

The role of animal personality in the pace-of-life of coexisting rodents

by

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Abstract

The pace-of-life syndrome hypothesis (POLS) predicts that life-history, behaviour, and physiology correlate along a fast to slow continuum. Relationships between POLS domains evolve in response to natural selection and energetic trade-offs at different phylogenetic levels. Access to resources is dependent on movement within a home-range, and differences in movement strategies should arise to accommodate competition among sympatric species and between conspecifics. I examined behaviours relating to home-range movement among sympatric rodents and between sexes. I tested two hypotheses: (1) sympatric rodents will express differences in movement behaviours to accommodate resource competition; and (2) differences in behaviour and physiology will arise between sexes because of differences in reproductive costs. I found differences in behaviour among species, and a uniform expression of traits relating to movement within a home-range between sexes. My results help to understand differences in animal personality, movement patterns and sex-specific strategies in rodents.

Keywords

Pace-of-Life, Personality, Life-history, Rodents, Stress, Sexual differences

Co-Authorship Statement

The information presented in this thesis is the result of a collaborative initiative between authors. Dr. Albrecht Schulte-Hostedde and Dr. Jeff Bowman have helped with the conceptualization and methodology formulated in chapters one and two and are both editors of any final manuscript. The laboratory methods used in chapter two are derived from methodology in collaboration with Dr. Gabriela Mastromonaco, Toronto Zoo. Methods and analyses of information presented in chapter two are a collaborative work between myself and Dr. Gabriela Mastromonaco.

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Table of Contents

Abstract	iii
Co-authorship statement	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	viii
List of Figures	x
List of Appendices	xiii
General introduction	1
Literature Cited.....	10

Chapter One – Exploratory and risk-taking behaviours in coexisting rodent species

Abstract.....	17
Introduction.....	18
Methods.....	22
Results.....	26
Discussion.....	28
Literature Cited.....	36
Tables and Figures.....	44

Chapter Two – Sexual differences in the pace-of-life of rodents

Abstract.....	53
Introduction.....	54
Methods.....	60
Results.....	63
Discussion.....	65
Literature Cited.....	71
Tables and Figures.....	79
General Discussion	93
Literature Cited.....	96

Appendix A	98
Appendix B	99
Appendix C	100
Appendix D	102

List of Tables

Chapter One

Table 1.1: The results from the repeatability analysis for the (Bag Test) BT and (Open Field Test) OFT measuring Docility and Exploration respectively. Results highlighted in bold were determined to be repeatable. All estimates were calculated using mixed effects model with age, sex, and reproductive condition as a fixed effect and individual ID as a random effect. 95% confidence intervals were calculated using parametric bootstrapping.....44

Table 1.2: Kruskal-Wallis results for each separated species (DM = Deer Mice, RBV = Red-Back Voles, WJM = Woodland Jumping Mice) for the impact of categorical predictor variables on total-docile behaviour. Docile behaviour includes any time that the individual spent static during the Bag Test.....46

Table 1.3 Kruskal-Wallis results for each separated species (DM = Deer Mice, RBV = Red-Back Voles, WJM = Woodland Jumping Mice) for the impact of categorical predictor variables on total non-docile behaviour. Non-docile behaviour includes any time the individual spent moving, trying to escape, or grooming during the Bag Test. * Denotes significant ($P < 0.05$) values..... 47

Table 1.4: Summary of top best fit linear mixed effects models predicting the relationships between personality (docility or exploration) pooled across species (n = 202 Bag Tests and 147 Open Field Tests)48

Chapter Two

Table 2.1: Summary for log₁₀-transformed values of fecal glucocorticoids and expression of docility in all three species (DM = Deer Mice, RBV = Red-Backed Voles, and WJM = Woodland Jumping Mouse, DM, n = 104; RBV, n = 52; WJM, n = 15). Bolded variables are those with $P < 0.05$ and are considered significant.....79

Table 2.2: The linear regression statistics for the relationship between docility, measured through the Handling Bag Tests (BT) and log₁₀ transformed fecal glucocorticoids in Deer Mice (n = 104)

and Woodland Jumping Mice (n = 15), using sex, age class (adult, sub-adult and juvenile), reproductive condition, and body mass as random effects.....81

Table 2.3: Summary of log₁₀-transformed values of fecal corticosterone/cortisol metabolites and total expression of explorative behaviour in all three species (DM, n = 47; RBV n = 27; WJM, n = 10), bolded values are P < 0.05 and are considered significant.....83

Table 2.4: Linear regression statistics for models examining the relationship between total exploration time and log₁₀ transformed fecal glucocorticoids in Deer Mice and Red-Backed Voles. Significant values (P < 0.05) are shown in bold.....85

List of Figures

Chapter One

Figure 1.1 Scatter plots showing the relationship between total time spent expressing docile and exploratory behaviours in Deer Mice ($r = -0.018$, $P = 0.26$), Red-Backed Voles ($r = -0.437$, $P = 0.090$), and Woodland Jumping Mice ($r = -0.2$, $P = 0.92$). Each data point shows the first Bag Test (BT) and Open Field Test (OFT) an individual performed. Negative correlation is depicted with the trendline, and shading represents the 95% confidence interval.....50

Figure 1.2 Boxplot showing the relationship between total docile time (static behaviour) during the Bag Test (BT) comparing all three species, DM = Deer Mice, RBV = Red-Backed Vole, WJM = Woodland Jumping Mice, across all reproductive condition (SCR = Scrotal, NSCR = non-scrotal, NR = non-reproductive, PREG = pregnant, LACT = lactating) using individual ID as a random effect. Whiskers represent standard error; box lines represent the median time spent motionless across samples and faded jitters represent the distribution of data.....51

Figure 1.3 Box plots showing the relationship between exploratory behaviour (moving) during the Open Field Test (OFT) for each species (DM = Deer Mice, RBV = Red-Backed Vole, WJM = Woodland Jumping Mice) for each reproductive phase (SCR = scrotal, NSCR = non-scrotal, PREG = Pregnant, LACT = Lactating, and NR = non=reproductive) using individual ID as a random effect. Whiskers represent standard error, box lines represent the median time spent expressing exploratory behaviours across samples, and faded jitters represent the distribution of individuals.....52

Figure 1.4 A conceptual model to depict the placement of Deer Mice, Red-Backed Voles and Woodland Jumping Mice along the fast-slow continuum according to the pace-of-life syndrome hypothesis. Also shown is an approximate home range movement or dispersal distance to depict how coexisting species may benefit from foraging in alternative sites. Faster species may benefit from dispersing to greater distances, or having increased movement within a home-range while species on the slower end of the spectrum may benefit from more localized foraging.....53

Chapter Two

Figure 2.1: Relationship between \log_{10} -transformed values of fecal metabolites in Deer Mice (total n = 105, males = 61, females = 45) Woodland Jumping Mice (total n = 16, males = 6, females = 10) and Red-Backed Voles (total n = 53, males = 31, female = 23) and docility. Individuals showed a negative relationship between docile behaviour and basal \log_{10} FCM in Deer Mice and male Jumping Mice, however Red-Backed Voles showed a positive relationship. Data points are jittered and represent individual test (females = black, circle, males = grey, triangle). 95% confidence intervals are shown by shading.87

Figure 2.2: The relationship between \log_{10} -transformed fecal metabolites and total exploration behaviour in Deer Mice (total n = 47, males = 29, females = 19; Red-Backed Voles total n = 28, males = 18, females = 10; woodland jumping mice total n = 11, males = 5, females = 6). Individuals showed a negative relationship in Jumping Mice, Red Backed Voles, and male Deer Mice, however a positive relationship in female Deer Mice. Data points are shown by jitters (Black circle for females, and grey triangles for males). Shading represents 95% confidence interval.88

Figure 2.3: The relationship between \log_{10} -transformed values of fecal metabolites and docility in pregnant Red-Backed Voles. Individuals that underwent a Handling Bag Test are shown via black dots, and 95% confidence interval extracted through linear mixed effects model is represented through shading. Red-Backed Voles show a positive relationship between docility and basal fecal metabolite concentrations, where individuals with a higher concentration of glucocorticoids express higher levels of immobilization, or docile behaviour consistent with the POLS hypothesis.89

Figure 2.4: The relationship between \log_{10} -transformed fecal metabolites and time spent expressing exploratory behaviours including increased movement during the Open Field Test (OFT) for Deer Mice classified as the sub-adult age class. Shown are 95% confidence intervals (shading) calculated from mixed linear models.....90

Figure 2.5: The relationship between \log_{10} -transformed fecal metabolites in Deer Mice (Top), Red-Backed Voles (Middle), and Woodland Jumping Mice (Bottom) across individual ID number. Shown are the median (black line) interquartile range (box) and minimum/maximum values (bars). All species show considerable variation in fecal metabolite concentration.91

Figure 2.6: A series of boxplots showing the total basal fecal glucocorticoid metabolites in Deer Mice (panel A), Red-Backed Voles (panel B) and Woodland Jumping Mice (panel c) based on reproductive status (NSCR = non-scrotal, SCR = scrotal for males and PREG = pregnant, LACT = lactating, PERF = perforate, or NR = non-reproductive for females). Dark lines represent the mean value of fecal metabolite (ng/g) while whiskers represent the standard deviation.92

List of Appendices

Appendix A: Common actions for all rodents in the Open Field Test (OFT). Actions refer to what an individual may do, while the definition is the interpretation for the observer. The code column refers to the code used in all statistical analysis to attribute behaviour to any given personality type. For the OFT, any behaviours marked as moved were counted as total exploration time, while freezing and hiding behaviours were considered non-exploratory...98

Appendix B: Ethogram for possible actions of all tested individuals during the one-minute Bag Test (BT), action refers to individual movements displayed by animals with the definition used by the observer to mark each action. The code refers to statistical analysis where behaviours are summed together to generate a cause of personality. Escape, movement, and grooming behaviours were considered non-docile while freezing behaviour was considered docile...99

Appendix C: The total sample size for each age category, sex, and reproductive status of all three species used in the statistical analyses of this study. Deer Mice (DM), Red-Backed Voles (RBV) and Woodland Humping Mice (WJM) are shown as a total sample size (n) for each group.....100

Appendix D: The relationships between total fecal glucocorticoid metabolites and % antibody binding compared to a standard curve generated using (Soper, 2021). Shown is the relationship for Deer Mice, Red-Backed Voles and Woodland Jumping Mice for corticosterone or cortisol to validate fecal metabolites.....102

General Introduction

An introduction to the pace-of-life syndrome hypothesis

Life history theory is an analytical framework that hypothesizes that genetic and phenotypic variation in organisms is mediated through natural selection and trade-offs in resource allocation and acquisition (Dammhahn et al., 2018; Roff, 1993). The pace-of-life Syndrome hypothesis (POLS) expands on life history theory and suggests that species coevolve physiological and behavioural traits to accommodate life-history strategies (Ricklefs and Wikelski, 2002; Wikelski et al., 2003). The Pace-of-life (POL) for an organism includes a suite of life-history, behavioural and physiological traits, while the syndrome refers to the correlation among traits (Dammhahn et al., 2018; Sih et al., 2004). The POLS is modelled along a fast-slow continuum predicting that strategies among species, populations or between individuals should favour strategies corresponding to one end (Réale et al., 2010). For example, slower species generally have a longer lifespan, reach sexual maturity later, and produce fewer offspring. Conversely, faster species reach sexual maturity sooner, produce more offspring per reproductive cycle, and have a shorter lifespan (Réale et al., 2010).

While the POLS hypothesis aims to evaluate patterns in the development of life-history traits and physiological phenotypes, there has been an increased emphasis on the addition and value of behavioural strategies as a component (Réale et al., 2007). Consistent differences in behaviours between individuals are commonly referred to as an animals personality (Réale et al., 2010a). Given that personality drives how species interact with conspecifics and more broadly, different components of an ecosystem, research has pivoted to evaluating the relationships between behaviour and POLS traits (Réale et al., 2010b). Indeed, animal personality has been

shown to influence ecological variables relating to seed distribution and land use (Boone et al., 2022; Brehm et al., 2019). Personality differences may also influence interspecific interactions among coexisting species and may help facilitate ecological trade-offs in environments where species inhabit similar ecological niches (Morris and Palmer, 2023; Sobral et al., 2023).

Personality types that involve how individuals react to energetically stressful situations are also sometimes referred to as an individual coping style (Koolhaas et al., 1999). Behavioural predictions within the POLS hypothesis suggest that behaviours are repeatable and can be modelled along the fast-slow continuum relating to proactive fast-paced coping styles or reactive slow-paced coping styles (Réale et al., 2010b). For example, increased risk-taking behaviour, including increased exploration and reduced docility, are associated with a reduced investment in individual survivorship (Hall et al., 2015). Given the importance of behaviour and personality in shaping environmental interactions, behaviour remains an integral component of the POLS hypothesis.

There is a growing literature that aims to dissect and expand on the predictions of the POLS hypothesis (Bielby et al., 2007; Dammhahn et al., 2018; Royauté et al., 2018; Mathoth and Frankhuit, 2018) with conflicting evidence to support predictions within populations or between individuals. Further, there remains mixed support for how behavioural traits correlate with other POLS domains. Substantial evidence shows variation in POLS traits can be sexually dimorphic (Hämäläinen et al., 2018; Tarka et al., 2018). Interestingly, it has been shown that in some vertebrates, females show correlations in POLS traits that would be the opposite of the correlation predicted by the current POLS hypothesis (Hämäläinen et al., 2018). There is also empirical evidence to support the POLS across an array of environments and factors, including thermal physiology (Goulet et al., 2017), latitudinal gradient (Debecker and Stoks, 2019),

climate (Lovegrove, 2003), and environmental productivity (Mueller and Diamond 2001). Despite this, empirical evidence to support the POLS remains mixed (Dammhahn et al., 2018), specifically the integration of behaviour into the POLS model does not always appear to follow initial assumptions (Royauté et al., 2018). Therefore, further investigation into specific syndromes and the relationships between behaviours and various ecological interactions remain important to fill knowledge gaps and for the future development of POLS research.

Behavioural syndromes and testing

The relationships between behaviour and other POLS characteristics are complex with few common trends currently reported in literature (Dammhahn et al., 2018). Because behaviour can facilitate ecological trade-offs (Réale et al., 2010b), it is important to understand how behavioural syndromes arise within species. There is evidence to support behavioural relationships with life-history or physiology across an array of taxa. For example, relationships between boldness and metabolic rate (Binder et al., 2016), proactive risk-taking behaviour and growth rate (Damsgård et al., 2019), immunological function and reproductive success (Monceau et al., 2017) and reactive behaviour and glucocorticoid concentration (Carbillet et al., 2022) have all been observed in a variety of different ecosystems and taxa. Despite some evidence supporting the POLS hypothesis and behaviour, evidence remains mixed, and the integration of other ecological components remains necessary to better understand the relationships between behaviour and various physiological phenotypes, and life-history strategies.

Personalities are repeatable observations of behaviours that may define an individual's place along the fast-slow continuum. Réale et al., (2007) proposed five categories for personality traits that model docility, exploration, activity, and social interactions, including sociability and

aggression. Docile interactions measure how an individual reacts to potentially risky yet non-novel situations and are sometimes noted as a concept of “fear” or “anxiety” especially in the context of animal to human interactions (Boissy and Bouissou, 1995; Finkemeier et al., 2018; Brehm et al., 2020). A Handling Bag Test, where an individual is left in the presence of an observer for a specific amount of time, is commonly used as a measure of docility under the assumption that an individual perceives the observer as a potential predator (Martin and Réale, 2008). Exploration and activity are personalities where exploration may measure movement within novel environments, and activity may measure locomotion and energy expenditure (Réale et al., 2007). Movement behaviours are typically measured through Open Field Tests or novel environment tests that measure the likelihood of an individual to engage or move around a particular environment (Gould et al., 2009; Hall, 1934; Wilson et al., 1976). Exploration-avoidance or activity may also be associated with boldness, or the likelihood of an individual to engage a novel environment (Réale et al., 2007). Dispersal is the primary mechanism to mitigate inter and intraspecific resource competition (Waser, 1985). However, movement within a home-range can also mitigate intra and interspecific competition for resources, particularly in sympatric species (Pérez-Barbería et al., 2015). Since exploration and docility measure how an individual will engage and move around an environment, these personalities can be used to predict the frequency of an individual or species to move or disperse within a given area. More explorative and less docile personalities are associated with a greater tendency to engage in potentially risky situations, and a greater likelihood of engaging in movement within a given area. Therefore, a greater expression of exploration and a lower expression of docility should indicate an individual or species is more likely to engage and move around a given area at a greater frequency than a more docile and less explorative counterpart.

