ISLAND SYNDROME AND STRESS PHYSIOLOGY
OF MICE IN THE GENUS *PEROMYSCUS*

A Thesis Submitted to the Committee on Graduate
Studies in Partial Fulfillment of the Requirements for the Degree of
Master of Science
in the Faculty of Arts and Science

TRENT UNIVERSITY
Peterborough, Ontario, Canada

© Copyright by Nathan D. Stewart 2017

Environmental and Life Sciences M.Sc. Graduate Program
September, 2017
ABSTRACT

ISLAND SYNDROME AND STRESS PHYSIOLOGY OF MICE IN THE GENUS *PEROMYSCUS*

Nathan D. Stewart

Biological differences between island and mainland conspecifics have been well studied, but few studies have addressed differences in stress physiology. Stressors, such as predation and competition for resources, cause the release of glucocorticoids (GCs). Characteristics of island wildlife, called “island syndrome”, are attributed to low levels of predators and competitors. I tested the hypothesis that island syndrome includes differences in GC levels between island and mainland rodents using two approaches; first, using white-footed mice (*Peromyscus leucopus*) from a near-shore archipelago (Thousand Islands, Ontario) and the nearby mainland; second, using study-skins of deer mice (*Peromyscus maniculatus*) from two archipelagos offshore of Vancouver Island, British Columbia. White-footed mice in the near-shore archipelago did not show characteristics of island syndrome, or changes in GC levels (feces and hair); however deer mice from both archipelagos in British Columbia were heavier and had lower hair GCs for their size than Vancouver Island mice.

**Keywords**: Island syndrome, island rule, stress physiology, glucocorticoids, *Peromyscus*
ACKNOWLEDGEMENTS

First, I would like to thank my supervisor, Dr. Gary Burness for providing me with support and encouragement, but for also challenging me during my graduate degree. You provided me with so many opportunities to learn and explore for which I am grateful. This work was made possible through the collaboration and guidance of Dr. Gabriela Mastromonaco, Christine Gillman, and Stephanie Matteer at the Toronto Zoo, who were incredibly good-natured and patient with me throughout the entire process. I would also like to thank my thesis advisory committee members, Drs. Jim Schaefer and Jeff Bowman for providing me with excellent advice on experimental design and data analysis during my time at Trent, and Jeff for lending me field gear.

Conducting field and lab work can be challenging, but it is made much easier by the good company provided by members of the Burness lab. I owe much to Chantelle Penny and Tess Ward for taking on the St. Lawrence River with me, and to Aoife Reilly for her diligent work with mice at Trent. Thank you to Lanna Desantis, Devin Fischer, and Simon Tapper, and everyone else in the lab, for providing me with advice and support during each stage of my research. I am grateful to Cyndi Forrest-Gregoire for welcoming me, and others, into her home in Gananoque during my fieldwork and never taking issue with our mouse-based work schedule. Thank you to Smolly Coulson and Ed Wilson for helping me with all things boat-related, and to Gabriel Huebsch for his guidance with geographic analysis and map-making. Jason Allen and Cynthia Grant at the Trent Animal Care Facility were very cooperative and supportive while working with mice at Trent. My fieldwork was made possible through the enthusiastic collaboration of staff at the Thousand Islands National Park in Mallorytown, including Josh VanWieren,
Prabir Roy, and Sheldon Lambert. Being able to work in the collection of the Royal
British Columbia Museum was a real privilege, and I would like to thank Lesley Kennes
and Gavin Hanke for helping to make that possible. I would also like to thank the many
collectors who contributed to the extensive collection of deer mice over the decades that I
was able to study. My stay in Victoria was very comfortable thanks to the hospitality of
John Cobb and Lorene Anderson, who both supported me in many ways during the
course of my graduate degree. I would like to thank my parents, Don Stewart and
Stephanie Smith, for their guidance and advice (and Dad, for his help in the field), and
my partner, Kathleen Cobb, for her support in all things. I appreciate the comments and
revisions of Aaron Shafer, Don Stewart, and Kathleen Cobb on components of this thesis.
This research was supported by a grant from the Natural Sciences and Engineering
Research Council of Canada (NSERC; G. B.), and scholarships from NSERC and from
the Government of Ontario (N.D.S).
TABLE OF CONTENTS

ABSTRACT .............................................................................................................. ii
ACKNOWLEDGEMENTS .................................................................................. iii
TABLE OF CONTENTS .................................................................................. v
LIST OF TABLES ................................................................................................. vi
LIST OF FIGURES ............................................................................................. viii
ABBREVIATIONS ................................................................................................. xi

CHAPTER 1: GENERAL INTRODUCTION ............................................................. 1
LITERATURE CITED .......................................................................................... 10

CHAPTER 2: STRESS AND BODY SIZE DO NOT DIFFER BETWEEN ISLAND AND MAINLAND WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) IN A NEAR-SHORE ARCHIPELAGO .......................................................... 16
ABSTRACT .......................................................................................................... 16
INTRODUCTION .................................................................................................. 17
METHODS .......................................................................................................... 20
RESULTS ............................................................................................................. 36
DISCUSSION ....................................................................................................... 45
LITERATURE CITED .......................................................................................... 55

CHAPTER 3: USING MUSEUM SPECIMENS TO QUANTIFY HAIR CORTICOSTERONE OF DEER MICE (PEROMYSCUS MANICULATUS) FROM TWO COASTAL ARCHIPELAGOS ........................................................................ 71
ABSTRACT .......................................................................................................... 71
INTRODUCTION .................................................................................................. 72
METHODS .......................................................................................................... 77
RESULTS ............................................................................................................. 86
DISCUSSION ....................................................................................................... 95
LITERATURE CITED .......................................................................................... 107

CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS ................................. 120
LITERATURE CITED .......................................................................................... 124

APPENDIX 1: SUPPLEMENTAL DATA FROM TRAPPING WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) AND OTHER SMALL MAMMALS IN THE THOUSAND ISLANDS NATIONAL PARK, ONTARIO .......................................................... 127

APPENDIX 2: ENZYME-IMMUNOASSAY VALIDATION FOR HAIR AND FECAL CORTICOSTERONE OF WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) ......................................................... 132

APPENDIX 3: REANALYSIS OF DATA FROM AN ADRENOCORTICOTROPIC HORMONE CHALLENGE IN WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) .......................................................... 134
INTRODUCTION ................................................................................................. 134
METHODS .......................................................................................................... 135
RESULTS AND DISCUSSION ........................................................................... 136
LITERATURE CITED .......................................................................................... 137
**LIST OF TABLES**

Table 2.1. Study sites where white-footed mice were trapped in the Thousand Islands, with abbreviation codes, island area (ha), and distance from the islands to the mainland (m). Check marks indicate when trapping occurred; x’s mark when trapping was not conducted, and dashes indicate when trapping occurred but no mice were caught. ............................................................... 23

Table 2.2. Island-mainland and within-archipelago comparisons of body size (PC1) of white-footed mice captured in Thousand Islands National Park during spring (May-June) and summer (July-August) of 2016. Significance of fixed effects was evaluated from linear mixed effects models for which trapping site (islands: n = 9, mainland sites: n = 5) was included as a random effect, and nested within habitat type (island or mainland) for the island-mainland comparison. Marginal (M) and conditional (C) pseudo \( R^2 (R^2_{GLMM}) \) values are provided. ................................................................. 38

Table 2.3. Island-mainland and within-archipelago comparisons of ln-transformed hair corticosterone of white-footed mice captured in Thousand Islands National Park during summer (July – August) of 2015 - 2016. Significance of fixed effects was evaluated using linear mixed effects models for which trapping site (islands: n = 7, mainland sites: n = 5) was included as a random effect, and nested within habitat type (island or mainland) for the island-mainland comparison. Marginal (M) and conditional (C) pseudo \( R^2 (R^2_{GLMM}) \) values are provided. ................................................................. 40

Table 2.4. Comparison of three models with ln-transformed body mass (g), body size (PC1), or condition index as predictors of ln-transformed hair corticosterone of white-footed mice (n = 140). The comparison was made for maximum likelihood linear mixed-effects models (fixed effects: sex and season; random effect: site). AICc, ∆AICc, and marginal (M) and conditional (C) pseudo \( R^2 (R^2_{GLMM}) \) values are provided to compare relative fits of the three different models. ................................................................. 41

Table 2.5. Linear mixed effects model results for ln-transformed hair corticosterone of white-footed mice (n = 147) captured in the Thousand Islands National Park during spring (May–June) and summer (July–August) of 2016. Trapping site was included as a random effect. Marginal (M) and conditional (C) pseudo \( R^2 (R^2_{GLMM}) \) values are provided. ................................................................. 42

Table 2.6. Island-mainland and within-archipelago comparisons of ln-transformed fecal corticosterone metabolites of white-footed mice captured in Thousand Islands National Park during summer (July – August) of 2015. Trapping site (island: n = 7, mainland: n = 3) was included as a random effect in all models, and nested within habitat type (island or mainland) for the island-mainland comparison. Marginal (M) and conditional (C) pseudo \( R^2 (R^2_{GLMM}) \) values are provided. ................................................................. 43

Table 2.7. Linear mixed effects model results for ln-transformed fecal corticosterone of white-footed mice (n = 71) captured in the Thousand Islands National Park during spring (May–June) and summer (July–August) of 2016. Trapping site (n = 14) was included as a random effect. Marginal (M) and conditional (C) pseudo \( R^2 (R^2_{GLMM}) \) values are provided. .......................................................................................... 44
Table 3.1. Study site names with island area (ha) and distance to Vancouver Island (m), sample sizes of skulls (SK), body mass (BM), and hair CORT (HC). Collection years are provided. .................................................................................................................. 80

Table 3.2. Comparison of skull size (PC1) of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region that others were compared to. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided. .............................................................................................................. 87

Table 3.3. Comparison of body mass (g) and condition scores of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region others were compared to. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided. .............................................................................................................. 88

Table 3.4. Comparison of ln-transformed hair corticosterone levels of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island without controlling for structural size and using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region others were compared to in the model. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided. .............................................................................................................. 90

Table 3.5. Comparison of ln-transformed hair corticosterone levels of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island controlling for structural size and using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region others were compared to in the model. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided. .............................................................................................................. 91

Table 3.6. Comparison of body mass (g), condition index, and skull size (PC1) as predictors of ln-transformed hair corticosterone for male deer mice (n = 90), based on maximum likelihood linear mixed-effects models (fixed effects: region, year, and calendar day; random effect: site, nested within region). AICc, ΔAICc, and marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided to compare relative fit. ................................................................................................................................. 92

Table 3.7. Results of body mass (g) as a predictor of ln-transformed hair corticosterone for male deer mice (n = 90) based on a linear mixed-effects model (random effect: site, nested within region). AICc, ΔAICc, and marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided to compare relative fits of the three different models. ................................................................................................................................. 92

Table 3.8. Evaluating the effect of degradation of hair corticosterone over time in specimens of deer mice from Vancouver Island (11 sites, 82 individuals) collected during 1915-1991 using a linear mixed-effects model (random effect: site). Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided. .......................................................... 93

Table 3.9. Evaluating the effect of degradation of hair corticosterone over time while controlling for the effect of structural size (PC1) in specimens of deer mice from Vancouver Island (10 sites, 68 individuals) collected during 1915-1991 using a linear mixed-effects model (random effect: site). Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided. ............................................................................................... 94
Table A1.1. Summary of trapping success of white-footed mice per site during each trapping period in the Thousand Islands National Park. Abbreviations provided below................................................................. 128

Table A1.2. Number of individuals of each species trapped at locations in the Thousand Islands National Park during 2015-2016. Species abbreviations are provided, however flying squirrels (Glaucomys spp.), shrews of the genus Sorex, and weasels (Mustela spp.) were identified only to genus................................................................. 130

LIST OF FIGURES

Figure 2.1. Trapping locations in the Thousand Islands National Park. Islands that were trapping sites are shaded in darker grey. Abbreviations relate to those used in Table 2.1.......................................................................................................................... 62

Figure 2.2. Relative abundance of white-footed mice, measured in catch per unit effort (captures per 100 trap nights) and corrected for trap disturbance for island (n = 11) and mainland (n = 5) trapping locations in the Thousand Islands National Park during three periods. Relative abundance was higher in summer 2016 than summer 2015, and higher during both summers than in the spring 2016. Several locations were not trapped during all three periods. .................................................................................. 63

Figure 2.3. Correlation of white-footed mouse abundance at trapping locations (n = 11) in the Thousand Islands National Park during summer months of 2015 and 2016 (r = 0.852, t9 = 4.875, p < 0.001). The solid black line represents the linear relationship in abundance between the two years, and the dashed line represents the predicted line if abundances were equal between years. Abundance was measured in catch-per-unit-effort (captures per 100 trap nights), which was corrected for tripped traps............. 64

Figure 2.4. There was no difference in white-footed mouse body size (PC1) between sexes or for individuals captured on islands and the mainland (p > 0.05 for both sex and habitat type; Table 2.2). Boxplots of principal component scores for body size of male and female white-footed mice (n = 175) captured during the summer of 2016 from islands and mainland trapping locations in the Thousand Islands National Park. The median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range. Filled circles represent values for individual mice. .............................................................................. 65

Figure 2.5. There was no difference in deer mouse ln-transformed hair corticosterone between sexes or for individuals captured on islands and the mainland (p > 0.05 for both sex and habitat type; Table 2.3). Boxplots of ln-transformed hair corticosterone of white-footed mice (n = 270) captured during the summers (July-August) of 2015-2016 from islands and mainland trapping locations in the Thousand Islands National Park. The median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range. Filled circles represent values for individual mice................................................................. 65

Figure 2.6. Significant positive relationship (β = 0.568, t261 = 3.306, p = 0.001) between ln-transformed hair corticosterone (ng/g) and ln body mass (g) of white-footed mice (n = 270) caught in the Thousand Islands National Park during 2015-2016. A linear-mixed model which included habitat type (island vs. mainland), sex, and year as
fixed factors and site as a random effect nested within habitat type was used to test this relationship (all other predictor variables were not significant).

Figure 2.7. Both sexes of white-footed mice had lower ln-transformed fecal corticosterone metabolite levels in spring than in summer ($p < 0.001$; Table 2.7), but only female ln-transformed hair corticosterone differed between seasons (sex*season interaction; $p < 0.001$; Table 2.5). Box plots of ln-transformed hair corticosterone (A) and fecal corticosterone metabolites (B) of white-footed mice during spring (May-June) and summer (July-August) of 2016 in the Thousand Islands National Park. The median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range...

Figure 2.8. There was no difference in fecal corticosterone metabolites between sexes or for white-footed mice (n = 151) captured on islands and the mainland ($p > 0.05$ for both sex and habitat type; Table 2.6) during summer (July-August) 2015 in the Thousand Islands National Park. For boxplots, the median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range. Filled circles represent values for individual mice...

Figure 2.9. Ln-transformed hair corticosterone plotted against ln-transformed fecal corticosterone metabolites (ng/g) for white-footed mice (n = 180) caught in the Thousand Islands National Park ($r = 0.16$, $t_{178} = 2.196$, $p = 0.015$).

Figure 3.1. Locations on Vancouver Island (mainland; Top), in Barkley Sound (bottom left), and in the Gulf Islands (bottom right) from which deer mice were collected. Individual study sites are indicated by points on the map (Vancouver Island) or by shading entire islands in the archipelagos.

Figure 3.2. Ventral view of deer mouse skull with measurements indicated: skull length (SL), cranium breadth (CB), zygomatic arch length (ZL), and zygomatic arch breadth (ZB). Photo of skull provided by Phil Myers.

Figure 3.3. Deer mice from Barkley Sound had larger skulls (PC1) than Vancouver Island deer mice (A; Table 3.2), and male deer mice from both archipelagos had greater body mass (B) and were in better condition than Vancouver Island deer mice (C; Table 3).

Figure 3.4. Hair corticosterone (ng/g) of deer mice from two archipelagos did not differ from Vancouver Island mice (A; Table 4) without controlling for skull size (PC1; Table 5; B). 95% confidence ellipses of the mean are provided on the scatterplot to highlight the distribution of individual points for each region. Hair corticosterone values were ln-transformed for analysis.

Figure 3.5. Hair corticosterone (ng/g) values of deer mouse specimens collected from Vancouver Island (11 sites, 82 individuals) demonstrate that earlier specimens had lower hair corticosterone.

Figure A1.1. Density distributions of white-footed mice (n = 182) with grey (juvenile), brown (subadult), and reddish-brown (adult) coats.

Figure A1.2. Hair corticosterone values (ng/g) of white-footed mice (n = 10) that were recaptured during different trapping periods in the Thousand Islands National Park. Of 10 individuals with multiple hair CORT values the hair CORT profile of six individuals either changed little or increased slightly. Hair CORT of two white-footed mice decreased between sampling periods, and two individuals increased by 400-500%.
Figure A2.1. There was a significant relationship (p < 0.01) between the amount of antibody bound to corticosterone in extractions of hair and feces of white-footed mice (*Peromyscus leucopus*) and standard solutions from synthetic stock for both hair corticosterone (A) and fecal corticosterone and its metabolites (B)............. 132

Figure A2.2. Recovery of exogenous corticosterone from white-footed mouse (*Peromyscus leucopus*) hair (A) and fecal (B) extracts, each demonstrating a significant relationship between the amounts of corticosterone recovered from spiked samples with varying amounts of corticosterone added (p < 0.01)............ 133

Figure A3.1. White-footed mice (*Peromyscus leucopus*) injected with adrenocorticotropic hormone (ACTH; n = 7) did not have higher final hair corticosterone (CORT) than saline injected mice (n = 9) after 6-8 weeks (A; redrawn as presented in Reilly 2017), but final hair CORT was significantly correlated with initial hair CORT (B; p < 0.01; $R^2 = 0.427$; generated using data from Reilly 2017). ................................................................................................................................. 137
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike’s information criterion</td>
</tr>
<tr>
<td>CORT</td>
<td>Corticosterone</td>
</tr>
<tr>
<td>CPUE</td>
<td>Catch per unit effort</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme-immunoassay</td>
</tr>
<tr>
<td>FCM</td>
<td>Fecal corticosterone metabolites</td>
</tr>
<tr>
<td>FGM</td>
<td>Fecal glucocorticoid metabolites</td>
</tr>
<tr>
<td>GC</td>
<td>Glucocorticoid</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>LMM</td>
<td>Linear mixed-effects model</td>
</tr>
<tr>
<td>ML</td>
<td>Maximum likelihood</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>REML</td>
<td>Restricted maximum likelihood</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>VI</td>
<td>Vancouver Island</td>
</tr>
</tbody>
</table>
CHAPTER 1: GENERAL INTRODUCTION

Island biogeography

Island flora and fauna provide remarkable examples of adaptation, but are often highly sensitive to anthropogenic disturbance (Whittaker and Fernandez-Palacios 2007). Island species have shown disproportionately high rates of extinction compared to continental species as habitat destruction has increased and invasive species have spread across the globe (Loehle and Eschenbach 2012). Island wildlife are often initially naïve in the face of novel predation threats, which is attributed to low levels of predation and competition on islands (Kavaliers 1990; Rödl et al. 2007; Swarts et al. 2009). Studying the physiology of island wildlife can increase our understanding of how they respond to stress and improve efforts to conserve these sensitive species. Biogeographic comparisons of the characteristics of island populations improve our understanding of how ecology influences adaptation and provide lessons that are applicable to both island and mainland habitats. The objective of my thesis was to study how island life affects the stress physiology of rodents by comparing glucocorticoid (“stress hormone”) levels of island rodents to their mainland conspecifics. I compared the morphology and physiology of island and mainland rodents using two study systems: white-footed mice (*Peromyscus leucopus*) captured in the Thousand Islands in the St. Lawrence River in Ontario and preserved deer mouse (*Peromyscus maniculatus*) specimens collected from islands adjacent to Vancouver Island, British Columbia.

The Theory of Island Biogeography proposed by MacArthur and Wilson (1967) demonstrated that species richness is related to island isolation and area. According to
their theory, species richness should be highest on large islands that are close to the mainland and lowest on small islands that are far from the mainland. The Theory of Island Biogeography has been applied beyond islands to test relationships between species richness and habitat size of forest fragments, mountain peaks, lakes, and reefs (Santos et al. 2016). Species characteristics, such as body size or predator avoidance behaviour, can also vary in relation to island area and isolation (Adler and Levins 1994; Cooper et al. 2014; Lister and Hall 2014).

Many studies of island fauna have focused on rodents (Adler and Levins 1994). Rodents provide excellent model species for island studies because they can occur in high numbers even on small islands, and their biology appears to be highly influenced by the ecology of islands (Gliwicz 1980; Adler 1996; Cuthbert et al. 2016). There is a significant body of research demonstrating morphological, demographic, and behavioural differences between island and mainland rodents (Gliwicz 1980; Adler and Levins 1994). For example, island rodent populations often exhibit gigantism (Pergams and Ashley 2001; Lomolino et al. 2012; Cuthbert et al. 2016), providing evidence for the “island rule” of mammalian body size (Foster 1964; Van Valen 1973). Gigantism of island rodents is attributed to several factors, including resource availability, immigrant selection (founder effect of large body size), and ecological release from the constraints of predators and competitors (Lomolino et al. 2012). Gigantism should be most pronounced in rodents that inhabit small, distant islands where competitors and predators are absent (Lomolino et al. 2012). The island rule also predicts that large mammalian species will exhibit dwarfism on islands. Although the generality of the island rule has been contested by some (Meiri et al. 2008), large data sets of mammal body size have
demonstrated consistent patterns of dwarfism and gigantism on islands (Lomolino et al. 2012, 2013; Durst and Roth 2015).

In addition to increased body size, island rodents tend to live at higher and more stable population densities than mainland populations (Sullivan 1977; Herman and Scott 1984). Because many island rodents lack sink environments for dispersal, neighbour familiarity and kin recognition is high on islands, which results in decreased aggression towards conspecifics (Halpin and Sullivan 1978). Adler and Levins (1994) referred to the suite of morphological, behavioural, and demographic characteristics demonstrated by island rodents as “island syndrome” and proposed that these characteristics vary based on island area and isolation. There have been many island-mainland comparisons of rodents in terms of demography (Crespin et al. 2012), behaviour (Halpin and Sullivan 1978), and body size (Durst and Roth 2015); however, few studies have examined physiological differences between island and mainland rodents.

