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Environmental modulation and physiological correlates of same-sex affiliative behavior in female meadow voles

Author

Ondrasek, Naomi

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Environmental modulation and physiological correlates of same-sex affiliative behavior in female meadow voles

by

Naomi Rose Ondrasek

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requirements for the degree of

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University of California, Berkeley

Committee in charge:

Professor Irving Zucker, Chair
Professor Lance Kriegsfeld
Professor Eileen Lacey

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Abstract

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The prevalence of female-biased affiliations in group living mammalian species suggests that same-sex relationships are of particular importance for females. However, little is known about the influence of environmental and physiological factors on same-sex social bonds. Female meadow voles present an interesting opportunity for the investigation of these questions because free-living females display seasonal variations in same-sex affiliation. From summer to winter, females transition from an aggressive, territorial phenotype to an affiliative, group living phenotype. The thermoregulatory advantages of huddling have been offered as an explanation for winter sociality in meadow voles; thus, I designed a study to assess the effects of ambient temperature, day length, food availability, and frequency of handling on same-sex affiliative behavior and several potential physiological correlates. Adult female pairs were housed in varying combinations of day length (short (SDs) or long days (LDs)), temperature (21˚C or 10˚C), and food availability (ad libitum or restricted) and regularly assessed for food intake and body mass. After seven weeks of treatment, females were evaluated for same-sex huddling behavior using 3 h partner preference tests. Uterine mass and serum concentrations of corticosterone and estradiol were assessed for all focal voles. In another study, group size and social preferences were evaluated in male and female meadow voles. Voles were housed in same-sex trios at weaning and behavior tested at 70-100 days of age. During behavior tests, focal voles were presented with the options of huddling with one familiar individual (a member of their trio) or a trio of strangers. My findings suggest that:

1) Day length, food availability, and ambient temperature interact to regulate same-sex affiliative behavior in female meadow voles.
2) Low temperature exposure can modify social preferences without increasing huddling behavior.

3) In SDs, lower ambient temperature augments the propensity to interact with strangers without interfering with existing social bonds, whereas lower temperature in LDs disrupts the retention of bonds.
4) Food restriction enhances affiliative behavior in SDs without causing significant body mass decline.
5) Differences in handling modulate uterine mass, plasma corticosterone, and plasma estradiol without modifying same-sex affiliation.
6) Under certain environmental conditions, variations in same-sex affiliative behavior are correlated with plasma corticosterone and estradiol.
7) The propensity to join a group consisting of novel individuals varies by day length and sex.

Collectively, the results described in this dissertation suggest that conditions associated with winter (food scarcity, low temperatures, and short day lengths) increase social tolerance and promote aggregation of females into groups. Corticosterone secretion and the reproductive axis are also responsive to the environmental factors examined; however, current concentrations of blood plasma corticosterone and estradiol do not fully account for the behavioral responses observed.

For Tommy and Logan

Acknowledgements

There are many people who deserve my heart-felt gratitude for their contributions to my personal and professional development. I cannot mention everyone here, but know that I count myself lucky to have interacted with such an abundance of intelligent and compassionate individuals.

First and foremost, I owe many thanks to my doctoral advisor, Irving Zucker. The title "doctoral advisor" hardly provides a true measure of the role you have played in my life as a graduate student. When I was struggling to choose a graduate school, I interviewed some of your current and former students about the quality of your mentorship. Every review was glowing, especially one that began with, “Go to Cali and work with Irv. He's one of the most genuinely good people out there—there's no other way to put it. He will treat you with respect and after talking with him, you can tell his work is his passion.” It has been a little over six years since I first arrived at Berkeley, and I can say with absolute conviction that I made the right decision. My personal life injected a number of obstacles into my progress towards this degree, but through it all, I never doubted that you were on my side and would see me through to the end as long as I brought desire and effort to the table. You helped me cultivate my ability to sift through the literature and lift out intriguing questions, a skill that you poetically refer to as “the ephemeral sense of taste." Rather than thrusting a research project upon me, as some advisors do when their students are lost in a sea of literature, you displayed an astonishing amount of patience, giving me ample freedom to develop my own interests and my voice as a scientist. I can't name every way in which I have benefitted from being your student, but I can say for certain that your approaches to mentorship—especially your calmness, humor, compassion, and honesty—will color the way in which I mentor my own students.

I have been lucky as a student, particularly in the assembly of professors I have worked with. Eileen Lacey, Lance Kriegsfeld, and George Bentley—thank you for your support and encouragement, for guiding me through the literature, and for helping me see the value of studying biology at multiple levels of analysis. Douglas Shedd and Ron Gettinger, even though it's been more than six years since I graduated from college, you continue to be two of my most influential mentors, role models, and biggest supporters. I came into college with my sights set on a degree in English, but your passion for science made it easy for me to change my mind. Karin Warren—your optimism and love of teaching is contagious; I’m so grateful that I weaseled my way out of spending a semester translating Cicero and into your introductory Environmental Studies course.

To my friends and fellow lab mates, thank you for giving me the two things a graduate student needs the most—humor and a listening ear. I have shared many stories, laughs, and frustrations with you, and it's not an understatement to say that I could not have made it this far without your support. Steve and David—I could always count on you, whether I needed schooling in a new technique or a dose of offbeat humor. Thank you to the women in my graduate department and my fellow Randolph-Macon alumnae for providing such a strong support network. In particular, a big thank

you to Erin and Mariska for the many cumulative hours of discussing dreams, advice, fears, and funny stories about marriage and academia.

Numerous undergraduates contributed to my research and made everything possible. Each of you gave me invaluable experience as a mentor and made my research much more enjoyable. Some of my fondest memories as a graduate student consist of our long conversations on topics ranging from science to music to philosophy. Thanks to Negar Foolad and Victry Mueller for assisting with management of the vole colony; to Kacie Hsu and Tracy Burkhard for sticking with me through a full year of what was at times a grueling experimental schedule; and to Jessica Post and Tiffany Nguyen for helping me bring it all to a close in the last year. Finally, special thanks to Adam Wade—without you my dissertation would not have been possible. Over a period of two years, you contributed hundreds of hours (most of them unpaid!) to my research and kept things running when unexpected issues drew me away on several occasions. Many thanks for your tireless efforts and the stellar quality of your work.

To my dad: thank you for teaching me the important life skill of laughing when it seems most inappropriate. Fear, anger, bitterness, and sadness—these can all be diffused by a well-timed (or very poorly-timed) joke. Without complaint, you sacrificed a great deal for my education and on many occasions reserved your opinion, opting instead to encourage me to follow my own mind. You taught me to treat others with respect and without discrimination, and to behave honorably. Daniel and Gabe: even when we were children, our exhaustingly long conversations about anything and everything taught me to think critically and craft strong arguments. When cordial debates failed to settle a disagreement, you taught me the value of practical jokes. To my family by marriage: although stories about nightmarish in-laws have always been plentiful, I can happily report that my experience has been the exact opposite. Thank you for welcoming me into your family, and for the support and concern you have shown for my wellbeing.

During my early childhood, my mom saw my fledgling passion for animal life and encouraged it. There was scarcely a time when our house did not contain a small zoo. Birds, cats, dogs, rabbits, and rodents flew, ran, and sometimes chewed their way around our home, but still she quietly added to my menagerie. She was a fierce and strong woman who insisted that I could be anything if I put forth my best effort. The faith that she placed in my abilities remains a major source of inspiration, even in her absence.

And lastly, I thank my husband, Tommy. How can I express your value to me in just a few lines? It has been ten years since we met, seven since we were married, and still strangers ask if we are newlyweds. You are my most trusted advisor and closest friend. Thank you for doing anything to get a laugh, for the sacrifices and the many hours you have spent reading, scoring, and critiquing my work. I have always admired your inner strength, generosity, solid sense of ethics, and the passion with which you pursue your goals. Being with you has made me into a better person. Thank you for joining me as my partner through graduate school and through life.

Curriculum Vitae

Professional Preparation

PhD, Integrative Biology (2012)
University of California, Berkeley
Research: Environmental modulation and physiological correlates of same-sex affiliative behavior in female meadow voles

BS, Biology (2006; GPA=4.0)
Randolph-Macon Woman's College
Research: Effects of atrazine on reproductive success in African clawed frogs

Appointments

Spring-Fall 2012
Graduate Student Researcher (NSF-funded)
University of California, Berkeley
- Curriculum development for evolution section of introductory biology class (Introductory Biology: Ecology, Evolution, and Plant and Fungal Diversity)

Summer 2011
Class Reader
University of California, Berkeley
- Principles of Biochemistry

Fall 2007
Graduate Student Instructor
University of California, Berkeley
- Introductory Biology: Ecology, Evolution, and Plant and Fungal Diversity

Spring 2007
Graduate Student Instructor
University of California, Berkeley
- Behavioral Ecology

Fall 2003-Spring 2006
Teaching and Laboratory Assistant
Randolph-Macon Woman's College
- Zoology, Ornithology and Mammalogy, Animal Behavior

Presentations

Spring 2012
Invited Talk: Ondrasek, N.R. "Female Relationships—Why We Should Study Them and What We Know."
AAUW (American Association of University Women) West Contra Costa County and Berkeley Joint Meeting, El Cerrito, California

Fall 2011
Invited Talk: Ondrasek, N.R. **"**Life as a Scientist with Autoimmune Disease."
Crohn's and Colitis Foundation of American Northern California Chapter Meeting, Fremont, California

Summer 2011
Contributed Poster: Ondrasek, N.R. "Environmental Modulation of Same-Sex Affiliative Behavior in Female Meadow Voles."
15th Annual Meeting of the Society for Behavioral Neuroendocrinology, Querétaro, Mexico

Fall 2008
Invited Talk: Ondrasek, N.R. "Thermosensitivity of Biological Rhythms."
Sigma Xi Speaker Series, Randolph College, Lynchburg, Virginia.