Study system: Coexisting rodents

Deer Mice (*Peromyscus maniculatus*), Red-Backed Voles (*Clethrionomys gapperi*), and Woodland Jumping Mice (*Napaeozapus insignis*) are three rodent species that inhabit Algonquin Provincial Park, Ontario, Canada. There is a significant relationship between population density and variation in behavioural tendencies observed in small mammal populations (Fryxell et al., 1998). Indeed, habitat usage and environmental interactions may be influenced by interspecific competition (Schulte-Hostedde and Brooks, 1997; Eccard and Ylönenm 2003; Braz et al., 2020). Given the significance of animal personality in shaping the ecosystem (Hunter Jr et al., 2022), evaluating interspecific interactions may help provide insight into more broad questions concerning evolution and how coexisting species interact within an ecosystem. Significant variation in behavioural syndromes is known to occur across coexisting species (Morris and Palmer, 2023). Since small mammals in Algonquin Park have been shown to demonstrate reliance on similar diets, habitat, and other ecosystem characteristics, it remains a reasonable assumption that behavioural syndromes should evolve to promote coexistence. For example, coexisting species that are reliant on similar diets may experience a trade-off in investment strategies to obtain adequate resources. Despite the relevance of competition in shaping environmental trade-offs (Wauters et al., 2019), there remains little investigation of how coexistence shapes species interactions within the environment. While there is ample empirical evidence to suggest a relationship between small mammal personality and shaping plant community assemblages (Schlägel et al., 2020; Brehm et al., 2019; Boone et al., 2022), how coexistence influences the expression and development of different personality phenotypes among species remains poorly understood. Further investigation into the complex array of morphological, physiological, and behavioural phenotypes that develop amongst coexisting

species may help further understand how animal communities influence and persist within an ecosystem. Similarly, metabolic costs, often denoted as energetic stress (Parsons, 2005), are associated with resource acquisition, competition and various species' interactions, and further study may help to better understand the complex integration of behaviour and POLS traits.

Fecal glucocorticoids as a non-invasive proxy of stress

Fecal samples are a robust non-invasive method for measuring glucocorticoid metabolites in captive and wild populations (Harper and Austad, 2000; Lane, 2006; Palme, 2012). Unlike plasma glucocorticoid samples, fecal sampling allows for periodic measurements of glucocorticoids that may be used to measure stress reactivity (Millspaugh et al., 2002; Palme, 2012). Glucocorticoids, including corticosterone and cortisol are released from the hypothalamic-pituitary-adrenal glands (HPA axis) to facilitate the regulation of homeostasis. While glucocorticoids have a greater function than just acting as “stress hormones”, glucocorticoids can be used as a proxy for measuring the energetic stress on an individual in a given environmental context (MacDougall-Shackleton et al., 2019; Palme et al., 2019). Because hormone activity can be subject to a variety of confounding factors, including diet and metabolism, and is species-dependent, it is recommended that a fecal glucocorticoid assay must be validated for each unique species (Palme, 2019; Touma and Palme 2005). Enzyme validations of corticosterone exist for Deer Mice (Eleftheriou et al., 2020) and the Red-Backed Vole (Harper and Austad 2000) but not yet for Woodland-Jumping Mice.

The POLS hypothesis predicts that individuals adjusted towards the faster side of the fast-slow continuum should express a lower HPA axis reactivity (Réale et al., 2010). Because long-term exposure to elevated glucocorticoid concentrations can ultimately be harmful

(SaPOLSky et al., 1990), fast-paced proactive species that encounter a greater occurrence of stressful stimuli should benefit from being more accustomed to novel situations, thus having a lower necessity for reactivity from the HPA-axis (Koolhaas et al., 1999). Across species, glucocorticoid concentrations may be a proxy for phenotypic adaptations involving behaviour and various movement strategies within the ecosystem. For example, species that invest more in movement within a home range should be exposed to a greater number of novel stimuli, including potential predators and pathogens (Bordes et al., 2009). Species that are more frequently exposed to novel stimuli should benefit from a lower stress reactivity and should have comparatively lower concentrations of glucocorticoids compared to less explorative species (Careau et al., 2009). Between individuals of the same species, glucocorticoids measurements can be used to predict trade-offs in resource allocation given varied reproductive roles. Because males and females often have different reproductive roles in an ecosystem, namely where males often invest more in movement and mate acquisition, while females invest in the care and development of young, differences in energetic cost and glucocorticoid concentration should arise between sexes.

Sexual differences in the pace-of-life hypothesis

Because males and females are subject to different energetic pressures during reproduction, the POLS hypothesis predicts sex-specific differences in the directionality of, or position of traits, along the fast to slow continuum (Hämäläinen et al., 2018; Immonen et al., 2018; Tarka et al., 2018; Moschilla et al., 2019). Sex differences in life history are dependent on the trade off between current and future reproductive potential as an ultimate result of anisogamy (Bateman, 1948; Lehtonen et al., 2016). Energetic costs of reproduction therefore differ between

males and females. Anisogamy suggests that differences in the energetic cost of gamete production should arise between males and females, such that males occupy a faster POLS given a higher necessity for mate acquisition, and lower energetic investment in reproduction compared to females (Clutton-Brock and park, 1992). However, in mating systems where female reproductive rate is equal to or exceeds the reproductive rate of males it is possible for the expression of uniform traits, or a sexual role reversal of POLS traits (Trivers 1972; Eens and Pinxten, 2000, Biro et al., 2014). In systems where a uniform POLS exists, there may be no sex specific selection along the fast to slow continuum, or between sexes there may be the same directionality of selection, albeit along different positions of the continuum (Hämäläinen et al., 2018). While species with a high level of POLS yamy often experience different reproductive costs between sexes, ultimately leading to sexual selection and a divergence of POLS traits (Jensen et al., 2008), in species with a low investment in parental care it remains possible that energetic cost of reproduction are similar between sexes, resulting in a convergent POLS (Hämäläinen et al., 2018, Immonen et al., 2018). It is also worth considering that sexual differences may be absent in the POLS of some species, where differences in energetic costs may have negligible impact on the directionality or position of POLS traits (Debecker et al., 2016). Because sexual differences can influence how individuals within a population interact with conspecifics and the surrounding environment, sex-specific variation in POLS traits are an important consideration for research on animal personality.

Project overview

To understand the development of different POLS phenotypes, I used three species of coexisting rodents from Algonquin Provincial Park. I aimed to (1) identify behavioural phenotypes across coexisting species; and (2) investigate potential relationships in behaviour and physiology between males and females of the same species: First, I used standardized behavioural tests, including a Handling Bag Test (Martin and Réale, 2008) and an Open Field Test (Carter et al., 2013), to model known personality types using an expert-based analysis approach (Mazzamuto et al., 2018). Each test aimed to model personality traits relating to dispersal and movement within an individual's home range, evaluating docility and exploration. I also collected fecal samples to measure total fecal glucocorticoid metabolites as a proxy for energetic stress (Palme et al., 2019). Given the importance of personality and POLS traits in shaping interactions between species and the ecosystem, this effort will help fill potential knowledge gaps within pace-of-life theory. Namely, while the existence of POLS is known to occur across species, there is little understood about how coexisting species evolve and how different phenotypes may play a role in shaping the ecosystem. Secondly, while sex-specific POLS traits are a rapidly increasing topic of interest (Moschilla et al., 2019), how energetic stress and personality may vary between males and females, and what the larger implications of these relationships may be, remain poorly understood.

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Chapter One

Exploratory and risk-taking-behaviours in coexisting rodent species

Abstract

There has been an increasing interest in modelling the influence of animal personality on species interactions within ecosystems. Personality types associated with dispersal and movement within a home range, including docility and exploration, have been shown to influence an array of environmental variables including seed dispersal and habitat availability. However, despite growing interest, little information is available to model personality phenotypes among coexisting species. Since coexisting or sympatric species often compete for the same resources, movement patterns can help mitigate the impact of intra and interspecific competition. I used a series of standardized behavioural tests with three species of coexisting rodents in Algonquin Provincial Park, Ontario, Canada to measure animal personality and movement patterns. To determine exploratory and docile personalities, I modelled plastic changes in behaviours within species and phenotypic variation in behavioural strategies among species. I show empirical evidence to support differences in personality phenotypes in coexisting species and consider the importance of alternative personality strategies in shaping community dynamics.

Introduction

Genetic and phenotypic variation in the life-history, behaviour and physiology of an organism are mediated through natural selection and ecological trade-offs in resource acquisition and allocation (Roff and Fairbairn, 2007). The pace-of-life syndrome hypothesis (POLS) is an analytical framework that suggests the life history, behaviour and physiology of an organism exhibit predictable correlations (Reale et al., 2010; Dammhahn et al., 2018). The patterns predicted by the POLS hypothesis follow a fast-slow continuum, where short-lived organisms with a high reproductive output demonstrate proactive or “fast” behavioural and physiological phenotypes. In contrast, long-lived organisms that have fewer offspring but invest more in the care of individual young demonstrate more reactive or “slow” phenotypes (Réale et al., 2010). While there is empirical evidence to support differences among species along the fast-slow continuum (Ricklefs and Wikelski, 2002), evidence to support the POLS hypothesis between individuals remains mixed, specifically, how similar environmental factors may influence the expression of alternative behavioural strategies within a population is seldom documented (Bielby et al., 2007; Dammhahn et al., 2018; Royaute et al., 2018; Hamalainen et al., 2021). By further investigating differences in animal personality and behaviour, we may gain insight into how species interact within an ecosystem.

Reproduction is an energetically expensive and potentially risky investment, and rodent populations may invest in seasonal or continuous breeding strategies to accommodate energetic costs (Bergeron et al., 2011; Bronson and Perrigo, 1987). For seasonally breeding rodents, both males and females experience an increase in hormone levels that facilitate mate acquisition and selection during the breeding season (Kavaliers and Choleris, 2017). Given the associations

predicted by the POLS hypothesis, variation in physiology during active breeding periods should result in a shift in behaviours associated with mate acquisition. Deer Mice (*Peromyscus maniculatus*), Red-Backed Voles (*Clethrionomys gapperi*), and Woodland Jumping Mice (*Napaeozapus insignis*) are three rodent species that often inhabit a similar ecological niche (Fryxell et al., 1998). Each of these three species displays polyandrous mating systems and have an active breeding season from May through August during which individuals may breed several times, although Deer Mice may also breed during the winter if there are adequate temperatures and resources available (Wolf and Sherman, 2008). Despite similarities, each species has varying life history traits that may be modelled along the proactive versus reactive fast-slow continuum outlined by the POLS hypothesis. Deer Mice generally display proactive behaviours (Careau et al., 2011), have litter sizes averaging 4-6 pups (Maser et al., 1981), and have an average lifespan of 1-2 years (Dice, 1936). Woodland Jumping Mice live longer than voles and deer mice, living up to 4-5 years (Wrigley, 1972). Woodland Jumping Mice also have a lower reproductive output than either Deer Mice or Red-Backed Voles, engaging in only 1-2 breeding events per season. While Jumping Mice have a greater gestation and weaning period than both Deer Mice and Red-Backed Voles, Jumping Mice only produce 2-4 pups per litter (Whitaker and Wrigley, 1972). Red-Backed Voles live 1-2 years and produce litter sizes that range from 4-5 pups, up to 2-3 times per reproductive season (Merritt, 1981). Based on the lifespan and reproductive output of these three species, Deer Mice should express the “fastest” behaviours, while jumping mice should express “slower” behaviours, meanwhile Red-Back Voles may express behavioural traits that are intermediate compared to deer mice and jumping mice.

The POLS of a species is heavily influenced by the evolutionary necessity to reproduce (Healy et al., 2019). Because animal personality is a driving mechanism for determining how

individuals and species react to environmental influences (Réale et al., 2010), it is important to understand how different behavioural strategies arise among species during different parts of the reproductive cycle. A species home range refers to the spatial area where a population or individual may forage for resources or compete for mate selection. Dispersal and movement patterns are the predominant mechanisms to mitigate potential intraspecific competition in mate selection; therefore, reproductive output is heavily influenced by dispersal and movement patterns within a species home range (Perrin and Maxalov, 2000). Evaluating behaviours that are associated with movement during different stages of a species reproductive season may help provide insight into factors mediating the expression of different behavioural strategies. In rodents, docility is considered a measure of how an individual reacts to a potential predatory or risky situation (Boice et al., 1968; Martin and Réale, 2008), while exploration is a measure of the engagement or movement of an individual within the environment (Careau et al., 2009). Therefore, actions that reflect docility and exploration are both indicators of an individual's movement potential (Dingemanse et al., 2003; Réale et al., 2007; Morris 1984; Petelle et al., 2013).

Despite the relevance of personality as a driver for how a species will interact to various components within an ecosystem, there are few and often inconclusive studies evaluating the expression of personality in wild populations (Réale and Dingemanse, 2012). My study aims to evaluate varied behavioural strategies among species during different stages of the reproductive cycle. Similarly, I evaluated how individuals of the same population may express differences in movement behaviour throughout the breeding season. Because each of these three species should benefit from the same correlation of traits, such that less docile individuals explore more, I predicted that behavioural syndromes that influence habitat usage should arise such that more

explorative species and individuals should also express less docile personality traits. Despite variation in microhabitat usage (Schulte-Hostedde, 1997) which also reduces intra-specific competition, populations of these three species often compete for similar habitat and resources when populations occupy the same area (Davidson and Morris, 2001; Dracup et al., 2016). Because these three species may compete for similar resources, each species should occupy a different position along the “fast” to “slow” continuum. I hypothesized that if coexistence promotes alternative behavioural strategies to accommodate intra and interspecific competition, then there should be measurable differences in personality among species. I further predicted that there should be measurable variation in behaviours relating to movement patterns at both the population and species level. If these hypotheses are supported, then there should be observable differences in behavioural strategies among species and between individuals during the active breeding season, such that some species or individuals will be more explorative and less docile. Further among different species, I predicted similar directionality of syndromes at different positions along the predicted “fast”- “slow” continuum. Between individuals of the same species, I predicted that actively breeding males should express a higher rate of proactive behaviours compared to non-scrotal or non-breeding males. Likewise, females that are actively investing in the care or development of young should express more reactive behavioural strategies, given the energetic investment required to facilitate the development and care of offspring (Rémy et al., 2011).

Methods

Animal sampling

Deer Mice, Woodland Jumping Mice and Red-Backed Voles were collected from May-September 2022 under animal handling procedures approved by Laurentian University (protocol #6011106). All animals were sampled from Algonquin Provincial Park, Ontario, Canada (45°54' N, 78°26' W) from across seventeen established traplines (Veitch et al., 2021 but see Fryxell 1998). Sherman traps (H.B. Sherman Traps, Inc., Tallahassee, Florida) were baited bi-weekly with water-soaked sunflower seeds over 3 consecutive nights and were checked at dawn. Because trap confinement may have an impact on individual behaviour (Brehm et al., 2020), each trapline was checked in the same order each morning, and the start time of any behavioural test was recorded to estimate the impact longer confinement may have on behaviour. To measure the potential impact of cumulative testing and handling on behaviour, I maintained a record of the total number of times any individual was captured (denoted as cumulative capture) and the total number of times an individual entered either the (Bag Test) BT or (Open Field Test) OFT throughout the season. Captured individuals were collected from the Sherman traps in a plastic handling bag. Individuals were weighed with a Pesola scale (0.1 g), sexed (male or female), assigned an age class (juvenile, sub-adult, adult) based on weight and identifiable morphological characteristics (Schmidt et al., 2019). Breeding status was assessed in individuals as scrotal or non-scrotal for males, and pregnant, lactating, or non-reproductive for females. All behavioural testing and animal handling took place directly in the field after capture.

Behavioural assays

Captured individuals were subject to either a Handling Bag Test or an Open Field Test each trap day. Handling Bag Tests (BT) are used to measure behaviours associated with animal docility (Martin and Reale 2008), while Open Field Tests (OFT) measure behaviours associated with exploration and activity (Carter et al., 2013).

All behavioural assays were recorded in the field using a camera (Sony HDR-CX405), and behaviours were later analyzed using the recording. The Handling Bag Test was conducted before animals were weighed and measured. Individuals were left in the plastic handling bag for one minute and held at arm's length from the observer. Docility during the Bag Test was measured as the total amount of time an individual spent motionless or static (Martin and Réale, 2008; Appendix A).