**Stress physiology**

Within conservation biology, stress physiology is becoming an increasingly prevalent discipline. How an animal responds to a stressful event can determine its likelihood of survival (Romero and Wikelski 2001). Activation of the hypothalamic-pituitary-adrenal (HPA) axis governs the response to a stressful event. Activation of the HPA axis causes the adrenal glands to release glucocorticoids (GCs), which are steroid hormones that facilitate the release of energy resources such as glucose into the bloodstream, increase heart rate, and alter behaviour to promote survival. GCs enhance the stress response, but they also mediate it through negative feedback with the HPA axis.
The primary GC in mice and rats is corticosterone (CORT), compared to cortisol in other mammals (Sheriff et al. 2011). GCs have historically been measured from blood samples, however, hormone levels in blood change rapidly in response to capture and handling stress (Romero and Reed 2005). Given this complication, along with concerns of invasive sampling methods, many researchers have used other sources, including feces, urine, saliva, hair, and feathers to evaluate stress in wildlife (Sheriff et al. 2011). GC metabolites in feces indicate stress levels related to the length of the digestive cycle of the animal (Touma et al. 2004). GC concentrations in hair are considered to be a longer-term measure of stress, as hormones are thought to be incorporated into the hair shaft over time as it grows (Meyer and Novak 2012).

Analysis of fecal GC metabolites has been broadly applied to evaluate the stress physiology of wildlife (Keay et al. 2006) and have been particularly prominent in studying the endocrinology of wild rodents (Harper and Austad 2001; Hayssen et al. 2002; Montiglio et al. 2012; Dantzer et al. 2016). Despite the common use of fecal GC metabolites, concerns have been raised regarding the effects that both internal (sex, diet, and gut bacteria) and external factors (time of day and season) can have on their measurement (Goymann 2012). Although evaluating the effects of diet and gut bacteria is difficult or impossible to control in the field, sex differences can be controlled statistically, and experimental design should ideally reduce variation caused by daily and seasonal differences in fecal GC metabolite concentrations.

Hair hormone analysis is a relatively new method of evaluating chronic stress in mammals. The validity of hair as a measure of GCs was first tested by comparing repeated salivary cortisol measurements of rhesus macaques over 12 weeks to hair
cortisol concentrations after that period (Davenport et al. 2006). Since then, hair has been used to demonstrate biological relationships in a variety of species, including lab mice (Jarcho et al. 2016), Antarctic fur seals (Arctocephalus gazella; Meise et al. 2016), polar bears (Ursus maritimus; Bechshoft et al. 2015), wolves (Canis lupus; Bryan et al. 2015), non-human primates (Chlorocebus aethiops; Fourie et al. 2015), and humans (Smy et al. 2016). Entire lengths of hair are collected and analyzed to evaluate integrated levels of GCs over time, and segmental analysis provides time-scales of GCs (Carlitz et al. 2014).

Physiological validations using adrenocorticotropic hormone (ACTH) challenges on captive lynx (Lynx canadensis; Terwissen et al. 2013), lab rodents (Scorrano et al. 2015), and on wild eastern chipmunks (Tamias striatus; Mastromonaco et al. 2014) have demonstrated the validity of hair GCs as a measure of HPA activity (but see Ashley et al. 2011 for an exception).

Despite validation experiments that demonstrate an increase in hair GCs in response to elevated HPA activity, there are reasons to be cautious about interpreting hair hormone data. Evidence of local production of GCs by the hair follicle has been found in humans and guinea pigs (Sharpley et al. 2009; Keckeis et al. 2012). External factors such as mechanical irritation, chemical washes, and sunlight have also been shown to affect hair GC levels (Salaberger et al. 2016; Wester et al. 2016). With these concerns in mind, hair hormone analysis still provides a promising non-invasive measure of stress in wildlife that has demonstrated meaningful biological relationships. Hair hormone analysis has not been greatly applied to field studies in rodents, with the exception of some work on eastern chipmunks (Mastromonaco et al. 2014; Lyons 2015) and deer mice (Hanselmann 2016). Among studies of island wildlife, rodents have featured prominently
as model species (Foster 1964; Adler and Levins 1994; Lomolino et al. 2012), and hair hormone analysis provides a promising tool to evaluate how rodent physiology is influenced by island life.

Study systems

I studied the potential relationship between island life and stress physiology using two study systems: live-trapped white-footed mice (Peromyscus leucopus) in Thousand Islands National Park in Ontario, and museum specimens of deer mice (Peromyscus maniculatus) from coastal islands of British Columbia. This achieved two goals: 1. To study stress physiology of Peromyscus on two different scales: a relatively near-shore, freshwater archipelago and on a larger scale using more isolated populations from coastal islands, and 2. To evaluate the utility of hair as a biomarker of stress from live-trapped rodents and preserved study skins. Additionally, each of these study systems has a relevant history in the literature of development of island biogeographic theory and island syndrome in rodents.

The Thousand Islands, located in the St. Lawrence River between southern Ontario, Canada, and New York, USA, have been featured in several studies of small mammal ecology (Lomolino 1982, 1989, 1990), most recently regarding the prevalence of Lyme disease in the region and its relationship to small mammal diversity and geography (Werden et al. 2014, 2015). Mammalian species richness in the Thousand Islands is positively related to island area (Lomolino 1982), and body size of meadow voles (Microtus pennsylvanicus) and short-tailed shrews (Blarina brevicauda) increases with isolation from the mainland (Lomolino 1984). These previously observed
biogeographic trends between species richness and morphology of mammals in the Thousand Islands indicate that it may be a suitable study system for testing potential relationships between island geography and stress physiology of rodents.

The coastal islands of British Columbia have provided study sites for many island-mainland comparisons of populations of *Peromyscus* spp., including Foster’s (1963) Ph.D. thesis on mammals of the Queen Charlotte Islands (Haida Gwaii). The island rule has sometimes been called “Foster’s rule” because of Foster’s observation that mammals demonstrate trends in body size evolution on islands. Several authors have investigated characteristics of deer mice from the Gulf Islands, located farther south than Haida Gwaii, in the Salish Sea, between south-eastern Vancouver Island and mainland British Columbia. Deer mice in the Gulf Islands show characteristics of island syndrome, including high population density (Sullivan 1977) and low aggression (Halpin and Sullivan 1978). Off the west coast of Vancouver Island, in Barkley Sound, island deer mice have larger body size than their mainland counterparts (Herman 1979; Marinelli and Millar 1989). Because deer mice in Barkley Sound and the Gulf Islands demonstrate characteristics of island syndrome, I determined that they would be suitable systems for studying potential eco-geographical relationships.

**Objectives**

The primary objective of my thesis was to study the effects of island life on the stress physiology of *Peromyscus* populations. Relatively few authors have performed island-mainland comparisons of the stress physiology of conspecifics, although some studies have demonstrated differences in baseline and stress-induced corticosterone levels.
between insular and mainland passerines (Clinchy et al. 2004; Müller et al. 2007). Such comparative studies of island wildlife are valuable for both applied conservation research and from a fundamental perspective of understanding selection pressures affecting HPA activity.

The second objective of my thesis was to evaluate the utility of hair as an integrative biomarker of HPA activity in both live-trapped and preserved *Peromyscus*. While fecal CORT metabolites have been used extensively to evaluate HPA activity in wild rodents, including *Peromyscus* (Harper and Austad 2000, 2001, 2004; Hayssen et al. 2002; Good et al. 2003; Brown and Fuller 2006; Pedersen and Greives 2008), less work has involved hair CORT. A member of our laboratory has recently performed an ACTH validation experiment of enzyme-immunoassay (EIA) of hair CORT as a measure of chronic stress in captive white-footed mice (Reilly 2017), results of which will be discussed in Chapter 2 of my thesis. The potential long-term stability of hormone levels in hair is perhaps the most attractive feature of hair hormone analysis, given the opportunities to evaluate temporal and spatial trends in hormone levels using museum collections. Studies have presented data that support long-term stability of hair hormone levels in polar bears (Bechshøft et al. 2012) and humans (Webb et al. 2010), however, more tests regarding the potential for degradation of hair hormones are required.

**Hypotheses and predictions**

In both Chapters 2 and 3 I tested the hypothesis that island syndrome includes differences in rodent GC levels between island and mainland populations. GC levels are affected by multiple environmental factors, including predation (Clinchy et al. 2011),
food availability (Kitaysky et al. 1999), and population density (Dettmer et al. 2014), all of which vary between mainland and island habitats (Lomolino et al. 2012). Although the expected directional effect of these factors may vary differently between island and mainland habitats (predation expected to decrease and population density expected to increase), I predicted that *Peromyscus* on islands would have lower levels of hair CORT and fecal CORT metabolites than mainland mice in response to decreased predator pressure and interspecific competition on islands. I reasoned that the expected positive effect on CORT of higher population density on islands would be mitigated by the reduced aggression characteristic of island rodents (Halpin and Sullivan 1978; Adler and Levins 1994).

To determine if *Peromyscus* display island syndrome, I first recorded measures of body size for mice from both study systems, and assessed relative abundance in the Thousand Islands. I predicted that mice on islands would be larger and (in the Thousand Islands) live at higher population densities than mainland mice. In both chapters, I quantified hair CORT, and in the Thousand Islands I also quantified fecal CORT metabolites. I predicted that these measures of CORT would be lower in island populations of *Peromyscus* compared to their mainland counterparts. In both chapters I used the available data to assess what internal (body mass, condition, and structural size) and external factors (relative abundance, season, and storage time) are strong predictors of hair CORT. In Chapter 2, I tested the hypothesis that hair GC levels are affected by long term storage using *Peromyscus* specimens collected during 1915-1991, and predicted that earlier specimens would have lower hair CORT than more recently collected specimens. The results of these studies will improve the understanding of how
the stress physiology of rodents is influenced by island life, and will identify strengths and weaknesses in the application of hair as an integrative biomarker of stress in wild rodents.

**LITERATURE CITED**


Harper, J. M., and S. N. Austad. 2001. Effect of capture and season on fecal glucocorticoid levels in deer mice (Peromyscus maniculatus) and red-backed


Lomolino, M. V., D. F. Sax, M. R. Palombo, and A. A. van der Geer. 2012. Of mice and
mammoths: evaluations of causal explanations for body size evolution in insular

Lyons, J. 2015. Anthropogenic impacts on life history traits on eastern chipmunks


Mastromonaco, G. F., K. Gunn, H. McCurdy-Adams, D. B. Edwards, and A. I. Schulte-
Hostedde. 2014. Validation and use of hair cortisol as a measure of chronic stress

Meiri, S., N. Cooper, and A. Purvis. 2008. The island rule: made to be broken?

reflect the maternal prenatal social environment: potential for foetal programming?


Noninvasive monitoring of fecal cortisol metabolites in the eastern chipmunk
(Tamias striatus): validation and comparison of two enzyme immunoassays.
Physiological and Biochemical Zoology: Ecological and Evolutionary

Jenni. 2007. Circulating corticosterone levels in breeding blue tits Parus
caeruleus differ between island and mainland populations and between habitats.

Pedersen, A. M., and T. J. Greives. 2008. The interaction of parasites and resources cause

112(1):245–256.

Redfield, J. A. 1976. Distribution, abundance, size, and genetic variation of Peromyscus
maniculatus on the Gulf Islands of British Columbia. Canadian Journal of

physiology in a predator-naive island species confronted with novel predation
threat. Proceedings of the Royal Society of London B: Biological Sciences
274(1609):577–582.

Reilly, A. 2017. Validation of the use of hair corticosterone to measure chronic stress in
white-footed mice (Peromyscus leucopus). Honours Thesis. Trent University.

Romero, L. M., and J. M. Reed. 2005. Collecting baseline corticosterone samples in the
field: is under 3 min good enough? Comparative Biochemistry and Physiology
Part A: Molecular & Integrative Physiology 140(1):73–79.


CHAPTER 2: STRESS AND BODY SIZE DO NOT DIFFER BETWEEN ISLAND AND MAINLAND WHITE-FOOTED MICE (*PEROMYSCUS LEUCOPUS*) IN A NEAR-SHORE ARCHIPELAGO

ABSTRACT

Island rodents are often larger and live at higher population densities than their mainland counterparts, characteristics that have been referred to as “island syndrome”. Island syndrome has been well studied, but few studies have demonstrated island-mainland differences in stress physiology. I evaluated island syndrome within the context of stress physiology of white-footed mice (*Peromyscus leucopus*) captured from islands (n = 11) and mainland sites (n = 5) in Thousand Islands National Park, Ontario. Stress physiology was evaluated by quantifying corticosterone (CORT), the primary glucocorticoid (stress hormone) of rodents, from hair and its related metabolites from fecal samples. White-footed mice captured in this near-shore archipelago did not display characteristics of island syndrome, nor differences in levels of hair CORT or fecal CORT metabolites compared to mainland mice. I suggest that island white-footed mice experience similar degrees of stress in the Thousand Islands compared to the mainland.

**Keywords:** Island syndrome, island rule, stress physiology, glucocorticoids, *Peromyscus*
INTRODUCTION

Islands have played a central role in ecological and evolutionary biology, in part because their relative isolation allows them to act as replicate natural laboratories. For example, trends in animal biodiversity, body size, and behaviour have been widely studied in relation to the geographic isolation and size of islands (Lomolino et al. 2012; Cooper et al. 2014; Warren et al. 2015). Island communities tend to have low species diversity compared with mainland systems (Losos and Ricklefs 2009), including fewer native predators (Blackburn et al. 2004). In response to decreased predator pressure and interspecific competition combined with changes in food availability on islands, small mammals evolve towards gigantism upon arrival to islands while larger species often display dwarfing (Lomolino 2005; Lomolino et al. 2012). This pattern, which is repeated across numerous archipelagos, contributes to the evolutionary trend called the “island rule”, a term coined by Van Valen (1973). The combination of increased body size with changes in behaviour and demography has been referred to as “island syndrome” in rodents (Adler and Levins 1994).

When an animal encounters a perceived stressor, its hypothalamic-pituitary-adrenal (HPA) axis is activated, resulting in the secretion of glucocorticoids (GCs) (Sapolsky et al. 2000). The primary GC in mice and rats is corticosterone (CORT), compared to cortisol in other mammals. Multiple environmental factors can influence GC levels, including predation (Clinchy et al. 2011; Sheriff et al. 2011a), food availability (Kitaysky et al. 1999; Walker et al. 2005), and population density (Dettmer et al. 2014; Blondel et al. 2016). Once secreted, GCs enhance the stress response by promoting cardiovascular activity, and mobilizing energy stores, but GCs also mediate the response
through negative feedback with the HPA axis (Sapolsky et al. 2000). Elevated GC levels are involved in preparation for future stressors by shifting resources from reproduction and digestion toward replenishing energy stores used during the initial stress response (Romero and Wingfield 2016).

GCs have historically been measured from blood samples, however, hormone levels in blood change rapidly in response to capture and handling stress (Romero and Reed 2005). Given this complication, along with concerns of invasive sampling methods, many researchers have used other sources, including feces, saliva, hair, and feathers to evaluate stress in wildlife (Sheriff et al. 2011b). Fecal GC metabolite (FGM) measurement is attractive to researchers because feces can be easily and non-invasively collected from wildlife. FGM concentrations are an integrative biomarker of HPA activity because they are thought to represent GC levels over the course of several hours prior to the collection of feces (Harper and Austad 2000; Blondel et al. 2016; Fauteux et al. 2017). Quantifying FGM concentrations has been broadly applied to the study of stress physiology in free-living mammals (Keay 2006), including Peromyscus spp. (Harper and Austad 2001; Hayssen et al. 2002; Brown and Fuller 2006).

GC concentrations in hair are thought to provide an integrative measure of GC levels over the time required for the sampled length of hair to grow, which may be weeks or months, depending on the species (Sheriff et al. 2011b). Concerns have been raised regarding the influence of external factors on hair GCs and the possibility of local production of GCs by the hair follicle (Sharpley et al. 2009; Keckeis et al. 2012). However, hair hormone analysis is a promising method of evaluating stress in wildlife, and has demonstrated biological relationships in a variety of species (Bryan et al. 2015;
Fourie et al. 2015; Meise et al. 2016; Jarcho et al. 2016; Smy et al. 2016). Hair GC analysis has been validated as a biomarker of HPA activity in eastern chipmunks (Mastromonaco et al. 2014) and laboratory rats (Scorrano et al. 2015).

The same ecological factors that affect GC levels in wildlife (predation, competition, and resource availability) are those that account for island syndrome in rodents. Therefore, I tested the hypothesis that island syndrome includes differences in GC levels between island rodents and their mainland conspecifics. To test the hypothesis that island syndrome includes changes in GC levels, I compared levels of corticosterone (CORT) in hair and its metabolites in the feces of white-footed mice (Peromyscus leucopus) captured at multiple island and mainland locations in Thousand Islands National Park in Ontario, Canada. I predicted that white-footed mice in the Thousand Islands would have higher relative abundance, greater body size, and lower hair CORT and fecal CORT metabolites (FCM) than mainland white-footed mice. Characteristics of island syndrome in rodents are affected by island area and distance from the mainland (Adler and Levins 1994), so I also predicted that white-footed mice would be more abundant, larger, and have lower GC levels on the smaller and more isolated islands in the archipelago. GC levels vary with both internal factors, such as age and condition (Walker et al. 2005), and external factors, such as season and population density (Romero 2002; Blondel et al. 2016). Given this, I also evaluated how body mass, structural size, and condition affected hair CORT and FCM in white-footed mice, and how GCs varied between seasons.
METHODS

The Trent University Animal Care Committee approved all procedures prior to working with the animals. Trapping in the Thousand Islands National Park was approved via a Parks Canada Research and Collection Permit (No. 22959).

Study species

The white-footed mouse, *Peromyscus leucopus*, is a small, nocturnal rodent that inhabits deciduous and mixed forests in the eastern United States and southern edge of Canada. White-footed mice are omnivorous, relying heavily on invertebrates and seeds for food (Manson and Stiles 1998). The white-footed mouse is the most abundant small mammal in the Thousand Islands (Werden et al. 2014) and has been the study species for island-mainland comparisons in other regions (Adler and Tamarin 1984; Adler et al. 1986). Although visual differentiation of white-footed mice and deer mice (*P. maniculatus*) is difficult in areas of overlap, genetic analysis of mice belonging to the genus *Peromyscus* in the Thousand Islands shows that all (or at least the vast majority) are white-footed mice (Werden et al. 2014).

*Peromyscus* spp., including white-footed mice, display both seasonal and developmental moults (Layne 1968). Juvenile white-footed mice are characterized by a grey coat that is retained until 40-50 days of age, at which point they begin to develop a brown subadult coat, which is retained until 60-75 days of age (Nicholson 1941; cited by Layne 1968). Adult white-footed mice, like other *Peromyscus* spp., are characterized by a reddish-brown coat (Drost and Fellers 1991). Near the Thousand Islands (i.e., New York, USA), most white-footed mice have developed their summer pelage by May, and their
winter pelage by October (Pierce and Vogt 1993). In addition to seasonal and developmental moults, some hair replacement may occur in absence of complete moults, as in deer mice (Tabacaru et al. 2011). This means that hair samples collected in May-June should be representative of the summer pelage grown in late winter and early spring (February-June). Samples collected in July-August will be influenced by moult and any additional growth occurring from February-August for second year adults, and approximately May-August for the young of the year adults.

**Study Area**

All trapping locations were located in Thousand Islands National Park in Ontario, Canada (previously called St. Lawrence Islands National Park; Figure 2.1). Thousand Islands National Park includes properties on islands of the St. Lawrence River and along its shoreline. I trapped on nine islands in 2015 and on ten islands in 2016, with island areas ranging from 1.7-555.9 ha (Table 2.1). Trapping was conducted at three mainland locations in 2015 and at five locations in 2016. Mainland sites were located within 2 km of the St. Lawrence River (Figure 2.1). The shortest distance between mainland sites was 800 m (Jones Creek 1 and 2); others were at least 2 km apart, so I assumed there was little movement of white-footed mice between trapping sites. Trapping grid sites were selected based on both privacy (distance from public paths to prevent visibility and disturbance) and habitat type. I targeted wooded areas as opposed to open fields both to maintain consistency and to increase trapping success.
GIS Analysis

I calculated the area of islands and their shortest distance from the mainland to evaluate the influence of these geographic characteristics on white-footed mice in the Thousand Islands. Geographical analysis and map-making were performed using ArcMap (Version 10.4.1). Data files were obtained from the National Hydrography Dataset of the United States Geological Survey. Island area (ha) was measured using the “erase” tool and distance from the mainland (m) was calculated using the “generate near table analysis” tool.

Small mammal trapping

I trapped during three periods: summer 2015 (July – August), spring 2016 (May-June) and summer 2016 (July – August). Sherman live-traps (H.B. Sherman Traps, Inc., Tallahassee, FL, USA) were set 10 m apart in rectangular grids of varying size. The majority of grids were arranged in a 7 by 7 formation (49 traps in total), however, some areas on small islands (Beaurivage Island, Constance Island) were limited by pedestrian paths, which necessitated using smaller grids (5 by 5), or in one case, transects (Mermaid Island). Hulled sunflower seeds were used as bait and natural cotton bedding was provided for warmth. Traps were set in the evening (ca. 1800 h) and checked in the morning (ca. 0700 h) to target the active period of white-footed mice. Trapping sessions generally consisted of two nights of consecutive trapping (Adler and Tamarin 1984), however, I continued to trap at some locations for 3-4 nights to achieve a minimum useful sample size of white-footed mice. Consecutive nights of trapping were also occasionally interrupted by poor weather.
Table 2.1. Study sites where white-footed mice were trapped in the Thousand Islands, with abbreviation codes, island area (ha), and distance from the islands to the mainland (m). Check marks indicate when trapping occurred; x’s mark when trapping was not conducted, and dashes indicate when trapping occurred but no mice were caught.