Fall 2008
Invited Talk: Ondrasek, N.R. "Surviving the Summer Blizzard: the Role of Temperature in the Seasonal Timing of Non-Sexual Social Behavior."
Achievement Rewards for College Scientists Foundation Visit, University of California, Berkeley

Outreach

2010-Present
Reader/Recording Editor
Learning Ally (formerly Recording for the Blind and Dyslexic)
- ❖ Produce voice recordings of published materials for students and professionals with learning and visual disabilities. To date, I have recorded over 100 hours of text, primarily biology-related.

2008-2012
Undergraduate Research Mentor
University of California, Berkeley
- ❖ Mentored 7 undergraduates in the conduct of research. Taught laboratory techniques and experimental design, hosted literature meetings and surgery labs, and provided career and academic advice. Four students worked with me for a year or more; one completed an honors thesis with our lab and

received a Biology Scholars Scholarship (UC Berkeley) that funded his continued participation in my work.

2010-2011
Science Writer
Berkeley Science Review
- ❖ Wrote 2 feature articles, one on Astrobiology and the other on the neuroscience of dreaming. Astrobiology piece featured on the cover of Fall 2010 issue.

Fall 2008
Women in Science Panelist
Randolph College
- ❖ Answered questions from audience members regarding life as a woman in science.

Summer 2008
Community College Student Research Mentor
Pierce College/University of California, Berkeley Partnership in Neuroscience Program
- ❖ Served as summer research mentor to a community college student (who eventually gained admission to UC Berkeley and continued working with me for over 2 years, until his graduation).

Honors and Awards

2012-15	**Minority Postdoctoral Research Fellowship** (National Science Foundation; $189,000)
2011-12	**Dissertation Fellowship** (AAUW; $20,000)
2011, 2012	**Summer Research Grant** (University of California, Berkeley; $3,000)
2008-11	**Graduate Research Fellowship** (National Science Foundation; $135,000)
2006-09	**Graduate Research Fellowship** (Achievement Rewards for College Scientists; $36,000)
2002-06	**Gottwald Full Tuition Scholarship** (Randolph-Macon Woman's College; $80,000)
2006	**Dean's Senior Leadership Award** (Randolph-Macon Woman's College)
2006	**Maude Huff Award to co-valedictorian** (Randolph-Macon Woman's College)
2004	**Udall Scholarship in Environmental Policy** (Morris K. Udall Foundation; $5,000)

Research Experience

Fall 2006-Fall 2012
PhD Researcher
University of California, Berkeley

Fall 2005-Spring 2006
Senior Honors Researcher
Randolph-Macon Woman's College

Summer 2005
NSF-REU Summer Research Intern
Virginia Marine Science Museum and Norfolk State University
- ❖ Managed team of high school-aged volunteers interested in educating the public about environmental threats to Chesapeake Bay ecosystems. Also cared for injured birds in museum aviary.

Summer 2004
NSF-REU Summer Research Intern
Berry College
- ❖ Examined effects of invasive red fire ants on reproductive success of threatened Eastern bluebird populations in northwestern Georgia.

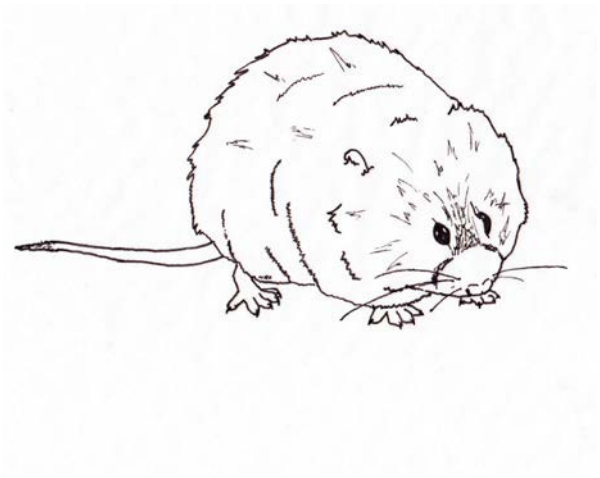
Summer 2003
Summer Research Intern
Randolph-Macon Woman's College
- ❖ Contributed to curriculum design for course in Environmental Studies.

Society Memberships

Society for Integrative and Comparative Biology
Society for Behavioral Neuroendocrinology
Sigma Xi Scientific Society
Phi Beta Kappa

If we have ever regarded our interest in natural history as an escape from the realities of our modern world, let us now reverse this attitude. For the mysteries of living things, and the birth and death of continents and seas, are among the great realities.

-Rachel Carson (1952)



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Introduction: Non-Reproductive Affiliations and Group Living

When we no longer look at an organic being as a savage looks at a ship, as something wholly beyond his comprehension; when we regard every production of nature as one which has had a long history; when we contemplate every complex structure and instinct as the summing up of many contrivances, each useful to the possessor, in the same way as any great mechanical invention is the summing up of the labour, the experience, the reason, and even the blunders of numerous workmen; when we thus view each organic being, how far more interesting—I speak from experience—does the study of natural history become!

-Charles Darwin (1859)

Social groups exist in a wide diversity of organisms. Rodents, insects, cetaceans, bats, primates, birds, fishes, and even some reptiles can be found in assemblages that vary in composition (relatives or non-relatives), size (from a few individuals to millions), and stability (lasting only temporarily or for entire lifetimes). Humans too are deeply social, so much so that scientists and poets alike can scarcely define what it means to be human without including descriptions of social behavior. The conspicuousness of many social groups and their existence across a great range of taxa clearly suggest that these gatherings offer some important benefit to individuals. Perhaps it is no surprise, then, that behavioral biologists have focused much intellectual effort on resolving the mysteries of sociality, or the assembly of conspecifics into social groups.

Our understanding of group living has increased markedly over the past several decades, particularly as multiple lines of investigation have converged, yielding a more complete picture of sociality's causes and consequences. Research in the field of behavioral ecology has revealed the costs and benefits associated with sociality and the mediating roles played by environmental factors. Of great importance are models that predict the probabilities of philopatry and dispersal in free-living animals (Alexander 1974; Emlen 1982; Mumme 1997; Koenig et al., 1992; Solomon 2003). By assessing the likelihood that individuals will depart from or remain within their natal territories,

these models effectively predict the formation of kin-based social groups. Such work provides an “outside-in” perspective on sociality—that is, how factors external to the animal regulate social behavior.

Equally important, the physiological perspective asks a different sort of question: within individual organisms, which factors influence the tendency to gravitate towards or avoid conspecifics? Today, better techniques with greater resolution at the molecular scale allow biologists to delve deeper into the inner workings of organisms, permitting more integrative investigations of the proximate factors underlying social behavior. Most recently, great strides have been made in elucidating the roles of steroid hormones and neuropeptides, including oxytocin and vasopressin, in the neural pathways that regulate sociality (Curtis et al., 2007; Goodson et al., 2006; Goodson 2008; Nelson 2005).

While these discoveries are of great importance, it is worth noting that most of them address only a few types of social behavior. No universal consensus for the precise categorization of various social behaviors exists, but for the sake of clarity, a brief discussion of definitions follows. In *Sociobiology*, author E.O. Wilson (1975) defines social behavior as a varied assortment of interactions in which one or more participants derive benefit. Social behavior may be further separated into the general categories of aggression, which drives individuals apart, and affiliation, which brings or keeps individuals together (Nelson 2005). Defined in this manner, the term “affiliation” encompasses parental, mating, and same-sex affiliative behaviors. Often, the term “affiliative” is reserved for descriptions of so-called “friendly” behaviors, such as allogrooming and huddling (e.g., Cameron et al., 2009; Parker and Lee 2003; Saltzman et al., 1991). To avoid anthropomorphizing animal behavior, I will assume the broad definition of affiliation as a category of behaviors that brings animals together, although I will maintain a clear distinction between non-reproductive and reproductive affiliative behaviors: “non-reproductive affiliative behavior” will refer to interactions that do *not* directly contribute to the production or rearing of young (e.g., social bonding between females or the formation of non-breeding aggregations), while “reproductive affiliative behavior” will refer to interactions that do (e.g., alloparenting or social bonding between mates).

In general, aggression and affiliative behaviors directly involved in reproduction (e.g., mate guarding, courtship, and parental interactions) have received considerably more attention than non-reproductive affiliative behavior (e.g., allogrooming between female group members; Cameron et al., 2009; Goodson et al., 2006; Nelson 2005; Tang-Martinez 2003). Studies on mating, parental, and aggressive behaviors extend back into the early 1900s, but interest in the physiological bases of non-reproductive affiliation and bond formation did not arise until the end of the 20th century (Nelson 2005; Parker and Lee 2003; Beery et al., 2008). On May 16, 2012, a search of the PubMed database using two groups of terms—1) “sexual,” “reproduc*,” “parental,” “aggress*,” combined with “social behavior” and 2) “same-sex,” “same-sex affiliat*,” “non-sexual,” “non-sexual affiliat*,” combined with “social behavior”—returned 63,888 and 821 results respectively (the “*” symbol represents wildcard terms). Individually, the categories of sexual, parental, and aggressive behaviors each contained more than 15,000 publications.

Non-reproductive affiliative behavior is quite common in the animal world, particularly in the form of same-sex interactions. In migrating birds, females and males

may form sex-segregated flocks that take flight several weeks apart and overwinter in different areas (Falls and Kopachena 2010). In mammals, the prevalence of male-biased dispersal and female-biased philopatry produces a predominance of female-based kin groups (Engelhaupt et al., 2009; Lacey and Sherman 2007; Nunes 2007; Solomon 2003). The fact that female groups are widespread across numerous mammalian species suggests that females gain significant benefits from interacting with one another (Hamilton 1964; Lacey and Sherman 2007; Silk 2007). This conclusion receives particularly strong support from studies on cooperatively-breeding species, in which group living offers several advantages to females, including communal nursing, group provisioning of offspring, shared defense of young and territory, and increased lifetime reproductive success (Konig 1994; Lewis and Pusey 1997; Packer et al., 2001; Silk 2007; Solomon and Keane 2007).