The OFT was conducted inside a plastic novel arena (51 x 41 x 74 cm) fitted with an 8.89 cm PVC opening and a mesh barrier on top to prevent an individual from leaving the arena, while still allowing for recording. Arena tests were filmed for a total of five minutes starting from when the individual entered the arena through the PVC pipe opening. Total exploration behaviour was measured as the time an individual spent moving around the arena, while non-exploratory time was measured as time spent static either in the arena, or the entrance (Appendix B). To reduce interference from potential pheromones left behind from the previous test, the arena was cleaned in between each trial using an 80 % vinegar solution and then rinsed with water.

Video processing:

All videos were recorded and later assessed using CowLog 3.0 (Pastell 2016) to quantify behaviours using a pre-designated ethogram (Appendices A and B). I analyzed 222 Handling Bag Tests and 156 Open Field Tests.

Statistical analyses:

Statistical analyses were conducted using statistical software analyses packages in R, version 4.2.3 (R Core Team 2023). I ran a repeatability analysis on behavioural variables for each species (Table 1.1) to determine if the behaviours observed in each video were repeatable and thus considered personality phenotypes (Wilson 2018). Because there were no repeated observations of Jumping Mice with the exploration test, we were unable to test for repeatability for this test. Each repeatability test was performed using the rptR package in R (Stoffel et al., 2017), using reproductive condition, age and sex as fixed effects, and individual ID as a random effect. Because recapture events may increase acclimation to behavioural tests, individuals that were retagged due to lost or unidentifiable tag numbers were removed from analyses, as cumulative capture count would be uncertain. I did not limit the repeatability test to individuals with only one observation (single capture count) because this has been shown to miss variation in individual plasticity (Martin 2011).

To first establish the relationship between exploration and docility, I ran Spearman's ranked correlation tests between behavioural tests and personality measurements from the first BT and OFT for each individual (n = 40 Deer Mice, 16 Red-Backed Voles, and 4 Woodland Jumping Mice). I only used the first observation of each test to reduce the impact of acclimation or learned behaviour from repeated testing (Cnops et al., 2022). To reduce the impact of

temporal variables, I only used individuals that underwent both behavioural tests during the same 3-day trapping period. Because retagged individuals may have already been subject to behavioural testing, individuals marked as retagged or having missing ear tag numbers were also excluded from this section of the analysis.

I used a Kruskal-Wallis test to quantify the relationship between personality type (either total docility time or total exploration time) and each categorical variable including age, sex, reproductive condition. I also used the Kruskal-Wallis test to quantify the relationship between variables associated with handling, including date of capture, and handling start time for both total exploration and total docile behaviour. For the Open Field Test, I included an additional binary variable to observe if the addition of adding an ear tag to an individual had an observable impact on exploratory behaviour.

I then used a linear mixed effect model using the R package lmer4 (Bates et al., 2015) for each species and behavioural test separately. For each test, either total time spent expressing docile behaviour, or total time spent expressing exploratory behaviour was used as a dependent variable. I used reproductive condition, age, weight, capture count and date as a fixed effect, and individual ID as a random effect. For this model, sex and reproductive condition were not included together because the two categories are dependent.

Results

Repeatability estimates:

Individuals that were retagged due to lost tag numbers were removed from analysis as the total number of tests that individual had undergone would be uncertain. I analyzed 202 Bag Tests (BT), including observations of 83 Deer Mice (135 total tests), 48 Red-Backed Voles (51 total tests) and 15 Woodland Jumping Mice (16 total test). I also analyzed a total of 147 Open Field Tests (OFT) including observations of 84 Deer Mice (93 total tests), 30 Red-Backed Voles (36 total tests) and 14 Woodland Jumping Mice (18 total tests). For the BT, static behaviours were repeatable in Deer Mice, but not in Red-Backed Voles or Jumping Mice. For the OFT, static behaviour was repeatable in Deer Mice and Red-Backed Voles (Table 1.1).

Correlation between docility and exploration:

To quantify the relationship between docility and exploration I used the first BT and OFT from each individual (n = 40 Deer Mice, 16 Red-Backed Voles and 4 Woodland Jumping Mice Figure 1.1). Between docility and exploration there was a weak negative correlation in Deer Mice ($R_s = -0.18$, $P = 0.26$) while the Red-Backed Vole had the strongest negative correlation ($R_s = -0.44$, $P = 0.091$).

Bag Test and docility:

When evaluating differences between individuals, there was no significant relationship between docility and measured variables including age, sex, reproductive status, or month of capture, for any of the three study species (Table 1.2). Similarly, I did not observe any significant relationship between docility and variables associated with animal handling including test start time or cumulative capture count. However, there was a weak negative correlation ($R_s = -0.28$, $P = 0.0066$) between static behaviour and cumulative capture count, and a weak positive correlation ($R_s = 0.091$, $P = 0.15$) between static behaviour and test start time in Deer Mice. Non-docile, or escape behaviour had a significant relationship with reproductive condition in Deer Mice, but not in Jumping Mice or Red-Backed Voles (Table 1.3).

The linear effects model showed significant differences in docility among species during different stages of the reproductive cycle ($F = 4.8$, $df = 216$, $P = 0.00098$, $R^2 = 0.081$). Non-scrotal males ($P = 0.031$) and pregnant females ($P = 0.021$) had a significant relationship with movement behaviours. Static or docile behaviour also had a significant relationship with reproductive condition ($F = 11.67$, $df = 214$, $P = 0.026$, $R^2 = 0.25$), where pregnant ($P = 0.048$) individuals had the most significant relationship with total time spent motionless (Table 1.4). Despite high variation between individuals, there were distinct differences among species during each reproductive phase. Deer Mice consistently expressed the lowest amount of static behaviour, while Red-Backed Voles displayed the greatest amount of static behaviour. Woodland Jumping Mice consistently expressed an intermediate amount of static behaviour compared to Deer Mice and Red-Backed Voles across all reproductive phases (Figures 1.2 and 1.3).

Open Field Test and exploration:

Between individuals there were no significant relationships between exploratory behaviour (time spent moving) in the OFT and any of the predictor variables - sex, age, reproductive condition, month of capture, date of receiving an ear tag - when using individual ID as a random effect ($F = 2.87$, $df = 141$, $P = 0.0014$, $R^2 = 0.19$, Table 1.3). Pooled across species, there was a weak positive correlation between capture count ($r = 0.22$, $P = 0.010$) and exploration ($n = 93$ Deer Mice, 44 Red-Backed Voles, 19 Woodland Jumping Mice). Among species there was variation in exploration time between individuals for each reproductive phase. Deer Mice were consistently the most explorative with the exception of lactating females which were similar to lactating Red-Backed Voles. Red-Backed Voles consistently expressed the lowest amount of exploration time while Jumping Mice were intermediate between the two (Figure 1.3).

Discussion

I hypothesized that coexistence among sympatric rodents will promote a divergence in behavioural strategy to accommodate for potential resource competition that occurs in coexisting populations, during the reproductive breeding season. I used reproductive status between individuals of the same species to evaluate changes in behaviour throughout the breeding season. However, there was little effect of reproductive status on behaviour within species. Given potential differences in energetic costs at different stages of the breeding season (Gittleman and Thompson, 1988), I predicted measurable differences in docility and exploration between individuals of the same species. While I did show a non-significant relationship between docility and exploration personality types, my results do not show evidence for differences between

individuals based on reproductive status that would support the POLS hypothesis or my predictions.

I found significant variation in behaviour and personality among species. Since docility and exploration are related to an individual's tendency to disperse and engage with the environment, I predicted that more explorative individuals should be less docile (Martin and Réale, 2008; Réale et al., 2010). I observed a weak, negative relationship between docility and exploration in all three species within this study. I also showed differences in the position each species occupies along the predicted fast to slow continuum. Deer Mice are consistently more explorative and less docile across all reproductive stages than Jumping Mice and Red-Backed Voles. Meanwhile, Jumping Mice are consistently more docile and less explorative than Deer Mice, with Red-Backed Voles providing relatively intermediate values between deer mice and woodland jumping mice, supporting the POLS hypothesis.

Empirical evidence to support the POLS hypothesis at the intraspecific level is mixed (Dammhahn et al., 2018; Royauté et al., 2018). My findings further suggest that POLS between individuals of the same species may be more complex than the definition of the POLS currently allows (Royauté et al., 2018). I found little evidence that different reproductive phases influenced exploration and docility between individuals. For all observed species, docility and exploration time remained consistent between scrotal and non-scrotal males. In seasonally breeding rodents, the gonadal state is associated with energy use (Bergeron et al., 2011). Given the strong association between exploration and home range or foraging patterns (Gharnit et al., 2020; Spiegal et al., 2017) and the increase in hormones to illicit breeding and change the gonadal state, one would expect potential differences in behavioural strategies between individuals at

different reproductive stages. Given the increased energetic demand for mate acquisition, males investing in reproduction through an enlarged gonadal state should have a higher level of exploration and a lower tendency to display docile behaviours (Hämäläinen et al., 2021). A low sample size for Red-Backed Voles and Jumping Mice may explain some of the lack of differences I observed within these species. However, for Deer Mice, I propose two reasons for my results. First, differences in movement behaviour may be negligible between non-scrotal and scrotal males. While an altered gonadal state is associated with an increased energetic cost, natal dispersal is a necessity in juvenile and sub-adult mice (King, 1968; Rémy et al., 2011). Therefore, if potentially non-scrotal individuals are dispersing at greater rates to alleviate competition, the trade-off in energetic investment between finding a mate and finding a new home may be comparable. Second, it remains possible that another factor strongly associated with dispersal and reproductive cost such as resource and habitat availability, is masking my results. Given the strong associations among mate acquisition, population abundance and resource availability (Bonte et al., 2012), it remains possible that there is a greater trade-off in the energetic costs of these population dynamics than the trade-offs presented solely by mate acquisition and reproductive investment during the breeding season.

There is an increased energetic cost in the investment of care and development of young for females. I anticipated a decrease in risk-taking behaviour in pregnant and lactating females compared to non-reproductive females. Indeed, several studies have observed an association between behaviour and reproductive status, including pheromone induced aggression (Martín-Sánchez et al., 2015), increased vigilance to protect against infanticide (Breedveld et al., 2019) and perhaps most significantly, hyporesponsiveness in lactating females (Chauke et al., 2011; Fleming and Luebke 1981; Lonstein 2005; Windle et al., 1997). Because I did not see the

expected decrease in risk-taking behaviour that may be anticipated with females undergoing various stages of reproduction, it remains possible that some other factor is more strongly associated with these behaviours. For example, pregnant or lactating females may have an increased energetic cost associated with the production of milk or the development of young, but non-reproductive females may also be subject to selective pressures from potential predators or conspecifics that have a comparatively equal cost.

The POLS hypothesis also postulates differences in behavioural strategies and personality among species (Réale et al., 2010). My results show differences in behaviour across observed species, such that Deer Mice were less docile and more explorative than Red-Backed Voles and Woodland Jumping Mice. Competition among coexisting species may illicit variation in reproductive strategy and ultimately personality phenotypes. Despite apparent competition, there are few studies that aim to model personality types in coexisting species (Wauters et al., 2019; Morris and Palmer, 2023). Docile behaviours are often associated with behaviours that reflect risk taking behaviour, where a lack of aggression, increased predator avoidance, and a tendency to react to shock or stressful environmental stimulus through prolonged periods of freezing or remaining static may be considered docile (Boice et al., 1968; Martin and Réale 2008; Réale et al., 2000). Because of the association between docile personality types, and risk avoidance, docile behaviour is understood to influence seed predation and dispersal (Boone et al., 2022), pathogen spread (Zahdy et al., 2017), individual reproduction and survival (Goulet et al., 2016), and has an overall impact on how species interact with various environmental variables. I also saw a pattern across species in which less docile individuals, or those that expressed less time immobile, were more exploratory in the OFT. Exploration behaviour, often measured through an OFT is described as an individual's tendency to move in a novel environment (Réale et al.,

2007). The exploration-activity personality phenotype largely evaluates individuals that are more willing to engage in an action under novel stimulus, ultimately influencing dispersal and home range movement patterns (Réale et al., 2007; Boone et al., 2022).

My results showing differences in behavioural phenotypes among species are consistent with the POLS hypothesis. Deer Mice showed a lower level of docility and greater rate of exploration across all reproductive categories compared to Red-Backed Voles and Jumping Mice. Red-Backed Voles consistently expressed the greatest docility and lowest exploration, while Jumping Mice expressed relatively intermediate values between Deer Mice and Voles. While I anticipated Red-Backed Voles should be more intermediate between Deer Mice and Woodland Jumping Mice, these results are perhaps not surprising given these two species have similar reproductive outputs and lifespans, while the Deer Mouse has a much greater reproductive output and shorter lifespan. Given the differences in coexisting species that abide by the POLS hypothesis, I suggest that coexistence may be a driving factor in the development of alternative evolutionary strategies. Deer Mice, Red-Backed Voles and Woodland Jumping Mice inhabit the same ecological niche, and population density is known to influence foraging and dispersal behaviour (Davidson and Morris, 2001). Given that each species is subject to the same environmental pressure, it remains likely that there is a benefit to alternative dispersal and movement strategies in sympatric species in the absence of niche partitioning of resources (Figure 1.4).

Animal behaviour and personality are influenced by an array of environmental conditions (Manning and Dawkins 2012). To help reduce potential causation from environmental and handling variables, I used four preliminary factors associated with animal handling and capture

including test start time, month of capture, cumulative capture count, and, for the Open Field Test only, if the individual received an ear tag before the test. Overall, I observed no significant correlation between start time and either docility or exploration. Similarly, I did not find any significant correlation between the month of capture and any of our personality traits. While seasonal variation in dispersal behaviours has been documented in laboratory settings using small rodents (Eccard and Herde 2013; Harrison et al., 2015), it is possible that the lack of seasonal variation I observed is the result of the timeframe of my study. Given that the majority of my samples occurred during the active breeding season it remains possible that more variation would be observed by comparing behaviours between spring and winter, or in months outside of the primary breeding season. It also remains possible that other seasonal environmental factors such as temperature or precipitation rate (Sergio 2003), or population dynamics including spatial competition or predator density (Bowler and Benton 2005) may instead have a greater impact on dispersal and movement behaviour, masking seasonal variation in my study.

Trap bias resulting from “trap happy” individuals, and the underrepresentation of potentially trap-shy individuals is an ongoing caution when discerning behavioural tests. While some researchers have shown that trap bias can not necessarily be a proxy for personality (Brehm and Mortelliti 2018), there are several studies that have noted a correlation in cumulative capture count and bold, exploratory personalities (Boon et al., 2008; Boyer et al., 2010). I used the cumulative capture count to assess possible correlations between trap count and behaviour. Overall, my results suggest that a higher cumulative trap count did not have a significant impact on docile behaviour. However, I did find a significant weak positive correlation between exploratory behaviour and capture count. Suggesting that individuals that are more likely to be recaptured may be more likely to engage in risk-taking behaviour, an observation commonly

reported in literature (Johnstone et al., 2023; Boon et al., 2008; Boyer et al., 2010; Carter et al., 2012).

For my final handling variable, I used a binary scale of whether or not an individual received an ear tag prior to the Open Field Test, to measure potential variation in behaviour associated with tagging. Ear tagging, while common in live-capture research (Fokidis et al., 2006) will cause discomfort (Wever et al., 2017) and may potentially be a greater stressor than taking handling measurements. While there was a weak non-significant negative correlation in exploratory behaviour in Deer Mice that received a tag compared to those that did not, I did not see this trend for either Red-Backed Voles or Jumping Mice. Comparing individuals that have received an ear tag to those that have not, is also a comparison of individuals that have been recaptured versus first time, or naive captures. Despite the lack of statistical evidence, I suggest that increased handling time during live-recapture studies is likely to cause additional stress in an animal. Therefore, caution is advisable as small changes to behaviour may occur from prolonged handling time, or stressful measurements.

While I did not show evidence to support the POLS hypothesis between individuals of the same species, I show significant differences in behavioural phenotypes among coexisting species. Because species inhabiting the same ecological niche must engage in increased competition for resources, I further postulate that competition can regulate the expression of alternative behavioural phenotypes (Morris and Palmer, 2023; Sobral et al., 2023). Because personality may be a driving mechanism for environmental processes such as the distribution of seeds through fecal droppings (Réale et al., 2010; Morris and Palmer, 2023; Boone et al., 2022;

Brehm and Mortelliti, 2022), I suggest that further evaluation into trade-offs in personality, population dynamics and changes in environmental structure will be valuable future research.