<table>
<thead>
<tr>
<th>Site</th>
<th>Code *</th>
<th>Area (ha)</th>
<th>Distance (m)</th>
<th>Summer 2015</th>
<th>Spring 2016</th>
<th>Summer 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aubrey Island</td>
<td>AI</td>
<td>6.8</td>
<td>756</td>
<td>✔</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beau Rivage Island</td>
<td>BI</td>
<td>4.9</td>
<td>349</td>
<td>✗</td>
<td>✔</td>
<td>✗</td>
</tr>
<tr>
<td>Camelot Island</td>
<td>CI</td>
<td>9.4</td>
<td>3636</td>
<td>✔</td>
<td>✗</td>
<td>✗</td>
</tr>
<tr>
<td>Constance Island</td>
<td>CE</td>
<td>3.4</td>
<td>463</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Georgina Island</td>
<td>GA</td>
<td>10.1</td>
<td>191</td>
<td>✔</td>
<td>✔</td>
<td>-</td>
</tr>
<tr>
<td>Grenadier Island</td>
<td>GI</td>
<td>554.3</td>
<td>1038</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Hill Island</td>
<td>HI</td>
<td>555.9</td>
<td>438</td>
<td>✔</td>
<td>✗</td>
<td>✔</td>
</tr>
<tr>
<td>Lindsay Island</td>
<td>LI</td>
<td>14.4</td>
<td>429</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Mermaid Island</td>
<td>MI</td>
<td>1.7</td>
<td>1046</td>
<td>✗</td>
<td>✔</td>
<td>✗</td>
</tr>
<tr>
<td>McDonald Island</td>
<td>MD</td>
<td>17.4</td>
<td>512</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Thwartway Island</td>
<td>TI</td>
<td>40.1</td>
<td>2837</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Escot Property</td>
<td>EP</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Jones Creek1</td>
<td>JC1</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Jones Creek2</td>
<td>JC2</td>
<td></td>
<td></td>
<td>✗</td>
<td>✗</td>
<td>✔</td>
</tr>
<tr>
<td>Landon Bay</td>
<td>LB</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Mallortown</td>
<td>MT</td>
<td></td>
<td></td>
<td>✗</td>
<td>✗</td>
<td>✔</td>
</tr>
</tbody>
</table>

*Abbreviation codes based on those used by Werden et al. (2014).

Upon capture, white-footed mice were removed from the trap and placed into a clear plastic bag. The total mass of the bag, including the mouse and other trap contents, was measured using a Pesola scale (± 1 g). The mass of the bag was recorded after the mouse was removed, and the mass of the mouse was calculated by subtraction. In 2015, I recorded tail length (± 1 mm) and right-hind foot length (±1 mm) with a ruler. In 2016, I recorded tail length (± 1 mm) with a ruler, and right-hind foot length, head width (behind the eyes), head length, and ear length with digital calipers (±0.1 mm; Schulte-Hostedde et
al. 2001). Before release, a patch of hair (ca. 1 x 1 cm) was shaved from the rump of each individual, above the right-hind limb using an electric razor (Remington™ Model PG6025), collecting the entire length of each shaft from the skin to the end of the shaft. Each white-footed mouse was ear-tagged to recognize recaptured individuals, and released. The razor blades were cleaned with alcohol swabs between shaving each animal. GC concentrations in hair are considered to be stable at room temperature over long periods (Meyer and Novak 2012), so hair samples were stored in Fisherbrand™ Snap-Cap™ Flat-Top Microcentrifuge Tubes in the dark at ambient temperature (approx. 22°C) until hair hormone analysis (2-5 months later). Coat colour was occasionally noted (grey, brown, reddish-brown or moulting) in 2015, and always noted in 2016. I excluded juveniles from analyses of body size and GC levels based on grey pelage, and, in absence of notes of pelage, for individuals ≤14 g (Appendix 1, Figure A1.1).

White-footed mouse feces were collected from traps. Fecal samples were stored in Fisherbrand™ Snap-Cap™ Flat-Top Microcentrifuge Tubes and placed in a cooler with ice packs until they could be stored in a liquid nitrogen-cooled dry-shipper (≤6 h post collection). They remained in the dry-shipper until transported to Trent University where they were stored at -80°C until hormone extraction. Samples always spent < 2 weeks in the dry-shipper before being placed into the freezer; fecal samples spent 2-9 months in the freezer prior to extraction. Soiled traps were cleaned with 70% ethanol between uses to ensure that the feces collected from each trap belonged to the animal caught in the trap that night.
**Relative Abundance**

Population density was evaluated by calculating relative abundance of white-footed mice as a proxy for their density during each trapping session. Relative abundance was calculated as catch-per-unit-effort (CPUE), presented in number of white-footed mice captured per hundred trap-nights (Nelson and Clark 1973; Parker et al. 2016). I corrected for tripped traps by using the following correction factor equation provided by Nelson and Clark (1973):

\[
CPUE = \frac{A \times 100}{(TU - S/2)}
\]

\[
TU = P \times N
\]

where \(A\) = number of white-footed mice caught; \(TU\) = trapping units, calculated as total number of trap nights per site session; \(P\) = number of nights in each trapping session; \(N\) = number of traps set each night; and \(S\) = total sprung traps.

**Body size and condition**

A body condition index was calculated for white-footed mice in 2016 by calculating the residuals of a linear regression between ln-transformed body mass and a principal component score of structural size (Schulte-Hostedde et al. 2005). The principal component analysis (PCA) was conducted on right-hind foot length, head length, and head width measurements of adults and subadults on their first capture (\(n = 175\)). All measurements were ln-transformed and I ran the PCA using a correlation matrix due to the range of variances of the different body measurements. I used the loadings from the first principal component to calculate a single variable (PC1) to represent structural size.
The first principal component (PC1) explained 48.9% of the variation in the three body measurements. PC1 was most highly correlated with head width ($r = 0.596$), closely followed by head length ($r = 0.576$), and right-hind foot length ($r = 0.560$). The residuals of the following linear regression ($F_{1,159} = 64.73, p < 0.0001; R^2 = 0.28$) were used to calculate condition index using the first principal component (PC1):

$$\text{Predicted Ln body mass} = 0.0881*(\text{PC1}) + 3.038$$

$$\text{Condition index} = \text{Actual Ln body mass} - \text{Predicted Ln body mass}$$

**Hormone extraction and analysis**

Methods for extraction and analysis of hair corticosterone and fecal corticosterone metabolites (FCM) follow Mastromonaco et al. (2014). Extractions were conducted at Trent University and EIA were performed at the Reproductive Physiology Lab at the Toronto Zoo.

Hair samples were placed in a tared 7 ml glass scintillation vial using forceps and weighed using an analytical balance (Model SI-124 Denver Instruments; ± 0.0001 g). The forceps were washed with 70% ethanol between samples to prevent cross-contamination. Hair samples were first washed with 0.5 ml 100% methanol, vortexed for 10 s, and all of the 100% methanol was then discarded. Washing was done to prevent any biological fluids present on the hair from affecting corticosterone measurements. After washing, the appropriate extraction volume of 100% methanol was added to the hair at a ratio of 0.005 g hair per 1 ml of 100% methanol. The 100% methanol was added in two volumes; after the first volume the sample was vortexed for 5 s and then a second volume was added down the inside of the vial to collect any stray hairs that may have stuck to the sides of
the vial. New pipette tips were used for each sample. Samples were then placed on a plate shaker for 24 h (VWR Mini Orbital Shaker) at room temperature, after which the vials were centrifuged at ca. 1800 g for 10 min at 21°C (Eppendorf Centrifuge 5810 R). The supernatants were transferred and stored at -20°C in clean vials with caps that were sealed with Parafilm M® for 1-2 months until they were dried down (600 µL per sample) by evaporation in a fume hood for 24 h. Dried-down extracts were then stored at -20°C until analysis (ca. 1 month later).

To extract fecal CORT metabolites, fecal samples were removed from the freezer (-80°C) and allowed to thaw at room temperature for 45 min. Fecal samples were placed in a 7 ml glass scintillation vial using forceps and weighed using an analytical balance (Model SI-124 Denver Instruments; ± 0.0001 g). Then, 80% methanol was added to the feces at a ratio of 0.05 g feces per 1 ml 80% methanol, also in two volumes as described above for hair samples. Fecal samples were then placed on a plate shaker overnight, at room temperature. The following day, fecal samples were centrifuged at ca. 1800 g for 10 min. For each sample the supernatant was then transferred to a clean vial and stored at -20°C until they were dried down (200 µL per sample) in a fume hood. Dried-down samples were then stored at -20°C until analysis (1-6 months).

Corticosterone assay for hair and feces

An enzyme immunoassay (EIA) was used to quantify hair CORT, and fecal CORT metabolites, following methods described by Baxter-Gilbert et al. (2014). This EIA was developed to evaluate GC metabolite levels across a broad variety of taxa, and has high cross reactivity with CORT (100%) and at least one of its metabolites,
desoxycorticostone (14.25%), in addition to other GC metabolites (Watson et al. 2013). Other cross-reactivities (<3%) are reported by Watson et al. (2013). Because CORT is the dominant GC white-footed mice, and nearly all CORT is metabolized prior to excretion (Touma et al 2003), I refer to the values obtained from the EIA of fecal extractions as fecal CORT metabolites.

Dried-down hair hormone extracts were reconstituted in 150 µl EIA buffer solution (0.1 mM sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1 g of bovine serum albumin per litre) resulting in a 4-fold concentration and vortexed twice, with a minimum of 1h between vortexing. Dried-down fecal extracts were reconstituted in 200 µl EIA buffer solution and diluted for a final 1:20 dilution.

EIA standards were created using synthetic corticosterone (Steraloids Q1550; 39–10 000 pg/ml). Pooled fecal extracts obtained from spotted-necked otters (Hydrictis maculicollis) were used as the control that was run at 65% binding to determine coefficient of variation (CV) values for inter-assay CV (variation between plates). Samples were run as duplicates, and only samples with < 10% CV were accepted. Intra-assay CV (variation within plates) was 5.6% at 50% binding as reported in Baxter-Gilbert et al. (2014).

In brief, microtitre plates (Nunc Maxisorp, VWR, Mississauga, ON, Canada) were coated with 0.25 µg/well goat anti-rabbit IgG polyclonal antibody (Sigma-Aldrich, Mississauga, ON, Canada) diluted in coating buffer at a dilution of 1:200 000 (50 mM bicarbonate buffer, pH 9.6). After overnight incubation at room temperature in the dark, plates were washed with 0.05% Tween 20, 0.15 M NaCl solution using a Bio-Tek ELx 405VR microplate washer (Bio-Tek Instruments, Winooski, VT) and blocked with 250 µl
EIA buffer per well for a minimum 1 h at room temperature. Next, 50 µl of reconstituted hair or fecal extracts, standards, and controls diluted in EIA buffer were added in duplicates. These were followed by 100 µl of horseradish peroxidase conjugate (C. Munro, University of California, Davis, CA, USA) and 100 µl corticosterone antiserum (CJM006; C. Munro, University of California, Davis, CA, USA) diluted in EIA buffer at 1:1 000 000 and 1:200 000, respectively. Plates were incubated overnight in the dark at room temperature. On the third day, plates were washed and 200 µl of substrate solution (0.5 ml of 4 mg/ml tetramethylbenzidine in dimethylsulphoxide and 0.1 ml of 0.176 M H₂O₂ diluted in 22 ml of 0.01 M sodium acetate trihydrate [C₂H₃NaO₂·3H₂O], pH 5.0) was added. After 30 min in the dark at room temperature, colour development was stopped with 50 µl H₂SO₄ (1.8M). Absorbance was measured at 450 nm using a spectrophotometer (MRXe microplate reader, Dynex Technologies, Chantilly, VA).

**Parallelism**

Parallel displacement is used to detect immunological similarities between standard and sample hormone. Parallelism was assessed by running (separately) pooled samples of hair and fecal extracts alongside the standard curve. Both hair and fecal extracts were serially diluted: the hair extract pool was concentrated and serially diluted 2-fold from 10X to 0.313X concentrations, and the fecal extract pool was concentrated then serially diluted 2-fold from 1:1 to 1:32 dilution. Linear regression was used to assess the variance in the bound antibody between the standard curve and sample extracts. Serial dilutions of pooled hair and fecal extract showed parallel displacement with the corticosterone standard curve (hair: \( r = 0.996, p < 0.01 \); fecal: \( r = 0.996, p < 0.01 \);
Appendix 2, Figure A2.1).

**Accuracy**

Recovery of hormone added to the extracts was calculated to determine if components within the extracts interfered with antibody binding. A pooled sample of hair extract was concentrated to the usual range for experimental samples (4-fold concentration), and a pooled sample of fecal extract was dried-down then diluted to the usual range for experimental samples (20-fold dilution). Aliquots of 75 µl of concentrated or diluted sample were added to 75 µl aliquots of the usual standards (39–10 000 pg/ml). The endogenous hormone levels in the concentrated or diluted pool alone were determined, as well as hormone levels in the spiked samples. The percent recovered was calculated by dividing the observed amount (concentration of the spiked sample) by the expected amount (calculated amount of standard hormone added plus the endogenous hormone in the unspiked sample). Regression analysis was used to determine if there was a significant relationship between hormone added vs. hormone recovered. The recovery of known concentrations of corticosterone from mouse hair and fecal extracts were 102.7 ± 4.3% and 83.5 ± 5.4% respectively (Appendix 2, Figure A2.1). The measured hormone concentrations in the spiked samples correlated with the expected concentrations (hair: r = 0.999, p < 0.01; fecal: r = 0.998, p < 0.01; Appendix 2, Figure A2.2).
Statistical analysis

I began by testing my first two predictions: that island mice would show higher relative abundance and larger body size than mainland mice. I then tested my third prediction, that hair CORT and FCM of mice would be lower on islands than on the mainland. I also evaluated whether island geography (island area and distance from the mainland) affected relative abundance, body size and GC levels, and I present those results following the island-mainland comparison of each variable. Following these analyses, I tested which individual measure (body mass, PC1 of body size, or condition index) best predicted GC levels, and how both measures of GCs differed between spring and summer of 2016.

I ln-transformed hair CORT, FCM, and body mass data, and log\(_{10}\) transformed island area and distance from the mainland to improve the normality of the model residuals. Normality of residuals was visually assessed using kernel density histograms, which were used to compare the model residuals to a normal distribution. I visually assessed models for homoscedasticity by plotting the residuals against the fitted values of the model. Juveniles were excluded and only subadults and adults were analyzed in tests of body size and GC levels. Any visibly pregnant females (n = 15) were excluded from all analyses involving body mass data. Only the first value of each measure for individuals for which multiple samples were collected was used in each set of analyses.

Analyses were conducted using RStudio (Version 0.99.484, RStudio, Inc). Linear-mixed effects models were fit with the lmer function of the “lme4” package (Bates et al. 2014) using restricted maximum likelihood (REML) in all cases except when comparing models with Akaike’s information criterion (Akaike 1974), corrected for small sample
size (AICc), when maximum likelihood (ML) was used. Interactions between fixed effects were included, but removed if they were not significant (at \( p < 0.05 \)) and results were reported for reduced models (without the insignificant interactions). Results, including \( p \)-values, \( t \)-values, and Satterthwaite approximations to degrees of freedom, were obtained using the “summary” function of the “lmerTest” package (Kuznetzsova et al. 2015). Goodness of fit was assessed using marginal (\( R^2_{\text{GLMM(M)}} \); Nakagawa and Schielzeth 2013) and conditional (\( R^2_{\text{GLMM(C)}} \)) pseudo \( R^2 \) values calculated with the “r.squaredGLMM” function in the “MuMIn” package (Bartoń 2011). \( R^2_{\text{GLMM(M)}} \) represents the proportion of the variation explained by the fixed effects alone, and \( R^2_{\text{GLMM(C)}} \) represents the proportion of variation explained by both the fixed and random effects (Nakagawa and Schielzeth 2013).

In all linear mixed models, trapping site (island or mainland location) was included as a random effect for each white-footed mouse to account for lack of independence between individuals caught at the same site. Site was nested within habitat type (island vs. mainland) in all island-mainland comparisons, however, it was not nested when comparing island mice to each other, or when assessing seasonal variation in GC levels, for which differences were assessed across habitat types.

**Relative abundance**

To test if my first prediction, that relative abundance (CPUE) of white-footed mice would be higher at island than mainland sites, I used a linear-mixed effects model with habitat type, year (2015 or 2016) and season (spring or summer) as fixed factors. Then, using only island data, I tested whether population abundance was affected by
island area and distance from the mainland, with year as a fixed factor. I also tested the correlation of white-footed mouse abundance between sites that were trapped during the summer of both years by calculating the Pearson correlation coefficient and significance of this correlation was determined using the “cor.test” function in R, which calculates a \( p \)-value based on Fisher's Z transformation.

*Body size*

I used the previously described PC1 scores for body size to test my second prediction, that island mice would be larger than mainland mice. I tested this prediction using a linear-mixed effects model with sex, habitat type, and season as fixed factors. Following this, using only island mice, I ran a linear mixed-effects model with island area and distance from the mainland as covariates and tested for their interaction to determine if island geography affected PC1.

*Hair corticosterone*

I began testing my third prediction, that island mice would have lower GC levels than mainland mice, using hair CORT data collected during the summers of 2015-2016. (Spring 2016 was excluded, because I did not trap in the spring of 2015.) In this linear mixed-effects model, habitat type (island vs. mainland), relative abundance, sex, and year were included as fixed effects. Preliminary analysis indicated that body mass was positively correlated with hair CORT, so mass was included as a covariate in all models (except where body size or condition index were used instead). Relative age (subadult vs.
adult) was previously tested in the following models, however, it was not a significant factor, and because it was correlated with body mass it was not included.

Following the island-mainland comparison, I ran a linear mixed model with only island mice, and included island area and distance from the mainland as covariates to test if hair CORT was affected by geographic characteristics of islands. Two ln-transformed hair CORT values that were suspected outliers of the ln-transformed data (< 10 ng/g, which was > 2.5 SD below the ln-transformed mean) reduced the normality of the residuals, so tests were run with and without these two values to evaluate their influence on the model. Because they did not influence the results, the results are reported for the models with these possible outliers retained.

I then used the spring and summer hair CORT data from 2016 to test whether body mass, condition index, or body size, was a better predictor of hair CORT. I then used the best predictor as a covariate to evaluate what factors contribute to seasonal variation in hair CORT levels. Sex, season, and their interaction were included as fixed effects in the model. Habitat type was initially included, however, it was removed after determining it was not significant, so results are presented without habitat type as a fixed effect. The three resulting models were compared using AICc and the model with the best predictor (evaluated using ΔAICc and $R^2_{GLMM}$) was used to evaluate seasonal differences in hair CORT.

**Fecal corticosterone metabolites**

I again tested my third prediction, that island mice would have lower GC levels than mainland mice, using FCM, and followed the same process as that described for hair
CORT. One value was removed from all analyses because it was a suspected outlier, and influenced certain models (1777.2 ng/g, which was > 3 SD above the mean of the ln-transformed data). Only 2015 values were used to compare island and mainland white-footed mice, because of small sample size (n = 5) of fecal samples from female white-footed mice caught during the summer of 2016, which caused heteroscedasticity, demonstrated by diagnostic plots of model residuals vs. fitted values. This small sample size was largely due to loss of samples caused by a processing error. There was still a sufficient sample size to evaluate seasonal differences in the 2016 fecal CORT metabolite data when habitat type was removed as a fixed effect. The following measurements were not correlated with FCM: body mass (r = 0.03, t_{57} = 0.219, p = 0.827), condition index (r = 0.05, t_{57} = 0.355; p = 0.724, and PC1 (r = -0.02, t_{57} = -0.162, p = 0.871), therefore, I did not include these measures in the linear mixed-effects models when evaluating seasonal variation in FCM.

The strength of the correlation between hair and FCM was assessed using the “cor.test” function in R. Both measures were ln-transformed to meet the assumption of normality required for the Pearson correlation. One hair CORT value was removed from this test because it was a suspected outlier of the ln-transformed data (< 10 ng/g; which was > 2.5 SD below the ln-transformed mean). Results from the test and the plot are presented without this value, because it increased the value of the correlation coefficient. I also re-ran the primary models for testing island-mainland differences in PC1, hair CORT, and FCM, but excluded the two largest islands (Grenadier and Hill), to determine if mice from the smallest islands differed from mainland mice. These results were not
different from the analysis with large islands included, so I present the complete analysis in the results section.

RESULTS

We caught 408 individual white-footed mice during 2015-2016; 17 individuals were recaptured between sessions. Trapping success was highly variable across sites. There were no captures made on Aubrey Island and Georgina Island in 2016, while Lindsay Island was consistently among the most successful trapping locations across years and seasons (Appendix 1, Table A1.1). Based on overall CPUE, trapping efforts were more successful on some islands than on the mainland, however, on some islands there were zero captures during some sessions and mainland sites always yielded captures (Appendix 1, Table A1.1). Trap disturbance likely contributed to the high degree of variation in trapping success. More traps were tripped at island sites (mean ± SD; 38% ± 10.8%) than mainland sites (23% ± 12.8%), and it was likely that disturbance from raccoons and from other mammals added error to the calculation of relative abundance.

Relative abundance of mice did not differ between islands and the mainland

There was no difference in relative abundance of island and mainland white-footed mice (habitat type: $t_{17} = 0.741, p = 0.469$). Relative abundance was 44% higher in summer 2015 than in summer 2016 across habitat types (year: $t_{22} = -2.74, p = 0.012$; Figure 2.2). Relative abundance was 74% higher in the summer than the spring of 2016 (season: $t_{22} = 2.253, p = 0.034$; Figure 2.2). Summer abundance of individual sites was positively correlated between years ($n = 11$, $r = 0.852$, $t_9 = 4.87$, $p < 0.001$; Figure 2.3). Based on capture data from only island sites, there was no effect of island area ($t_7 =$
0.542, \( p = 0.603 \) nor distance from the mainland (\( t_8 = -0.805, p = 0.442 \)) on relative abundance of white-footed mice, nor was there an interaction between these two variables (\( t_7 = 0.755, p = 0.474 \)).