Despite the prevalence of female associations in mammals, the determinants and effects of affiliative behavior between females remain poorly understood (Goodson et al., 2006; Silk 2007; Tang-Martinez 2003). In general, affiliative behavior has positive impacts on individuals, including reductions in stress and heart rate, enhanced immune function, and improved infant survival (Feh and de Mazières 1993; Hennessy et al., 2008; Silk et al., 2003). However, only a small number of studies, many conducted on nonhuman primates, have directly addressed the consequences of same-sex affiliation for females. Squirrel monkeys (*Saimiri sciureus*) live in sexually segregated groups and associate primarily with individuals of the same sex. While agonistic interactions occur commonly amongst males, commingling females are strongly attracted to one another and predominantly display affiliative behaviors. Establishment of same-sex dyads results in a significant and prolonged decline in basal cortisol levels in females; this is not the case for males (Saltzman et al., 1991). Additionally, social facilitation by same-sex partners enhances a female's reproductive potential by increasing the likelihood that she will display ovarian cyclicity (Schiml et al., 1996).

Given that many social groups are kin-based, investigations of social behavior have focused heavily on the costs and benefits of interactions between genetic relatives. However, in the case of same-sex affiliation, both related and unrelated females may benefit from same-sex interactions. Female horses live in groups with multiple unrelated members and form strong, long-lasting relationships with one another, often remaining in close proximity to preferred partners and engaging in allogrooming. Socially-integrated females experience less harassment by males and show increases in foal birth rates and survival, independent of habitat quality, dominance status, and age (Cameron et al., 2009). In rodents, less is known regarding the incidence of same-sex affiliative behaviors because many of the field studies examining female associations rely on trapping data, which reveal little information about female interactions (Silk 2007; West and Dublin 1984). Much of the relevant research has been conducted on arvicoline (vole) species and will be considered later.

Seasonal Variations in Same-Sex Affiliative Behavior—An Opportunity

In the animal world, a multitude of behaviors varies on a seasonal basis. Comparisons between seasonally varying phenotypes have uncovered a wealth of information regarding the environmental and neuroendocrine regulators of animal behavior. For instance, comparisons of summer and winter phenotypes in mammalian and avian species have elucidated the roles of gonadotropin-releasing hormone (GnRH) and androgens in regulating reproduction and male aggression, respectively (Ball and Bentley 2000; Nelson 2005; Prendergast et al., 2002). Despite the usefulness of this approach, very few studies have examined the determinants of affiliative behaviors within the context of seasonality (e.g., Beery et al., 2008; Beery et al., 2009; Ferkin and Seamon 1987). Much of what is known regarding the proximate mechanisms underlying affiliative behavior derives from comparisons of between-sex affiliation in monogamous and promiscuous species (Goodson et al., 2006). While interspecies comparisons are certainly necessary for uncovering the evolutionary origins of group living, they possess inherent drawbacks—namely the difficulty of controlling for differences in other assortments of behaviors (e.g., mating systems, parental care patterns, and habitat preferences). The physiological mechanisms that affect affiliative behaviors independently of these confounding factors remain as one author says, “almost wholly unexplored” (Goodson et al., 2006). Comparisons of seasonal phenotypes are unfettered by these confounds, since all experimental groups consist of individuals from the same species.

The *modus operandi* of comparing species with different mating systems (see Curtis et al., 2007) as a means of understanding sociality possesses an additional drawback—it promotes evaluation of sociality based on behaviors expressed during the breeding period, at the expense of affiliations established at other times of the year. This may partly explain why physiologists have largely ignored winter sociality—a behavioral phenomenon in which individuals transition from an aggressive or solitary phenotype, displayed during the summer breeding season, into a group living phenotype, displayed during the winter non-breeding season. Winter sociality is widespread across numerous taxa, including rodents, birds, insects, and some reptiles (Berkvens et al., 2010; Davis et al., 2011; Lee et al., 2010; Madison 1984). Many winter-social rodent species, which express promiscuous, territorial, and aggressive behaviors during the breeding season, are classified as “asocial,” despite the fact that they display a social, group-nesting phenotype under winter conditions. This seasonal variation in social behavior occurs commonly in voles (genus *Microtus*) and, it is thought, most other small rodents in temperate or polar latitudes (Ishibashi et al., 1998; Madison 1984; West and Dublin 1984; Wolff and Lidicker 1981).

Several hypotheses regarding the general benefits of group living have been applied to winter sociality, among them predator avoidance and defense (Alexander 1974; Elgar 1989), increased foraging efficiency (Alexander 1974, Mock et al., 1988; Richner and Hebb 1995), and shared access to resources in areas with habitat scarcity (Alexander 1974). However, the most commonly proposed and widely accepted impetus for winter sociality is the thermoregulatory benefit derived from huddling (Getz and

McGuire 1997; Madison 1984; Nelson and Klein 2000; Prendergast et al., 2002; West and Dublin 1984; Wolff 1985). During the breeding season, territoriality and aggression permit control of resources necessary for survival and successful reproduction. During winter, lower temperatures and diminishing food availability decrease the benefits of resource defense and increase the benefits of reproductive quiescence and group living (Madison 1984; Prendergast et al., 2002; West and Dublin 1984).

Huddling under low temperatures proffers significant thermoregulatory and metabolic advantages. House mice maintained in groups consisting of various sex ratios and dominance hierarchies display increased huddling levels under 5˚C when compared to animals tested under 26˚C (Batchelder et al., 1983). Wild mice housed in pairs survive exposure to temperatures well below 0˚C twice as long as those housed singly; survival time increases by smaller increments when group size is increased to 3 and 4 individuals (Sealander 1952). Other studies suggest that huddling behavior decreases the metabolic demand associated with thermogenesis and that the benefits of huddling increase as ambient temperature decreases (Andrews et al., 1987; Hansson 1970; Hayes et al., 1992; Kauffman et al., 2003; Sealander 1952).

In many free-living rodents, winter sociality is facultative and varies according to environmental conditions, particularly ambient temperature and snowfall (Madison 1984; West and Dublin 1984; Wolff 1985). Huddling likely provides thermoregulatory and energy conservation benefits by elevating microclimate temperatures and reducing the amount of surface area exposed to the external environment (Hayes et al., 1992). Winter sociality may constitute an important survival tactic for numerous rodent species and contribute to selection of the genetic founders emerging each spring (Madison 1984). However, despite its potential adaptive significance, little is known regarding the proximate causes of winter sociality. The classification of heat and energy conservation as the major benefits of huddling prompted West and Dublin (1984) to speculate that the degree of winter sociality is highly correlated with ambient temperature; however, the influence of temperature on social bonding requires more explicit evaluation. Thus far, investigations of the environmental mediators of social bonding have focused primarily on social cues; the possibility that ambient temperature may also influence social bonding and its neuroendocrine foundations is exciting and, as of yet, wholly uninvestigated. One obvious prediction is that exposure to low temperatures will enhance social affiliation by modifying the hormones and neural pathways involved in the regulation of social behavior.

Female Meadow Voles—A Model for Non-Reproductive Affiliation

Winter sociality is particularly well documented in free-living populations of meadow voles (*Microtus pennsylvanicus*), which inhabit much of the northern half of North America (Hoffmann and Koeppl 1985; Wolff 1985). The utility of *M. pennsylvanicus* as a model for population dynamics and mating systems has generated a considerable amount of field data on seasonal variations in meadow vole population structure, reproduction, and behavior (e.g., Beer and MacLeod 1961; Madison et al.,

1984; McShea 1990; McShea and Madison 1984, 1986, 1989; Turner et al., 1983; Webster and Brooks 1981a, 1981b). During summer, solitary males establish large territories that overlap substantially with the territories of other males and contain the home ranges of several females. In contrast, females rarely enter areas defended by other females and exclude males from their territories, except during mating (Madison 1980; Webster and Brooks 1981b). Mating behavior in meadow voles is promiscuous; in one study, nearly 80% of females copulated with more than one male (Berteaux et al., 1999). During the breeding season, young of both sexes disperse from maternal territories shortly after weaning.

The exclusivity of maternal territories lessens in autumn, when females retain their juvenile offspring and allow immigrant males to join their home ranges and nests (Madison and McShea 1987). Group members sleep together in clusters of 2-5 individuals, synchronize their activity patterns, avoid other group territories, and exhibit little aggression towards one another (Webster and Brooks 1981b; Madison 1985; Madison et al., 1984; McShea 1990). By December, predation and subsequent movement of voles between groups leads to the replacement of extended maternal family territories with mixed lineage groups. Winter dispersal events appear to be contact-seeking in nature and occur after the loss or removal of nest mates, suggesting that dispersing animals are attempting to reacquire an optimal number of huddling partners (Madison and McShea 1987; McShea 1990). By spring, groups disintegrate and females either establish solitarily defended territories or engage in cooperatively-breeding, dyadic associations with other females. Dyads largely disappear by early summer, possibly as a result of predation (McShea 1990; McShea and Madison 1984).

The transition between summer territoriality and winter sociality may be of particular importance for female voles. The solitary lifestyle of females during summer provides protection against the infanticidal attempts of male and female conspecifics (Madison 1980; Webster et al., 1981). However, during fall, reproductive cessation minimizes the threat of infanticide, while declining temperatures and food availability maximize the benefits of group living. Laboratory and field studies suggest that females maintained under a winter-like photoperiod develop social bonds and that same-sex social preferences amongst females may exist in winter groups (Beery et al., 2008; Parker and Lee 2003; Ferkin and Seamon 1987).

