SD (1969) On territorial behavior and other factors influencing habitat distribution in birds: III. breeding success in a local population of field sparrows. Acta Biotheoretica 19: 45-52.

Fretwell SD (1972) *Populations in a seasonal environment*. Monographs in population biology. Princeton: Princeton University Press.

Fretwell SD, Calver JS (1969) On territorial behavior and other factors influencing habitat distribution in birds: II. sex ratio variation in the Dickcissel (*Spiza americana* Gmel). Acta Biotheoretica 19: 37-44.

Fretwell SD, Lucas HL (1969) On territorial behavior and other factors influencing habitat distribution in birds: I. Theoretical development. Acta Biotheoretica 19: 16-36.

Gause GF (1934) The Struggle for Existence. Williams and Wilkins, Baltimore, Maryland.

Goldberg DE, Turkington R, Olsvig-Whittaker L, Dyer AR (2001) Density dependence in an annual plant community: variation among life history stages. Ecological Monographs 71: 423-446.

Gilchrist GW (1995) Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. The American Naturalist 146: 252-270.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293: 2248-2251.

Goldberg DE, Turkington R, Olsvig-Whittaker L, Dyer AR (2001) Density dependence in an annual plant community: variation among life history stages. Ecological Monographs 71: 423-446.

Good NE (1936) The flour beetles of the genus *Tribolium*. USDA Technical Bulletin 5: 27-28.

Grand TC, Dill LM (1999) Predation risk, unequal competitors and the ideal free distribution. Evolutionary Ecology Research 1: 389-409.

Hairston NG, Smith FE, Slobodkin LS (1960) Community structure, population control, and competition. The American Naturalist 94: 421-425.

Halliday WD, Blouin-Demers G (2014) Red flour beetles balance thermoregulation and food acquisition via density-dependent habitat selection. Journal of Zoology 294: 198-205.

Halliday WD, Blouin-Demers G (2015A) A stringent test of the thermal coadaptation hypothesis in flour beetles. Journal of Thermal Biology 52: 108-116.

Halliday WD, Blouin-Demers G (2015B) Efficacy of coverboards for sampling small northern snakes. Herpetology Notes 8: 309-314.

Halliday WD, Gilmour KM, Blouin-Demers G (2015B) Faecal corticosterone metabolite levels are not a good predictor of habitat suitability for Common Gartersnakes. Conservation Physiology 3: doi: 10.1093/conphys/cov047.

Halliday WD, Thomas AS, Blouin-Demers G (2015A) High temperature intensifies negative density dependence of fitness in red flour beetles. Ecology and Evolution 5: 1061-1067.

Harms KE, Wright SJ, Calderón O, Hernández A, Herre EA (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. Nature 404: 493-495.

Harvey DS, Weatherhead PJ (2006) A test of the hierarchical model of habitat selection using eastern massassauga rattlesnakes (*Sistrurus c. catenatus*). Biological Conservation 130: 206-216.

Harvey DS, Weatherhead PJ (2010) Thermal ecology of Massasauga Rattlesnakes (*Sistrurus catenatus*) near their northern range limit. Canadian Journal of Zoology 89: 60-68.

Haugen TO, Winfield IJ, Vøllestad LA, Fletcher JA, James JB, Stenseth NC (2006) The ideal free pike: 50 years of fitness-maximizing dispersal in Windermere. Proceedings of the Royal Society of London B: Biological Sciences 273: 2917-2924.

Herczeg G, Saarikivi J, Gonda A, Perälä J, Tuomola A, Merilä J (2007) Suboptimal thermoregulation in male adders (*Vipera berus*) after hibernation imposed by spermiogenesis. Biological Journal of the Linnaean Society 92: 19-27.

Hertz PE, Huey RB, Stevenson RD (1993) Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. The American Naturalist 142: 796-818.

Hixon MA, Carr MH (1997) Synergistic predation, density dependence, and population regulation in marine fish. Science 277: 946-949.

Holt RD (1996) Adaptive evolution in source-sink environments: direct and indirect effects of density-dependence on niche evolution. Oikos 75: 182-192.

Howe RW (1956) The effect of temperature and humidity on the rate of development and mortality on *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). Annals of Applied Biology 44: 356-368.

Howe RW (1962) The effects of temperature and humidity on the oviposition rate of *Tribolium castaneum* (Hbst.) (Coleoptera, Tenebrionidae). Bullutin of Entomological Research 53: 301-310.

Huey RB (1991) Physiological consequences of habitat selection. The American Naturalist 137: S91-S115.

Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology and Evolution 4: 131-135.