**Body size did not differ between island and mainland mice**

There was no difference in body size (PC1, \( n = 175 \)) between male and female white-footed mice, and there was no difference in PC1 scores between island and mainland mice (Table 2.2; Figure 2.4). For the first model, when both island and mainland mice were analyzed together, results indicated that white-footed mice captured in the spring were larger than summer mice (Table 2.2). However, season was not a significant factor when island mice were analyzed separately from mainland mice (Table 2.2). The significant difference in PC1 between spring and summer for white-footed mice across habitats was likely the result of individuals captured in spring being relatively older than individuals captured in the summer months. Among white-footed mice (\( n = 106 \)) from nine of the islands, there was no effect of island size on PC1, however, isolation had a marginally significant negative effect (Table 2.2). The farthest island from shore for which I had body size data, Thwartway Island, was only 400 m from a much larger island, Grindstone, which may have acted as a source population. If the value for shortest distance to the mainland for Thwartway was substituted with the distance between Thwartway and Grindstone (400 m), then the effect of isolation on PC1 was no longer marginally significant (\( t_8 = -0.800, p = 0.445 \)).
Table 2.2. Island-mainland and within-archipelago comparisons of body size (PC1) of white-footed mice captured in Thousand Islands National Park during spring (May-June) and summer (July-August) of 2016. Significance of fixed effects was evaluated from linear mixed effects models for which trapping site (islands: n = 9, mainland sites: n = 5) was included as a random effect, and nested within habitat type (island or mainland) for the island-mainland comparison. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Fixed effects</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M),(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Island and mainland mice</td>
<td>(Intercept)</td>
<td>0.301</td>
<td>0.224</td>
<td>33</td>
<td>1.342</td>
<td>0.189</td>
<td>0.07, 0.15</td>
</tr>
<tr>
<td></td>
<td>Type (Mainland)</td>
<td>0.144</td>
<td>0.281</td>
<td>11</td>
<td>0.513</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>0.061</td>
<td>0.177</td>
<td>165</td>
<td>0.343</td>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Season (Summer)</td>
<td>-0.646</td>
<td>0.184</td>
<td>170</td>
<td>-3.513</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Island mice</td>
<td>(Intercept)</td>
<td>3.346</td>
<td>1.408</td>
<td>8</td>
<td>2.376</td>
<td>0.042</td>
<td>0.12, 0.15</td>
</tr>
<tr>
<td></td>
<td>Log10(Island area)</td>
<td>-0.275</td>
<td>0.167</td>
<td>5</td>
<td>-1.648</td>
<td>0.157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Log10(Distance)</td>
<td>-0.984</td>
<td>0.515</td>
<td>8</td>
<td>-1.909</td>
<td>0.089</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Season (Summer)</td>
<td>-0.349</td>
<td>0.235</td>
<td>99</td>
<td>-1.485</td>
<td>0.141</td>
<td></td>
</tr>
</tbody>
</table>

Hair corticosterone did not differ between island and mainland mice

Median hair CORT of white-footed mice included in the analysis was 79.8 ng/g, and ranged widely from 5.1-398.6 ng/g, although only 1.5% of hair samples yielded CORT concentrations greater than 250 ng/g. The right-skewed nature of the data was what necessitated ln-transformation for analysis. Based on the values from the summers of 2015-2016, there was no difference between hair CORT of island and mainland white-footed mice (Table 2.3; Figure 2.5). Sex, year of capture, and relative abundance also had no effect on hair CORT (Table 2.3). Removing insignificant terms from the model (sex
and relative abundance) did not result in any other factors achieving significance, so the results of the full models are presented.

Body mass was a significant positive predictor of hair CORT (Table 2.3; Figure 2.6). There was a small effect size for this model, and the higher conditional $R^2_{GLMM}$ relative to the marginal $R^2_{GLMM}$ indicates that the model had more explanatory power with the addition of the random effect (site) than with fixed effects alone ($R^2_{GLMM(M)} = 0.05$, $R^2_{GLMM(C)} = 0.14$; Table 2.3). This suggests that there was similarity between individuals from the same sites that was not explained by the fixed effects in the model. There was no significant interaction between body mass and sex ($t_{257} = -0.434$, $p = 0.665$), and analyzing the sexes separately demonstrated that there was a significant positive effect of body mass on hair CORT for both males ($n = 166$, $\beta = 0.570$, $t_{161} = 2.658$, $p < 0.01$), and females ($n = 104$, $\beta = 0.595$, $t_{95} = 2.093$, $p < 0.05$).

For island mice, neither island area nor distance from the mainland predicted hair CORT levels (Table 2.3) and there was no interaction between these variables ($t_4 = 1.652$, $p = 0.169$). However, body mass continued to be a significant predictor of hair CORT for the model testing only island mice (Table 2.3).
Table 2.3. Island-mainland and within-archipelago comparisons of ln-transformed hair corticosterone of white-footed mice captured in Thousand Islands National Park during summer (July – August) of 2015 - 2016. Significance of fixed effects was evaluated using linear mixed effects models for which trapping site (islands: n = 7, mainland sites: n = 5) was included as a random effect, and nested within habitat type (island or mainland) for the island-mainland comparison. Marginal (M) and conditional (C) pseudo $R^2 (R^2_{GLMM})$ values are provided.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Fixed effects</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Island and mainland mice (n = 270)</td>
<td>(Intercept)</td>
<td>2.813</td>
<td>0.539</td>
<td>253</td>
<td>5.216</td>
<td>&lt;0.001</td>
<td>0.05, 0.14</td>
</tr>
<tr>
<td></td>
<td>Type (Mainland)</td>
<td>-0.104</td>
<td>0.120</td>
<td>10</td>
<td>-0.861</td>
<td>0.408</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPUE</td>
<td>-0.004</td>
<td>0.004</td>
<td>20</td>
<td>-0.890</td>
<td>0.384</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>-0.003</td>
<td>0.064</td>
<td>261</td>
<td>-0.049</td>
<td>0.961</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year (2016)</td>
<td>0.051</td>
<td>0.078</td>
<td>104</td>
<td>0.652</td>
<td>0.516</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ln body mass</td>
<td>0.568</td>
<td>0.172</td>
<td>261</td>
<td>3.306</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Island mice (n = 188)</td>
<td>(Intercept)</td>
<td>2.692</td>
<td>0.863</td>
<td>18</td>
<td>3.120</td>
<td>0.006</td>
<td>0.07, 0.14</td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>0.002</td>
<td>0.077</td>
<td>181</td>
<td>0.025</td>
<td>0.980</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Log$_{10}$ (Area)</td>
<td>0.083</td>
<td>0.090</td>
<td>3</td>
<td>0.922</td>
<td>0.409</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Log$_{10}$ (Distance)</td>
<td>-0.123</td>
<td>0.229</td>
<td>4</td>
<td>-0.535</td>
<td>0.616</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year (2016)</td>
<td>0.110</td>
<td>0.081</td>
<td>180</td>
<td>1.366</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ln body mass</td>
<td>0.633</td>
<td>0.210</td>
<td>178</td>
<td>3.008</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

CPUE – Catch-per-unit-effort (CPUE), presented in number of white-footed mice captured per hundred trap-nights, corrected for tripped traps.

*Body mass was a better predictor of hair corticosterone than body size or condition*

I used only data from spring and summer of 2016 to test the effects of body condition, mass, and size on hair CORT, and for seasonal variation of hair CORT and FCM. I evaluated the fit of three separate models with body mass, body size (PC1), and condition index as predictors of hair CORT. Because these three variables were correlated with each other (and condition index is a product of body mass and PC1), they
could not be included in the same model. Each model had sex and season as fixed factors and site as a random effect. Body mass, body size (PC1), and condition index were each significant positive predictors of hair CORT (Table 2.4). The model with body mass had the best fit, indicated by the lowest AICc value and the highest pseudo $R^2_{GLMM}$ values (Table 2.4). Condition index provided the second-best model, followed by the model including PC1, which had the least explanatory power (Table 2.4).

**Table 2.4.** Comparison of three models with ln-transformed body mass (g), body size (PC1), or condition index as predictors of ln-transformed hair corticosterone of white-footed mice ($n = 140$). The comparison was made for maximum likelihood linear mixed-effects models (fixed effects: sex and season; random effect: site). AICc, ΔAICc, and marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided to compare relative fits of the three different models.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\hat{\beta}$</th>
<th>$t$</th>
<th>$p$</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>$R^2_{GLMM}$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln body mass</td>
<td>0.868</td>
<td>4.606</td>
<td>$&lt; 0.001$</td>
<td>157.95</td>
<td>0.00</td>
<td>0.22, 0.33</td>
</tr>
<tr>
<td>Condition index</td>
<td>0.768</td>
<td>3.538</td>
<td>$&lt; 0.001$</td>
<td>165.70</td>
<td>7.75</td>
<td>0.18, 0.30</td>
</tr>
<tr>
<td>Body size (PC1)</td>
<td>0.079</td>
<td>2.417</td>
<td>$&lt; 0.05$</td>
<td>178.72</td>
<td>13.96</td>
<td>0.15, 0.26</td>
</tr>
</tbody>
</table>

*Hair corticosterone differed between seasons for females but not for males*

After determining that body mass was a better predictor of hair CORT than condition or body size, I ran the model on data from 2016 ($n = 147$; Table 2.5) to test for any variation in hair CORT between spring and summer. This model showed that there was an interaction between sex and season, which indicated that female hair CORT was
lower in spring than in summer, but male hair CORT did not differ between seasons (Table 2.5; Figure 2.7A). This model had a greater effect size than other models of factors affecting hair CORT (Table 2.5), indicating a relatively strong effect of season on hair CORT in white-footed mice.

**Table 2.5.** Linear mixed effects model results for ln-transformed hair corticosterone of white-footed mice (n = 147) captured in the Thousand Islands National Park during spring (May-June) and summer (July – August) of 2016. Trapping site was included as a random effect. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M)</th>
<th>$R^2_{GLMM}$ (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>1.258</td>
<td>0.596</td>
<td>138</td>
<td>2.111</td>
<td>0.0366</td>
<td>0.22, 0.35</td>
<td></td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>0.553</td>
<td>0.117</td>
<td>136</td>
<td>4.735</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season (Summer)</td>
<td>0.472</td>
<td>0.119</td>
<td>137</td>
<td>3.956</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln body mass</td>
<td>0.891</td>
<td>0.186</td>
<td>136</td>
<td>4.783</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex*Season</td>
<td>-0.496</td>
<td>0.145</td>
<td>135</td>
<td>-3.412</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fecal corticosterone metabolites did not differ between island and mainland mice**

Median FCM of white-footed mice included in the analysis was 335.4 ng/g and ranged widely from 30.5-1239.8 ng/g, demonstrating a similarly positively skewed distribution to hair CORT. Based on the values from the summer of 2015, there was no difference between ln-transformed FCM of island and mainland white-footed mice (Table 2.6; Figure 2.8). Relative abundance also had no effect on FCM (Table 2.6). Although there was a trend toward females having higher FCM in the summer of 2015 (Figure 2.8), there was no significant difference between sexes. Unlike hair CORT, FCM levels were
not influenced by body mass (Table 2.6). Among island mice, there was also no effect of either island size or isolation on FCM (Table 2.6).

**Table 2.6.** Island-mainland and within-archipelago comparisons of ln-transformed fecal corticosterone metabolites of white-footed mice captured in Thousand Islands National Park during summer (July – August) of 2015. Trapping site (island: n = 7, mainland: n = 3) was included as a random effect in all models, and nested within habitat type (island or mainland) for the island-mainland comparison. Marginal (M) and conditional (C) pseudo $R^2 (R^2_{GLMM})$ values are provided.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Fixed effects</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Island and mainland mice (n = 151)</td>
<td>(Intercept)</td>
<td>6.035</td>
<td>0.685</td>
<td>120</td>
<td>8.809</td>
<td>&lt;0.001</td>
<td>0.02, 0.27</td>
</tr>
<tr>
<td></td>
<td>Type (Mainland)</td>
<td>-0.078</td>
<td>0.223</td>
<td>7</td>
<td>-0.350</td>
<td>0.736</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPUE</td>
<td>-0.002</td>
<td>0.007</td>
<td>6</td>
<td>-0.239</td>
<td>0.818</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>-0.121</td>
<td>0.079</td>
<td>141</td>
<td>-1.531</td>
<td>0.128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ln body mass</td>
<td>-0.085</td>
<td>0.212</td>
<td>143</td>
<td>-0.401</td>
<td>0.689</td>
<td></td>
</tr>
<tr>
<td>Island mice (n = 119)</td>
<td>(Intercept)</td>
<td>6.198</td>
<td>0.848</td>
<td>19</td>
<td>7.311</td>
<td>&lt;0.001</td>
<td>0.03, 0.12</td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>-0.132</td>
<td>0.082</td>
<td>112</td>
<td>-1.614</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ln body mass</td>
<td>-0.032</td>
<td>0.225</td>
<td>110</td>
<td>-0.143</td>
<td>0.887</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Log$_{10}$(Area)</td>
<td>-0.047</td>
<td>0.091</td>
<td>4</td>
<td>-0.515</td>
<td>0.633</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Log$_{10}$(Distance)</td>
<td>-0.107</td>
<td>0.223</td>
<td>4</td>
<td>-0.481</td>
<td>0.651</td>
<td></td>
</tr>
</tbody>
</table>

– Catch-per-unit-effort (CPUE), presented in number of white-footed mice captured per hundred trap-nights, corrected for tripped traps.

**Fecal corticosterone metabolites of both sexes were lower in spring than summer**

The interaction between sex and season, which influenced hair CORT levels, did not influence FCM levels so it was dropped from the model ($t_{63} = -1.423, p = 0.160$).
Based on the resulting model for FCM data from 2016, both sexes of white-footed mice had lower FCM levels in spring than in summer (Table 2.7; Figure 2.7B).

Hair CORT and FCM of white-footed mice were significantly, although weakly, positively correlated ($r = 0.16, t_{178} = 2.196, p = 0.015$; Figure 2.9).

**Table 2.7.** Linear mixed effects model results for ln-transformed fecal corticosterone of white-footed mice ($n = 71$) captured in the Thousand Islands National Park during spring (May-June) and summer (July–August) of 2016. Trapping site ($n = 14$) was included as a random effect. Marginal (M) and conditional (C) pseudo $R^2 (R_{GLMM}^2)$ values are provided.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R_{GLMM}^2$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>5.904</td>
<td>0.091</td>
<td>39</td>
<td>64.754</td>
<td>&lt; 0.001</td>
<td>0.30, 0.44</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>0.117</td>
<td>0.084</td>
<td>62</td>
<td>1.396</td>
<td>0.168</td>
<td></td>
</tr>
<tr>
<td>Season (Summer)</td>
<td>0.451</td>
<td>0.088</td>
<td>67</td>
<td>5.129</td>
<td>&lt; 0.001</td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>
DISCUSSION

Wildlife often demonstrate morphological and physiological adaptations to insularity (Matson et al. 2014; Holding et al. 2014; Spencer et al. 2017). In accordance with typical characteristics of island syndrome in rodents, I predicted that white-footed mice in the Thousand Islands would display higher abundance and greater body size than white-footed mice from the nearby mainland. I also predicted that these island syndrome characteristics would include decreased GC levels. My predictions were not supported, given that I did not find evidence that white-footed mice in the Thousand Islands differed in any of these characteristics. Taken together, these results suggest that these islands were not sufficiently isolated to produce an island effect, either due to the presence of predators and competitors on islands, or frequent immigration of mainland white-footed mice to the islands, or both of these factors. There was, however, a notable relationship between hair CORT and body mass, as well as seasonal differences in GC levels across habitat types. First, I will discuss explanations for the lack of an island-effect on demography, morphology, or physiology on white-footed mice in the Thousand Islands, then evaluate internal and external sources of variation in CORT levels. I will close my discussion by identifying future directions for research on small mammal ecology and physiology in the Thousand Islands and in other systems, and evaluate the utility of hair as a measure of HPA activity in rodents.

Greater population fluctuation on islands, but no overall difference in relative abundance

My first prediction, that white-footed mice would be more abundant than mainland mice, was not supported. Although the highest relative abundances were found
on islands (McDonald Island and Lindsay Island particularly), so too were the lowest (Aubrey Island and Camelot Island). These findings challenge the general prediction that rodents exhibit particularly high densities on islands (Adler and Levins 1994; Crespin et al. 2012; Cuthbert et al. 2016), which has been observed in other prey species as well (Novosolov et al. 2013; Sale and Arnould 2013). However, previous work in the Thousand Islands has also shown that some islands have relatively low numbers of white-footed mice (Werden et al. 2014), which could be due to more pronounced population fluctuations occurring on islands.

Populations of white-footed mice demonstrate annual and multi-year cycles in response to food supply and mortality (Wang et al. 2009). I found that abundance was lower in the spring than the summer months, and that summer abundance was higher in 2015 than 2016. The drop in abundance in 2016 occurred mainly on islands and levels on the mainland were essentially equal between years (Figure 2.3). Higher annual variability in abundance of island white-footed mice compared to the mainland challenges Adler and Levins’ (1994) description of island syndrome, including studies showing that island rodents are more stable, and less prone to cycling than mainland populations (Gliwicz 1980; Herman and Scott 1984; Tamarin et al. 1987). However, other authors have described insular *Peromyscus* populations that exhibit more pronounced population swings than on the adjacent mainland (Drost and Fellers 1991). Similarly, the reverse of predictions based on island syndrome have been found for insular lizards (Raia et al. 2010). When observed, the stability of high population densities on oceanic islands has been partially attributed to marine resource subsidies and climate stability compared to continental systems (Stapp and Polis 2003; Barrett et al. 2005; Sale and Arnould 2013).
These factors do not apply when comparing rodents on the near-shore Thousand Islands to the adjacent mainland.

*Island mice were not larger than mainland mice*

Small mammals on islands often exhibit large body size (Lomolino et al. 2012; Sale and Arnould 2013; Harper and Rutherford 2016), however my second prediction, that insular white-footed mice would be larger than mainland mice, was not supported. I found no difference in body size of island and mainland white-footed mice in the Thousand Islands. A high degree of isolation is not necessarily required for demographic (Adler et al. 1986) or body mass (Nupp and Swihart 1996) differences between *Peromyscus* populations to occur, so I was surprised to find no island-mainland differences in these measures from white-footed mice in this near-shore archipelago. I also predicted that body size would be negatively affected by island area and positively affected by isolation, of which I did not find evidence.

My negative results regarding patterns between body size and island geography do not agree with results for other small mammals in the Thousand Islands. Lomolino (1984) found that the body size of meadow voles (*Microtus pennsylvanicus*) and short-tailed shrews (*Blarina brevicauda*) in the Thousand Islands increased as distance from mainland increased. Lomolino attributed this pattern to the ability of larger individuals to cross greater distances on ice during the winter, and subsequent founder effects of large individuals reaching more distant islands. My analysis of body size for island mice showed that distance from the mainland had a marginally negative effect on body size; however, substituting the distance of Thwartway Island with its distance to a nearby large
island removed this effect, suggesting it was not robust. Those islands sampled by Lomolino (1984) may be better suited to investigating patterns related to isolation because they were less closely clustered together than those that I sampled. The clustered nature of many of the islands in my study makes their true degree of isolation difficult to determine.

No difference in GC levels between island and mainland mice

I predicted that white-footed mice on islands would have lower GC levels than on the mainland based on both hair CORT and its related metabolites in feces. This prediction was not supported, given that there was no difference in either of these measures of HPA activity between island and mainland white-footed mice, and no effect of either island size or distance from the mainland. This result may not be surprising, given that my first two predictions related to abundance and body size were not supported. The lack of an island effect on any of these characteristics in the Thousand Islands suggests either that island white-footed mice experience similar selection pressures to mice on the mainland, or that there is a high degree of gene flow between island and mainland white-footed mice.

Gene flow among islands and the mainland could be attributed to the ability of white-footed mice to swim short distances (Evans et al. 1978), cross ice in the winter (Lomolino 1989), and to disperse via transport onboard boats. White-footed mice may also use a bridge that crosses the St. Lawrence River at Hill Island as a dispersal mechanism. Despite these dispersal mechanisms, genetic dissimilarity between Peromyscus populations can occur at short distances (< 500 m from mainland or large
island) in freshwater archipelagos (Landry and Lapointe 2001; Vucitech et al. 2001). An additional explanation to high gene-flow for lack of an island effect in the Thousand Islands is that white-footed mice experience similar degrees of predation and competition across the island and mainland sites that I sampled. I discuss these factors below.

Similar levels of competition and predation on islands

How near these islands are to the mainland and to one another may mean that insular white-footed mice experience similar levels of inter-specific competition to mainland mice. Although the diet of white-footed mice is based primarily on insects, they also forage heavily on seeds (Manson and Stiles 1998). Release from competition with larger granivores, such as squirrel species (Sciuridae), could result in increased body size of *Peromyscus* (Nupp and Swihart 1996). However, I caught red squirrels (*Tamiasciurus hudsonicus*) and flying squirrels (*Glaucomys* spp.) on near-shore islands (Constance Island and Georgina Island Appendix 1, Table A1.2), and observed grey squirrels (*Sciurus carolinensis*) on more isolated islands (McDonald Island and Thwartway Island). Although I did not catch eastern chipmunks on any islands, they have previously been caught on multiple islands in the archipelago (Coleman 1979; Werden et al. 2014).

The proximity of these islands to shore might cause equal predation risk on the islands and the mainland. Small terrestrial predators, such as weasels (*Mustela* spp.), might occur in low numbers on some of the islands, however, avian predators can readily access islands to prey on mice. The existence of tracks of larger predators, such as coyotes (*Canis latrans*) and red foxes (*Vulpes vulpes*), has demonstrated that they access islands during the winter when the St. Lawrence River freezes (Coleman 1979).
Raccoons (*Procyon lotor*), which are common in the Thousand Islands (Coleman 1979), will prey on white-footed mice (Rulison et al. 2012). It has been shown that mammalian species richness in the Thousand Islands is affected by island size and isolation (Lomolino 1982), but others have failed to find such a pattern (Coleman 1979; Werden et al. 2014). My trapping methods and other recent work (Werden et al. 2014) were primarily designed to target white-footed mice; however, a comprehensive survey of mammalian species in the Thousand Islands would be useful to understand the current distributions of these species in the archipelago.