During the breeding season, reproductively active females prefer their own odors and the odors of males over those of other females, while males prefer the odors of females. In contrast, during the non-breeding season, non-reproductively active females prefer the odors of other females over their own odors or those of male conspecifics. Non-reproductively active males show no preference for their own odor or those of other males or females (Ferkin and Zucker 1991). In addition, paired encounters reveal that during the breeding season, female interactions contain more agonistic acts than male-male or male-female encounters; in contrast, during winter, female interactions contain fewer agonistic acts than male-male or male-female encounters (Ferkin and Seamon 1987). The existence of same-sex social bonds between over-wintering females is also suggested by the persistence of female dyads following the dissolution of winter groups (Madison and McShea 1987; McShea and Madison 1984). In the laboratory, breeding females have been observed co-nesting and co-nursing and generally interact more amicably with one another than with a breeding male (Bamshad and Novak 1992).

Although meadow voles were initially popular amongst field biologists as a model for population dynamics, towards the end of the 20th century physiologists began using *M. pennsylvanicus* to investigate the neuroendocrine origins of parental behavior and opposite-sex pair bonding. The utility of meadow voles for the latter purpose arises from the interspecific diversity of mating systems present in the genus *Microtus*. In particular, comparisons of meadow voles and prairie voles (*M. ochrogaster*) have done much to clarify the social behavior networks in the brain that control affiliation, and the roles played by the neuropeptides oxytocin and arginine vasopressin in these networks.

Female meadow voles were selected for this dissertation for several reasons. First, the wealth of biological knowledge available about meadow voles means that new findings can be interpreted within the context of an existing framework of species-specific behavioral and physiological data. Second, and perhaps most importantly, the natural history of female meadow voles—primarily their seasonal variations in aggression and affiliation—makes them particularly suitable for studies on same-sex interactions and group living, and the responsiveness of these behaviors to changes in the environment.

The Significance of Multiple Environmental Cues

The vast majority of laboratory investigations overlook interaction among multiple signals as an important regulator of seasonality, focusing instead on day length as the most potent of all environmental cues (Lee and Gorman 2000; Prendergast et al., 2002). However, the integration of multiple environmental signals may be necessary for the appropriate timing of seasonal changes in the physiology and behavior of free-living rodents (Lee and Gorman 2000).

In meadow voles, seasonal variations in social behavior are likely influenced by several environmental factors. Field and laboratory data suggest that interactions among temperature, diet, and photoperiod may affect social behavior, at least partly by modifying reproductive status (Keller 1985). Although reproduction in the laboratory may be activated and deactivated using photoperiod manipulations alone, breeding in natural populations of meadow voles has been noted during every month of the year, indicating that factors other than day length contribute to reproductive control (Beer and MacLeod 1961). In addition, extensions of the breeding period in free-living meadow voles are associated with mild winters, suggesting the potential importance of temperature and food availability, two importance challenges faced by overwintering animals (Webster and Brooks 1981b).

Research Goals and Organization of this Dissertation

Although social bonds certainly play a major role in the maintenance of cooperative behavior and group cohesion, biologists have thus far largely ignored key social relationships that bind groups together—namely, non-reproductive relationships. Female aggregations are especially common amongst mammals and carry important

consequences for group members. Thus, it is surprising that so little is known regarding the proximate determinants of female bonding and affiliation. Equally intriguing and poorly understood are the social interactions occurring within non-breeding, winter-social populations. Practically nothing is known about the environmental and physiological regulation of winter sociality, despite the prevalence of this phenomenon across a diversity of taxa. The aims of this dissertation were: 1) to contribute to our understanding of same-sex affiliations and their potential role in the formation of social groups, 2) to investigate same-sex affiliation in a manner that acknowledges that wild populations of meadow voles live in complex environments in which a multitude of environmental signals vary; and 3) to determine if thermoregulatory demand can facilitate changes in social bonding.

Chapter 2 describes the general methodology used for the research discussed in this dissertation. Chapters 3 through 5 describe different aspects of one large, multifaceted experiment. At the core of this experiment were two broad alternative hypotheses: 1) that day length, in conjunction with food availability and ambient temperature, regulates the physiological underpinnings and expression of same-sex affiliative behavior, or 2) that day length constitutes the primary regulator of same-sex affiliative behavior in female meadow voles. Each chapter examines different components of these hypotheses. Specifically, Chapters 3 and 4 explore the influence of environmental stimuli (day length, ambient temperature, and food availability) on female meadow vole behavior and physiology, respectively. Chapter 5 focuses on the integration of environment, behavior, and physiology.

Chapters 3-5 also include an examination of the effects of my animal handling procedures. This portion of my research was added after preliminary data analyses yielded unexpected results. In short, Beery et al. (2008) demonstrated day length-dependent differences in huddling behavior and uterine mass that this research did not replicate. Animal handling procedures became a primary suspect for the disparate outcomes of my work and that of Beery et al. for two major reasons: 1) the animals in my research were handled and their home cages disturbed far more frequently, and 2) other studies have demonstrated that handling by experimenters can elicit changes in social behavior, presumably by modulating the hypothalamo-pituitary-gonadal (HPG) and hypothalmo-pituitary-adrenal (HPA) axes. At the core of this investigation were the following hypotheses: 1) extensive handling eliminated day length-dependent differences in same-sex affiliative behavior by modifying plasma concentrations of corticosterone and/or estradiol, 2) other factors (to be considered later) eliminated the day length-dependent difference in same-sex affiliation in female meadow voles, or 3) day length-dependent differences in huddling behavior do not occur in the absence of other environmental signals.

Chapter 6 relates the outcomes of a separate experiment that investigated the effects of day length and sex on group size preferences. With this experiment, I sought to determine if males and females differ in their expression of winter sociality, and if females display a seasonal variation in the tendency to affiliate with groups of strangers.

2

General Methods

There are some really hard problems, some high-information problems, ahead of us…It seems to me that the method of most rapid progress in such complex areas, the most effective way of using our brains, is going to be to set down explicitly at each step just what the question is, and what all the alternatives are, and then to set up crucial experiments to try to disprove some.

-John R. Platt (1964)

Materials and methods that apply to specific chapters are identified in bolded section titles. Sections without references to specific chapters contain information that applies to all experiments.

Animals

General Colony Maintenance

All voles were descendants of original stock that was generously provided by Michael Ferkin of the University of Memphis. Breeding pairs of males and females were co-housed under 21±1˚C in long day lengths (LDs; 14:10 light:dark cycle) in opaque plastic cages (48 x 25 x 15 cm), each containing pine bedding, cotton squares (Nestlets) for nest building, one breeder box, a paper nest tent, and one opaque refuge tube (Fig. 2.1). All voles, breeders and experimental alike, were provided tap water and food (mouse chow no. 5015, Purina Mills, St. Louis, MO) and housed with a dark onset of 17:00 PDT. Breeders were supplemented biweekly with lettuce and alfalfa.

Experimental Animals

Pups were weaned at 18-20 days of age into same-sex pairs or trios (group huddling experiment only; see Chapter 6) and housed in clear plastic cages (48 x 25 x 15 cm), each containing pine bedding, two Nestlets, and an opaque plastic refuge tube large enough for two animals (Fig. 2.1). Unless otherwise specified, voles were provided

tap water and food ad libitum. Animal care and experimental procedures were approved by the Animal Care and Use Committee of the University of California, Berkeley (protocol number R084-0912C).

Influence of Photoperiod, Food Availability, and Temperature on Same-Sex Affiliation (Chapters 3-5)

Experimental Design (Fig. 2.2, Table 2.1)

This study employed a full factorial design that included 8 treatment groups with varying combinations of day length (short (SDs) or long day lengths (LDs)), ambient temperature (10°C or 21°C), and food availability (ad libitum or food-restricted). The groups were: 1) LDs, food ad libitum, low ambient temperature (10˚C; LDadlib10); 2) LDs, food-restricted, 10˚C (LDfr10); 3) LDs, food ad libitum, mild temperature (21˚C; LDadlib21); 4) LDs, food-restricted, 21˚C (LDfr21); 5) SDadlib10; 6) SDfr10; 7) SDadlib21; and 8) SDfr21. Sample size was 12 female pairs per group. This design was adopted for two primary reasons: 1) to facilitate planned comparisons between groups differing in one treatment factor, which would permit targeted analyses of the effects of specific environmental treatments, and 2) to allow for investigations of interactions between day length, food availability, and temperature.

Experimental Timeline (Fig. 2.3)

Females were paired at weaning and, beginning at 30-45 days of age, assessed for food intake and body mass at weekly intervals for 8 weeks. After the first 3 weeks of body mass and food intake measurements, pairs were either transferred to SDs (10:14 light:dark cycle) or maintained in LDs at 10±2˚C or 21±1˚C, where they remained for 7 weeks. During the final 2 weeks of treatment, body mass and food intake were monitored daily. Voles were observed for behavioral signs of aggression and anxiety and cages were examined for evidence of nest maintenance and food caching. All voles were fed ad libitum until the 7th week of treatment, when females in food-restricted groups underwent a reduction in food availability.

After 7 weeks in their respective treatment conditions, voles were tested for social preference. On the day after social preference testing, from 13:00-14:00 PDT, focal voles were sacrificed for uterine mass measurement and the collection of brains and blood samples.

Body Mass and Food Intake Measurements

All body mass and food intake measurements (± 0.1 g) were conducted between 14:30 and 16:30 PDT. To minimize disturbance to animals, weekly measurements occurred concurrently with cage changes.