Humphries MM, Thomas DW, Speakman JR (2002) Climate-mediated energetic constraints on the distribution of hibernating mammals. Nature 418: 313-316.

Hutchinson GE (1959) Homage to Santa Rosalia or why are there so many kinds of animals? The American Naturalist 93: 145-159.

Jensen WE, Cully JF (2005) Density-dependent habitat selection by brown-headed cowbirds (*Molothrus ater*) in tallgrass prairie. Oecologia 142: 136-149.

Kennedy M, Gray RD (1993) Can ecological theory predict the distribution of foraging animals? A critical analysis of experiments on the ideal free distribution. Oikos 68: 158-166.

King CE, Dawson PS (1973) Habitat selection by flour beetles in complex environments. Physiology Zoology 46: 297-309.

Kitchell JF (1969) Thermophilic and thermophobic responses of snakes in a thermal gradient. Copeia 1969: 189-191

Knight TW, Morris DW, Haedrich RL (2008) Inferring competitive behavior from population census data: site pre-emption by brook trout (*Salvelinus fontinalis*). Israel Journal of Ecology and Evolution 54: 345-360.

Korona R (1990) Travel costs and the ideal free distribution of ovipositing female flour beetles, *Tribolium confusum*. Animal Behaviour 40: 186-187.

Krasnov BR, Khokhlova IS, Fielden LJ, Burdelova NV (2001) Effects of air temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: Pulicidae). Journal of Medical Entomology 38: 629-637.

Krasnov BR, Khokhlova IS, Shenbrot GI (2003) Density-dependent host selection in ectoparasites: an application of isodar theory to fleas parasitizing rodents. Oecologia 134: 365-372.

Krasnov BR, Khokhlova IS, Burdelova NV, Mirzoyan NS, Degen AA (2004) Fitness consequences of host selection in ectoparasites: testing reproductive patterns predicted by isodar theory in fleas parasitizing rodents. Journal of Animal Ecology 73: 815-820.

Krivan V (1997) Dynamic ideal free distribution: effects of optimal patch choice on predator-prey dynamics. The American Naturalist 149: 164-178.

Krivan V (2014) The Allee-type ideal free distribution. Journal of Mathematical Biology 69: 1497-1513.

Ladyman M, Bradshaw D (2003) The influence of dehydration on the thermal preferences of the Western tiger snake, *Notechis scutatus*. Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology 173: 239-246.

Lamb RJ, Loschiavo SR (1981) Diet, temperature, and the logistic model of developmental rate for *Tribolium confusum* (Coleoptera: Tenebrionidae). Canadian Entomologist 113: 813-818.

Legendre P (2014) lmodel2: model II regression. R package version 1.7–2. url: http://CRAN.R-project.org/package=lmodel2.

Lelièvre H, Blouin-Demers G, Pinaud D, Lisse H, Bonnet X, Lourdais O (2011) Contrasted thermal preferences translate into divergences in habitat use and realize performance in two sympatric snakes. Journal of Zoology 284: 265-275.

Lelièvre H, Le Hénanff M, Blouin-Demers G, Naulleau G, Lourdais O (2010) Thermal strategies and energetics in two sympatric colubrid snakes with contrasted exposure. Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology 180: 415-425.

Lerner A, Sapir N, Erlick C, Meltser N, Broza M, Shashar N. 2011. Habitat availability mediates chironomid density-dependent oviposition. Oecologia 165: 905-914.

Lillywhite HB (1980) Behavioral thermoregulation in Australian elapid snakes. Copeia 1980: 452-458.

Lin YTK, Batzli GO (2001) The influence of habitat quality on dispersal, demography, and population dynamics of voles. Ecological Monographs 71: 245-275.

Lin YK, Batzli GO (2002) The cost of habitat selection in prairie voles: an empirical assessment using isodar analysis. Evolutionary Ecology 16: 387-397.

Lourdais O, Guillon M, DeNardo D, Blouin-Demers G (2013) Cold climate specialization: Adaptive covariation between metabolic rate and thermoregulation in pregnant vipers. Physiology and Behavior 119: 149-155.

Malthus TR (1798) An essay on the principle of population. J Johnson, London.

Massot M, Clobert J, Pilorge T, LeComte J, Barbault R (1992) Density dependence in the common lizard: demographic consequences of a density manipulation. Ecology 73: 1742-1756

Matthiopoulos J, Fieberg J, Aarts G, Beyer HL, Morales JM, Haydon DT (2015) Establishing the link between habitat selection and animal population dynamics. Ecological Monographs 85: 413-436.