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Tables and Figures

Table 1.1. The results from the repeatability analysis for the (Bag Test) BT and (Open Field Test) OFT measuring Docility and Exploration respectively. Results highlighted in bold were determined to be repeatable. All estimates were calculated using mixed effects model with age, sex, and reproductive condition as a fixed effect and individual ID as a random effect. 95% confidence intervals were calculated using parametric bootstrapping.

Docility - ID random effect					
Behavioural Variable	Mean	R	SE	CI (95%)	P
Deer Mouse					
Movement	0.341	0.269	0.143	0.0351, 0.6	0.044
Escape	0.148	0.066	0.13	0, 0.443	0.33
Grooming	0.0998	0.031	0.093	0, 0.313	0.35
Foraging	0.109	0	0.122	0, 0.407	1
Freezing	0.404	0.339	0.137	0.116, 0.655	0.020
Red-Backed Vole					
Movement	0.34	0	0.268	0, 0.837	1
Escape	0.369	0	0.259	0, 0.805	1
Grooming	0.348	0	0.243	0, 0.761	0.5
Freezing	0.354	0	0.268	0, 0.819	1
Woodland Jumping Mouse					
Movement	0.0001	0	0	0, 0	1
Escape	1	1	0	1, 1	0.30
Freezing	1	1	0	1, 1	0.30

Exploration - ID random effect

Behavioural Variable	Mean	R	SE	CI (95%)	P
Deer Mice					
Move	0.284	0.066	0.207	0, 0.691	0.37
Groom	0.237	0	0.214	0, 0.66	1
Freezing	0.842	0.809	0.067	0.695, 0.943	0.0019
Hide	0.227	0	0.209	0, 0.658	1
Red-Backed Vole					
Move	0.836	0.315	0.232	0, 1	0.34
Groom	0.741	0	0.308	0, 1	0.5
Freezing	0.856	0.391	0.205	0.187, 1	0.097
Hide	1	1	0	1, 1	0.000067

Table 1.2: Kruskal-Wallis results for each separated species (DM = Deer Mice, RBV = Red-Back Voles, WJM = Woodland Jumping Mice) for the impact of categorical predictor variables on total-docile behaviour. Docile behaviour includes any time that the individual spent static during the Bag Test.

Species	Variable	Chi-Squared	df	P
DM	Age	2.07	2	0.36
	Repro	8.03	4	0.090
	Sex	2.23	1	0.14
	Date	2.04	3	0.57
RBV	Age	0.135	1	0.71
	Repro	4.91	4	0.30
	Sex	0.238	1	0.63
	Date	2.57	3	0.46
WJM	Age	0.117	2	0.94
	Repro	7.99	4	0.092
	Sex	1.21	1	0.27
	Date	0.939	3	0.82

Table 1.3: Kruskal-Wallis results for each separated species (DM = Deer Mice, RBV = Red-Back Voles, WJM = Woodland Jumping Mice) for the impact of categorical predictor variables on total non-docile behaviour. Non-docile behaviour includes any time the individual spent moving, trying to escape, or grooming during the Bag Test. * Denotes significant ($P < 0.05$) values.

Species	Variable	Chi-Squared	df	P
DM	Age	2.22	2	0.33
	Repro	9.57	4	0.048 *
	Sex	3.18	1	0.074
	Date	1.13	3	0.77
RBV	Age	0.0727	1	0.79
	Repro	5.33	4	0.26
	Sex	0.369	1	0.54
	Date	2.14	3	0.55
WJM	Age	0.119	2	0.94
	Repro	7.94	4	0.094
	Sex	0.524	1	0.47
	Date	0.548	3	0.90

Table 1.4: Summary of top best fit linear mixed effects models predicting the relationships between personality (docility or exploration) pooled across species (n = 202 Bag Tests and 147 Open Field Tests).

Behavioural Model	Predictor Variables	Coefficient	Standard Error	t	P
Docility – Non-Docile Behaviour + Age + Reproductive Condition + Body Mass	AgeJ	-4.4	6.2	-0.72	0.48
	AgeSA	8.93	4.7	1.9	0.057
	ReproNR	-2.4	7.7	0.31	0.76
	ReproNSCR	-14.3	7.6	-1.9	0.031
	ReproPREG	-18.3	9.3	1.9	0.021
	ReproSCR	-8.2	7.6	-1.08	0.28
	Body Mass	-0.78	0.53	-1.5	0.14
Docility – Docile Behaviour + Age + Reproductive Condition + Body Mass	AgeJ	6.2	6.2	0.99	0.32
	AgeSA	-7.7	4.7	-1.6	0.10
	ReproNR	2.5	7.7	0.33	0.75
	ReproNSCR	12.7	7.6	1.7	0.097
	ReproPREG	18.8	9.5	1.9	0.048
	ReproSCR	8.65	7.7	1.1	0.26
	Body Mass	0.89	0.53	1.7	0.099

Exploration –	AgeJ	16.6	27.6	0.60	0.55
Explorative					
Behaviour + Age +	AgeSA	21.2	20.7	1.02	0.30
Reproductive					
Condition + Date +	ReproNR	-28.2	27.05	-1.04	0.30
Body Mass	ReproNSCR	-31.01	27.1	-1.1	0.26
	ReproPREG	-33.06	47.03	-0.703	0.48
	ReproSCR	-32.6	27.03	-1.2	0.23
	DateMay	-68.6	33.2	-2.06	0.040
	Body Mass	-4.06	2.5	-1.6	0.11

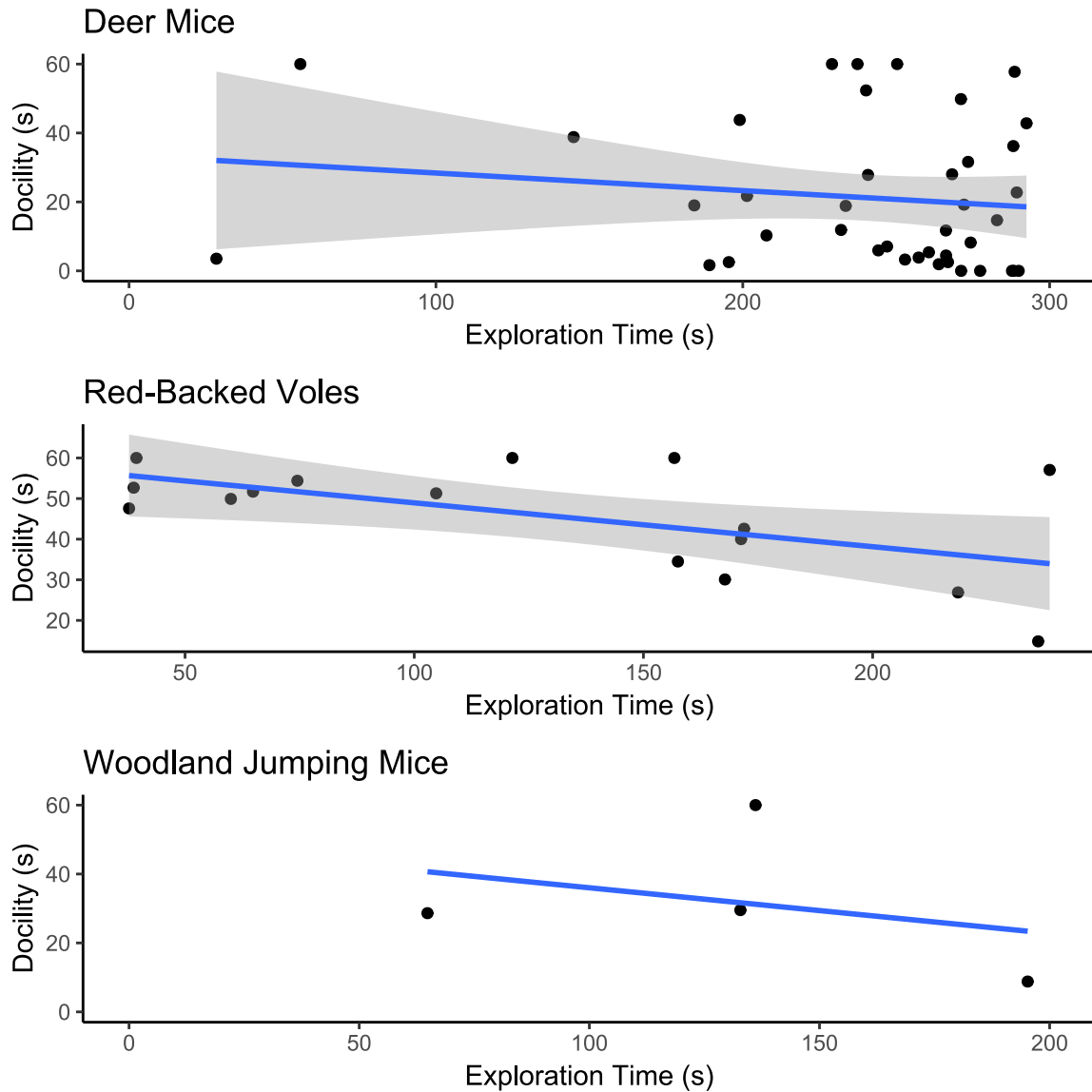


Figure 1.1: Scatter plots showing the relationship between total time spent expressing docile and exploratory behaviours in Deer Mice ($r = -0.018$, $P = 0.26$), Red-Backed Voles ($r = -0.437$, $P = 0.090$), and Woodland Jumping Mice ($r = -0.2$, $P = 0.92$). Each data point shows the first Bag Test (BT) and Open Field Test (OFT) an individual performed. Negative correlation is depicted with the trendline, and shading represents the 95% confidence interval.

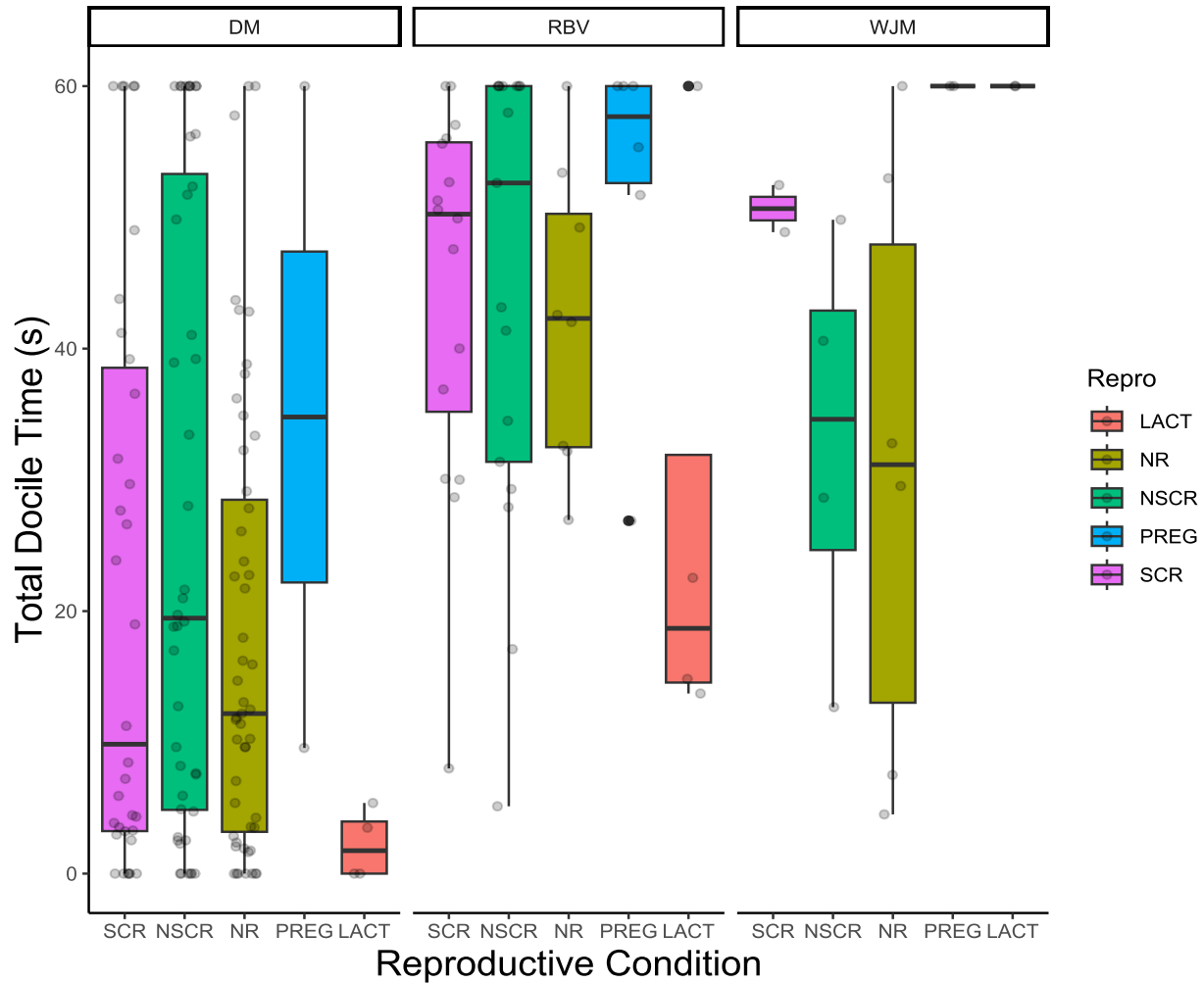


Figure 1.2: Boxplot showing the relationship between total docile time (static behaviour) during the Bag Test (BT) comparing all three species, DM = Deer Mice, RBV = Red-Backed Vole, WJM = Woodland Jumping Mice, across all reproductive condition (SCR = Scrotal, NSCR = non-scrotal, NR = non-reproductive, PREG = pregnant, LACT = lactating) using individual ID as a random effect. Whiskers represent standard error; box lines represent the median time spent motionless across samples and faded jitters represent the distribution of data.

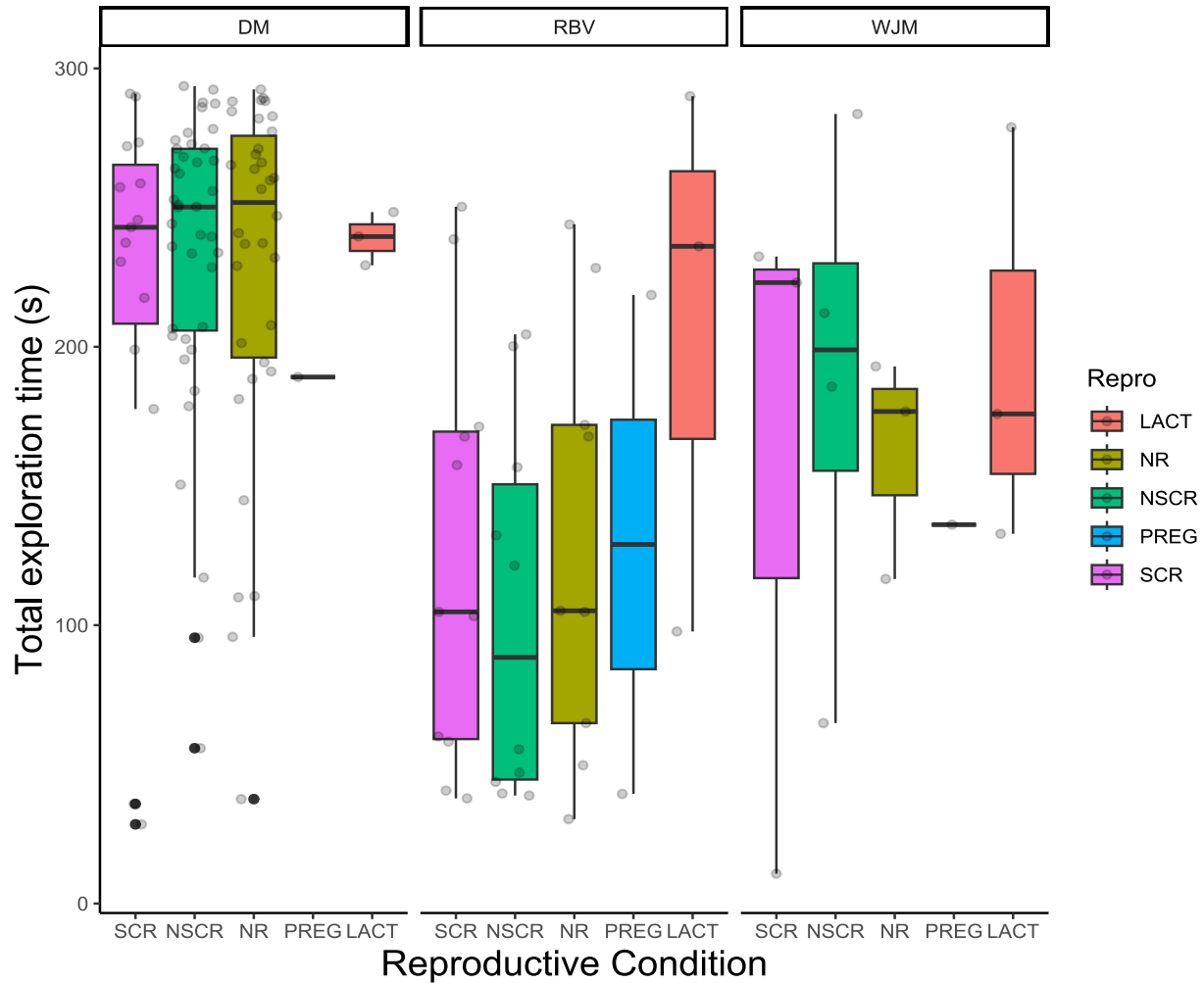


Figure 1.3: Box plots showing the relationship between exploratory behaviour (moving) during the Open Field Test (OFT) for each species (DM = Deer Mice, RBV = Red-Backed Vole, WJM = Woodland Jumping Mice) for each reproductive phase (SCR = scrotal, NSCR = non-scrotal, PREG = Pregnant, LACT = Lactating, and NR = non-reproductive) using individual ID as a random effect. Whiskers represent standard error, box lines represent the median time spent expressing exploratory behaviours across samples, and faded jitters represent the distribution of individuals.