Body mass was measured by temporarily placing each vole into a pitcher seated atop a zeroed scale. Voles spent less than 1 min in the pitcher and were subsequently transferred into a fresh cage. For daily measurements, paired voles were either transferred into a clean cage after body mass measurements (during once weekly cage changes) or temporarily placed into a clean holding chamber while experimenters searched through bedding for food caches in home cages. Voles generally spent no more than 2-3 minutes in holding chambers. In total, cage changing and all measurements required less than 5 minutes per cage. All voles were handled using the opaque refuge tubes present in their home cages.

Weekly food intake measurements for paired females were conducted by placing a known amount of food (~100 g) into the hopper at the beginning of each week and weighing the remaining pellets (including caches) one week later. Food intake was calculated by subtracting the weight of remaining food from the weight of food provided one week previously. Food pellets absorb moisture at low temperatures; thus, food was acclimated to the 10˚C chamber for 7 days before being offered to voles in 10˚C treatment groups.

Food restriction occurred throughout the 7th week of treatment and continued until the completion of behavior tests. Voles in food-restricted groups were offered a diet reduced to 90% of daily ad libitum intake. To acclimate animals to daily measurements prior to food restriction and obtain an estimate of daily ad libitum intake, food intake (including caches) was measured throughout the 6th week of treatment by placing a known amount of food (~20 g) into the hopper daily and weighing the remaining pellets 24-h later. Data from the 6th week were then used to calculate daily ad libitum food intake for paired females. Animal health during food restriction was monitored on the basis of daily body mass measurements and behavioral observations. To maintain consistency in handling protocol, all experimental groups (including those fed ad libitum throughout the experiment), were subjected to daily body mass and food intake measurements during weeks 6 and 7.

Influence of Handling on Same-Sex Affiliation (Chapters 3-5)

Females weaned into pairs at 18-20 days of age were provided water and food ad libitum and minimally handled throughout the experiment. At 50-65 days of age, pairs were either transferred to SDs or maintained in LDs (*n*=12/day length group). Social preference tests occurred 7 weeks later, when pairs were 100-115 days of age. Cage changes were performed weekly throughout the experiment and completed in 1-2 minutes per cage. To minimize animal disturbance, voles were transferred between cages using opaque refuge tubes.

Social Preference Testing

Behavioral tests lasted for 3 h and occurred in an apparatus previously employed by other researchers (Beery et al., 2008). The set-up consisted of a single rear chamber

connected to two fore-chambers by tubes (5 cm diameter, 5 cm length); each chamber was equally sized (17 x 28 x 12.5 cm; Fig. 2.4). After light anesthetization with isoflurane vapor, the focal vole's partner was tethered in one of the fore-chambers. Stranger(s) from the same treatment condition, but unfamiliar to the focal vole was (were) tethered in the other fore-chamber. Tethered animals were given 5-10 minutes to acclimate before the start of each test. Tethers, consisting of a 10 cm nylon tie (Radio Shack) bound by a swivel to a flexible steel fishing leader (South Bend Sporting Goods, Northfield, IL), were secured to the chamber lids and permitted voles to move throughout half of their respective chambers. The placement of strangers and partners into either the left or right fore-chamber was randomized to avoid bias. After each test, the apparatus was thoroughly washed with soap and water.

Footage of tests was recorded using a digital video camera (Panasonic SDR-SW20) and converted to 4x speed video using movie editing software (iMovie 2009, Apple Inc., Cupertino, CA). Videos were scored using the program Intervole Timer (Annaliese Beery), which records counts and durations of chamber entries and huddling. Activity is measured as the number of entries into the rear chamber. Data from voles that failed to explore both fore-chambers at least once during the first 100 min of the 180 min test were excluded from final analysis.

Data Analysis

Time spent huddling with the partner versus a stranger was compared within treatment groups using paired *t*-tests for normally distributed data and Wilcoxon signed-rank tests for non-normally distributed data. Sign tests were used instead of Wilcoxon signed-rank tests for groups in which differences between paired samples were non-normally distributed. Partner preference was inferred when a focal vole spent at least twice as much time in side-by-side contact with a familiar versus a novel vole (as in Beery et al., 2008 and Insel et al., 1995). Since instances in which groups did *not* display partner preferences are of heuristic value, I report both significant and non-significant statistical outcomes for paired comparisons.

To minimize the risks of inflating the type I error rate, group comparisons with the greatest likelihood of yielding useful information were selected *a priori*. Specifically, comparisons of experimental groups differing in one treatment factor (e.g., LDadlib21 versus LDadlib10) were planned and executed using two-tailed *t*-tests or Wilcoxon rank sum tests (see Ruxton and Beauchamp 2008). The decision to use *t*-tests assuming equal or unequal variances was made after performing the Levene test for unequal variance.

Post hoc testing for unplanned comparisons was accomplished using variants of the ANOVA test, followed by comparisons of specific groups when the null hypothesis (homogeneity across all groups) could be rejected. All statistical analyses were performed using JMP 9.0.2 (SAS Institute Inc., Cary, NC). Means ± SEM are reported throughout.

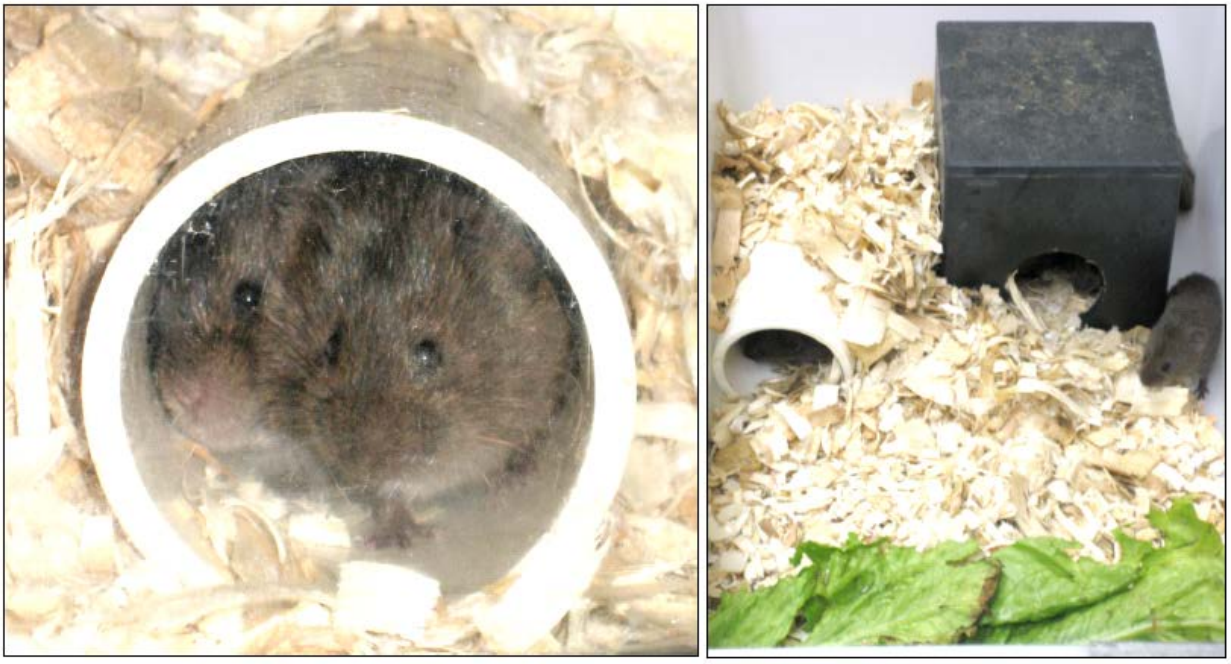

Figure 2.1 Female meadow voles in laboratory housing. Left: Co-housed, same-sex voles were commonly found in tight huddles, sometimes within opaque refuge tubes. Right: Black breeder boxes, refuge tubes, and twice weekly alfalfa and lettuce supplements were supplied to every breeding pair. A vole pup is visible on the right.