McConnachie S, Greene SN, Perrin MR (2011) Thermoregulation in the semi-aquatic yellow anaconda, *Eunectes notaeus*. Journal of Thermal Biology 36: 71-77.

McDonald D (1968) The response of. *Tribolium confusum* to variation in the volume of its environment. Ecology 49: 1166-1168.

Milinksi M (1994) Ideal free theory predicts more than only input matching – a critique of Kennedy and Gray's review. Oikos 71:163-166.

Moody AL, Houston AI, McNamara JM (1996) Ideal free distributions under predation risk. Behavioral Ecology and Sociobiology 38: 131-143.

Morita K, Tsuboi J, Matsuda H (2004). The impact of exotic trout on native charr in a Japanese stream. Journal of Applied Ecology 41: 962-972.

Morris DW (1988) Habitat-dependent population regulation and community structure. Evolutionary Ecology 2: 253-269.

Morris DW (1989) Density-dependent habitat selection: testing the theory with fitness data. Evolutionary Ecology 3: 80-94.

Morris DW (1990) Temporal variation, habitat selection and community structure. Oikos 59: 303-312.

Morris DW (2003) Toward an ecological synthesis: a case for habitat selection. Oecologia 136: 1-13.

Morris DW (2011) Adaptation and habitat selection in the eco-evolutionary process. Proceedings of the Royal Society of London B: Biological Sciences 278: 2401-2411.

Morris DW (2014) Can foraging behaviour reveal the eco-evolutionary dynamics of habitat selection? Evolutionary Ecology Research 16: 1-18.

Morris DW, Dupuch A, Halliday WD (2012) Climate-induced habitat selection predicts future evolutionary strategies of lemmings. Evolutionary Ecology Research 14: 689-705.

Nowak EM, Theimer TC, Schuett GW (2008) Functional and numerical responses of predators:

where do vipers fit in the traditional paradigm? Biological Reviews 83: 601-620.

Ovadia O, Abramsky Z (1995) Density-dependent habitat selection: evaluation of the isodar method. Oikos 73: 86-94.

Park T (1932) Studies in population physiology: the relation of numbers to initial population growth in the flour beetle *Tribolium confusum* Duval. Ecology 13: 172–181.

Park T (1934) Observations on the general biology of the flour beetle, *Tribolium confusum*. Quarterly Review of Biology 9: 36-54.

Park T, Frank MB (1948) The fecundity and development of the flour beetles, *Tribolium confusum* and *Tribolium castaneum* at three constant temperatures. Ecology 29: 368–374.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2015) nlme: linear and non-linear mixed effects models. R package version 3.1-120. url: http://CRAN.R-project.org/ package=nlme

Porter WP, Tracy CR (1974) Modeling the effect of temperature on the ecology of the garter snake and leopard frog. In: Gibbons JW, Sharitz R (eds) Thermal Ecology of Oak Ridge, Tennessee. pp 595-609.

Pulliam HR (1988) Sources, sinks, and population regulation. The American Naturalist 132: 652-661.

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. url: http://www.R-project.org/

Rangeley RW, Kramer DL (1998) Density-dependent antipredator tactics and habitat selection in juvenile pollock. Ecology 79: 943-952.

Reinert HK (1984) Habitat variation within sympatric snake populations. Ecology 65: 1673-1682.

Rich ER (1956) Egg cannibalism and fecundity in Tribolium. Ecology 37: 109-120.

Ricker WE (1954) Stock and recruitment. Journal of the Fisheries Research Board of Canada 11: 559-623.

Robertson IC, Weatherhead PJ (1992) The role of temperature in microhabitat selection by northern water snakes (Nerodia sipedon). Canadian Journal of Zoology 70: 417-422.

Rodenhouse NL, Sillett TS, Doran PJ, Holmes RT (2003) Multiple density-dependence mechanisms regulate a migratory bird population during the breeding season. Proceedings of the Royal Society of London B: Biological Sciences 270: 2105-2110.

Rodríguez MA (1995) Habitat-specific estimates of competition in stream salmonids: a field test of the isodar model of habitat selection. Evolutionary Ecology 9: 169-184.

Rosenzweig ML (1981) A theory of habitat selection. Ecology 62: 327-335.

Rosenzweig ML, Abramsky Z (1986) Centrifugal community organization. Oikos 46: 339-348.

Rossman DA, Myer PA (1990) Behavioral and morphological adaptations for snail extraction in the North American brown snakes (Genus *Storeria*). Journal of Herpetology 24: 434-438.

Row JR, Blouin-Demers G (2006) Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. Oecologia 148: 1-11.

Schwab FE, Pitt MD (1991) Moose selection of canopy cover types related to operative temperature, forage, and snow depth. Canadian Journal of Zoology 69: 3071-3077.