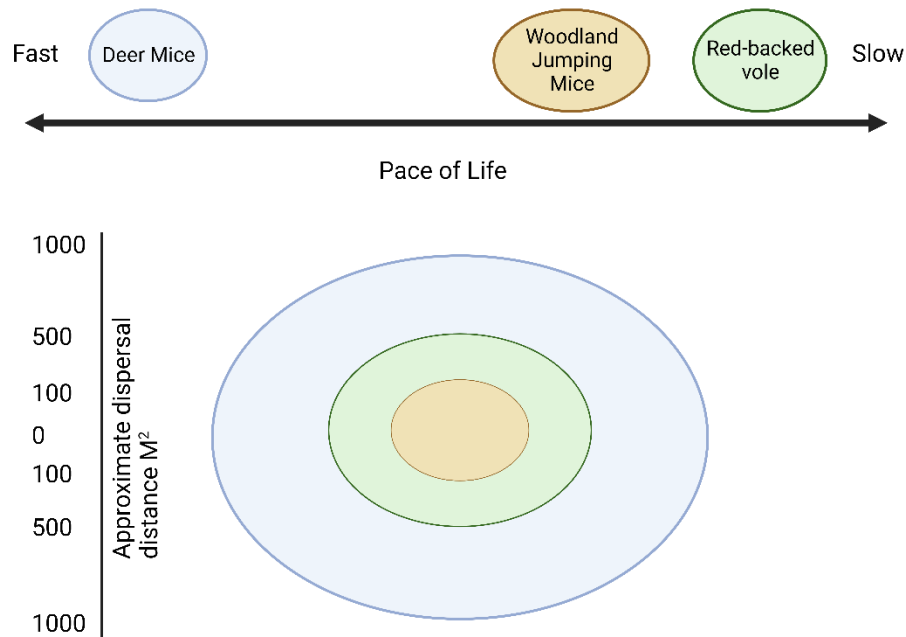


Figure 1.4: A conceptual model to depict the placement of Deer Mice, Red-Backed Voles and Woodland Jumping Mice along the fast-slow continuum according to the pace-of-life syndrome hypothesis. Also shown is an approximate area of movement within a home range, to depict how coexisting species may benefit from foraging in alternative sites. Faster species may benefit from dispersing to greater distances, or having increased movement within a home-range while species on the slower end of the spectrum may benefit from more localized foraging.

Chapter Two

Sex differences and behaviour in the pace-of-life of rodents

Abstract

Because male and female rodents experience different selective pressures associated with reproductive costs, we may expect the expression of different Pace-of-life (POLS) strategies between the sexes. Further, the POLS hypothesis, and anisogamy predict differences in the costs of gamete production, where variation in life history trait expression should follow the fast-slow continuum such that males and females may exist on opposite ends of the spectrum. However, in systems where reproductive potential is consistent between sexes, or where selective pressures force a similar directionality of traits, males and females may express a similar POLS strategy. I used a series of standardized behavioural tests and fecal glucocorticoids to measure potential differences in POLS strategies among three species of rodent in Algonquin Provincial Park. I hypothesized that differences in reproductive costs between males and females would result in differences of POLS traits along the fast-slow continuum. I predicted that males would express more explorative behaviours and have a lower level of energetic stress (measured through fecal glucocorticoids) compared to females. I found little support for the POLS hypothesis and instead suggest that the species observed within my study may express a uniform directionality of selection, where both sexes express similar relationships in POLS traits resulting from alternative selective pressures. Males may be more explorative to accommodate the increase of energetic stress associated with mate acquisition, while females may share similar trait expression to accommodate the increased energetic demand for the care and development of young.

Introduction

The pace-of-life syndrome (POLS) hypothesis suggests that trade-offs between energy investment and environmental pressure should result in differences in behavioural, physiological, and life-history strategies. Relationships among POLS traits are predicted to evolve along a predictable fast-slow continuum (Réale et al., 2010). Fast-paced species are predicted to favour strategies including a high reproductive output with low investment in parental care, behavioural strategies that include a high rate of exploration and boldness, and a lower investment in survival, resulting in a comparatively weakened immunological function and lower stress sensitivity (Réale et al., 2010; Santostefano et al., 2017; Hall et al., 2015). Slow-paced species display traits that reflect the opposite, with a lower reproductive output yet higher investment in individual young (Réale et al., 2010). Because males and females fulfill different reproductive roles within a population, there is sex-specific variation in the energetic trade-offs concerning self-preservation and reproduction (Hämäläinen et al., 2018; Tarka et al., 2018). Differences in energetic trade-offs drive natural and sexual selection resulting in sexually dimorphic traits or sexual differences in behaviour and physiology that may influence the placement of individuals along the predicted fast-slow continuum (Shutler, 2010; Hedrick and Temeles 1989; Fairbairn et al., 2007). Indeed, there is variation in life-history traits between sexes, including size, age of maturation, growth, reproductive potential, and mortality (Martin, 2004; Clutton-Brock and Vincent, 1991; Bonduriansky et al., 2008; Maklakov and Lummaa, 2013; Adler and Bonduriansky, 2014; Arso Civil et al., 2019). While there are sex-specific differences in life history for many taxa, the directionality and expression of sex-specific traits and syndromes remain relatively poorly understood (Tarka et al., 2017).

Differences in male and female trait expression may stem from sex-specific reproductive roles influenced by anisogamy (Lehtonen et al., 2016). Anisogamy suggests that sex differences in behaviour and physiology should arise such that males invest more in mate acquisition, whereas females invest more heavily in the care and development of offspring (Schärer et al., 2012; Lehtonen et al., 2016). Because of these differences in reproductive roles, males are expected to have faster life strategies than females (Tarka et al., 2018). Sex-specific variation has been proposed to be one of the underlying mechanisms responsible for a lack of empirical support for the assumptions presented by the POLS hypothesis within taxa (Royauté et al., 2018; Hämäläinen et al., 2018; Immonen et al., 2018). Given the association between POLS traits, sex can be an important variable to include when evaluating various physiological or behavioural phenotypes. Differences in selective pressures should result in alternative physiological patterns, including differences in immunity (Monceau et al., 2017; Love et al., 2008; Lee, 2006), metabolism (Shingleton and Vea, 2023; Rønning et al., 2016), hormone production (Nelson, 2005), and thermoregulation (van Jaarsveld et al., 2021) between sexes. Consequently, different selective pressures should also favour differences in behavioural strategies between the sexes, including differences in risk-taking behaviour (Holtby and Healey, 1990), foraging patterns (De Pascalis et al., 2020) and overall behavioural syndromes (Fresneau et al., 2014). Given the assumptions of anisogamy, males should express behavioural and physiological traits differently than females, including higher exploration and risk-taking and reduced physiological reactivity to environmental stimuli.

The POLS syndrome predicts a correlation between physiological mechanisms and behavioural coping strategies (Réale et al., 2010). In ecology, stress is the energetic change and physiological response to environmental stimuli (Romero, 2004; Hobfoll, 1988; Costantini,

2008). Fecal glucocorticoid metabolites are a common non-invasive proxy of species or individual stress (Palme 2019; Palme 2012; but see MacDougall-Shackleton et al., 2019). Glucocorticoids such as corticosterone and cortisol are a group of steroid hormones released from the hypothalamic-pituitary-adrenal axis (HPA-axis) that assist in regulating metabolic function in response to external stimulus (Palme, 2019; Toufexis et al., 2014). Because species or individuals with a faster life history are predicted to invest less in energetic reactivity to environmental stimuli, faster individuals should have a higher basal concentration and lower fluctuation of glucocorticoid metabolites during various stimuli (Boyce and Ellis, 2005). Between sexes, males should therefore show higher basal concentrations in fecal glucocorticoids than females. Indeed, a greater investment in physiological response, most prominently seen in pregnant and lactating females, suggests females follow the slower predicted life pace (Reeder and Kramer, 2005). Behaviours associated with movement and risk-taking, such as docility and exploration, can also be modelled along the fast-slow continuum (Réale et al., 2010). Because fast-paced species or individuals should invest more in movement and less in self-maintenance, faster coping styles should include more explorative and less docile behaviours. Between sexes, this may suggest that males should be more exploratory and less docile compared to female conspecifics. Given the role of proactive and reactive coping strategies on the expression of species-specific life history and physiology, there is increasing interest in evaluating the correlation between behavioural coping strategy and physiological reactivity (Damsgård et al., 2019; Koolhaas et al., 2010).

Hämäläinen et al. (2018) provide a framework that suggests differences in POLS may evolve from a variety of circumstances where predicted covariance may be expressed differently between sexes. Anisogamy has been well described in rodents (Ramm et al., 2005; Dewsbury

1982; Roldan et al., 1992), where males and females often express differences in coping strategy and physiology based on energetic demands during reproduction (Eccard and Herde, 2013; Immonen et al., 2018). I quantified sexual differences in movement behaviour, and energetic investment, measured through fecal glucocorticoids (Palme et al., 2019), between sexes in three species of rodent including, the Deer Mouse (*Peromyscus maniculatus*), the Red-Backed Vole (*Clethrionomys gapperi*) and the Woodland Jumping Mouse (*Napaeozapus insignis*). I hypothesized that the different reproductive roles of the sexes should lead to different expression of traits between sexes according to the POLS. If my hypothesis is supported, I predicted that males should express more reactive behavioural phenotypes, including being more exploratory and less docile than females within the same species. Males should also have higher basal concentrations of glucocorticoids because of the increased cost associated with mate acquisition. Understanding sex-specific variation in POLS traits is one step in answering more broad questions concerning the evolution of personality and how species mitigate competition between conspecifics given different environmental and selective pressures.

Methods

Capture and handling of animals

Deer Mice, Red-Backed Voles, and Woodland Jumping Mice were sampled in Algonquin Provincial Park, Ontario, Canada, from May through September 2022 across 17 traplines following animal care protocol (#6011106) approved by Laurentian University. Each trapline consisted of 20 Sherman traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) baited with water-soaked sunflower seeds. Traplines consisted of 100 m transects consisting of two traps placed every 10 m and covering an array of forest habitat (see Fryxell et al., 1998 for detailed trapline methodology and habitat description). Traps were baited at dusk and checked the following morning, and each trapline was baited biweekly for 3 consecutive nights. Individuals received metal ear tags with unique alphanumeric codes (National Band and Tag Co., Newport, Kentucky, USA) for identification. For each individual, I recorded the sex (Male or Female), Age class (Juvenile, Sub-adult, or Adult) based on weight (Schmidt et al., 2019), body mass using a 0.01 g Pesola scale, and reproductive status measured as scrotal or non-scrotal for males, and non-reproductive, pregnant, perforate or lactating for females.

Behavioural tests

Individuals were subjected to one behavioural test per trap day. Each test aimed to quantify a different personality phenotype including docility from the Handling Bag Test (Martin and Réale 2008) or exploration and activity from the Open Field Test (Carter et al., 2013). I recorded each test using a camera (Sony HDR-CX405), and later analyzed behaviours using CowLog 3.0 (Pastell 2016, see Appendix A and B for ethograms).

Fecal collection, extraction, and immunoassay analyses

I used fecal samples as a non-invasive measure of glucocorticoid levels in all three species. Because small mammals often defecate in response to handling, fecal samples were collected immediately after defecation during the handling process and placed in an Eppendorf tube filled with 80% methanol. Eppendorf tubes were then placed on ice in a cooler and were later transferred to a -20 C freezer. Where not possible to collect fecal samples during handling due to insufficient sample volume or lack of defecation, fecal samples were collected from the Sherman trap, no later than 19 hours after defecation. While traplines were checked in the same order each week to reduce decay of fecal samples, the 19-hour collection period has no significant impact on total fecal metabolite concentrations (Veitch et al., 2021).

Corticosterone metabolites were extracted using methods detailed by Veitch et al. (2021) and quantified by enzyme immunoassay as described in Baxter-Gilbert et al. (2014), and Stewart et al. (2020). All materials used were purchased from Fisher Scientific Inc. I transferred fecal samples and any methanol from the collection Eppendorf tube and added them to a 7 ml glass vial, I then added an additional 1 ml of 100% methanol. I left samples under a fume hood to completely evaporate to obtain a fecal sample weight. I then added fresh 80 % methanol to the samples using a ratio of 0.0005 g/ml before vortexing samples for 10 s. Vortexed samples were placed in a centrifuge for 5 minutes at 2400 rpm to remove steroid from the glass, before being placed on 100 rpm orbital shaker for approximately 20 hours. The following day I placed samples on a second centrifuge for 10 minutes at 2400 rpm. I then extracted the remaining sample into a fresh glass vial, added parafilm to the seal and placed the samples back in a -20 freezer until the immunoassay analyses.

To validate fecal metabolite concentrations, I used a parallel displacement between the standard curve and serial dilutions of fecal extract as an indirect measure of assay specificity. Pooled reconstituted fecal extracts were serially diluted two-fold in assay buffer and visually compared to the respective standard curve. The data were plotted as log (relative dose) vs. percent antibody bound (Microsoft Excel). The slopes of the lines within the linear portion of the curves were determined using linear regression analysis and compared (Soper 2021) where $p > 0.05$ indicates that the slopes are not significantly different and thus interpreted as parallel. Samples were assayed at the dilution factor that corresponded to 50% binding of the serially diluted fecal pool for each assay.

Statistical analyses

All statistical analyses were conducted using the statistical software program R version 4.2.3 (R Core team 2023). A \log_{10} transformation was applied to the total fecal corticosterone or cortisol metabolites (FCM) variable for all models to normalize distribution of data. Fecal glucocorticoid values outside the high and low cut-off values obtained using the Soper (2021) standard curve calculation are considered inaccurate and were thus removed as outliers (Deer Mice = > 9500 or < 90 ng / g, Woodland-Jumping Mice > 2500 or < 20 ng / g, Red-Backed Voles = > 6200 or < 60 ng / g). Low cut-offs were determined as the limit of quantitation (LOQ) using the blank determination method described in Shrivastava and Gulpa (2011), high cut-off values were determined through visual assessment of where values exceeded the standard curve of the parallelism (Appendix E).

I fitted linear mixed effects models using individual ID as a random effect for each species separately using the lme4 package in R (Bates et al 2015). First, we tested for variation in

FCM between males and females of each species using age class (adult, sub-adult or juvenile), reproductive status (scrotal, non-scrotal, pregnant, lactating, perforate or non-reproductive), body mass and date (May through August). I used a linear mixed effect model with all predictor variables to show the relationship between docile and exploratory behaviour and the \log_{10} transformed FCM.