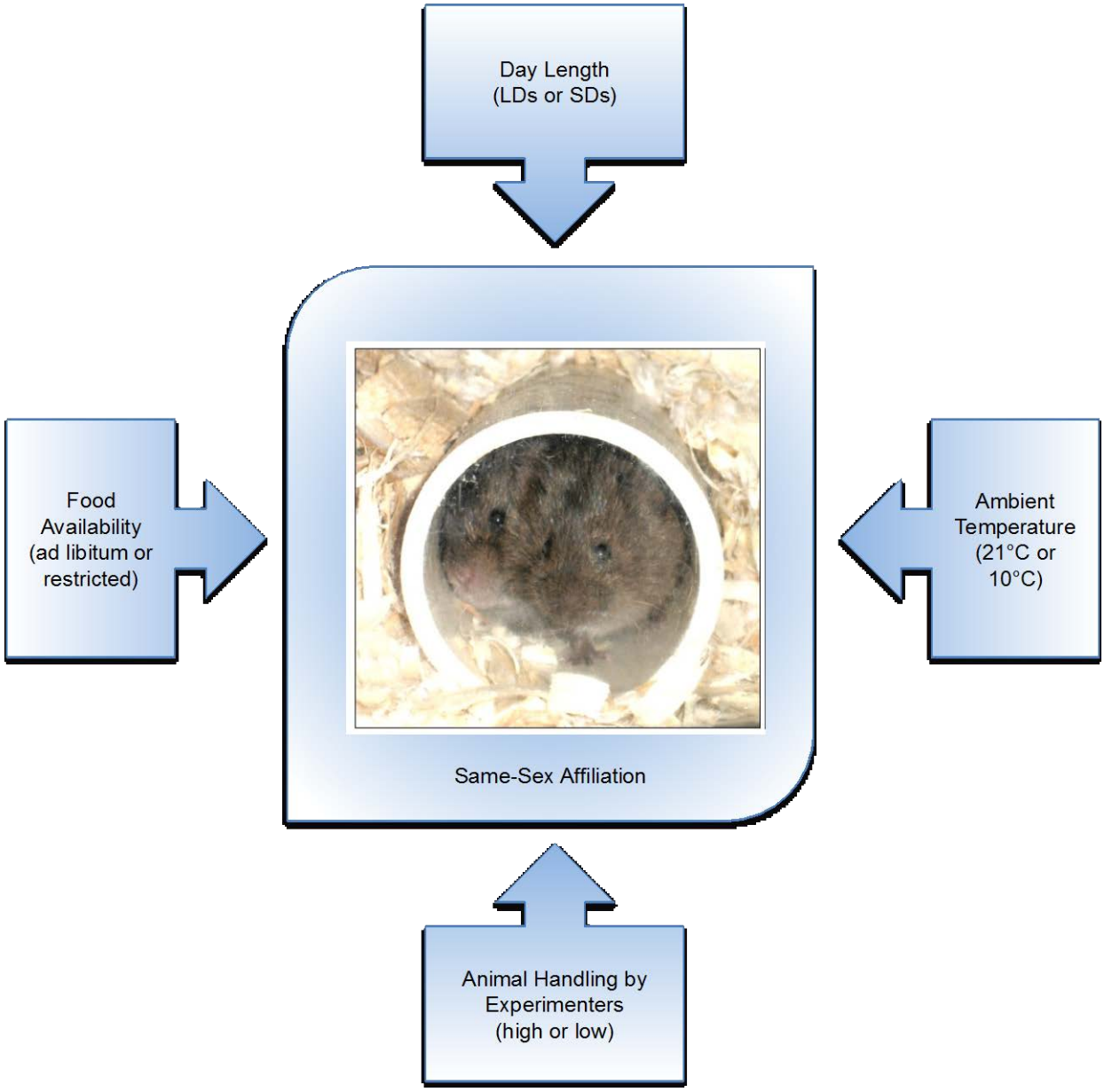

Figure 2.2 Experimental design for work detailed in Chapters 3-5. The aim of these studies was to examine the influence of several different environmental inputs on same-sex affiliative behavior and its physiological correlates.

Abbrev.	Treatment
LD	long day length
SD	short day length
fr	food-restricted
ad lib	ad libitum
10	10˚C
21	21˚C
high	frequent handling
low	infrequent handling

Table 2.1 Environmental treatment abbreviations used in tables and figures.

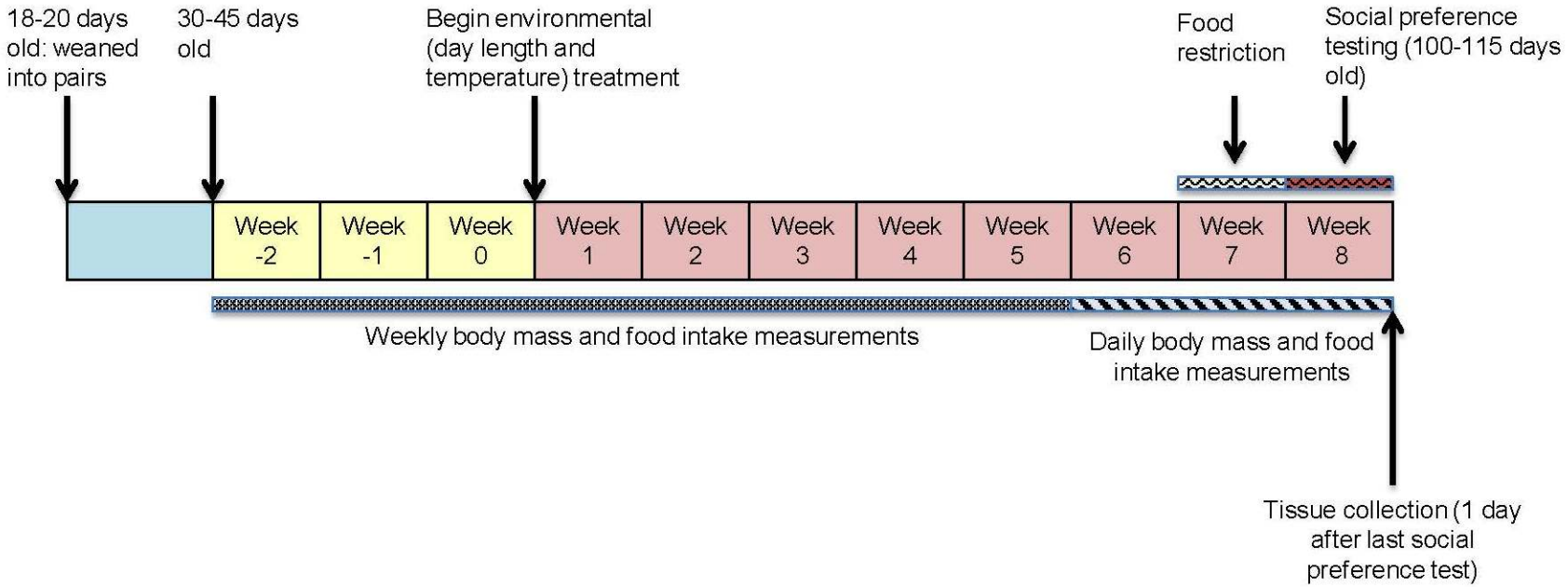

Fig. 2.3 Experimental timeline (Chapters 3-5) for frequently-handled voles. Infrequently-handled voles were fed ad libitum, housed in 21˚C, and did not undergo body mass or food intake measurements.

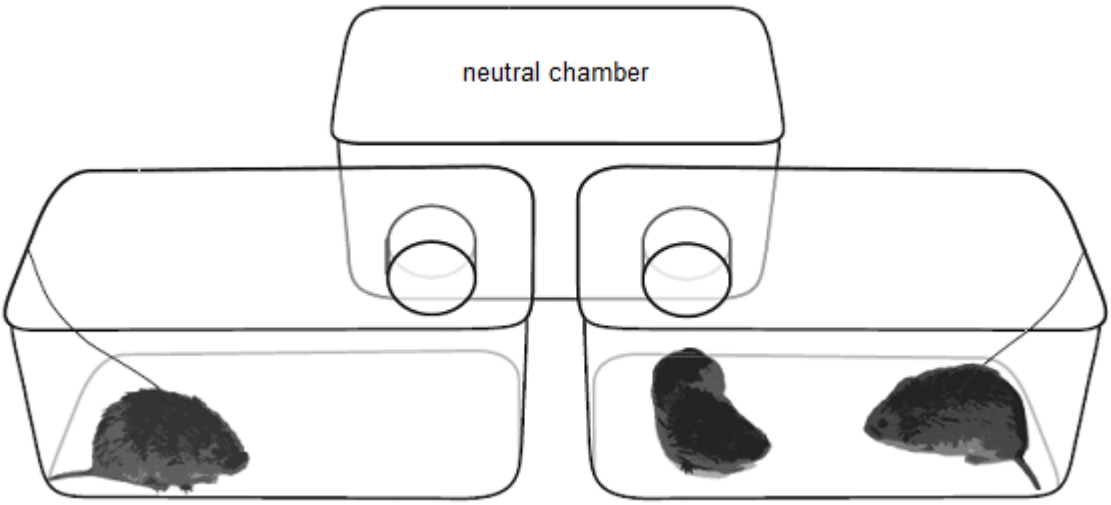

Figure 2.4 General layout of a social preference test. A focal vole is placed into the neutral chamber and allowed to roam freely throughout all chambers for the duration of the 3 h test. Fore-chambers contain tethered voles that are either strangers or the focal vole's cage-mate.

3

Environmental Modulation of Same-Sex Affiliative Behavior

I have gradually discovered that my particular type of behavior studies, those of the way in which animals are and have become adapted to the environments they choose to live in, is relatively rare, and yet occupies a niche which must not be left unoccupied.

-Nikolaas Tinbergen (quoted in Hinde 1988)

Until the mid-twentieth century, scientific investigations of animal behavior were largely description-based and fell under the purview of comparative psychologists, many of whom considered behavior the product of experience. A more systematic approach, one that acknowledged the biological origins of behavior and combined both experimentation and observation, did not become commonplace until pioneering work by researchers such as Konrad Lorenz and Niko Tinbergen established behavioral biology as a legitimate branch of science (Hinde 1990; Tinbergen 1939, 1963). The collective move to bring behavior into the folds of biology was well founded, given that scientists now acknowledge links between animal behavior and evolution, the central and defining concept of modern biology. Today, biologists recognize that behavior, like physical traits, can both influence and be influenced by evolutionary processes. The term “behavior” encompasses the myriad ways that animals respond to their surroundings, which are characterized by factors such as temperature, day length, food and water availability, conspecifics, and other species. By modifying the internal milieu of an animal’s physiology, these factors may independently or collectively influence behavior, forming complex webs of cause and effect that in many cases remain poorly understood.

Social behaviors are diverse and provide a plethora of intriguing opportunities for investigation. Across the animal kingdom, conspecifics engage in a wide variety of ways—they fight, mate, and cooperate, establish and maintain social bonds, form complex dominance hierarchies, and communicate using means that often elude human observers. Despite this rich variety, biologists have focused most heavily on certain categories of social behavior, namely aggressive (e.g., mate competition, defense of mates and breeding territory), parental, and sexual. This partiality arises perhaps

because these behaviors all have clear links to reproductive success, which is commonly used to evaluate the adaptive value, or evolutionary worth, of a behavior.