*Hair corticosterone increased with body mass, size, and condition*

I found that body mass, body condition, and structural size all had positive relationships with hair CORT, but body mass was the best predictor among these measures. Curiously, however, although heavier white-footed mice had higher hair CORT levels, heavier mice did not exhibit higher FCM. Body mass is positively correlated with age in *Peromyscus* (Chappell et al. 2003), and is therefore often used to identify age classes in the field (Vandegrift et al. 2008). If circulating CORT increased with age, I would expect to see a positive relationship between FCM and body mass. Others have reported no effect of body mass on FCM in white-footed mice (Brown and Fuller 2006), and a lack of age group differences in FCM levels for deer mice (Harper and Austad 2004). In a survey of eight rodent species, no relationship was found between body mass and FGM for any species (St. Juliana et al. 2014). Based on my results, and those from other studies, I suggest that the positive relationship between hair CORT and
body mass indicates a relationship between moulting schedule and hormone deposition in hair.

Moulting in *Peromyscus* occurs before or following energetically demanding time periods, such as breeding (Pierce and Vogt 1993; Tabacaru et al. 2011). Moulting might be partially regulated by CORT, because steroid hormones have an inhibitory effect on moulting in *Peromyscus* (Garwood and Rose 1995). As a result, hairs grown during complete moults might have relatively low CORT concentrations. Based on observations for deer mice (Tabacaru et al. 2011), and from my own observations of shaved white-footed mice in the field and laboratory re-growing their hair, hair growth can occur in absence of a complete moult. If hairs grown during complete moults have low CORT concentrations, replacement hairs grown following a moult should have relatively higher CORT levels. This would result in heavier individuals (which are likely older and have grown since their last moult) having higher hair CORT compared to younger mice that have more recently grown their adult pelage. This relationship with body mass as a proxy of age and hair CORT might be particularly relevant when sampling multiple generations of adult mice during the summer months. To appropriately test this proposed relationship, knowledge of age, start date of moult, hair growth, and hair CORT would be required.

White-footed mice in better condition had higher hair CORT, but there was no relationship between FCM and condition. GC levels are generally thought to be elevated in animals that are in poor condition (Romero and Wikelski 2001; Suorsa et al. 2003; Busch et al. 2011), however, previous studies have shown that fecal GCs were positively associated with condition in both deer mice (Harper and Austad 2000) and eastern chipmunks (Mastromonaco et al. 2014). In contrast, hair cortisol was not related to
condition in eastern chipmunks (Mastromonaco et al. 2014). The positive relationship that I found between body condition and hair CORT may be indicative of the same relationship that I described between hair CORT and body mass.

Hair GC levels are an integrative measure of HPA activity, because they will reflect both an individual’s phenotype related to their baseline GC levels (Fairbanks et al. 2011), but can also be influenced by an animal’s exposure to stressors (Bryan et al. 2015; Scorrano et al. 2015). It is therefore not entirely surprising that CORT in hair and its related metabolites in feces are not both correlated with the same measures (body mass and condition), and demonstrate different seasonal patterns. The relatively weak correlation that I found (n = 180, r = 0.16, $t_{178} = 2.196$, $p = 0.015$) was similar to results from the equivalent measures in wild eastern chipmunks (n = 62, r = 0.25, $p = 0.055$; Mastromonaco et al. 2014), and may be because they are representative of different time-frames of GC secretion. The significance of these relationships may represent the underlying influence of individuals’ baseline GC levels on both hair CORT and FCM.

The hair CORT values that we measured for white-footed mice (median: 79.82 ng/g, range: 5.10-398.56 ng/g) were similar to those reported for deer mice (median: 97.5 ng/g, range: 0.2-290.2 ng/g; Hanselmann 2016). Although a recent ACTH challenge in our lab failed to demonstrate a difference in hair CORT between saline control and ACTH treatment in white-footed mice (Reilly 2017; Appendix 3, Figure A3.1A), there was a significant positive relationship between pre- and post-treatment hair CORT ($F_{1,14} = 10.44$, $p < 0.01$, $R^2 = 0.427$; Appendix 3, Figure A3.1B). This positive relationship demonstrates that individuals exhibited a consistent phenotype in their hair corticosterone profile over time. Reilly (2017) suggested that the type of ACTH drug used was less
appropriate for replicating conditions of chronic GC elevation than slow-release ACTH used in successful validations of hair GCs (Terwissen et al. 2013; Mastromonaco et al. 2014).

*Seasonal variation in corticosterone*

Seasonal changes in GC levels are common among vertebrates (Romero 2002). FCM levels of white-footed mice of both sexes were higher in the summer than the spring, in agreement with other studies of *Peromyscus* (Harper and Austad 2001; Hayssen et al. 2002). High summer FCM levels could be attributed to changes in CORT levels associated with reproduction (Harper and Austad 2001), however breeding occurs in white-footed mice during early spring and lasts throughout the summer months (Pierce and Vogt 1993). Alternatively, high summer FCM levels could be caused by increased abundance of white-footed mice relative to the spring (Hayssen et al. 2002), which is consistent with my observations given that abundance was higher in the summer than the spring in the Thousand Islands. Although I did not find a relationship between CORT and relative abundance across sites, CORT and population density may be correlated within areas as densities change during annual cycles.

Although FCM levels differed between seasons for both sexes, only female white-footed mice showed seasonal variation in hair CORT. Female hair CORT was higher in the summer than the spring. The contrasting sex-specific results between FCM and hair CORT patterns are likely the result of the two measures differing in their representative time-scales of CORT secretion (Mastromonaco et al. 2014). Sex differences in GC levels between seasons occur in other wild rodents and are attributed to interactions between
GCs and sex hormones, and differences in parental behaviour (Romero et al. 2008; Schradin 2008; Bauer et al. 2014). These results suggest that female white-footed mouse CORT levels were lower during late winter and early spring than during the summer. This could be attributed to decreased CORT during pregnancy in the spring or elevated CORT in response to lactation during the summer (Reeder and Kramer 2005), and/or increased aggression among territorial females when population density increases during the summer (Wolff 1993).

Conclusion and future directions

In conclusion, local populations of white-footed mice in the Thousand Islands did not differ systematically in their abundance, body size, or hair and fecal GC levels, compared with white-footed mice on the nearby mainland. I did find evidence that body mass was an important predictor of hair CORT levels in white-footed mice, and suggest that this could indicate a relationship between deposition of CORT in replacement hairs grown as mice age, relative to initially low CORT levels during moulting. I also found temporal patterns in hair CORT and FCM. FCM levels were lower in spring than summer for both sexes, and female hair CORT was lower in spring, but males did not differ in hair CORT levels between seasons. Together, these results demonstrate the importance of accounting for age and moulting schedule when measuring hair GCs in free-living mammals.

Because I found no island-mainland differences in either body size or GCs, my results leave open the possibility that on more isolated islands, where the community structure is distinctly different from mainland habitats, decreased interspecific
competition and predation may cause changes in the stress physiology of rodents. Despite this, near-shore archipelagos make an attractive option for island-mainland comparisons, because many variables (temperature, precipitation, and day length) are similar between closely spaced island and mainland habitats. Although differences may not be apparent from quantifying chronic measures of GC section (hair), evaluating the stress response of rodents may be informative, as shown for other island wildlife (Rödl et al. 2007; Müller et al. 2007). Islands have been important natural laboratories for advancing the field of ecology, and studying island rodents as model species has been central to the development of evolutionary theory. Continuing to study island rodents could advance our understanding of factors affecting stress in vertebrates, and our ability to conserve both island and mainland wildlife.

**LITERATURE CITED**


Reilly, A. 2017. Validation of the use of hair corticosterone to measure chronic stress in white-footed mice (Peromyscus leucopus). Honours Thesis. Trent University.


Figure 2.1. Trapping locations in the Thousand Islands National Park. Islands that were trapping sites are shaded in darker grey. Abbreviations relate to those used in Table 2.1.
Figure 2.2. Relative abundance of white-footed mice, measured in catch per unit effort (captures per 100 trap nights) and corrected for trap disturbance for island (n = 11) and mainland (n = 5) trapping locations in the Thousand Islands National Park during three periods. Relative abundance was higher in summer 2016 than summer 2015, and higher during both summers than in the spring 2016. Several locations were not trapped during all three periods.
Figure 2.3. Correlation of white-footed mouse abundance at trapping locations (n = 11) in the Thousand Islands National Park during summer months of 2015 and 2016 (r = 0.852, $t_9 = 4.875$, $p < 0.001$). The solid black line represents the linear relationship in abundance between the two years, and the dashed line represents the predicted line if abundances were equal between years. Abundance was measured in catch-per-unit-effort (captures per 100 trap nights), which was corrected for tripped traps.
There was no difference in white-footed mouse body size (PC1) between sexes or for individuals captured on islands and the mainland ($p > 0.05$ for both sex and habitat type; Table 2.2). Boxplots of principal component scores for body size of male and female white-footed mice ($n = 175$) captured during the summer of 2016 from islands and mainland trapping locations in the Thousand Islands National Park. The median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range. Filled circles represent values for individual mice.
Figure 2.5. There was no difference in deer mouse ln-transformed hair corticosterone between sexes or for individuals captured on islands and the mainland ($p > 0.05$ for both sex and habitat type; Table 2.3). Boxplots of ln-transformed hair corticosterone of white-footed mice ($n = 270$) captured during the summers (July-August) of 2015-2016 from islands and mainland trapping locations in the Thousand Islands National Park. The median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range. Filled circles represent values for individual mice.
Figure 2.6. Significant positive relationship ($\beta = 0.568$, $t_{261} = 3.306$, $p = 0.001$) between ln-transformed hair corticosterone (ng/g) and ln body mass (g) of white-footed mice ($n = 270$) caught in the Thousand Islands National Park during 2015-2016. A linear-mixed model which included habitat type (island vs. mainland), sex, and year as fixed factors and site as a random effect nested within habitat type was used to test this relationship (all other predictor variables were not significant).
Figure 2.7. Both sexes of white-footed mice had lower ln-transformed fecal corticosterone metabolite levels in spring than in summer ($p < 0.001$; Table 2.7), but only female ln-transformed hair corticosterone differed between seasons (sex*season interaction; $p < 0.001$; Table 2.5). Box plots of ln-transformed hair corticosterone (A) and fecal corticosterone metabolites (B) of white-footed mice during spring (May-June) and summer (July-August) of 2016 in the Thousand Islands National Park. The median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range.
Figure 2.8. There was no difference in fecal corticosterone metabolites between sexes or for white-footed mice (n = 151) captured on islands and the mainland ($p > 0.05$ for both sex and habitat type; Table 2.6) during summer (July-August) 2015 in the Thousand Islands National Park. For boxplots, the median line is provided within the hinges, which indicate first and third quartiles and whiskers represent 1.5 times the inter-quartile range. Filled circles represent values for individual mice.
Figure 2.9. Ln-transformed hair corticosterone plotted against ln-tranformed fecal corticosterone metabolites (ng/g) for white-footed mice (n = 180) caught in the Thousand Islands National Park (r = 0.16, t_{178} = 2.196, p = 0.015).
CHAPTER 3: USING MUSEUM SPECIMENS TO QUANTIFY HAIR CORTICOSTERONE OF DEER MICE (*PEROMYSCUS MANICULATUS*) FROM TWO COASTAL ARCHIPELAGOS

ABSTRACT

Studying island ecosystems and species has been central to the development of ecological and evolutionary theory. I evaluated the effect of island life on hair corticosterone (CORT) levels of museum specimens of deer mice (*Peromyscus maniculatus*). Deer mice were collected during 1964-1991 from two coastal archipelagos (13 islands in Barkley Sound and 14 of the Gulf Islands) offshore of Vancouver Island (VI), British Columbia, which served as the mainland (6 sites) for this comparison. Deer mice from the two archipelagos were heavier, and Barkley Sound mice were structurally larger than VI mice, consistent with island syndrome. Hair CORT was lower for deer mice from both archipelagos compared to VI mice, after controlling for the effect of structural size. In addition, hair CORT for deer mice collected during 1915-1991 from VI (11 sites) demonstrated a decline in hair CORT with increasing specimen age, providing evidence of long-term degradation of hair CORT.

**Keywords:** Island syndrome, island rule, stress physiology, glucocorticoids, hair-hormone analysis, *Peromyscus*
INTRODUCTION

Introduction

Studying island ecosystems and species has been central to the development of ecological and evolutionary theory (Foster 1964; MacArthur and Wilson 1967; Van Valen 1973; Lomolino et al. 2012; Warren et al. 2015). Islands are of interest both for the utility of the discrete replicate sites they provide, and more recently because of conservation concerns related to the sensitivity of island species (Whittaker and Fernandez-Palacios 2007; Loehle and Eschenbach 2012). The vulnerability of island species is in part attributed to low levels of predation on islands, which initially leave island wildlife naïve to novel sources of stress (Rödl et al. 2007; Swarts et al. 2009). How island vertebrates respond to stressors, such as predators, and changes in the environment, can therefore determine their survival (Romero and Wikelski 2001, 2010), and suggests the potential for selection on aspects of their stress physiology (Patterson et al. 2014).

The effects of island life on the biology of vertebrates have been demonstrated in numerous taxa by comparing the characteristics of island and mainland conspecifics, or closely related species (Müller et al. 2007; Raia et al. 2010; Sale and Arnould 2013; Matson et al. 2014; Harper and Rutherford 2016). Rodents have featured prominently in island-mainland comparisons (Foster 1964; Adler and Levins 1994a; Lomolino et al. 2012). Island rodents are often larger than their mainland counterparts; this pattern has been attributed to immigrant selection, and ecological release from predators and competitors on islands (Adler and Levins 1994a; Crespin et al. 2012; Lomolino et al. 2012). In addition to increased body size, island rodents are often less aggressive and live
at higher population densities than their mainland counterparts (Gliwicz 1980; Adler and Levins 1994b). Adler and Levins (1994) grouped the characteristics of island rodents under the term “island syndrome”, and since then, island syndrome has been well-studied in terms of demography, behaviour, and morphology across several taxa (Goltsman et al. 2005; Russell et al. 2011; Crespin et al. 2012b; Novosolov et al. 2013; Sale and Arnould 2013; Blanco et al. 2014), but few studies have considered how stress physiology might differ between island and mainland conspecifics (but see Clinchy et al. 2004; Müller et al. 2007).

Vertebrates respond to stress in part through activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of glucocorticoid hormones (GCs). GCs enhance the stress response by promoting cardiovascular activity, and mobilizing energy stores, but they also mediate the response through negative feedback with the HPA axis (Sapolsky et al. 2000). Elevated GC levels are involved in preparation for future stressors by shifting resources from reproduction and digestion toward replenishing energy stores used during the initial stress response (Romero and Wingfield 2016). The primary GC in mice and rats is corticosterone (CORT), compared to cortisol in most other mammals (Sapolsky et al. 2000). A variety of environmental factors affect GC levels, including predation (Clinchy et al. 2011; Sheriff et al. 2011a), interspecific competition (Narayan et al. 2012), and resource availability (Walker et al. 2005; Herring et al. 2011).

Studies of island birds have demonstrated that decreased predation risk on islands results in lower baseline and stress-induced GC levels compared with their mainland conspecifics (Clinchy et al. 2004; Müller et al. 2007). Such differences in GCs might be a
result of phenotypic plasticity, in response to differences in habitat characteristics (Clinchy et al. 2004), or due to genetic isolation and selection (Müller et al. 2007). In Chapter 2, I showed that GC levels did not differ between white-footed mice (Peromyscus leucopus) captured in a near-shore archipelago with those on the nearby mainland. I suggested that this was due to high gene-flow or similar ecological conditions between the islands and the mainland, but proposed that differences in GCs might occur on more isolated islands where community structure is different from the mainland. Maintaining high GC levels and reacting strongly to stressors might be costly on islands where predation is limited, thus selecting for low GC levels.

GCs have historically been quantified from blood samples, however, hormone levels in blood change rapidly in response to capture and handling stress (Romero and Reed 2005). Given this complication, along with concerns with invasive sampling methods, other sources such as feces, urine, and saliva have been used to evaluate stress in vertebrates (Sheriff et al. 2011b). Most recently, GCs have been quantified from keratinized structures such as claws (Baxter-Gilbert et al. 2014), feathers (Bortolotti et al. 2009; Fairhurst et al. 2012), and hair (Carlitz et al. 2014). Hair is thought to provide a biomarker of long-term HPA activity because GCs are integrated into hairs as they grow (Sherriff et al. 2011b). Analysis of GCs in hair provides several benefits, including ease of collection from both living and dead animals, and because concentrations are considered to be relatively stable during storage over time (Meyer and Novak 2012).

If hormones are stable in hair over time, it would facilitate studies of large-scale spatial and temporal trends in GC levels using museum collections, or from other preserved specimens (Meyer and Novak 2012). The idea that hair GCs are stable over
time has been supported by observations that hair cortisol levels from historical human (ca. 500-1500 years old) and polar bear specimens (Ursus maritimus; ca. 80 – 120 years old; Bechshøft et al. 2012) were higher than recently collected samples. While studies have shown that external factors such as mechanical irritation, chemical washes, and sunlight affect hair GC levels (Salaberger et al. 2016; Wester et al. 2016), the effect of storage over long periods of time (decades) remains unclear.

Although island-mainland differences in baseline and stress-induced GC levels have been reported in birds (Clinchy et al. 2004; Müller et al. 2007), to my knowledge, only one study has compared the GC levels of island and mainland rodents (Chapter 2). To make an island-mainland comparison of the stress physiology of isolated rodent populations, I quantified CORT in hair samples from museum specimens of deer mice (Peromyscus maniculatus) collected from Vancouver Island, British Columbia, and two archipelagos offshore of this large island: the Gulf Islands and islands in Barkley Sound. I also recorded body mass and collected cranial measurements to determine if deer mice were heavier and structurally larger on the islands of the two archipelagos than on Vancouver Island, in accordance with island syndrome. Vancouver Island (hereafter VI) is sufficiently large to serve as a mainland site for this comparison (31,285 km² compared to the next largest island in the analysis at 182 km²), and has been considered ecologically to be the equivalent of the mainland in previous studies (Herman 1981; Marinelli and Millar 1989; Clinchy et al. 2004). Redfield (1976) suggested that deer mice have been isolated in the Gulf Islands for ca. 12,000 years and that there is little movement between islands. Deer mice from both archipelagos display characteristics of island syndrome that distinguish them from deer mice from VI and the mainland of
British Columbia (Redfield 1976; Sullivan 1977; Halpin and Sullivan 1978; Marinelli and Millar 1989), suggesting these archipelagos are suitable for testing differences in stress physiology.

I tested the hypothesis that island syndrome includes differences in GC levels between island and mainland deer mice. I predicted that deer mice from the two archipelagos would be larger (indicated by skull size), heavier, and have lower hair CORT than VI mice in response to decreased predation risk on islands. To evaluate sources of individual variation in hair CORT (and to compare to results for white-footed mice in Chapter 2), I evaluated which of the following measurements was the best predictor of hair CORT: body mass, condition score, or structural size. Following this evaluation, I tested the hypothesis that hair GC levels are affected by long term storage, and predicted that hair CORT would be lower in earlier samples due to degradation over time.
METHODS

Study species

The deer mouse is a small omnivorous rodent that is likely the most widespread and abundant small mammal in North America, and among the most well studied of any small wild mammal species (Bedford and Hoekstra 2015). The ecology of deer mice, and other *Peromyscus* species, has been the focus of many island-mainland comparisons, which have demonstrated that their biology is heavily influenced by island life (Sullivan 1977; Halpin and Sullivan 1978; Herman and Scott 1984; Adler and Tamarin 1984; Vucetich et al. 2001; Kuhn et al. 2016). Deer mice occupy a variety of habitats in British Columbia, but have been most well studied on coastal islands (Nagorsen 2005). The Royal British Columbia Museum houses a large collection of *Peromyscus* specimens from the coastal islands of British Columbia, which provided the samples for this chapter.

Study site

I collected morphological and physiological data from museum specimens of deer mice that were captured in the Gulf Islands (14 islands, 8-18,247 ha), islands in Barkley Sound (13 islands, 4-1,703 ha), and from 11 sites across VI (Table 3.1). Sites on VI were approximated from locations provided on specimen labels, and although some may have been near (< 5 km apart) each other (trapping site in Victoria could be near Albert Head or Mt. Douglas), all other sites were separated by at least 10 km and were therefore considered independent replicates. The Gulf Islands are in the Salish Sea, between the lower mainland of British Columbia, and southeastern VI (Figure 3.1). The Gulf Islands are located within the Coastal Douglas-fir biogeoclimatic zone, which is dominated by
Douglas-fir (*Pseudotsuga menziesii*), with contributions from western red cedar (*Thuja plicata*), and grand fir (*Abies grandis*). A rain-shadow effect is caused by the Vancouver Island and Olympic mountain ranges surrounding the Gulf Islands, creating the relatively dry summers and mild winters (average annual rainfall 1961-1990: 902.5 mm; Environment Canada 2017) that are required by vegetation of the Coastal Douglas-fir forests (Klassen and Burton 2015).

Islands in Barkley Sound occur in two clusters: the Broken Group and the Deer Group (Figure 3.1). I analyzed these two groups of islands together within the archipelago of the Barkley Sound islands, as they are located adjacent to one another and are ecologically similar. Islands in Barkley Sound are located within the Coastal Western Hemlock biogeoclimatic zone, and characterized by western hemlock (*Tsuga heterophylla*), western red cedar, and Sitka spruce (*Picea sitchensis*; Cody 2006). Barkley Sound receives high amounts of precipitation (average annual rainfall 1961-1990: 3,356 mm). Across VI, the forest type and levels of precipitation vary. Although Coastal Western Hemlock forests characterize most of VI, a small part of the southeastern corner of the Island, adjacent to the Gulf Islands, is characterized by Coastal Douglas-fir forest. The north and western coasts of VI generally receive more precipitation (Port Hardy average annual rainfall 1961-1990: 1,870.6 mm) than the eastern and southern coasts of the Island (Victoria at Gordon Head average annual rainfall 1961-1990: 708.2 mm).