Results

To validate FCM concentration detection, I performed a parallelism on each species to measure linearity in diluted samples. For Jumping Mice, serial dilutions of pooled fecal extract showed parallel displacement with the cortisol standard curve ($t=0.10$, $p=0.92$, $df=5$; Appendix E). Serial dilutions of pooled fecal extract did not show parallel displacement with the corticosterone standard curve ($t=5.97$, $df = 7$, $P = <0.0001$, Appendix E). Serial dilutions of pooled fecal extract showed parallel displacement with the corticosterone standard curve for Deer Mice ($t = 0.71$, $df = 9$, $P = 0.50$, Supplementary Fig A2), and Red-Backed Voles ($t = 0.03$, $df = 9$, $P = 0.98$, Appendix E).

I measured the relationship between total docility (time spent immobile) and \log_{10} -transformed FCM using linear models for a total of 104 Deer Mice ($F = 1.605$, $df = 92$, $P = 0.11$, $R^2 = 0.16$), 15 Woodland Jumping Mice ($F = 0.89$, $df = 38$, $P = 0.56$, $R^2 = 0.22$) and 52 Red-Backed Voles ($F = 18.59$, $df = 4$, $P = 0.0062$, $R^2 = 0.98$). My results showed non-statistically significant values for most predictor variables (date, reproductive status, body mass, sex, and age class), in Deer Mice and Red-Backed Voles (Table 2.1 and Figure 2.1). Body mass was significantly associated with docility in Deer Mice ($P = 0.045$), while reproductive condition ($P = 0.0044$), date ($P = 0.0092$) and mass ($P = 0.0039$) were significantly associated with docility in

Woodland Jumping Mice (Table 2.2). None of my examined species had a significant relationship between total docility and \log_{10} transformed glucocorticoid concentrations. Among species, Red-Backed Voles showed a significant relationship between sexes for explorative behaviours where males were less explorative than females (Table 2.3 and Figure 2.2).

When comparing within-category effects for the predictor variables (date, reproductive status, body mass, sex, and age class), pregnant individuals had the most significant relationship with total docility ($P=0.012$; Figure 2.3); however, this result was only observable in Red-Backed Voles. Pregnant Red-Backed Voles showed a positive relationship between total fecal metabolites and docility, such that individuals with a higher concentration of fecal glucocorticoid expressed more frequent motionless behaviour (Figure 2.3). I also measured the relationship between total exploratory behaviour (time spent moving around an Open Field Test) and the \log_{10} transformed fecal metabolites across a total of 46 Deer Mice ($F = 1.34$, $df = 35$, $P = 0.25$, $R^2 = 0.276$), 10 Woodland Jumping Mice ($F = 0.36$, $df = 3$, $P = 0.86$, $R^2 = 0.36$) and 27 Red-Backed Voles ($F = 2.654$, $df = 15$, $P = 0.042$, $R^2 = 0.63$). Of the three species, only Red-Backed Voles showed sexual differences in exploration behaviour, such that male Red-Backed Voles were less explorative than female counterparts. Sub-adult Deer Mice also had a significant relationship with total exploration time ($P = 0.0028$) such that individuals expressed a greater amount of exploration compared to juvenile and adult Deer Mice (Table 2.4 and Figure 2.4). Sub-adult Deer Mice also had the most notable relationship between exploration and \log_{10} transformed fecal metabolites, such that individuals with a higher concentration of fecal metabolites were more explorative (Figure 2.4). I also found a high degree of variation in the fecal metabolite concentrations between individuals for all three species (Figure 2.5).

I compared the differences in fecal metabolite values among all three study species based on reproductive status to determine potential directionality of selective pressure on energetic stress between sexes (Figure 2.6). I showed that there were no significant differences in fecal metabolite values among pregnant, or lactating females and scrotal males. Similarly, I saw no significant differences in fecal metabolite values between non-reproductive females and non-scrotal males in any of the three study species.

Discussion

While my results suggest significant variation in fecal metabolite concentrations among individuals, animal personality and fecal glucocorticoid concentrations showed no relationship between sexes that would support my hypotheses that individuals should express traits along the fast-to-slow continuum. While I do show evidence that reproductive status or age may affect the expression of behaviour and ultimately the syndrome showing the relationship between physiological adaptations and behaviours, I note that there was a low difference in fecal glucocorticoids between sexes. Given the similarities between basal fecal glucocorticoid concentrations between sexes, regardless of reproductive state, I propose that investment in sex-specific POLS strategies may evolve as a result of different directionality of selective pressures. In such circumstances, males may express an increased energetic stress influenced by mate acquisition, while female conspecifics express a similar rate of energetic stress to accommodate the reproductive costs associated with the care and development of young (Hämäläinen et al., 2018). While this result does not support the direct assumptions of the POLS hypothesis for males and females along the anticipated fast-slow continuum, my findings coincide with previous literature to suggest the emergence of similar POLS traits between sexes (Hämäläinen et al., 2018; Réale et al., 2010, Tarka et al., 2018).

Because of the different costs associated with reproduction, I predicted that there should be a difference in the expression of POLS traits between sexes (Moshilla et al., 2019; Prabh et al., 2023; Immonen et al., 2018). Generally, males are predicted to express faster strategies because of differential costs in mate and resource acquisition (Clutton-Brock and Isvaran 2007). Meanwhile, females should express slower strategies and a greater investment in self-preservation that accommodates the increased energetic costs associated with the development and care of young (Rogowitz 1996; Moschilla et al., 2019; Réale et al., 2010). Because scrotal males and pregnant or lactating females have an increased energetic cost associated with reproduction, I predicted that individuals in this phase of reproduction should have greater concentrations of fecal glucocorticoids compared to non-scrotal and non-reproductive counterparts. My results show little difference in basal fecal glucocorticoid concentrations between pregnant or lactating females, and scrotal males. Similarly, non-reproductive females, and non-scrotal males of the same species share similar concentrations of fecal glucocorticoids. Similar Pace-of-life strategies may be expected when reproductive rate and energetic demands of breeding are approximately equal for both sexes (Hämäläinen et al., 2018). Therefore, while the source of selective pressure between sexes may vary, the resulting relationship between behavioural and physiological traits may show little difference.

The results I show may further be explained by species-specific life-history strategies. In POLSygynous systems where females may mate with several males, males may invest in alternative mating strategies to as a consequence of competition (Mank et al., 2013). However, in systems where females are forced to compete for mates or resources at an equal or greater rate than males, the directionality and position of POLS traits in females may mimic males (Jenni 1974; Emlen and Oring 1977; Eens and Pinxten 2000). Further, environmental variables

including predation pressure, population abundance and resource availability can shape the expression of POLS traits (Krause et al., 2017). The lack of expected adherence to the POLS between sexes could be explained as a result of similar energetic costs. Between sexes, mating system, and similar energetic demand during various reproductive phase may drive the uniform distribution in POLS traits between sexes I have observed here.

Beyond the differences in physiological mechanisms between sexes, the POLS also predicts differences in behaviour and personality evolve from trade-offs in energetic demand (Réale et al., 2010; Réale et al., 2007). Males should express a lower level of docility and a higher rate of exploration in response to the increased necessity to find a mate (Montiglio et al., 2012; Réale et al., 2007). Similarly, females should express a higher level of docility, and a lower level of exploration to resulting in greater individual survivorship (Yao et al., 2023; Réale et al., 2007). However, in polyandrous mating systems, selective pressure may differ between sexes, allowing for alternative or even non-existent relationships in POLS traits (Hämäläinen et al., 2018). In systems where reproductive output remains consistent between males and females, such as mealworm beetles (*Tenebrio molitor* L.) (Krams et al., 2013), POLS traits may covary in a uniform pattern (Hämäläinen et al., 2018 Immonen et al., 2018). Given the lack of difference in docility and exploration within the present study, I further suggest that my study species may express a covaried syndrome due to differences in reproductive costs. Namely, males may invest more heavily in exploration for mate acquisition; however, because the reproductive output and energetic demand for resource acquisition is consistent between sexes, females may also show similar personalities regarding movement behaviour.

Understanding the relationship between environmental pressures and the expression of behavioural and physiological traits is important to understanding how POLS traits arise. Indeed, resource quality and availability have been shown to impact trait expression (Bright-Ross et al., 2020; Prabh et al., 2023; Finn et al., 2018). Similarly, seasonal variation shown in Common Voles (*Microtus arvalis*) may further correlate with resource availability (Eccard and Herde 2013). Given that resource availability will fluctuate based on season and will certainly depend on population abundance, these are important factors to consider when evaluating POLS traits. Given the significant variation across species, I further postulate that coexistence and ultimately population abundance of co-occurring species may have strong impacts on the expression of POLS traits. Indeed, intra- and interspecific competition may result in the expression of different behavioural strategies amongst species, altering movement patterns such that individuals, regardless of sex, must disperse and forage in alternative patterns (Hassel et al., 1994; Wauters et al., 2019). Therefore, combined with resource and habitat availability may further contribute to the relative expression of POLS traits observed in my study.

The relationship between behaviour and fecal glucocorticoids may be further masked by changes in seasonal temperatures, photoreception and consequently diet which has been shown to alter glucocorticoid concentrations (Demas and Nelson 1996; Reeder and Kramer 2005). My results suggested only pregnant females, and more specifically pregnant Red-Backed Voles, had a significant relationship between docility and total basal fecal glucocorticoid levels. I also did not see any significant relationship between fecal glucocorticoids and month of sample collection, suggesting low seasonal variation. However, further investigation into seasonal variation across winter and summer months where energetic costs differ, may highlight a more significant relationship (Moffatt et al., 1993). Fecal metabolites should increase from spring to

summer months in most rodents, given the increase in energetic demand for reproductive task (Stewart et al., 2020; Romero 2002). Veitch (2021) found a significant negative correlation in fecal corticosterone metabolites and date, attributing the plausible cause as consistent energetic cost in reproduction from May through August. My results further support this conclusion, as behaviour showed no significant relationship with date in the present study. The lack of sexual differences observed within my study may also be explained by similar environmental cost. While females actively investing in the care or development of young should express an increased level of energetic stress (Künkele 2000) similar increased cost may be incurred for males experiencing the associated cost of increased necessity for defense, dispersal, spatial movement, or hormone investment (Millar 1975; Romero 2002).

Fecal glucocorticoids and exploration time were positively related in sub-adult Deer Mice. This relationship may be explained by immune system maturation (Holt and Jones 2000; Simon et al., 2015), and the necessity of dispersal from individual natal area (King 1968), further support by Veitch (2021). Interestingly, I also found a negative relationship between exploration time and glucocorticoid concentrations for adults of all three species. While increased dispersal and movement within a home-range should increase environmental interactions associated with conflict between conspecifics and potential predators (Mayer et al., 2020), adults should have a greater level of immunocompetence (Webster et al., 2002). Therefore, while exploratory behaviours in adults may be greater or equal to younger conspecifics, total glucocorticoid production may be comparatively lower.

There was a high degree of variation in fecal glucocorticoid concentration and behaviour across individuals, a well-documented phenomenon (Veitch et al., 2021; St. Juliana et al., 2014;

Stedman et al., 2017). Indeed, fecal glucocorticoid concentration can be influenced by an array of environmental and genetic factors (Touma and Palme 2005) making conclusions on single observation samples difficult. While I did not observe the expected POLS and sexual differences between males and females of the same species, I did see a clear difference in syndrome among species. Further investigation into genetic differences which are seldom reported (Niemelä et al., 2013) between individuals may help reveal insight into potential differences in POLS strategies. While these observations conflict with the predictions of the POLS hypothesis, I note that there was an observable difference in behaviours and glucocorticoid concentrations amongst species. Given the similarities I show between male and female rodents during similar breeding conditions, I suggest that the expression of personality and accommodating physiological demand may be the result of a uniform selective pressure. To conclude, uniform syndromes in the observed species may arise from the directionality of various external selective pressures, including similar reproductive costs, similar environmental pressure from competition and resource availability, and species-specific life history. While these results do not abide by the fast-slow continuum predictions for males and females postulated by the POLS hypothesis, I suggest that the directionality in selective pressure may be similar between sexes. Thus, further investigation into the expression of sexual differences remains an intriguing endeavor for future research on sexual difference in life history.

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Tables and Figures

Table 2.1: Summary for log₁₀-transformed values of fecal glucocorticoids and expression of docility in all three species (DM = Deer Mice, RBV = Red-Backed Voles, and WJM = Woodland Jumping Mouse, DM, n = 104; RBV, n = 52; WJM, n = 15). Bolded variables are those with P<0.05 and are considered significant.

Variable	Df	Sum Sq	Mean Sq	F	P
DM					
Sex	1	1044	1043.58	2.418	0.12
Age	2	938	469.25	1.088	0.34
Repro	4	3020	755.11	1.751	0.15
Date	3	829	276.49	0.641	0.59
Body Mass	1	1782	1781.62	4.131	0.045
RBV					
Sex	1	29.2	29.22	0.106	0.75
Age	2	262.3	131.13	0.474	0.63
Repro	5	1200.4	240.08	0.868	0.51
Date	3	756.4	252.14	0.912	0.45
Body Mass	1	720.4	720.41	2.61	0.12

WJM

Sex	1	202.21	202.21	11.521	0.027
Age	2	153.79	76.90	4.381	0.098
Repro	3	1363.75	454.58	25.899	0.0044
Date	3	918.53	306.18	17.444	0.0092
Body	1	623.80	623.80	35.541	0.0040
Mass					

Table 2.2: Linear regression statistics for the relationship between docility, measured through the Handling Bag Tests (BT) and \log_{10} transformed fecal glucocorticoids in Deer Mice (n = 104) and Woodland Jumping Mice (n = 15), using sex, age class (adult, sub-adult and juvenile), reproductive condition, and body mass as random effects.

Predictor Variable	Coefficient	Standard Error	t	p
Deer Mice				
<i>Age</i>				
Juvenile	-8.826	9.026	-0.978	0.33
Sub-Adult	-10.513	6.816	-1.542	0.13
<i>Reproductive Condition</i>				
Non-Reproductive	18.296	12.806	1.429	0.16
Non-Scrotal	16.419	19.770	0.831	0.408
Pregnant	81.194	26.285	3.089	0.0027
Scrotal	13.781	20.252	0.680	0.49
Body Mass	-2.228	1.096	-2.03	0.045
Woodland Jumping Mice				
<i>Age</i>				
Juvenile	11.50	8.45	1.36	0.25
Sub-adult	6.90	8.28	0.84	0.45

*Reproductive**Condition*

Non-Reproductive	-10.8	3.54	-3.04	0.038
Non-Scrotal	-47.7	5.15	-9.27	0.00075
Pregnant	46.6	5.76	8.08	0.0013
<i>Body Mass</i>	-4.207	0.706	-5.96	0.0039

Table 2.3: Summary of log₁₀-transformed values of fecal glucocorticoids and total expression of explorative behaviour in all three species (Deer Mice = DM, n = 47; Red-Backed Voles = RBV n = 27; Woodland Jumping Mice = WJM, n = 10), bolded values are P>0.05 and are considered significant.

Variable	Df	Sum Sq	Mean Sq	F	P
DM					
Sex	1	2401	2401.2	0.673	0.42
Age	2	20685	10342.6	2.898	0.068
Repro	2	2464	1232	0.345	0.71
Date	4	19855	4963.8	1.39	0.26
Body Mass	1	2225	2224.8	0.623	0.44
RBV					
Sex	1	29688	29688.5	8.947	0.0091
Age	1	2622	2621.6	0.790	0.39
Repro	4	38078	9519.4	2.8687	0.060
Date	3	16323	5441	1.639	0.22
Body Mass	1	1350	1349.9	0.4068	0.53
WJM					
Sex	1	2754	2754.5	0.2304	0.66

Repro	2	6549	3274.6	0.273	0.78
Date	2	4005	2002.4	0.168	0.85
Body Mass	1	12389	12389.3	1.036	0.38

Table 2.4: Linear regression statistics for models examining the relationship between total exploration time and log₁₀ transformed fecal glucocorticoids in Deer Mice and Red-Backed Voles. Significant values (P > 0.05) are shown in bold.