Although its link to reproductive success is less immediately obvious, non-reproductive affiliative behavior is also worth investigating. To illustrate the points of this argument, we will focus on the two types of non-reproductive interactions that figure prominently in this dissertation—same-sex social bonding between female mammals and winter sociality:

1. Non-reproductive affiliative behavior exists in a diverse assortment of taxa, including mammalian, reptilian, avian, and insect groups. Winter sociality appears in birds (Western bluebird, Dickinson and McGowan 2005; Eastern bluebird, Frazier and Nolan 1959; Vinous-throated parrotbill, Lee et al., 2010), lizards (Desert Night lizard, Davis et al., 2011; tree lizard, Boykin and Zucker 1993, Elfström and Zucker 1999), insects (Harlequin ladybird beetle, Berkvens et al., 2010) and numerous rodent species (Madison 1984). Female affiliations are particularly common in rodents (Lacey and Sherman 2007), but they also exist in cetaceans (sperm whale, Gero et al., 2009), primates (baboon, Wittig et al., 2008; squirrel monkey, Saltzman et al., 1991), elephants (de Silva et al., 2011), and horses (Cameron et al., 2009). The spread of winter sociality and same-sex affiliation across diverse vertebrate and mammalian groups, respectively, suggests that these behaviors confer adaptive benefits and do not arise exclusively due to factors related to evolutionary constraints or history.
2. Animal behavior carries evolutionary significance only when it influences the passage of genes from one generation to the next (or, more simply put, reproduction). Although it may seem counter-intuitive, non-reproductive affiliations may influence reproductive fitness via indirect means. In winter-social species, social aggregations that are restricted to the non-breeding season may enhance an individual's chance of surviving periods of severe resource scarcity and facilitate future reproductive opportunities by "introducing" potential mates (Elfström and Zucker 1999; Lee et al., 2010; Madison 1984). Overwintering mortality is quite high in some species and potentially plays a role in selecting the individuals and, more importantly from an evolutionary perspective, genes that will persist within a population (Madison 1984). In female mammals, affiliative behaviors that maintain same-sex social bonds may confer benefits that opposite-sex interactions do not, such as communal nursing (Gero et al., 2009), reduced harassment by males (Cameron et al., 2009), and decreased stress during periods of instability in male hierarchies (Wittig et al., 2008). Same-sex social bonds between females may also help dictate patterns of infanticide and alloparenting within social groups (e.g., socially-bonded females may care for each others' young more frequently than non-bonded females and be less likely to injure one another's offspring) and elevate the potential for reciprocation of offspring care (Wittig et al., 2008).
3. There is reason to suspect that the environmental factors responsible for regulating non-reproductive and reproductive affiliative behaviors are not identical. In many cases, the two classes of behavior occur at different

times of year under different environmental circumstances. For instance, in squirrel monkeys, same-sex interactions are more common during the non-breeding season, while opposite-sex interactions are more common during the breeding season (Schiml et al., 1996). In winter-social species, territoriality and conspecific aggression predominantly occur during the spring and summer, while affiliation and group living occur under the conditions of winter (Madison 1984).

Although non-reproductive affiliative behavior potentially plays an important role in some animal social groups, little is known about its responsiveness to environmental factors. Same-sex affiliative behavior and winter sociality in female meadow voles constitute promising candidates for investigation because existing data suggest that these behaviors are regulated by environmental factors. Beery et al. (2008) demonstrated that day length influences same-sex huddling behavior in female meadow voles, such that females housed in SDs spend significantly more time huddling with partners and strangers than females housed in LDs. Free-living adult females display increased social tolerance during winter, when they retain their offspring (rather than forcing offspring dispersal) and huddle with adults from surrounding territories (Madison and McShea 1987). Winter huddling potentially occurs because it proffers thermoregulatory benefits, which are frequently associated with ambient temperature and the availability of thermogenic fuel (i.e., food). In addition, the formation of winter groups appears to be facultative, varying with snowfall (Madison 1984; West and Dublin 1984; Wolff 1985); moreover, meadow voles disperse and seek out new huddling aggregations following declines in the size of their winter groups (Madison and McShea 1987; McShea 1990). Given that thermoregulatory benefits are positively correlated with group size (Sealander 1952), the latter observation is consistent with the hypothesis that group living helps meadow voles deal with the challenges of winter, namely low temperatures and food scarcity.

This study utilized female meadow voles to examine the effects of day length, ambient temperature, and food availability on same-sex affiliative behavior. An experiment was designed to test the following alternative hypotheses: 1) along with day length, environmental conditions that affect thermoregulation (food availability, ambient temperature) modulate same-sex affiliative behavior, or 2) day length, but not ambient temperature or food availability, regulates variations in same-sex affiliation. Specific predictions, assuming the validity of the first hypothesis, included the following: 1) that low temperature and food restriction would increase total time spent huddling and elevate social tolerance of strangers in both day lengths, but that these effects would be more pronounced in SDs, and 2) that low temperature and food restriction would elicit short day-like patterns of huddling behavior in LD females.

The use of low temperatures and food restriction in this research necessitated, for animal welfare reasons, extensive and regular monitoring of food intake and body mass. To examine the effects of handling methods on vole behavior, additional experimental groups were included to test the hypothesis that extensive handling by experimenters eliminates the day length-dependent difference in same-sex affiliative behavior.

Materials and Methods

Behavioral Observations Prior to Social Preference Testing

At the time of food intake and body mass measurements, cages were inspected for the presence of nests and food caches and voles were observed for behaviors indicating aggression towards cage-mates and anxiety. Aggressive behaviors included rearing, boxing, chasing, lunging, hopper guarding, and vocalizing. The following behaviors were considered indicative of anxiety: climbing, swinging, or chewing on cage lid bars, and repetitive, rapid locomotion (e.g., jumping, running back and forth across the cage floor). Nests were examined for evidence of poor maintenance—specifically trampling, disbursement of cotton nesting material throughout the cage, and absence of a nest mound.

Social Preference Testing

After the 7th week of treatment (100-115 days of age), voles were tested for social preference in 10˚C or 21˚C, in accordance with their temperature treatment groups. Focal voles were given the option of either huddling with their cage-mate or a stranger of similar age and from the same treatment condition.

Data Analysis

The number of pairs that had nests, food caches, or displayed aggressive or anxious behaviors was tallied for each week, including the week of behavior testing (designated as “week 8”). The influence of day length, ambient temperature, and food availability on the resulting frequencies was assessed with Pearson’s Chi-Squared test. Frequencies for groups differing in one treatment factor were compared using Fisher’s Exact Test.

Results

Social Preference (Table 3.1)

Data from one SDadlib21*high* vole that did not enter either fore-chamber during the first 100 min of the test were excluded from statistical analyses of huddling behavior. Social interactions in LD females varied with ambient temperature; those housed under 21˚C spent more time huddling with partners over strangers, regardless of food availability. This effect was significant for LD food-restricted voles (S=30.00, P<0.05) and borderline significant for LD voles fed ad libitum (S=24.00, P=0.06). LD females housed under 10˚C showed no difference in time spent huddling with partners versus strangers, regardless of food availability (ad libitum, M=1.00, P=0.77; food restricted, S=5.00, P=0.73). In SD females, social interactions varied with food availability and ambient temperature; under 21˚C, SD voles fed ad libitum spent more time huddling

with partners than strangers (S=30.00, P<0.01), but voles fed a restricted diet displayed no difference in time spent huddling with partners versus strangers (S=5.00, P=0.73). Under 10˚C, SD females spent more time huddling with partners than strangers; this effect was significant for food-restricted (S=30.00, P<0.05), but not ad lib voles (t=1.88, P=0.09). Like their frequently-handled counterparts, infrequently-handled LD and SD females spent greater durations of time huddling with partners than strangers (LDs, S=27.00, P<0.05; SDs, M=5.00, P<0.01).

All treatment groups displayed a partner preference (i.e., spent at least twice as much time with the partner versus a stranger) except for LD females housed at 10˚C and SD females that were food-restricted and housed at 21˚C.

Planned Between-Group Comparisons

Time Huddling with a Partner (Table 3.1, Fig. 3.1)

Planned comparisons revealed no between-group differences in time spent huddling with a partner, except when voles were food-restricted and housed under 10˚C. Short day females held in these conditions spent significantly more time huddling with the partner than did LD voles (Z=2.34, P<0.05).

Time Huddling with a Stranger (Table 3.1, Fig. 3.1)

Both SD and LD females housed in 10˚C spent more time huddling with strangers than females housed in 21˚C. This effect was statistically significant for SD females fed ad libitum (Z=2.19, P=0.03) and food-restricted females housed in LDs (Z=2.68, P<0.01). LD females fed ad libitum also tended to spend more time huddling with strangers when housed in 10˚C versus 21˚C, but this effect was not significant (Z=1.82, P=0.07). When housed in 21˚C, SD females that were food-restricted spent significantly more time huddling with strangers than their ad lib counterparts (Z=3.05, P<0.01). In voles that were food-restricted and housed under 21˚C, day length moderated the amount of time spent huddling with strangers, such that SD females spent more time huddling with strangers than their LD counterparts (Z=2.80, P<0.01).

Total Time Huddling

No between-group differences in total time spent huddling were evident, except when voles were food-restricted and housed under 21˚C. Under these environmental conditions, total time spent huddling was significantly higher in SD females than in LD females (t=2.66, P=0.01).

Activity

One SDadlib21*high* vole that displayed unusually frantic activity during behavioral testing was considered an outlier. Her activity count was more than 2.5 standard deviations away from the group mean and 1.9 standard deviations away from the nearest data point and thus excluded from statistical analyses of activity data. SD

females that were food-restricted and housed under 21˚C were significantly less active than their LD counterparts (t=2.25, P=0.04). SD females also tended to display more investigatory activity when housed under 10˚C versus 21˚C, but this effect fell short of significance (SDfr21*high* versus SDfr10*high*: t=-1.95, P=0.06; SDadlib21*high* versus SDadlib10*high*, t=-1.83, P=0.08). A similar trend was not noted in LD females.