**Sample selection**

Deer mouse specimens were selected from islands and sites across VI with the intention of collecting data from at least two adult mice of each sex from each site.
Trapping methods were not clearly described on the specimen labels, however most deer mice were likely collected by snap-traps. Deer mouse specimens had been collected during April-August from 1915-1991, however all samples collected prior to 1964 were from VI (Table 3.1).

Maturity (adult vs. juvenile) was assessed based on coat colour, and juveniles were excluded based on their slate grey pelage so that only adults were included in the analysis (Drost and Fellers 1991). Although I could not find a description of moulting schedule for deer mice from VI, deer mice in Alberta and California develop a winter coat in the fall (September-October) and partial moulting can occur year-round (Collins 1923; Tabacaru 2011). This means that hair samples collected from samples captured during April-August would be influenced by hair growth that occurred from September of the previous year, up to the date of collection, depending on the age of the mouse.

Cranial measurements were made using digital calipers (± 0.01 mm) for any skulls that were available for the same study skins that were sampled. Cranial measurements were also opportunistically collected from skulls that were not paired with study skins. Skull length, cranial breadth, zygomatic arch breadth, and zygomatic arch length were measured (Koh and Peterson 1983; Rinderknecht and Blanco 2008; Figure 3.2). Broken skulls were excluded from the analysis. I recorded body mass data from specimen labels (rounded to ± 1 g for all individuals) and analyzed these data for males only; I excluded females out of concern for influence of pregnancy on body mass.

I shaved a patch of hair (1 x 1 cm) from the rump of each individual, above the right-hind limb using an electric razor (Remington™ Model PG6025), collecting the entire length of each shaft from the skin to the end of the shaft. Hair CORT was
quantified by methanol extraction and enzyme-immunoassay (EIA) using identical methods as those described in Chapter 2.

GIS Analysis

Individual trapping sites were approximated from notes on specimen labels (Figure 3.1). For each island, I calculated area and distance from VI (Table 3.1). Geographical analysis and map-making was performed using ArcMap (Version 10.4.1). Island area (ha) was measured using the “erase” tool and distance from the mainland (m) was calculated using the “generate near table analysis” tool (Table 3.1).

Table 3.1. Study site names with island area (ha) and distance to Vancouver Island (m), sample sizes of skulls (SK), body mass (BM), and hair CORT (HC). Collection years are provided.
Table 3.1. Description on previous page.

<table>
<thead>
<tr>
<th>Site</th>
<th>Area</th>
<th>Dist</th>
<th>SK</th>
<th>BM</th>
<th>CORT</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vancouver Island</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sahtlam</td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>1915</td>
<td></td>
</tr>
<tr>
<td>Comox</td>
<td>8</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>1934/35/37</td>
<td></td>
</tr>
<tr>
<td>Albert Head</td>
<td>6</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>1936</td>
<td></td>
</tr>
<tr>
<td>Cowichan Lake</td>
<td>4</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>1940/41/48/49</td>
<td></td>
</tr>
<tr>
<td>Markale Point</td>
<td>10</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>1958</td>
<td></td>
</tr>
<tr>
<td>Mount Douglas</td>
<td>5</td>
<td>3</td>
<td>10</td>
<td>-</td>
<td>1971</td>
<td></td>
</tr>
<tr>
<td>Port Hardy</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>-</td>
<td>1974/76</td>
<td></td>
</tr>
<tr>
<td>Rosewall Creek</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1979</td>
<td></td>
</tr>
<tr>
<td>Brooks Peninsula</td>
<td>8</td>
<td>4</td>
<td>10</td>
<td>-</td>
<td>1981</td>
<td></td>
</tr>
<tr>
<td>Victoria</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>-</td>
<td>1986</td>
<td></td>
</tr>
<tr>
<td>Woss</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td><strong>Barkley Sound</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haines Isl.</td>
<td>18</td>
<td>2434</td>
<td>6</td>
<td>10</td>
<td>1964/65</td>
<td></td>
</tr>
<tr>
<td>Diana Isl.</td>
<td>199</td>
<td>1910</td>
<td>9</td>
<td>1</td>
<td>18</td>
<td>1964/72</td>
</tr>
<tr>
<td>Edward King Isl.</td>
<td>116</td>
<td>2426</td>
<td>2</td>
<td>10</td>
<td>1965</td>
<td></td>
</tr>
<tr>
<td>Effingham Isl.</td>
<td>227</td>
<td>8309</td>
<td>6</td>
<td>4</td>
<td>1967</td>
<td></td>
</tr>
<tr>
<td>Fleming Isl.</td>
<td>315</td>
<td>2433</td>
<td>8</td>
<td>5</td>
<td>1967</td>
<td></td>
</tr>
<tr>
<td>Helby Isl.</td>
<td>82</td>
<td>1792</td>
<td>9</td>
<td>1</td>
<td>1967</td>
<td></td>
</tr>
<tr>
<td>Sanford Isl.</td>
<td>38</td>
<td>3316</td>
<td>3</td>
<td>1</td>
<td>1967</td>
<td></td>
</tr>
<tr>
<td>Fry Isl.</td>
<td>4</td>
<td>3957</td>
<td>4</td>
<td>1</td>
<td>1969</td>
<td></td>
</tr>
<tr>
<td>Tzartus Isl.</td>
<td>1703</td>
<td>1252</td>
<td>5</td>
<td>3</td>
<td>1969</td>
<td></td>
</tr>
<tr>
<td>Dodd Isl.</td>
<td>83</td>
<td>4746</td>
<td>7</td>
<td>5</td>
<td>1970</td>
<td></td>
</tr>
<tr>
<td>Gilbert Isl.</td>
<td>26</td>
<td>9455</td>
<td>3</td>
<td>1</td>
<td>1970</td>
<td></td>
</tr>
<tr>
<td>Howell Isl.</td>
<td>41</td>
<td>11344</td>
<td>5</td>
<td>3</td>
<td>1970</td>
<td></td>
</tr>
<tr>
<td>Wouwer Isl.</td>
<td>43</td>
<td>10179</td>
<td>5</td>
<td>2</td>
<td>1970</td>
<td></td>
</tr>
<tr>
<td><strong>Gulf Islands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galiano Isl.</td>
<td>5804</td>
<td>11140</td>
<td>7</td>
<td>1</td>
<td>5</td>
<td>1974</td>
</tr>
<tr>
<td>James Isl.</td>
<td>334</td>
<td>937</td>
<td>6</td>
<td>10</td>
<td>1974</td>
<td></td>
</tr>
<tr>
<td>Mandarte Isl.</td>
<td>8</td>
<td>7172</td>
<td>7</td>
<td>4</td>
<td>10</td>
<td>1974</td>
</tr>
<tr>
<td>Mayne Isl.</td>
<td>2333</td>
<td>16950</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>1974</td>
</tr>
<tr>
<td>Pender Isl.</td>
<td>2678</td>
<td>11128</td>
<td>8</td>
<td>5</td>
<td>9</td>
<td>1974</td>
</tr>
<tr>
<td>Sidney Isl.</td>
<td>901</td>
<td>3454</td>
<td>6</td>
<td>3</td>
<td>7</td>
<td>1974</td>
</tr>
<tr>
<td>Saltspring Isl.</td>
<td>18247</td>
<td>500</td>
<td>15</td>
<td>4</td>
<td>12</td>
<td>1974/84</td>
</tr>
<tr>
<td>D'Arcy Isl.</td>
<td>86</td>
<td>5666</td>
<td>7</td>
<td>3</td>
<td>9</td>
<td>1975</td>
</tr>
<tr>
<td>Gabriola Isl.</td>
<td>5239</td>
<td>976</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>1975</td>
</tr>
<tr>
<td>Penalakut Isl.</td>
<td>951</td>
<td>4143</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>1975</td>
</tr>
<tr>
<td>Samuel Isl.</td>
<td>200</td>
<td>18579</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>1975</td>
</tr>
<tr>
<td>Saturna Isl.</td>
<td>3219</td>
<td>17548</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>1975</td>
</tr>
<tr>
<td>Thetis Isl.</td>
<td>1125</td>
<td>3342</td>
<td>8</td>
<td>4</td>
<td>10</td>
<td>1975</td>
</tr>
<tr>
<td>Valdes Isl.</td>
<td>2450</td>
<td>6460</td>
<td>8</td>
<td>5</td>
<td>9</td>
<td>1975</td>
</tr>
</tbody>
</table>
Data analysis

In all analyses “region” refers to either archipelago (Gulf Islands and Barkley Sound) or VI, within which collection sites (specific islands or VI location) were nested when comparing regions. I tested my hypotheses using linear mixed-effects models (LMMs), which were fitted with the lmer function of the “lme4” package (Bates et al. 2014). Restricted maximum likelihood (REML) models were used in all cases except when comparing models with Akaike’s information criterion (Akaike 1974) when maximum likelihood (ML) was used. Results, including p-values, t-values and Satterthwaite approximations to degrees of freedom (rounded down to nearest whole number), were obtained using the “summary” function of the “lmerTest” package (Kuznetsova et al. 2015). Goodness of fit was assessed using marginal and conditional pseudo $R^2$ values (Nakagawa and Schielzeth 2013) calculated with the “r.squaredGLMM” function in the “MuMIn” package (Bartoń 2011). The normality of the model residuals was visually assessed using kernel-density histograms, which compared the residual distribution to an expected normal distribution. Homoscedasticity was visually assessed by plotting the model residuals against the predicted values. Interactions were included in models but removed if they were not significant (at $p < 0.05$).

I began by testing my three predictions for my hypothesis that island syndrome includes difference in GC levels between island and mainland mice. I excluded all pre-1964 data, because all of these samples were from VI, which created heteroscedasticity based on model diagnostic plots. After excluding pre-1964 data, I tested my three predictions relating to island-mainland differences in skull size, body mass, and hair CORT. I then assessed which of the following was the best predictor of hair CORT: body
mass, condition score, or skull size. Following this analysis, I tested the hypothesis that hair GC levels are affected by long term storage using data from specimens collected on VI during 1915-1991. In all models, site was included as a random effect to account for lack of independence among individuals captured from the same location. Hair CORT was ln-transformed in all analyses to improve normality of model residuals. Unless otherwise stated, other measures were not transformed.

**Island-mainland comparison of skull size, body mass, and hair CORT**

**Principal component analysis of skull size**

I used a principal component analysis (PCA) to calculate an index of skull size using four cranial measurements. Cranial measurements were ln-transformed and the PCA was run using a correlation matrix, because skull length had higher variation than the other three measures. I used the first principal component scores (PC1) as an index of skull size, and therefore structural size, for each deer mouse (Landry and Lapointe 2001). PC1 explained 64.4% of the variation in the four cranial measurements, and was most highly correlated with zygomatic arch breadth ($r = 0.562$) and skull length ($r = 0.530$), followed by zygomatic arch length ($r = 0.455$) and cranial breadth ($r = 0.444$). PC1 was significantly positively correlated with male ln-transformed body mass ($n = 90$, $r = 0.61$, $t_{88} = 7.320$, $p < 0.001$) demonstrating that it was a good indicator of structural size.

**Testing Prediction 1: Island mice will be larger than mainland mice**

To test my first prediction, that deer mice from the two archipelagos would be larger than VI mice, I ran a LMM with PC1 as the response variable, and sex and region,
and their interaction, as fixed effects (year and calendar day of capture had been included, but were removed when they were not significant). This model compared PC1 scores between all three regions, first using VI as the reference level (the region with which the other two regions were compared to). I then re-arranged the factor levels of the model to provide the test statistics comparing the archipelagos to each other, which are provided with the results of each model that compares the three regions.

Testing Prediction 2: Island mice will be heavier than mainland mice

To test my second prediction, that deer mice from the two archipelagos would be heavier than VI mice, I ran a LMM with body mass as the response variable, region, as a fixed effect (year and calendar day of capture had been included, but were removed when they were not significant). I ran the same analysis on the residuals of a linear regression between body mass and PC1 of skull size ($F_{1,88} = 54.35, p < 0.0001, R^2 = 0.37$) to determine if deer mice were heavier for their structural size:

\[
\text{Predicted Ln body mass} = 0.0736 \times (\text{PC1}) + 3.108
\]

\[
\text{Condition index} = \text{Actual Ln body mass} - \text{Predicted Ln body mass}
\]

I subsequently used these residuals as condition scores (Schulte-Hostedde et al. 2005) to assess their relationship with hair CORT and to assess differences between island and mainland mice.

Testing Prediction 3: Island mice will have lower hair corticosterone than mainland mice

To test my third prediction, that deer mice from the two archipelagos would have lower hair CORT than VI mice, I first ran a LMM with hair CORT as the response
variable, region and sex as fixed effects, and year and calendar day as covariates. In
Chapter 2, I found that body mass, condition score, and body size were each significant
positive covariates of hair CORT in white-footed mice. To account for this in the current
analysis, I re-ran the model and included PC1 scores for skull size as a covariate of hair
CORT on all deer mouse specimens for which I had PC1 scores (198 of 302 individuals).
To determine the effect of PC1 on the model, I interpreted this new model with and
without PC1 as a covariate for the subset of deer mice with available cranial
measurements.

**Effects of body mass, condition, and skull size on hair corticosterone**

In Chapter 2, I determined that for white-footed mice, body mass was a better
predictor of hair CORT than condition score or structural size. To determine if this was
also true for deer mice, I compared the fit of three separate maximum likelihood models
for predicting hair CORT of male deer mice, with body mass, condition score, or skull
size (PC1) as a covariate. Each model included region as a fixed effect, year and calendar
day as covariates, and site as a random effect that was nested within region. The three
resulting models were compared using AICc, and the model with the best predictor was
identified based on ΔAICc and $R^2_{GLMM}$.

**Testing prediction that hair corticosterone will be lower in earlier specimens**

Analysis of the hypothesis that hair GC levels are affected by long-term storage
was restricted to samples from VI. This was done to eliminate any regional effects, and
because most of the earlier specimens were collected from VI. Prior to testing for an
effect of time on hair CORT, I re-ran the PCA on cranial measurements for VI deer mice collected during 1915-1991. The results were similar to the previous PCA. PC1 explained 67.4% of the variation in the four cranial measurements from VI mice. PC1 was again most highly correlated with zygomatic arch breadth (r = 0.538) and skull length (r = 0.534), followed by cranial breadth (r = 0.479), and zygomatic arch length (r = 0.443).

Finally, I tested my prediction that hair CORT would be lower in earlier samples. To test this prediction, I ran a LMM with hair CORT as the response variable, sex as a fixed effect, and year and calendar day as covariates. Site was included as a random effect to account for lack of independence between individuals collected from the same sites on Vancouver Island. Because hair CORT is positively affected by body size (or mass), I ran the same model but used only samples for which PC1 scores of skull size were available. Also, to determine if skull size changed during 1915-1991, I ran a separate model with PC1 as the response variable.

RESULTS

Island mice from Barkley Sound had larger skulls than mainland mice

I found partial support for my first prediction. The largest 10% of deer mouse skulls belonged to island deer mice (Figure 3.3A), and Barkley Sound deer mice had significantly larger skulls than VI deer mice (Table 3.2). There was no difference in skull size between deer mice from the Gulf Islands and VI (Table 3.2). There was a significant interaction between sex and region when comparing deer mice from Barkley Sound and the Gulf Islands, which indicated that females had greater skull size than males in Barkley
Sound, but that sexes did not differ in the Gulf Islands (Table 3.2; Figure 3.3A). This interaction term did not affect the comparison of deer mice from VI and either of the archipelagos (Table 3.2). The large difference between marginal and conditional $R^2_{GLMM}$ values suggests that there was a high degree of similarity in skull size among individuals from the same sites that was not explained by the fixed effects of the model (Table 3.2).

**Table 3.2.** Comparison of skull size (PC1) of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region that others were compared to. Marginal (M) and conditional (C) pseudo $R^2 (R^2_{GLMM})$ values are provided.

<table>
<thead>
<tr>
<th>RL</th>
<th>Fixed effect</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>(Intercept)</td>
<td>-0.616</td>
<td>0.534</td>
<td>39</td>
<td>-1.153</td>
<td>0.256</td>
<td>0.07, 0.44</td>
</tr>
<tr>
<td></td>
<td>Sex(Male)</td>
<td>-0.079</td>
<td>0.414</td>
<td>174</td>
<td>-0.191</td>
<td>0.849</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Region(GI)</td>
<td>0.699</td>
<td>0.624</td>
<td>39</td>
<td>1.120</td>
<td>0.269</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Region(BI)</td>
<td>1.318</td>
<td>0.636</td>
<td>41</td>
<td>2.072</td>
<td>0.045</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Sex(Male)*Region(GI)</td>
<td>0.296</td>
<td>0.483</td>
<td>174</td>
<td>0.614</td>
<td>0.540</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex(Male)*Region(BI)</td>
<td>-0.659</td>
<td>0.508</td>
<td>176</td>
<td>-1.298</td>
<td>0.196</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Region(BI)</td>
<td>0.619</td>
<td>0.472</td>
<td>43</td>
<td>1.312</td>
<td>0.197</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex(Male)*Region(BI)</td>
<td>-0.955</td>
<td>0.385</td>
<td>177</td>
<td>-2.481</td>
<td>0.014</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Abbreviations and sample sizes: VI – Vancouver Island (mainland: 5 sites, 35 individuals), GI – Gulf Islands (14 islands, 99 individuals), and BI – Barkley Sound Islands (13 islands, 72 individuals).

*Island mice were heavier and in better condition than mainland mice*

Male deer mice from both archipelagos had greater body mass than VI mice, supporting my prediction (Table 3.3). On average, male deer mice from Barkley Sound
were approximately 5.0 grams (26.4%) heavier than VI mice and 2.5 grams (12.5%) heavier than mice from the Gulf Islands (Figure 3.3B). Deer mice from the Gulf Islands were 2.5 grams (12.4%) heavier on average than VI mice (Figure 3.3B). Deer mice from both archipelagos also had higher condition scores than VI mice, indicating that they were heavier for their size (Table 3.3; Figure 3.3C).

Table 3.3. Comparison of body mass (g) and condition scores of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region others were compared to. Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>RL</th>
<th>Fixed effect</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>t</th>
<th>p</th>
<th>$R^2_{GLMM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass</td>
<td>VI</td>
<td>(Intercept)</td>
<td>19.607</td>
<td>1.135</td>
<td>23</td>
<td>17.27</td>
<td>&lt;0.001</td>
<td>0.27, 0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Region(GI)</td>
<td>2.932</td>
<td>1.339</td>
<td>23</td>
<td>2.19</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Region(BI)</td>
<td>5.651</td>
<td>1.412</td>
<td>25</td>
<td>4.002</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GI</td>
<td>Region(BI)</td>
<td>2.72</td>
<td>1.099</td>
<td>27</td>
<td>2.474</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>VI</td>
<td>(Intercept)</td>
<td>-0.089</td>
<td>0.026</td>
<td>87</td>
<td>-3.372</td>
<td>0.001</td>
<td>0.28, 0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Region(GI)</td>
<td>0.065</td>
<td>0.031</td>
<td>87</td>
<td>2.086</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Region(BI)</td>
<td>0.188</td>
<td>0.034</td>
<td>87</td>
<td>5.521</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GI</td>
<td>Region(BI)</td>
<td>0.123</td>
<td>0.027</td>
<td>87</td>
<td>4.511</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations and sample sizes: VI – Vancouver Island (mainland: 5 sites, 18 individuals), BI – Barkley Sound Islands (11 islands, 27 individuals) and GI – Gulf Islands (13 islands, 45 individuals).
Island mice had lower hair corticosterone than mainland mice, but only after correcting for structural size

Hair CORT was successfully quantified from museum specimens of deer mice (1964-1991; median = 56.45 ng/g, range = 11.14 - 203.08 ng/g) at levels similar to those reported for live-captured deer mice in Oregon, USA (median: 97.5 ng/g, range: 0.2-290.2 ng/g; Hanselmann 2016). I found partial support for my prediction that deer mice from the two archipelagos would have lower hair CORT than VI mice. Mean hair CORT (± SD) of deer mice from both archipelagos (GI: 57.6 ± 23.0, BI: 60.9 ± 32.3 ng/g) was lower than for deer mice from VI (69.4 ± 34.3 ng/g), but these absolute levels were not significantly different (Table 3.4, Figure 3.4A). However, hair CORT did differ between regions after adding PC1 scores of skull size as a covariate to the model (Table 3.5, Figure 3.4B). Results from this model showed that after controlling for structural size, deer mice from both the Gulf Islands and Barkley Sound had lower hair CORT than VI deer mice (Table 3.5, Figure 3.4B). Adding a measure of structural size to the model for deer mouse hair CORT improved its explanatory power, based on the higher $R^2_{GLMM(M)}$ after adding PC1 as a covariate (Table 3.4, 3.5). Both models showed evidence of a difference in hair CORT between sexes, with males having higher hair CORT than females (Table 3.4; Figure 3.4A), although this difference was only marginally significant when PC1 was added as a covariate (Table 3.5). Among these hair samples, which were collected between 1964-1991, there was no effect of year, or calendar day on hair CORT (Table 3.4, 3.5).
Table 3.4. Comparison of ln-transformed hair corticosterone levels of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island without controlling for structural size and using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region others were compared to in the model. Marginal (M) and conditional (C) pseudo $R^2 (R^2_{GLMM})$ values are provided.