Shine R (1994) Sexual size dimorphism in snakes revisited. Copeia 1994: 326-346.

Shine R, Elphick MJ, Harlow PS, Moore IT, LeMaster MP, Mason RT (2001) Movements, mating, and dispersal of red-sided gartersnakes (Thamnophis sirtalis parietalis) from a communal den in Manitoba. Copeia 2001: 82-91.

Shochat E, Abramsky Z, Pinshow B, Whitehouse MEA (2002) Density-dependent habitat selection in migratory passerines during stopover: what causes the deviation from IFD? Evolutionary Ecology 16: 469-488.

Sillett TS, Holmes RT (2005) Long-term demographic trends, limiting factors, and the strength of density dependence in a breeding population of a migratory songbird. In: *Birds of two worlds: the ecology and evolution of migration* (Greenberg R, Marra PP, eds), pp 426-436. John Hopkins University Press, Baltimore, Maryland.

Sonleitner FJ (1961) Factors affecting egg cannibalism and fecundity in populations of adult *Tribolium castaneum* Herbst. Physiological Zoology 34: 233-255.

Sowig P (1995) Habitat selection and offspring survival rate in three paracoprid dung beetles: the influence of soil type and soil moisture. Ecography 18: 147-154.

Spiess A-N (2014) qpcR: modelling and analysis of real-time PCR data. R package version 1.4-0. url: http://CRAN.R-project.org/package=qpcR

Stevens TO, Holbert BS (1995) Variability and density dependence of bacteria in terrestrial subsurface samples: implications for enumeration. Journal of Microbiological Methods 21: 283-292.

Stevenson RD, Peterson CR, Tsuji JS (1985) The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. Physiological Zoology 58: 46-57.

Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. Nature Climate Change 2: 686-690.

Suzuki T (1980) 4,8-dimethyldecanal – the aggregation pheromone of the flour beetles, *Trobolium-castaneum* and *Tribolium-confusum* (Coleoptera, Tenebrionidae). Agriculture Biology and Chemistry 44: 2519-2520.

Tadesse SA, Kotler BP (2010) Habitat choice of Nubian ibex (*Capra nubiana*) evaluated with a habitat suitability modeling and isodar analysis. Israel Journal of Ecology and Evolution 56: 55-74.

Tanaka K (2007) Thermal biology of a colour-dimorphic snake, *Elaphe quadrivirgata*, in a montane forest: do melanistic snakes enjoy thermal advantages? Biological Journal of the Linnaean Society 92: 309-322.

Taylor NW (1965) A theoretical study of population regulation in *Tribolium confusum*. Ecology 46: 334-340.

Travis JMJ, Murrell DJ, Dytham C (1999) The evolution of density-dependent dispersal. Proceedings of the Royal Society of London B: Biological Sciences 266: 1837-1842.

Tregenza T (1995) Building on the ideal free distribution. Advances in Ecological Research 26: 253-307.

Tsai T, Tu M (2005) Postprandial thermophily of Chinese green tree vipers, *Trimeresurus s. stejnegeri*: Interfering factors on snake temperature selection in a thigmothermal gradient. Journal of Thermal Biology 30: 423-430.

van Beest F, van Moorter BFA, Milner JM (2012) Temperature-mediated habitat use and selection by a heat-sensitive northern ungulate. Animal Behaviour 84: 723-735.

Volterra V (1926) Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. Memorie Accademia Lincei Roma 2: 31-113.

Weatherhead PJ, Sperry JH, Carfagno JLH, Blouin-Demers G (2012) Latitudinal variation in thermal ecology of North American ratsnakes and its implications for the effect of climate warming on snakes. Journal of Thermal Biology 37: 273-281.

Webb JK, Shine R (1998) Thermoregulation by a nocturnal elapid snake (*Hoplocephalus* *bungaroides*) in southeastern Australia. Physiological Zoology 71: 680-692.

Whittier JM, Crews D (1990) Body mass and reproduction in female red-sided garter snakes (*Thamnophis sirtalis parietalis*). Herpetologica 46: 219-226.

Willott SJ (1997) Thermoregulation in four species of British grasshoppers (Orthoptera: Acrididae). Functional Ecology11: 705-713.

Winne CT, Willson JD, Andrews KM, Reed RN (2006) Efficacy of marking snakes with disposable medical cautery units. Herpetological Review 37: 52-54.

Zimmerman GS, Gutiérrez RJ, Thogmartin WE, Banerjee S (2009) Multiscale habitat selection by ruffed grouse at low population densities. Condor 111: 294-304.