Predictor Variable	Coefficient	Standard Error	t	P
<i>Deer Mice</i>				
<i>Sex</i>				
Male	-13.24	53.578	-0.25	0.80
<i>Age</i>				
Juvenile	-8.29	30.298	-0.274	0.79
Sub-Adult	42.01	26.657	1.576	0.012
<i>Reproductive Condition</i>				
Non-Reproductive	-26.076	52.308	-0.499	0.62
Non-Scrotal	-5.244	32.82	-0.160	0.87
<i>Body Mass</i>	2.975	3.768	0.790	0.44

**Red-Backed
Voles**

Sex

Male	-146.91	46.34	-3.170	0.0063
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Age

Sub-Adult	0.01709	59.55	0.0001	0.99
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*Reproductive
Condition*

Non-	-102.93	57.95	-1.78	0.096
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Reproductive

Non-Scrotal	-58.38	32.04	-1.82	0.89
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Pregnant	-72.38	67.96	-1.07	0.30
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<i>Body Mass</i>	-2.28	4.44	-0.64	0.53
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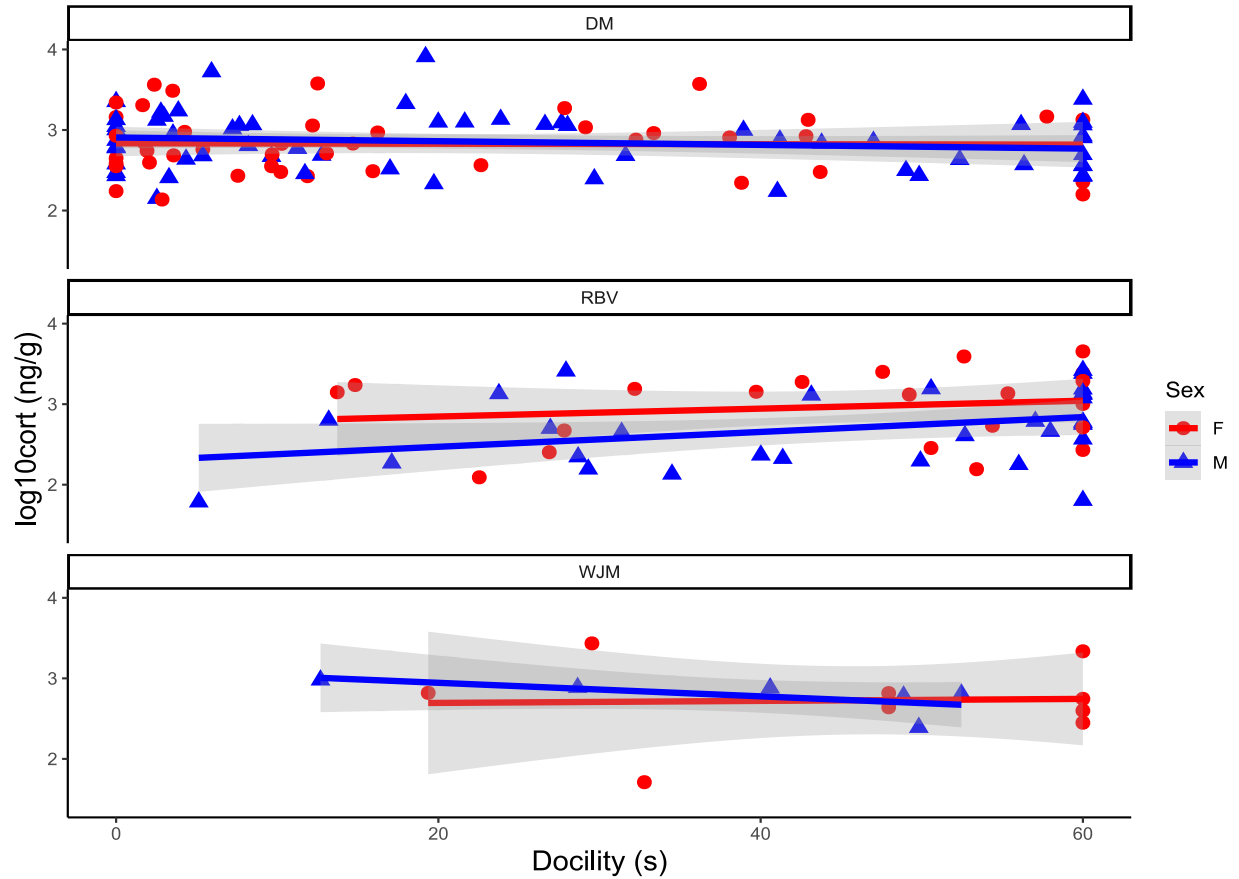


Figure 2.1: Relationship between log₁₀-transformed values of fecal metabolites in Deer Mice (total n = 105, males = 61, females = 45) Woodland Jumping Mice (total n = 16, males = 6, females = 10) and Red-Backed Voles (total n = 53, males = 31, female = 23) and docility. Individuals showed a negative relationship between docile behaviour and basal log₁₀ FCM in Deer Mice and male Jumping Mice, however Red-Backed Voles showed a positive relationship. Data points are jittered and represent individual test (red circle for females, and blue triangle for males). 95% confidence intervals are shown by shading.

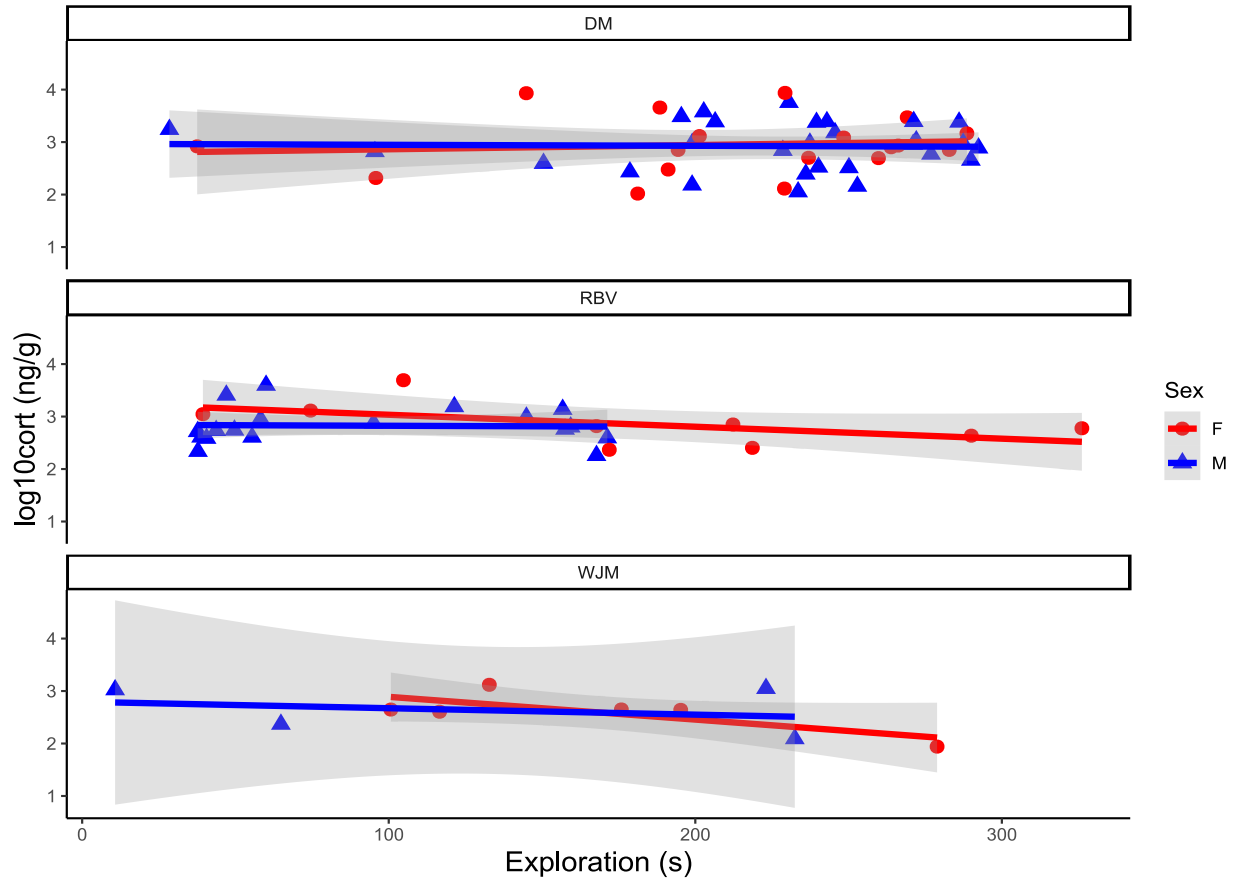


Figure 2.2: The relationship between \log_{10} -transformed fecal metabolites and total exploration behaviour in Deer Mice (total $n = 47$, males = 29, females = 19; Red-Backed Voles total $n = 28$, males = 18, females = 10; Woodland Jumping Mice total $n = 11$, males = 5, females = 6). Individuals showed a negative relationship in Jumping Mice, Red Backed Voles, and male Deer Mice; however, a positive relationship in female Deer Mice. Data points are shown by jitters (red circle for females, and blue triangles for males). Shading represents 95% confidence interval.

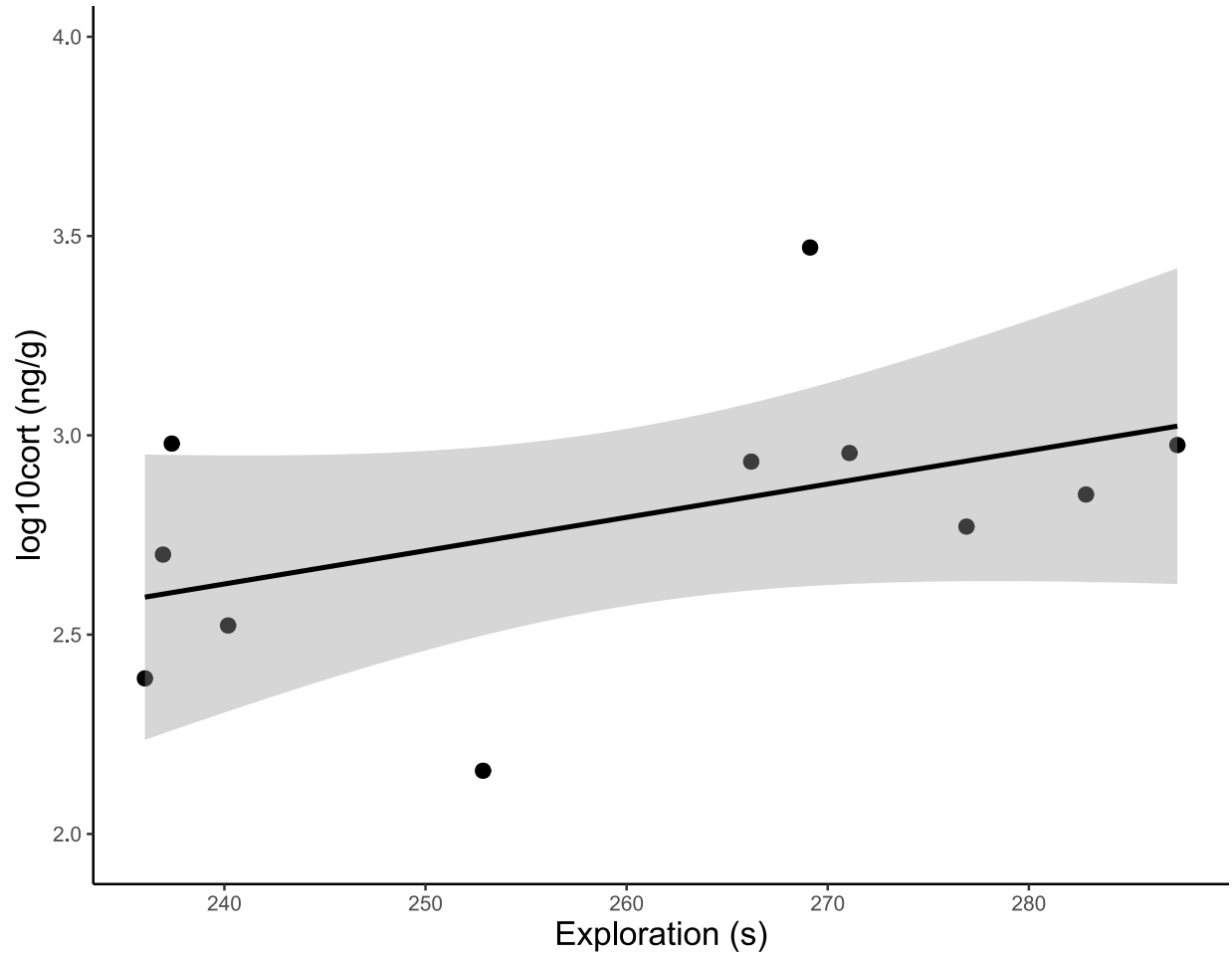


Figure 2.3: The relationship between \log_{10} -transformed values of fecal metabolites and docility in pregnant Red-Backed Voles. Individuals that underwent a Handling Bag Test are shown via black dots, and 95% confidence interval extracted through linear mixed effects model is represented through shading. Red-Backed Voles show a positive relationship between docility and basal fecal metabolite concentrations, where individuals with a higher concentration of glucocorticoids express higher levels of immobilization, or docile behaviour consistent with the POLS hypothesis.

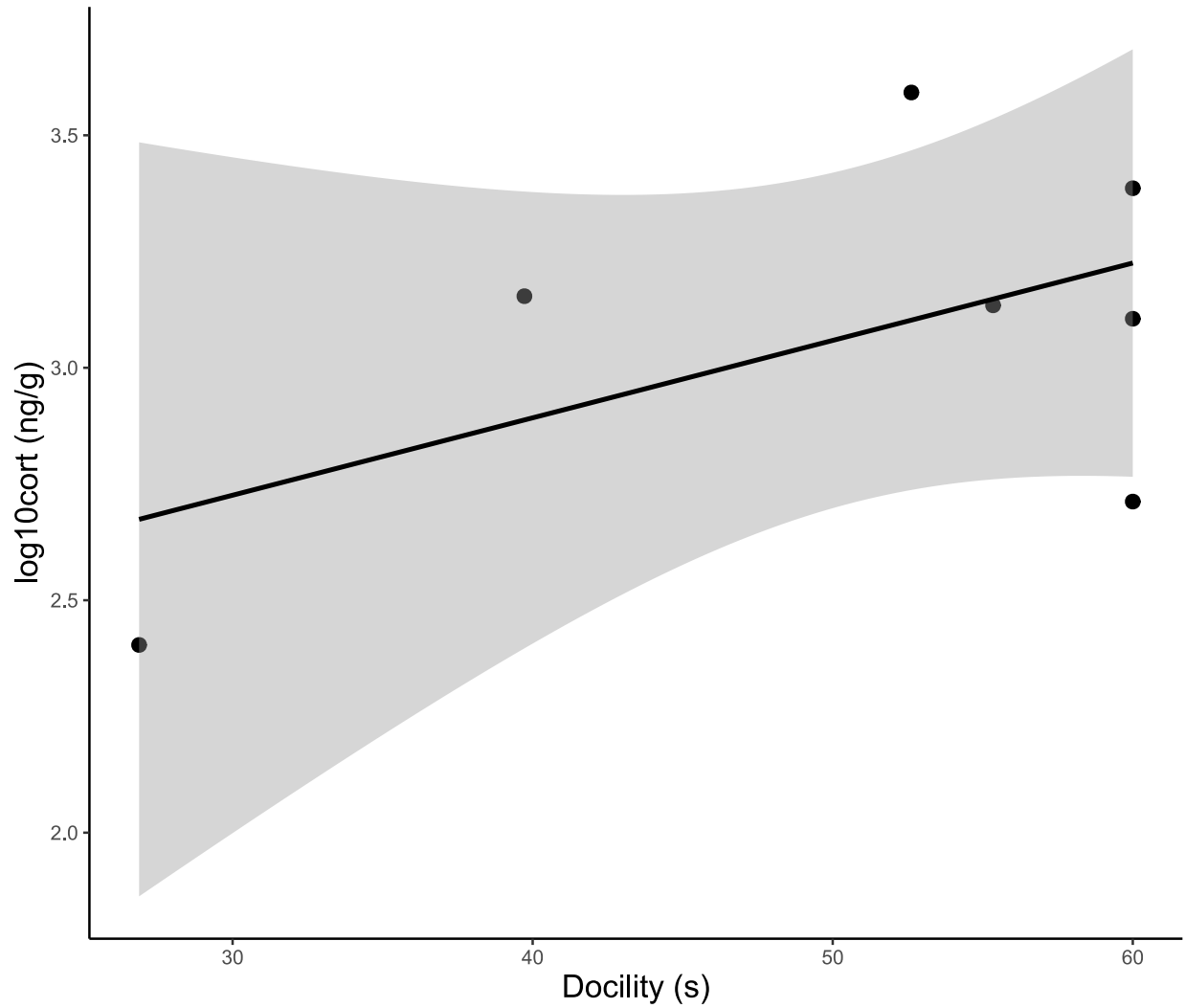


Figure 2.4: The relationship between \log_{10} -transformed fecal metabolites and time spent expressing exploratory behaviours including increased movement during the Open Field Test (OFT) for Deer Mice classified as the sub-adult age class. Shown are 95% confidence intervals (shading) calculated from mixed linear models.