<table>
<thead>
<tr>
<th>RL</th>
<th>Fixed effect</th>
<th>$B$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>(Intercept)</td>
<td>13.831</td>
<td>23.407</td>
<td>119</td>
<td>0.591</td>
<td>0.556</td>
<td>0.02, 0.30</td>
</tr>
<tr>
<td></td>
<td>Region(GI)</td>
<td>-0.154</td>
<td>0.157</td>
<td>37</td>
<td>-0.984</td>
<td>0.332</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Region(BI)</td>
<td>-0.217</td>
<td>0.204</td>
<td>49</td>
<td>-1.063</td>
<td>0.293</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>0.099</td>
<td>0.045</td>
<td>272</td>
<td>2.182</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>-0.005</td>
<td>0.012</td>
<td>118</td>
<td>-0.418</td>
<td>0.677</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>89</td>
<td>-0.030</td>
<td>0.976</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Region(BI)</td>
<td>-0.063</td>
<td>0.132</td>
<td>39</td>
<td>-0.476</td>
<td>0.637</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations and sample sizes: VI – Vancouver Island (mainland: 6 sites, 49 individuals), BI – Barkley Sound Islands (13 islands, 128 individuals) and GI – Gulf Islands (14 islands, 126 individuals).
Table 3.5. Comparison of ln-transformed hair corticosterone levels of deer mice collected from two archipelagos (Gulf Islands and Barkley Sound) and Vancouver Island controlling for structural size and using a linear mixed-effects model (random effect: site, nested within region). “RL” is the reference level region others were compared to in the model. Marginal (M) and conditional (C) pseudo $R^2 (R^2_{GLMM})$ values are provided.

<table>
<thead>
<tr>
<th>RL</th>
<th>Fixed effect</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>(Intercept)</td>
<td>25.911</td>
<td>27.066</td>
<td>75</td>
<td>0.957</td>
<td>0.341</td>
<td>0.12, 0.30</td>
</tr>
<tr>
<td></td>
<td>Region(GI)</td>
<td>-0.346</td>
<td>0.161</td>
<td>33</td>
<td>-2.149</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Region(BI)</td>
<td>-0.468</td>
<td>0.222</td>
<td>43</td>
<td>-2.110</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>0.106</td>
<td>0.060</td>
<td>174</td>
<td>1.774</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>-0.011</td>
<td>0.014</td>
<td>74</td>
<td>-0.801</td>
<td>0.426</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>-0.001</td>
<td>0.001</td>
<td>62</td>
<td>-0.601</td>
<td>0.550</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC1</td>
<td>0.087</td>
<td>0.023</td>
<td>170</td>
<td>3.712</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Region(BI)</td>
<td>-0.122</td>
<td>0.136</td>
<td>36</td>
<td>-0.898</td>
<td>0.375</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations and sample sizes: VI – Vancouver Island (5 mainland sites, 35 individuals), BI – Barkley Sound Islands (13 islands, 71 individuals) and GI – Gulf Islands (14 islands, 92 individuals).

*Body mass was a better predictor of hair corticosterone than condition index or skull size*

Body mass was a better predictor of hair CORT than condition index or structural size (PC1 scores of skull size) for male deer mice. The model with body mass as a covariate had both the lowest AICc value, and the greatest $R^2_{GLMM}$ values (Table 3.6).

Body mass, condition score, and skull size each had a positive effect on hair CORT, but the effect of PC1 was only marginally significant (Table 3.6). The full model using body mass as a predictor did not result in the same differences in hair CORT between island and mainland mice found previously (Table 3.5), however the $\beta$ values for both archipelagos showed a trend toward lower hair CORT (Table 3.7).
Table 3.6. Comparison of body mass (g), condition index, and skull size (PC1) as predictors of ln-transformed hair corticosterone for male deer mice (n = 90), based on maximum likelihood linear mixed-effects models (fixed effects: region, year, and calendar day; random effect: site, nested within region). AICc, ΔAICc, and marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided to compare relative fit.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$</th>
<th>$t$</th>
<th>$p$</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>$R^2_{GLMM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass</td>
<td>0.055</td>
<td>3.573</td>
<td>&lt; 0.0001</td>
<td>117.21</td>
<td>0</td>
<td>0.23, 0.39</td>
</tr>
<tr>
<td>Condition index</td>
<td>1.037</td>
<td>2.488</td>
<td>0.015</td>
<td>123.13</td>
<td>5.92</td>
<td>0.16, 0.35</td>
</tr>
<tr>
<td>Skull size (PC1)</td>
<td>0.072</td>
<td>1.914</td>
<td>0.059</td>
<td>125.60</td>
<td>8.39</td>
<td>0.14, 0.31</td>
</tr>
</tbody>
</table>

Table 3.7. Results of body mass (g) as a predictor of ln-transformed hair corticosterone for male deer mice (n = 90) based on a linear mixed-effects model (random effect: site, nested within region). AICc, ΔAICc, and marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided to compare relative fits of the three different models.

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-48.930</td>
<td>38.040</td>
<td>38</td>
<td>-1.286</td>
<td>0.206</td>
<td>0.21, 0.45</td>
</tr>
<tr>
<td>Region(GI)</td>
<td>-0.296</td>
<td>0.206</td>
<td>19</td>
<td>-1.436</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td>Region(BI)</td>
<td>-0.394</td>
<td>0.286</td>
<td>25</td>
<td>-1.376</td>
<td>0.181</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>0.026</td>
<td>0.019</td>
<td>38</td>
<td>1.374</td>
<td>0.178</td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>41</td>
<td>0.034</td>
<td>0.973</td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>0.055</td>
<td>0.016</td>
<td>80</td>
<td>3.411</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations and sample sizes: VI – Vancouver Island (5 mainland sites, 18 individuals), BI – Barkley Sound Islands (11 islands, 27 individuals) and GI – Gulf Islands (13 islands, 45 individuals).
Hair corticosterone levels were lower in earlier specimens

My prediction that hair CORT would be lower for earlier specimens was supported. There was a significant positive relationship between year and hair CORT in the LMM (Table 3.8). No deer mice from VI after 1945 had hair CORT concentrations lower than 20 ng/g, but half of the specimens collected before 1945 had levels that low (Figure 3.5). The LMM for comparing hair CORT of deer mice from VI across years also showed that males had higher corticosterone than females, which was found in previous models (Table 3.8). This model had a higher effect size ($R^2_{GLMM (M)} = 0.46$; Table 3.8) than the other models investigating predictors of hair CORT, suggesting that the temporal trend (potentially caused by degradation) had a strong effect on hair CORT.

**Table 3.8.** Evaluating the effect of degradation of hair corticosterone over time in specimens of deer mice from Vancouver Island (11 sites, 82 individuals) collected during 1915-1991 using a linear mixed-effects model (random effect: site). Marginal (M) and conditional (C) pseudo $R^2$ ($R^2_{GLMM}$) values are provided.

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM (M), (C)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-49.997</td>
<td>13.267</td>
<td>6</td>
<td>-3.768</td>
<td>0.008</td>
<td>0.46, 0.73</td>
</tr>
<tr>
<td>Year</td>
<td>0.027</td>
<td>0.007</td>
<td>6</td>
<td>4.044</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>-0.001</td>
<td>0.003</td>
<td>29</td>
<td>-0.552</td>
<td>0.585</td>
<td></td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>0.349</td>
<td>0.113</td>
<td>68</td>
<td>3.104</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

I was able to run the same model, including PC1 of skull size as a covariate, for a subset of samples for which I had cranial and hair CORT measurements. This model had somewhat unexpected results, showing that year had a significant effect, but PC1 did not (Table 3.9), unlike in previous models where PC1 had a significant positive effect on hair
CORT. This result may have been caused by the strong effect of time masking the
relationship between body size and hair CORT that was found in previous models. It is
unlikely that this was caused by collinearity between year and PC1, because the separate
model that I ran with PC1 as the response variable showed that there was not a significant
effect of year on PC1 scores of skull size ($t_8 = 1.726, p = 0.121$).

Table 3.9. Evaluating the effect of degradation of hair corticosterone over time while
controlling for the effect of structural size (PC1) in specimens of deer mice from
Vancouver Island (10 sites, 68 individuals) collected during 1915-1991 using a linear
mixed-effects model (random effect: site). Marginal (M) and conditional (C) pseudo $R^2$
($R^2_{GLMM}$) values are provided.

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>$\beta$</th>
<th>se</th>
<th>df</th>
<th>$t$</th>
<th>$p$</th>
<th>$R^2_{GLMM}$ (M), (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-54.026</td>
<td>14.194</td>
<td>8</td>
<td>-3.806</td>
<td>0.005</td>
<td>0.49, 0.73</td>
</tr>
<tr>
<td>Year</td>
<td>0.029</td>
<td>0.007</td>
<td>8</td>
<td>4.069</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>-0.002</td>
<td>0.002</td>
<td>54</td>
<td>-1.020</td>
<td>0.312</td>
<td></td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>0.357</td>
<td>0.132</td>
<td>53</td>
<td>2.713</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>-0.009</td>
<td>0.060</td>
<td>60</td>
<td>-0.153</td>
<td>0.879</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

I found partial support for my hypothesis that island syndrome includes differences in GC levels between island (two archipelagos) and mainland (Vancouver Island) deer mice. VI was considered the mainland in this comparison due to its large size. Mice from both Barkley Sound and the Gulf Islands had greater body mass than VI mice, in accordance with island syndrome. However, only deer mice in Barkley Sound were structurally larger than VI mice. Hair CORT differed between deer mice from the two archipelagos and VI after controlling for structural size. Additionally, I demonstrated that earlier museum specimens had lower hair CORT than more recently collected specimens of deer mice. These findings provide evidence for the effect of island life on HPA activity in rodents, and provide useful information concerning the use of hair from museum specimens as a measure of GCs.

Island-mainland comparisons

Barkley Sound deer mice were structurally larger than mainland mice

Adherence to the island rule in mammals is more often evaluated using body mass than with linear measurements of size (Lomolino 2005). However, differing results based on body mass and structural size demonstrate the importance of interpreting the two different measures (Strickland and Norris 2015). Characteristics of island syndrome are predicted to increase as island area decreases (Adler and Levins 1994), so island effects may act more strongly upon deer mice in Barkley Sound, given that those islands I sampled were generally much smaller (average area = 223 ha) than the Gulf Islands (average area = 3113 ha). Increased body size of island rodents has been attributed to...
ecological release from predators, and increased resource availability through marine subsidies on islands (Adler and Levins 1994; Lomolino et al. 2012), both of which may contribute to large body size of deer mice in Barkley Sound.

*Island deer mice were heavier and in better condition than mainland mice*

Male deer mice from both archipelagos were heavier, and in better condition than VI mice. Studies have reported greater body mass for deer mice from these islands compared to the VI mice (Herman 1979; Redfield 1976), which is a pattern that is repeated across rodent species (Lomolino et al. 2012). Characteristics of island populations may be the result of evolutionary change following isolation (Clegg et al. 2002), or due to phenotypic plasticity in response to conditions on islands (Strickland and Norris 2015). Distinguishing between the mechanisms can be difficult, particularly without habitat data, genetic analyses, or common garden experiments. Results for Barkley Sound and the Gulf Islands suggest that both types of change may occur for deer mouse populations in these archipelagos.

Deer mouse populations on islands in Barkley Sound and the Gulf Islands have likely been sufficiently isolated from VI for long enough for divergent evolution of traits to occur. Redfield (1976) suggested that deer mouse populations in the Gulf Islands were isolated from the mainland (both VI and mainland BC) for ca. 12,000 years and that there is little movement between islands. This time frame relates to when sea levels were ca. 45 m lower, and islands, including those in Barkley Sound, were connected by land bridges to VI (Cody 2006). Therefore, deer mouse populations on islands in Barkley Sound have likely been isolated for a similar period as the Gulf Islands. Upon colonizing islands,
mammals can experience rapid morphological evolution (Millien 2006). Immigrant selection, and subsequent founder effects, can also play a role if larger individuals are more likely to reach isolated islands (Lomolino 1984; Lomolino et al. 2012). Determining whether characteristics exhibited by island vertebrates are the result of genetic drift is also important (Clegg et al. 2002), however I demonstrated predictable island-mainland differences in body mass of deer mice across numerous islands for two archipelagos, suggesting a non-random pattern of selection toward larger body mass.

Although deer mice from the Gulf Islands were heavier than VI mice, they did not differ in structural size. Whether this means that selection acts on mass and not structural size, or if it indicates phenotypic responses to greater resource availability on islands, requires more direct experimentation. Condition scores of deer mice from both Barkley Sound and the Gulf Islands were higher than those of VI mice, which might be a phenotypic response to conditions on islands. There is evidence that deer mice from coastal islands of British Columbia forage heavily on amphipods in the intertidal zone (Thomas 1971), which might account for their increased body condition relative to VI mice.

*Island deer mice had lower hair corticosterone than mainland mice, after correcting for structural size*

After controlling for structural size, deer mice from both archipelagos had lower hair CORT than VI mice. Low GC levels on islands are a result of phenotypic plasticity (Clinchy et al. 2004), an evolved response to island life (Müller et al. 2007), or likely some combination of the two. For example, hair GC levels have a genetic basis
(Fairbanks et al. 2011), but can be influenced by an individual’s exposure to stressors (Mastromonaco et al. 2014; Bryan et al. 2015; Scorrano et al. 2015). Hair CORT might also be influenced by baseline and stress-induced CORT levels, both of which can be the object of selection (Patterson et al. 2014). I suggest that similar factors, which select for larger body size (and potentially body mass) of island deer mice, might also select for lower CORT levels.

Environmental differences must exist between islands and the mainland for selection, and not simply genetic drift, to systematically influence the physiology of island mice. Predators of deer mice that occur on VI, such as short-tailed weasels (*Mustela erminea*) and American marten (*Martes americana*), are absent or occur in low numbers on islands in Barkley Sound (Guiguet 1974; Herman 1981). Although American mink (*Neovison vison*) are abundant in Barkley Sound and the Gulf Islands (Redfield 1976; Herman 1981), they rarely prey on deer mice. (Just one of 1752 mink scat samples in Barkley Sound contained remains of deer mice; Hatler 1976). Island deer mice still face predation from avian predators, such as Western screech owls (*Megascops kennicottii*). The presence and diversity of predators is also likely island-specific, given that Saltspring Island (18,247 ha) has greater potential to support predators than Mandarte Island (8 ha). Overall, deer mice from these two archipelagos likely face a lower variety and intensity of predation threats than deer mice on VI.

Responding to a perceived threat on an island, where actual threats are low, may be non-adaptive (Cooper et al. 2014). Mounting an unnecessary stress response can be costly, because it diverts resources from reproduction toward survival (Wingfield and Sapolsky 2003). Therefore, lower baseline and stress-induced GC levels may be selected.
for on islands where there are few predators. “Island tameness”, which is the tendency for island species to have low reactivity to predation threats, has been described in multiple taxa (Swarts et al. 2009; Vitousek et al. 2010; Cooper et al. 2014). For example, deer mice from Moresby Island in the Gulf Islands (not sampled in the present study) were less reactive to the scent of a short-tailed weasel than individuals from mainland British Columbia (Kavaliers 1990). This evidence suggests that island deer mice might have behavioural adaptations to low-predator conditions on islands. Given that behavioural and physiological traits are closely linked (Carere et al. 2010), decreased sensitivity of island deer mice to predation threats may be accompanied by changes in their average GC levels.

In addition to lower predation, island-mainland differences in food availability and subsequent changes in population dynamics of deer mice may play a role in the CORT differences I detected. Elevated GC levels stimulate foraging behaviour in response to low resource availability (Pravosudov et al. 2001; Kitaysky et al. 2007). Intertidal invertebrates provide an important food source for island deer mice of coastal British Columbia (Thomas 1971). Marine subsidies, in addition to low predation levels, are linked to high population densities of island deer mice in the Gulf of California (Stapp and Polis 2003). High population density is a central characteristic of island syndrome as described by Adler and Levins (1994), but why low GC levels would occur under high population densities is an important question to address.

Adler and Levins (1994) proposed that island rodents are characterized by reduced aggression and high population density compared to their mainland counterparts, which they attributed to neighbour familiarity and social stability on islands. Low
aggression has been described in deer mice in the Gulf Islands (Halpin and Sullivan 1978; Halpin 1981) and other insular rodents (Grey and Hurst 1998). In contrast, Herman (1979) demonstrated that deer mice in Barkley Sound were more aggressive than mainland deer mice. Given the contradicting evidence of intraspecific aggression for deer mice from these two archipelagos, I suggest that the island-mainland differences in hair CORT are caused primarily by differences in predator pressure, as opposed to interactions between resources and intraspecific competition. However, direct measurement of variables such as predation, population density, and food availability are necessary to determine exactly what accounts for island-mainland differences in hair CORT for deer mice of a given size in these two archipelagos, and if they indicate an evolved response.

*Hair corticosterone increased with skull size*

Deer mouse body mass, condition score, and skull size each had a positive relationship with hair CORT. Although body mass was a better predictor of hair CORT than skull size in male deer mice, I used skull size as a covariate for island-mainland comparisons (including both sexes) because pregnancy influences body mass in females. In Chapter 2, I found a positive relationship between body mass and hair CORT in white-footed mice, and proposed a relationship between time since moult and hair CORT levels associated with age of the mice. Because cranial measurements differ between deer mouse age-classes (Dice 1936; Koh and Peterson 1983), skull size may provide a proxy for the same relationship. Steroid hormones have an inhibitory effect on moulting in *Peromyscus* (Garwood and Rose 1995); therefore, moulting generally occurs before or
following energetically demanding time periods, such as breeding (Pierce and Vogt 1993; Tabacaru et al. 2011). As a result, any hairs grown following a full seasonal or developmental moult should have higher CORT concentrations, which would result in older (larger) individuals having higher hair CORT compared to younger mice that have more recently grown their adult pelage. In American pika (Ochotona princeps), hair CORT was strongly influenced by body size (measured by cranial diameter; Waterhouse et al. 2017), but in the opposite direction compared to deer mice and white-footed mice. Larger American pikas had lower hair CORT, which the authors attributed to the negative relationship between mass-specific metabolic rate and GCs described by Haase et al. (2016). These conflicting descriptions of relationships between hair CORT and body size for small mammals demonstrate the need for more studies concerning internal factors affecting hair GCs.

**Sex differences in structural size and hair CORT**

*Female deer mice had larger skulls than males in Barkley Sound*

My analysis of skull size showed a significant interaction between sex and region, indicating that females were larger than males in Barkley Sound, but not in the other two regions. There is evidence of sexual dimorphism in body mass of deer mice (Bowers and Smith 1979; Schulte-Hostedde et al. 2001), however there is little evidence of sex differences in structural size (Holbrook et al, 1982; Koh and Peterson, 1983; Schulte-Hostedde et al. 2001). Bowers and Smith (1979) suggested that greater female body mass is favoured in areas with variable habitat quality, where large females defend small, high quality home ranges against males. Such a situation might occur in Barkley Sound,
possibly for competition for territory near the beaches where they feed on intertidal invertebrates (Thomas 1971). Marinelli and Millar (1989) found that pregnancy rates of deer mice were greater on beaches than inland on islands in Barkley Sound, demonstrating that beaches are higher quality areas, but they did not describe differences in body size between sexes. Herman (1979) surveyed 24 of the islands in Barkley Sound and found no difference in body mass between sexes, and did not report any difference in body length, although he measured it. Therefore, my result showing that females were structurally larger in Barkley Sound should be treated with caution, but presents an interesting opportunity for further investigation of sexual size dimorphism in deer mice.

*Female deer mice had lower hair CORT than males*

I found evidence that female deer mice had lower hair CORT than males, however this difference was only marginally significant when controlling for structural size. Sex differences in GCs for other wild rodents have been attributed to interactions between GCs and sex hormones, and differences in parental behaviour (Romero et al. 2008; Schradin 2008; Bauer et al. 2014). Although in mammals females typically have higher GC levels than males (Reeder and Kramer 2005), no sex differences in fecal corticosterone metabolite levels have been reported in free-living *Peromyscus* (Harper and Austad 2001, 2004; Hayssen et al. 2002). In Chapter 2, I found that female white-footed mice had lower hair CORT levels in spring (May-June) than males, but sexes did not differ during the summer months (July-August). The main breeding season of deer mice in British Columbia occurs from May to late autumn, however deer mice in Barkley Sound and the Gulf Islands may breed from April through to December (Nagorsen 2005).
The specimens used in the present study were collected during April-August, thus encompassing the breeding season. Hair samples may represent hair growth that occurred from September of the previous year, up to the date of collection, depending on the age of the mouse. Collins (1923) described female deer mice that were pregnant while developing their winter pelage, however moulting ceased following birth of their young. If hair growth in deer mice occurs during pregnancy, low hair CORT in females may be caused by decreased free CORT during pregnancy, which is common in laboratory rodents (Reeder and Kramer 2005; Brunton et al. 2008). It will be important to address whether low female hair CORT in two *Peromyscus* species represents a general sex difference in GC levels that differs from other mammals, or if it is the result of different moulting schedules between sexes and thus a result of using hair to evaluate GCs in wild *Peromyscus*.

**Hair corticosterone levels were lower in earlier specimens**

Using deer mice specimens collected from Vancouver Island over 76 years, I showed that specimens collected earlier in the 20th century had lower hair CORT than more recently collected individuals. Although there are multiple possible explanations for this trend, I suggest that this relationship was caused by degradation of hair CORT. To my knowledge, this study provides the first evidence of degradation of hair GCs over long periods of storage. If there is systematic degradation of hair GCs over time, use of museum specimens for assessing temporal trends between GCs and environmental variables may be compromised. In contrast to my study, other authors have provided evidence of the long-term stability of GCs in hair, based on detecting higher levels in
earlier samples (Webb et al. 2010; Bechshøft et al. 2012). Similar evidence has been provided for CORT in feathers (Bortolotti et al. 2009). In addition, feather CORT of samples collected over a 153 period showed no temporal trend indicative of degradation (Fairhurst et al. 2015). However, it is possible that feathers provide stability for hormones that hair does not.

Although temporal trends in GCs may be responses to changes in the environment, I think this is unlikely for the pattern that I observed. GC levels within populations can vary across years in response to changing predator pressure (Sheriff et al. 2011a), resource availability (Kitaysky et al. 2007), or unusual weather (Sheriff et al. 2012). Precipitation can affect food availability and population dynamics in deer mice (Reed et al. 2007); however, rainfall levels for Vancouver Island during the 20th century were far more cyclical than the relatively linear trend that I observed between hair CORT and time (Tuller 1990). Evolutionary changes that affect the morphology of rodents have been observed over relatively short time-scales (100-200 years; Pergams and Ashley 1999, 2001; Yom-Tov and Geffen 2011); however, I did not find a significant temporal effect on skull size of deer mice on VI. The temporal trend in hair CORT masked the relationship between hair CORT and skull size during 1915-1991, further providing evidence that degradation occurred.

The temporal trend that I observed could be explained by varying preservation treatments used over time, although this seems unlikely as well. For example, Reilly (2017) exposed white-footed mouse hair samples to three curatorial treatments (air-drying, borax, and turpentine), and found no effect of the treatments on hair CORT. Importantly, she also demonstrated that CORT in the preserved specimens did not differ
from samples collected at the time those individuals were still alive. However, earlier
treatments, such as arsenic soap washes, may have a stronger effect on hair GCs.
Although time alone may not cause significant degradation, exposure to light is known to
decrease hair GCs (Wester et al. 2016). Hair GC levels of museum specimens may be
compromised by time spent under lights on lab benches or in display cases.

It is important to consider whether the evidence of degradation, or at least change
over time, compromises the island-mainland differences that I found for hair CORT in
deer mice. I addressed this concern by limiting my analysis to samples collected during
1964-1991, however this meant that samples from VI were generally more recently
collected (1971-1991) than those from the two archipelagos (GI: 1974-1984; BI: 1964-
1970; Table 3.1). Although I cannot rule out the possibility that degradation of hair
CORT may have influenced my results, I suggest two reasons why it was not responsible
for the observed pattern. First, I found no effect of year as a covariate within the time-
frame that I made the island-mainland comparison (1964-1991), suggesting degradation
did not strongly influence the results. Second, adjusting the time-frame of the comparison
to include VI samples from 1958-1986 (excluding 1991 samples) resulted in a similar
pattern of archipelago deer mice having lower hair CORT for their structural size
compared to VI mice; however, the island-mainland differences were marginally
significant in this comparison (p-values < 0.1). I recommend that future studies involving
the analysis of hair CORT from museum specimens collecting samples from individuals
captured during narrow time-frames to eliminate the potentially confounding effect of
degradation.
The suggestion that hair GCs degrade over time, and that treatment and storage may compound this effect, should not rule out the use of museum specimens for analysis of hair hormones. Further analysis should be done to determine rates of degradation, and how it may be influenced by different treatments and storage conditions. Based on linear regression across 76 years, hair CORT in deer mouse specimens collected from VI degraded at an approximate rate of 0.8 ng/g a year, however the exact rate may be obscured by natural variation between years and sites. Determining specific rates of degradation may allow for temporal trends to be studied while controlling for decay associated with storage or exposure to light. One of the most rigorous ways to evaluate the possibility of degradation would be to use preserved specimens from mammals raised (lab or captive individuals), treated, and stored under similar conditions.

**Conclusion and future directions**

Many island-mainland studies are conducted on populations of one island compared to their mainland counterparts (Müller et al. 2007; Raia et al. 2010; Matson et al. 2014; Strickland and Norris 2015). In contrast, I demonstrated differences in body mass, condition, structural size (for one group of islands), and GC levels across multiple islands in two archipelagos compared to multiple mainland sites. Differences in HPA activity of deer mice between these two archipelagos and multiple mainland sites adds to the understanding that island life has physiological consequences (Lovegrove 2000; Clinchy et al. 2004; Müller et al. 2007; Rödl et al. 2007; Vitousek et al. 2010; Noakes et al. 2013; Matson et al. 2014). Further analyses could be done using this dataset to investigate within-archipelago patterns in body size and hair CORT, and test predictions.
relating to island area and isolation. This type of large-scale study was facilitated by the use of museum specimens to quantify hair CORT. This relatively new method of evaluating temporal and spatial patterns in HPA activity in mammals requires further investigation to reliably distinguish between real biological patterns and potential effects of storage.

**LITERATURE CITED**


**Figure 3.1.** Locations on Vancouver Island (mainland; Top), in Barkley Sound (bottom left), and in the Gulf Islands (bottom right) from which deer mice were collected. Individual study sites are indicated by points on the map (Vancouver Island) or by shading entire islands in the archipelagos.
Figure 3.2. Ventral view of deer mouse skull with measurements indicated: skull length (SL), cranium breadth (CB), zygomatic arch length (ZL), and zygomatic arch breadth (ZB). Photo of skull provided by Phil Myers.
Figure 3.3. Deer mice from Barkley Sound had larger skulls (PC1) than Vancouver Island deer mice (A; Table 3.2), and male deer mice from both archipelagos had greater body mass (B) and were in better condition than Vancouver Island deer mice (C; Table 3).
Figure 3.4. Hair corticosterone (ng/g) of deer mice from two archipelagos did not differ from Vancouver Island mice (A; Table 4) without controlling for skull size (PC1; Table 5; B). 95% confidence ellipses of the mean are provided on the scatterplot to highlight the distribution of individual points for each region. Hair corticosterone values were ln-transformed for analysis.
Figure 3.5. Hair corticosterone (ng/g) values of deer mouse specimens collected from Vancouver Island (11 sites, 82 individuals) demonstrate that earlier specimens had lower hair corticosterone.
Both insular and continental wildlife currently face major challenges from habitat degradation, the spread of invasive species, and climate change (Loehle and Eschenbach 2012; Venter et al. 2016; Ducatez and Shine 2017). While islands have become less isolated by the influence of humans through introductions and exploitation, mainland environments have become more fragmented and island-like through habitat destruction (Whittaker and Fernandez-Palacios 2007). By studying the effects of insularity on the biology of island vertebrates, we can increase our understanding of how evolution may affect continental wildlife as well (Yom-Tov and Geffen 2011). How animals respond to stress can determine their survival (Romero and Wikelski 2010; Rivers et al. 2012), and using islands as natural laboratories provides excellent opportunities for studying the evolution of mechanisms associated with the stress response (Müller et al. 2007). Islands provide replicate sites with varying degrees of predation, competition, and other sources of stress, which can be compared to each other and to the mainland (Clinchy et al. 2004, 2011). Quantifying glucocorticoid (GC) levels from hair and other keratinized structures may provide useful methods for studying such large-scale spatial and temporal trends in GC levels across landscapes (Bortolotti et al. 2009; Bryan et al. 2015; Fairhurst et al. 2015).

Small vertebrates on islands often demonstrate characteristics of island syndrome, including large body size, high population density, and reduced aggression (Adler and Levins 1994; Goltsman et al. 2005; Russell et al. 2011; Crespin et al. 2012; Novosolov et al. 2013; Sale and Arnould 2013; Blanco et al. 2014). In addition to morphological and behavioural adaptations to island life, island vertebrates may also have reduced GC levels...
compared to their mainland conspecifics (Müller et al. 2007). In Chapters 2 and 3 of this thesis, I tested the hypothesis that island syndrome includes differences in GC levels between island and mainland rodents. In Chapter 2, I successfully quantified corticosterone (CORT) from hair, and its related metabolites from the feces (FCM) of white-footed mice (*Peromyscus leucopus*). There was no evidence of island-mainland differences in either measure of GCs, or in terms of relative abundance and body size for white-footed mice in the Thousand Islands, Ontario. I suggested that the lack of any island effect on GCs of white-footed mice in this near-shore archipelago was the result of similar predator and competitor levels with the mainland. I did, however, identify a positive relationship between body mass and hair CORT, which I suggested was the result of replacement hairs grown following a moult having higher CORT than those grown during the complete moult. I also demonstrated that both sexes of white-footed mice have lower spring FCM compared to summer levels, but only females differed between seasons in their hair CORT levels. The negative results for the island-mainland comparison in Chapter 2 suggested that changes in CORT levels of island rodents might occur only on more isolated islands, where community structure differs from the mainland, and rodents display characteristics of island syndrome.

In Chapter 3, I tested the same hypothesis as that tested in Chapter 2, but on a larger scale, using two coastal archipelagos in British Columbia. I compared hair CORT from museum specimens of deer mice (*Peromyscus maniculatus*) collected from multiple islands in Barkley Sound and the Gulf Islands to those collected on Vancouver Island (VI; used as the mainland in this comparison). Deer mice from the archipelagos were heavier, and in better condition than VI mice. Deer mice from Barkley Sound were also
structurally larger than VI mice. These results were in agreement with previous studies for live deer mice, captured in these two archipelagos (Redfield 1976; Halpin and Sullivan 1978; Herman 1981; Marinelli and Millar 1989). Island deer mice from both archipelagos had lower hair CORT than VI mice, after controlling for the effect of structural size, providing support for my hypothesis. Lower corticosterone levels of island birds compared to their mainland conspecifics have been described as either a phenotypic response to low predator levels (Clinchy et al. 2004) or as an evolved response to island life (Müller et al. 2007). Reduced responsiveness to predation threats is characteristic of vertebrates on islands (Kavaliers 1990; Rödl et al. 2007; Swarts et al. 2009; Cooper et al. 2014), and I suggest that decreased CORT levels may accompany this adaptive response to low predator conditions. Further experiments would be required to definitively state whether low hair CORT of island deer mice is the result of phenotypic plasticity or an adaptive response to island life.

Although I did not find island-mainland differences for white-footed mice in the Thousand Islands, it still has great potential as an experimental system. There was similarity in GC levels among individuals from the same sites that was not explained by the explanatory variables that I measured. This means that quantifying other variables at each site, such as food availability, community structure, and aspects of demography, should explain more of the variation in GC levels of white-footed mice. Knowledge of each individual’s age, reproductive history, and moulting schedule may also be necessary to more accurately evaluate hair GCs in wild rodents. Detailed descriptions of moulting schedules are particularly useful for interpreting hair GCs in wild rodents, although such
studies are unfortunately few (but see Collins 1923; Layne 1968; Pierce and Vogt 1993; Tabacaru et al. 2011).

Studying deer mice from islands surrounding Vancouver Island provides the potential for further island-mainland comparisons, beyond which I explored. For example, the dataset I analyzed in Chapter 3 could also be used to investigate within-archipelago patterns in body size and hair CORT levels (e.g., related to degree of isolation or island area). The differing levels of precipitation for islands in Barkley Sound (higher rainfall) and the Gulf Islands (lower rainfall), and potential differences in predator and competitor levels within and between these archipelagos, provides the opportunity to tease apart the variables affecting the evolution of body size and GC levels in rodents. Although museum specimens provide an attractive option for evaluating spatial patterns in GC levels, more studies are needed to evaluate factors affecting their measurement in hair. In particular, I suggest that degradation may occur in museum specimens over time, as demonstrated by lower hair CORT in earlier specimens compared to more recently collected deer mice on Vancouver Island. Hair hormone analysis from museum specimens could be accompanied by chemical analysis of hairs to detect presence of arsenic, mercury, or other compounds that may affect the stability and measurement of GC levels. I suggest that a possible mechanism of degradation of hair GCs in museum specimens is exposure to light (Wester et al. 2016), which likely varies based on conditions of collection, preparation, and storage. Hair GC levels in collections of other species could be evaluated to determine if the trend that I observed was truly caused by degradation, or was due to long-term change in CORT levels of wild deer mice.
While there have been island-mainland comparisons of morphology and behaviour across various taxa (Raia et al. 2010; Sale and Arnould 2013; Harper and Rutherford 2016), comparisons of stress physiology have so far been limited to studies in birds (Clinchy et al. 2004; Müller et al. 2007). By comparing the results of Chapters 2 and 3, I suggest that the stress physiology of rodents may also change in response to island life, however a sufficient degree of isolation is required for differences in the community structure of islands to affect GC levels. Adler and Levins (1994) noted that although there was an explosion of studies on island rodents during the 1970s, research had slowed in the area due to a perception that the relevant patterns had been identified. Using rodents as model species for island-mainland comparisons should continue to provide opportunities to evaluate factors affecting the morphology, behaviour, and physiology of island wildlife.

**LITERATURE CITED**


APPENDIX 1: SUPPLEMENTAL DATA FROM TRAPPING WHITE-FOOTED MICE (*PEROMYSCUS LEUCOPUS*) AND OTHER SMALL MAMMALS IN THE THOUSAND ISLANDS NATIONAL PARK, ONTARIO

Age group classification

Different criteria have been used to classify *Peromyscus* into age groups. Adler and Tamarin (1984) classified white-footed mice ≤ 13 g as juveniles, those 14-16 g as subadults and individuals ≥ 17 g as adults. Drost and Fellers (1991) separated *P. maniculatus* into age groups based on pelage. Groupings based on mass can lead one to misclassify individuals that are visibly sexually mature as subadults or juveniles, while individuals that are visibly sexually mature never display the recognizable grey juvenile pelage (pers. observation). Therefore, I used pelage to classify individuals into age groups for those mice that had coat colour records. White-footed mice that were grey were considered juveniles, those that were brown or were moulting (either from grey to brown or brown to reddish-brown) were considered subadults and those that were reddish-brown were considered adults (Drost and Fellers 1991).

To classify white-footed mice for which I lacked coat colour observations (n = 161), I generated a density histogram of those individuals for which coat colour observations were made on primary capture in 2016 (n = 182) to create distributions of coat colour based on body mass (Figure A1.1). I designated the age groups based on where the body mass frequency distributions crossed; juveniles were considered ≤14 g, subadults were classified as 15-19 g and adults were considered ≥20 g (Figure A1.1). These classifications were then applied to the white-footed mice lacking coat colour descriptions.
Figure A1.1. Density distributions of white-footed mice (n = 182) with grey (juvenile), brown (subadult), and reddish-brown (adult) coats.

Table A1.1. Summary of trapping success of white-footed mice per site during each trapping period in the Thousand Islands National Park. Abbreviations provided below.
<table>
<thead>
<tr>
<th>Site</th>
<th>Summer 2015</th>
<th>Spring 2016</th>
<th>Summer 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tn</td>
<td>Trip</td>
<td>Ind</td>
</tr>
<tr>
<td>Aubrey Island</td>
<td>289</td>
<td>112</td>
<td>2</td>
</tr>
<tr>
<td>Beau Rivage Island</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Camelot Island</td>
<td>147</td>
<td>82</td>
<td>2</td>
</tr>
<tr>
<td>Constance Island</td>
<td>50</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Georgina Island</td>
<td>123</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Grenadier Island</td>
<td>100</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>Hill Island</td>
<td>98</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Lindsay Island</td>
<td>98</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>Mermaid Island</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>McDonald Island</td>
<td>98</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Thwartway Island</td>
<td>147</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>1150</td>
<td>295</td>
<td>155</td>
</tr>
<tr>
<td>Escot Property</td>
<td>98</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Jones Creek1</td>
<td>120</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Jones Creek2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Landon Bay</td>
<td>196</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>Mallorytown</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>414</td>
<td>39</td>
<td>48</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1564</td>
<td>334</td>
<td>203</td>
</tr>
</tbody>
</table>

* Tn – total number of trap nights during trapping session, Trip – total number of disturbed traps during trapping session, Ind – number of individually tagged/identified white-footed mice caught during trapping sessions, and Capt – total number of white-footed mice captured during trapping session.
Table A1.2. Number of individuals of each species trapped at locations in the Thousand Islands National Park during 2015-2016. Species abbreviations are provided, however flying squirrels (*Glaucomys* spp.), shrews of the genus *Sorex*, and weasels (*Mustela* spp.) were identified only to genus.

<table>
<thead>
<tr>
<th>Site</th>
<th>Pl</th>
<th>Mp</th>
<th>Ts</th>
<th>Th</th>
<th>Glaucomys</th>
<th>Bb</th>
<th>Sorex</th>
<th>Mustela</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aubrey Island</td>
<td>2</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beau Rivage Island</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Constance Island</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Escot Property</td>
<td>45</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jones Creek1</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jones Creek2</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Landon Bay</td>
<td>18</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mallorytown</td>
<td>21</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>408</td>
<td>28</td>
<td>17</td>
<td>2</td>
<td>4</td>
<td>23</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: White footed mice (*Pl – Peromyscus leucopus*), meadow voles (*Mp – Microtus pennsylvanicus*), eastern chipmunks (*Ts – Tamias striatus*), red squirrel (*Th - Tamiasciurus hudsonicus*), and short-tailed shrews (*Bb - Blarina brevicauda*).
Figure A1.2. Hair corticosterone values (ng/g) of white-footed mice (n = 10) that were recaptured during different trapping periods in the Thousand Islands National Park. Of 10 individuals with multiple hair CORT values the hair CORT profile of six individuals either changed little or increased slightly. Hair CORT of two white-footed mice decreased between sampling periods, and two individuals increased by 400-500%.
APPENDIX 2: ENZYME-IMMUNOASSAY VALIDATION FOR HAIR AND FECAL CORTICOSTERONE OF WHITE-FOOTED MICE (*PEROMYSCUS LEUCOPUS*)

**Figure A2.1.** There was a significant relationship (p < 0.01) between the amount of antibody bound to corticosterone in extractions of hair and feces of white-footed mice (*Peromyscus leucopus*) and standard solutions from synthetic stock for both hair corticosterone (A) and fecal corticosterone and its metabolites (B).
Figure A2.2. Recovery of exogenous corticosterone from white-footed mouse 
(Peromyscus leucopus) hair (A) and fecal (B) extracts, each demonstrating a significant 
relationship between the amounts of corticosterone recovered from spiked samples with 
varying amounts of corticosterone added ($p < 0.01$).
INTRODUCTION

Glucocorticoid (GC) concentrations in hair are thought to provide an integrative measure of GC levels over the course of time required for the sampled length of hair to grow, which may be weeks or months, depending on the species (Sheriff et al. 2011). Hair hormone analysis is a relatively recently developed method of quantifying GCs in wildlife, and concerns have been raised regarding the influence of external factors on its measurement (Salaberger et al. 2016; Wester et al. 2016), and the evidence of local production of GCs by the hair follicle (Sharpley et al. 2009; Keckeis et al. 2012). Nonetheless, hair hormone analysis is a promising method of evaluating stress in wildlife, and has demonstrated biological relationships in a variety of species (Bryan et al. 2015; Fourie et al. 2015b; Meise et al. 2016). Adrenocorticotropic hormone (ACTH) challenges have demonstrated the validity of hair as a biomarker of HPA activity in several species, including wild eastern chipmunks (Tamias striatus; Mastromonaco et al. 2014), Canada lynx (Lynx canadensis; Terwissen et al. 2013) and laboratory rats (Scorrano et al. 2015). The failure of an ACTH challenge to validate hair cortisol as a biomarker of stress in caribou (Rangifer tarandus; Ashley et al. 2011) shows the importance of validation studies when applying a method of hormone analysis to a new species.

Aoife Reilly, an undergraduate thesis student in our lab, recently performed an ACTH challenge on wild-caught white-footed mice that were housed in captivity in the Animal Care Facility at Trent University (Reilly 2017). The purpose of this experiment
was to demonstrate that white-footed mice injected with ACTH, which stimulates corticosterone (CORT) secretion, would have higher hair CORT levels after 6-8 weeks of injections compared to a saline injected control group. Reilly (2017) reported that there was no difference in final hair CORT values after the treatments, and attributed the negative results to use of the fast-acting drug used as the ACTH treatment, as opposed to a slow-releasing intramuscular ACTH drug that have been successfully used to simulate chronic stress in other species (Terwissen et al. 2013; Mastromonaco et al. 2014). Although this experiment failed to demonstrate that hair CORT becomes elevated with increased HPA activity in white-footed mice, we feel this relationship has been well established in other rodent species (Mastromonaco et al. 2014; Scorrano et al. 2015) and other mammals (Davenport et al. 2006; Terwissen et al. 2013). We feel that the negative results we found for white-footed mice show more about the way stress was simulated than the efficacy of hair CORT as a biomarker of stress in white-footed mice.

In her thesis, Reilly did not test the relationship between pre- and post-treatment hair CORT for individual white-footed mice, but I will present an analysis of those data here to demonstrate that individuals displayed consistent hair CORT levels over time. I also provide a brief overview of the methods she used, for completeness (modified from Reilly 2017).

**METHODS**

Briefly, white-footed mice were randomly assigned to two treatment groups. An ACTH injection group (n = 10; 100 µL IP liquid ACTH, Sigma-Aldrich®, ACTH Fragment 1-24) was compared to a saline injected control group (n = 10; 100 µL IP saline
solution, Sigma-Aldrich®, 0.9% buffered NaCl solution, pH 7.4), both of which had equal numbers of individual males and females. Initial hair samples were collected, and then the white-footed mice received injections twice a week for 6-8 weeks. The control group grew their hair back more quickly than the ACTH group, three of which never grew their hair back entirely. To determine if initial hair CORT influenced final hair CORT, I ran an analysis of covariance (ANCOVA) using data from Reilly (2017), with final hair CORT as the response variable, sex and treatment as factors and initial hair CORT as a covariate. After this test showed that there were no differences between treatment groups or sexes, as reported by Reilly (2017), I ran a linear regression between pre- and post-treatment scores. In both tests, hair CORT values were ln-transformed to improve normality of the residuals.

RESULTS AND DISCUSSION

Reilly (2017) reported that initial hair CORT levels differed between treatment groups by chance ($F_{1,17} = 5.822, p = 0.027$), but there was no difference between final hair CORT levels between treatment groups ($F_{1,17} = 1.909, p = 0.184$; Figure A3.1A). I found, however, that there was a significant relationship between pre- and post-treatment hair CORT ($F_{1,14} = 10.44, p < 0.01, R^2 = 0.427$; Figure A3.1B), suggesting that individuals maintained similar individual hair CORT profiles over time.
Figure A3.1. White-footed mice (*Peromyscus leucopus*) injected with adrenocorticotropic hormone (ACTH; n = 7) did not have higher final hair corticosterone (CORT) than saline injected mice (n = 9) after 6-8 weeks (A; redrawn as presented in Reilly 2017), but final hair CORT was significantly correlated with initial hair CORT (B; $p < 0.01; R^2 = 0.427$; generated using data from Reilly 2017).

LITERATURE CITED


Reilly, A. 2017. Validation of the use of hair corticosterone to measure chronic stress in white-footed mice (Peromyscus leucopus). Honours Thesis. Trent University.