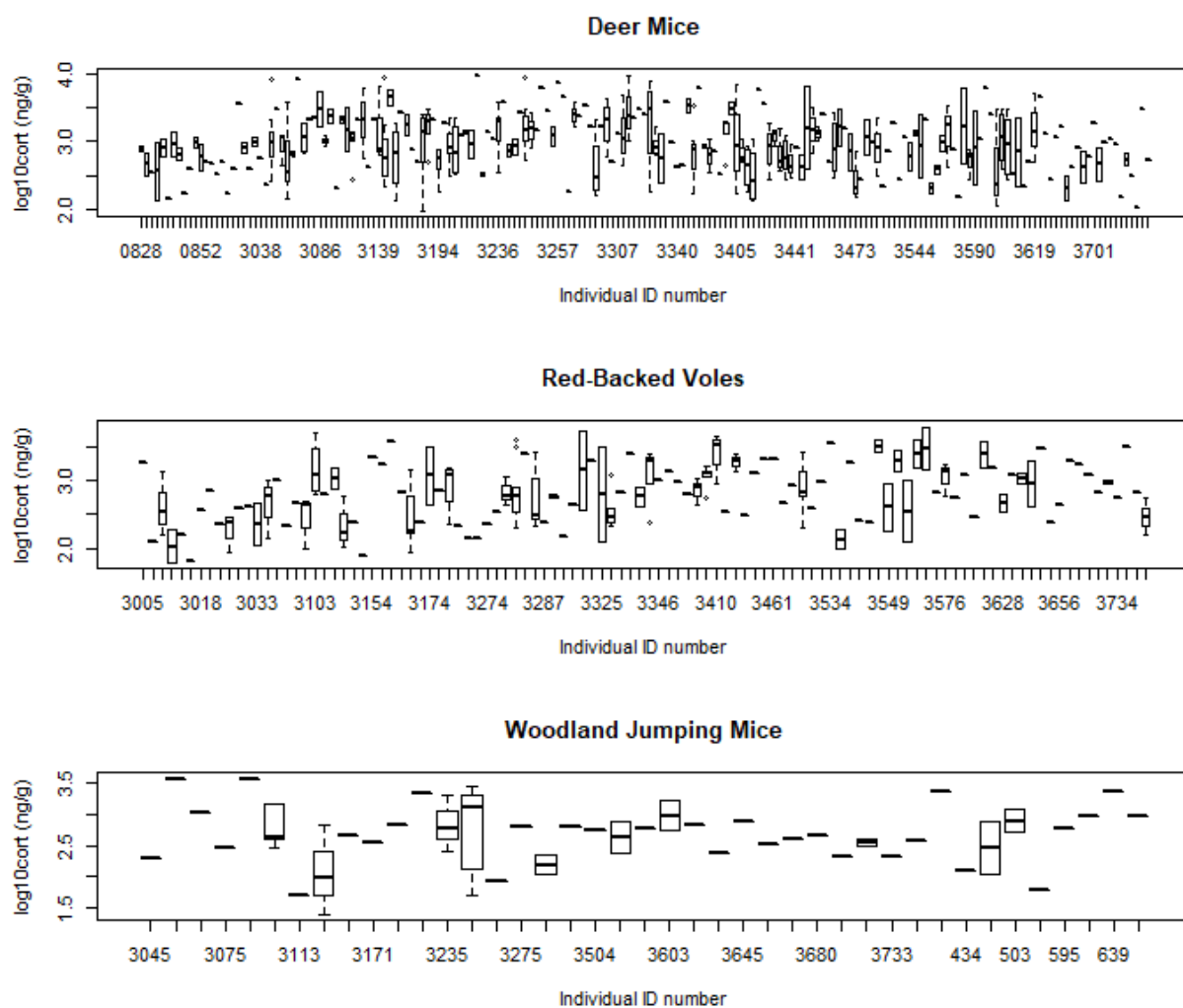


Figure 2.5: The relationship between \log_{10} -transformed fecal metabolites in Deer Mice (Top), Red-Backed Voles (Middle), and Woodland Jumping Mice (Bottom) across individual ID number. Shown are the median (black line) interquartile range (box) and minimum/maximum values (bars). All species show considerable variation in fecal metabolite concentration.

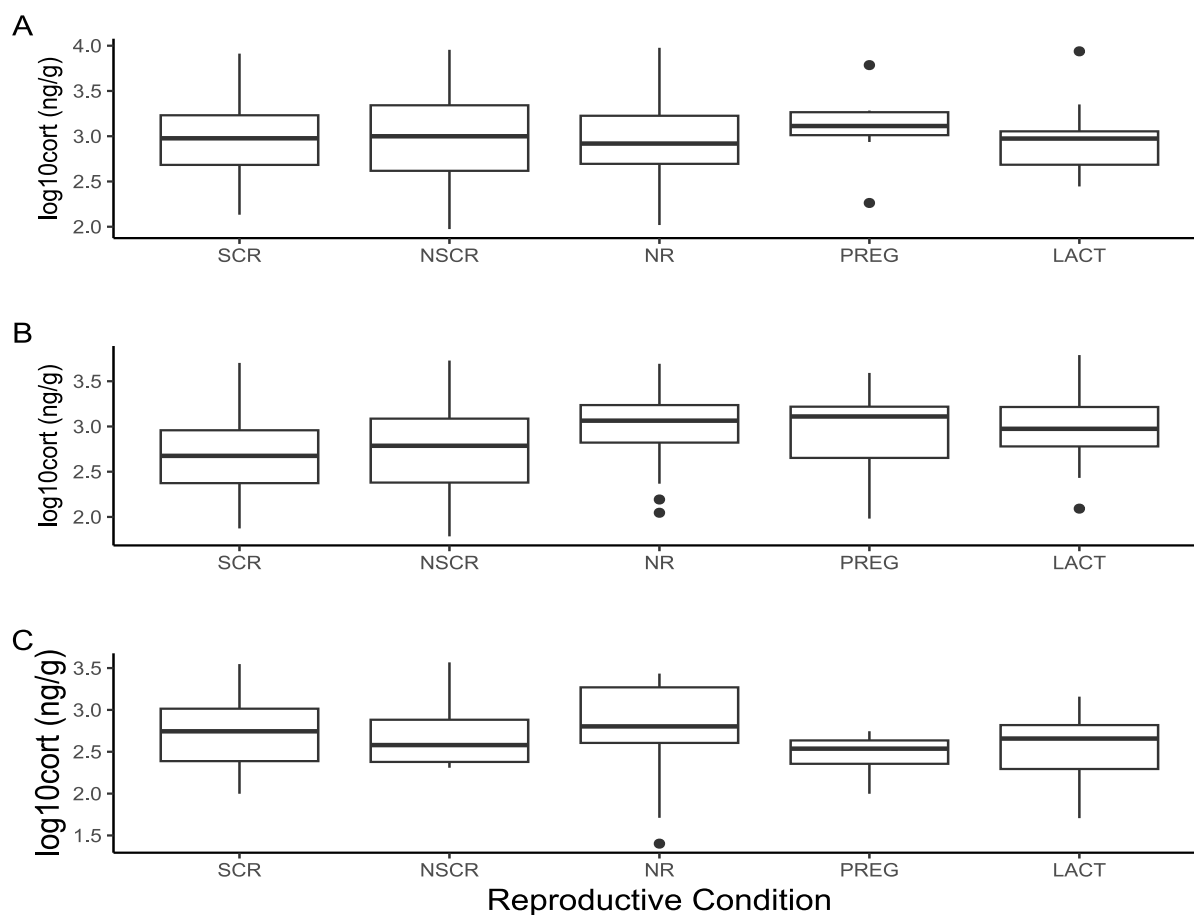


Figure 2.6: A series of boxplots showing the total basal fecal glucocorticoid metabolites in Deer Mice (panel A), Red-Backed Voles (panel B) and Woodland Jumping Mice (panel c) based on reproductive status (NSCR = non-scrotal, SCR = scrotal for males and PREG = pregnant, LACT = lactating, PERF = perforate, or NR = non-reproductive for females). Dark lines represent the mean value of fecal metabolite (ng/g) while whiskers represent the standard deviation.

General Discussion

How species interact within an environment is governed by a complex suite of relationships between behavioural, physiological and life history traits that arise from energetic trade-offs (Dammhahn et al., 2018; Réale et al., 2010). The fast-to-slow continuum predicted by the Pace-of-life syndrome (POLS) hypothesis suggests that there are measurable relationships in traits such that the directionality and strength of traits are observable among species, between populations, and individuals (Mathor and Frankenhuis, 2018; Montiglio et al., 2018; Réale et al., 2010). While there is support for the POLS hypothesis across different phylogenetic levels because syndromes are highly influenced by environmental conditions and population dynamics, evidence to support the POLS is not ubiquitous between individuals or populations (Royauté et al., 2018). The overall objective of my research was to determine the relationships between behaviour and physiology in three species of rodents. To investigate my objectives, I examined (1) behaviours relating to movement within a home-range, including docility and exploration, and (2) total fecal glucocorticoid metabolites as a proxy for energetic stress. I used evaluated differences among species and between individuals, regarding behaviours and physiology.

In chapter one, I tested the hypothesis that species with the same ecological niche will express differences in behaviour at different stages of the reproductive cycle. While there was the predicted correlation between docility and exploration in all species, I found limited evidence to support the predictions of the POLS hypothesis between individuals. However, I did find that among species there were clear differences in behavioural strategy across all stages of the reproductive cycle. Based on these findings, I propose that the coexistence of species inhabiting a similar ecological niche should promote the divergence of behavioural strategies. These results are perhaps not surprising, given dispersal is the primary mechanism to alleviate inter and

intraspecific competition within an environment (Stearns, 2003). Further, the results of my research supports observations that coexistence promotes divergence in personality, since personality has been observed to mediate species co-existence (Morris and Palmer 2023; Palmer et al., 2003).

In chapter two, I examined sexual differences in behaviour and physiology using movement behaviours, docility and exploration, and total fecal glucocorticoids as a measure of energetic stress. I hypothesized that differences in reproductive costs should result in the divergence of POLS traits along the fast to slow continuum between sexes, with a similar directionality in selective pressure. I found that the directionality and position of POLS traits remained relatively uniform across all three species. A uniform distribution of POLS traits is not uncommon in systems where reproductive investment is consistent between males and females, or there is an exceedingly low level of parental investment (Hämäläinen et al., 2018). Uniform expression of POLS traits is also possible in systems where external environmental variables, such as resource availability, mask selective pressures on energetic investment (Hämäläinen et al., 2021). Because I observed a uniform POLS between sexes, yet a difference in POLS strategies among species, I further suggests that the evolutionary relationships between coexisting species are complex and warrant further investigation.

Overall, my study highlights the complexity in the POLS hypothesis and highlights the necessity to understand individual components of species behaviour to better understand how species interact within an ecosystem. Animal personality and extending POLS have been shown to influence an array of ecosystem functions including habitat availability and seed dispersal (Boone et al., 2022; Brehm and Mortelliti, 2022; Réale and Dingemanse 2012). Such that,

differences in personality influence how individuals move and interact with conspecifics and various components of the ecosystem. Therefore, it is important to understand evolutionary relationships and the expression of different POLS traits, to better understand how species react and function within an environment.

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Appendices

Appendix A. Common actions for all rodents in the Open Field Test (OFT). Action refers to what an individual may do, while the definition is the interpretation for the observer. The code column refers to the ID used in all statistical analysis to attribute behaviour to any given personality type. For the OFT, any behaviours marked as moved are counted as total exploration time, while freezing and hiding behaviours are considered non-exploratory.

Action	Definition	Code
Total time spent walking, running, or jumping around.	Any form of forward locomotion	Move
Time spent performing other locomotion (scratching, sniffing, scanning)	Any form of stationary locomotion	Move
Time spent stopped/doing nothing (visible)	Individual stopped.	Freezing
Time spent stopped (hiding)	Individual is obscured from the camera by hiding in the entrance	Hide

Appendix B. Ethogram for possible actions of all tested individuals during the one-minute Bag Test (BT). Action refers to individual movements displayed by animals with the definition used by the observer to mark each action. The code refers to statistical analysis where behaviours are summed together to generate a cause of personality. Escape, movement, and grooming behaviours are considered non-docile while freezing behaviour is considered docile.

Action	Definition	Code
Flee, running, digging at bag	Individual attempts escape through bag by biting, scratching at bag.	Escape
Freezing/immobile (visible)	Individual stops	Freezing
Grooming	Individual raises paws over head and continues to lick/scratch at self	Grooming
Foraging	Individual continues to gather and eat seeds	Movement
Other locomotion	Individual is walking around bag with no distinct escape attempt	Movement

Appendix C. The results from the repeatability analysis for the (Bag Test) BT and (Open Field Test) OFT measuring Docility and Exploration respectively. Results highlighted in bold were determined to be repeatable. All estimates were calculated using mixed effects model with age, sex, and reproductive condition as a fixed effect and individual ID as a random effect. 95% confidence intervals were calculated using parametric bootstrapping.

Docility - ID random effect

Behavioural Variable	Mean	R	SE	CI (95%)	P
Deer Mouse					
Movement	0.341	0.269	0.143	0.0351, 0.6	0.044
Escape	0.148	0.066	0.13	0, 0.443	0.33
Grooming	0.0998	0.031	0.093	0, 0.313	0.35
Foraging	0.109	0	0.122	0, 0.407	1
Freezing	0.404	0.339	0.137	0.116, 0.655	0.020
Red-Backed Vole					
Movement	0.34	0	0.268	0, 0.837	1
Escape	0.369	0	0.259	0, 0.805	1
Grooming	0.348	0	0.243	0, 0.761	0.5
Freezing	0.354	0	0.268	0, 0.819	1
Woodland Jumping Mouse					
Movement	0.0001	0	0	0, 0	1
Escape	1	1	0	1, 1	0.30
Freezing	1	1	0	1, 1	0.30

Exploration - ID random effect

Behavioural Variable	Mean	R	SE	CI (95%)	P
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Deer Mice					
Move	0.284	0.066	0.207	0, 0.691	0.37
Groom	0.237	0	0.214	0, 0.66	1
Freezing	0.842	0.809	0.067	0.695, 0.943	0.0019
Hide	0.227	0	0.209	0, 0.658	1
Red-Backed Vole					
Move	0.836	0.315	0.232	0, 1	0.34
Groom	0.741	0	0.308	0, 1	0.5
Freezing	0.856	0.391	0.205	0.187, 1	0.097
Hide	1	1	0	1, 1	0.000067

Appendix C. The total sample size for each age class, sex, and reproductive status of all three species used in the statistical analyses of this study. Deer Mice (DM), Red-Backed Voles (RBV) and Woodland Jumping Mice (WJM) are shown as a total sample size (n) for each group. Age includes juveniles (J), sub-adults (SA) and adults (A). Reproductive class contains individuals marked as lactating (LACT), pregnant (PREG) or non-reproductive for females (F) or scrotal (SCR) and non-scrotal (NSCR) for males (M).

Species Age Sex Repro n

DM A F LACT 3

DM A F NR 22

DM A F PREG 2

DM A M NSCR 16

DM A M SCR 24

DM J F NR 17

DM J M NSCR 9

DM SA F LACT 1

DM SA F NR 12

DM SA M NSCR 19

DM SA M SCR 12

RBV A F LACT 5

RBV A F NR 10

RBV	A	F	PREG	8
RBV	A	M	NSCR	18
RBV	A	M	SCR	22
RBV	J	F	NR	1
RBV	J	M	NSCR	1
WJM	A	F	LACT	4
WJM	A	F	NR	6
WJM	A	F	PREG	2
WJM	A	M	NSCR	2
WJM	A	M	SCR	3
WJM	J	M	NSCR	1
WJM	SA	M	NSCR	1

Appendix D: The relationships between total fecal glucocorticoid metabolites and % antibody binding compared to a standard curve generated using Soper, 2021. Shown is the relationship for Deer Mice, Red-Backed Voles and Woodland Jumping Mice for corticosterone or cortisol to validate fecal metabolites.