Handling Effects

Like their frequently-handled counterparts, infrequently-handled LD and SD females displayed a partner preference. Differences in handling had no effect on huddling behavior or activity.

Aggressive and Anxiety Behaviors Observed During Cage Changes

No differences were found for groups differing in one treatment factor. Day length and temperature did not influence the incidence of aggressive or anxious behaviors observed at any point in the experiment except during food restriction (week 7), when females housed under 10˚C displayed more anxious behaviors (4/48 pairs versus 0/48 pairs, X^2=5.72, P<0.05). Anxious behaviors were more common in food-restricted voles than in females fed ad lib during week 7 (4/48 pairs versus 0/48 pairs, X^2=5.72, P<0.05) and the week of behavior testing (5/48 pairs versus 0/48 pairs, X^2=7.21, P<0.01). Food restriction resulted in heightened aggression towards cage-mates during Week 7 (3/48 pairs versus 0/48 pairs, X^2=4.26, P<0.05) and the week of behavior testing (11/48 pairs versus 2/48 pairs, X^2=7.84, P<0.01).

Food Caches and Nests Observed During Cage Changes

No differences were found for groups differing in one treatment factor. Food-restricted females trampled their nests more frequently than ad lib counterparts during the week of behavior testing (6/48 pairs versus 0/48 pairs, X^2=8.72, P<0.01). During the first week of daily measures (Week 6), food caching was more commonly observed in females housed under SDs than LDs (4/48 pairs versus 0/48 pairs, X^2=5.72, P<0.05) and in voles fed ad lib than voles fed food-restricted diets (4/48 pairs versus 0/48 pairs, X^2=5.72, P<0.05). Food caching was more frequently observed in voles housed at 21˚C than 10˚C during Weeks 4, 6, and 7, but this effect was only significant for Week 4 (3/48 pairs versus 0/48 pairs, X^2=4.26, P<0.05).

Treatment Interactions

Time spent huddling with a stranger was significantly affected by interactions between day length and temperature (P<0.05; Fig. 3.2). Subsequent analysis of all combinations of day length and temperature using the Kruskal-Wallis test revealed significant between-group differences (P<0.05), which pairwise comparisons confirmed. Females maintained in LDs and 21˚C spent less time huddling with strangers than LD

females housed in 10˚C (Z=-3.17, P<0.01) and SD females housed in 21˚C, although the latter effect was not significant (Z=1.82, P=0.07).

Interactions between day length and food availability significantly affected total time spent huddling (P<0.05; Fig. 3.3). Follow-up analysis of all combinations of day length and food availability using the Kruskal-Wallis test revealed significant between-group differences (P<0.05) that were confirmed by pairwise comparisons. Food-restricted voles housed in SDs spent more total time huddling than food-restricted LD voles (t=3.01, P<0.01) and SD females fed ad libitum (Z=-2.18, P<0.05).

Discussion

This research supports the hypothesis that day length, ambient temperature, and food availability modulate same-sex affiliative behavior in female meadow voles. Some aspects of my predicted outcomes materialized. First, exposure to low temperature, but not food restriction, increased social tolerance of strangers in both day lengths. In SDs, females fed ad lib spent significantly more time huddling with strangers when housed in 10°C versus 21°C. Food restriction of SD voles housed in 10°C did not eliminate this increase in time spent huddling with a stranger. Similarly, LD females fed a restricted diet spent significantly more time huddling with strangers when housed in 10°C versus 21°C; this effect was not eliminated when voles were fed ad lib. In addition, interactions between day length and temperature influenced the amount of time voles spent in side-by-side contact with strangers, such that voles housed in LDs and 10°C spent significantly more time with strangers than LD females in 21°C. These findings suggest that in both day lengths, exposure to 10°C promotes an increase in the time that voles spend with strangers, and that this increase is neither dependent upon, nor affected by, mild food restriction.

Interestingly, although housing at 10°C elevated social tolerance of strangers in both day lengths, the impact that low temperature had on social preference was influenced by photoperiod. In LDs, low temperature eliminated the strong partner preference apparent in LD voles housed in moderate temperature. LD females in moderate temperature spent 4.8 (ad lib) and 7.7 (food-restricted) times as long huddling with partners versus strangers, whereas LD voles in low temperature spent 1.4 (ad lib) and 0.8 (food-restricted) times as long huddling with partners than with strangers. In contrast, SD females exposed to low temperature retained their partner preference. At low temperature, SD females spent 2.2 (ad lib) and 3.7 (food-restricted) times as long huddling with partners versus strangers. Exposure to low temperature evidently interferes with the maintenance of social bonds in LD, but not SD females.

Food restriction did not have a discernible impact on the huddling behavior of females in LDs. However, food-restricted SD voles housed in moderate temperature displayed a marked increase in time spent huddling with strangers. In addition, food-restricted voles housed in 21°C were the only treatment group in SDs that did not display a partner preference. In SDs and moderate temperature, voles fed ad lib spent 13.9 times as long huddling with partners versus strangers, whereas voles that were food-restricted spent 1.3 times as long huddling with partners versus strangers. Females in the SDfr21 treatment group also spent significantly more total time huddling

than SD voles fed ad lib and food-restricted LD voles. Food restriction was not associated with similar increases in time spent huddling with a stranger and total huddling time when voles were housed in 10°C. This suggests that in 21°C, but not 10°C, the expression of various aspects of affiliative behavior—including partner preference, social tolerance of strangers, and total time spent in physical contact with another animal—is influenced by day length and food availability.

Surprisingly, low temperature did not increase total huddling time in any group, even when voles were also food-restricted, which presumably should have elevated the degree of thermoregulatory challenge. This finding contradicts the negative correlation between huddling duration and ambient temperature that has been demonstrated in other studies; it is also unexpected because voles had no other external means of warming themselves aside from huddling during behavior tests. Possible explanations are that the degree of thermoregulatory challenge was insufficient to induce an increase in huddling behavior, or that voles did not derive enough benefit from huddling with another individual to warrant an increase in huddling time. In either case, this outcome suggests that the modifications in affiliative behavior induced by food restriction and low temperature were not solely motivated by an immediate need to meet thermoregulatory demand; if this were the case, one would expect that voles would have spent more total time huddling and increased the duration of time spent huddling with either a stranger or a partner (or both). Additionally, if thermoregulatory demand simply prompted voles to huddle for warmth without altering social bonds, one would expect that low temperature, food-restricted groups would display a significant increase in total time spent huddling while retaining a partner preference. Interestingly, the only group to display a significant increase in total huddling time, SDfr21, did not display partner preference.

Females that were food-restricted during housing in 10°C were arguably exposed to the most pronounced thermoregulatory challenge of all treatment groups, which may explain why anxious and aggressive behaviors were more common under these conditions. During the final week of treatment, the increase in aggression and anxiety was significant in food-restricted groups, suggesting that these behaviors were related to food defense or food anticipation, respectively. Although both LD and SD females displayed anxiety and aggression towards cage-mates when food-restricted and housed in 10°C, SD females retained a partner preference, whereas LD females did not. The relationship between the display of aggression in the week preceding behavior testing and subsequent social preference is unclear, but one potential interpretation is that aggression induced by thermoregulatory challenge negatively affected social bonds in LDs, but not SDs.

Unlike Beery et al. (2008), I did not find that the huddling behavior of females fed ad libitum and housed in 21°C differed according to day length. Specifically, Beery et al. demonstrated that SD females spent significantly more time huddling with partners relative to LD females. However, my results suggest that the huddling behavior of LD and SD females differs only when voles are subjected to food restriction or low temperature; in no case did I observe significant differences in time spent huddling with the partner. Infrequently- and frequently-handled voles did not display significant differences in any measured aspect of huddling behavior, indicating that differences in the handling protocols of this study and that of Beery et al. (2008) are unlikely to account for the discrepant outcomes. Other potential explanations for our different

results include: 1) genetic differences between our colony populations, or 2) differences in the age of our voles at the time of transfer to SDs. Regarding the first possibility, although my work utilized the same colony as Beery et al., their study preceded mine by several years. In the intervening period, the colony was not outbred. Mates are cohoused; thus, breeding voles are selected partially for their amicability towards cohousing with another adult. Over time, this may have produced a more social LD phenotype. Regarding the second possible explanation, Beery et al.'s voles were placed into SDs at weaning (18-20 days of age), whereas my females were 50-65 days of age at the time of transfer to SDs.

The ability of arvicoline rodents to display facultative reproductive and social strategies is well documented and prompted one group of authors to suggest the value of investigating the impact of environmental variables, such as food restriction and low ambient temperatures, on social preferences (Parker et al., 2001). This research demonstrates that along with day length, food availability and ambient temperature are potentially important regulators of affiliative behavior in natural populations of meadow voles.

Group abbrev.	Huddling with partner (min)	Huddling with stranger (min)	Huddling with partner vs. stranger	*P* value
LDadlib21*low*	36.0±9.9	8.4±6.3	*	0.03
LDadlib21*high*	37.3±9.6	7.8±3.9		0.06
LDfr21*high*	34.7±8.1	4.5±2.2	*	0.02
LDadlib10*high*	28.7±10.7	20.8±8.7		0.77
LDfr10*high*	16.0±4.8	20.7±7.0		0.73
SDadlib21*low*	33.7±8.9	10.2±6.6	**	0.006
SDadlib21*high*	44.6±9.5	3.2±1.2	**	0.005
SDfr21*high*	39.5±11.3	30.5±9.8		0.73
SDadlib10*high*	28.2±7.3	12.8±3.6		0.09
SDfr10*high*	41.8±7.9	11.3±3.9	*	0.02

Table 3.1 Huddling times (min) by treatment group. "**" and "*" denote $P<0.01$ and $P<0.05$, respectively. "Low" and "high" refer to the amount of handling voles received throughout the course of the experiment.

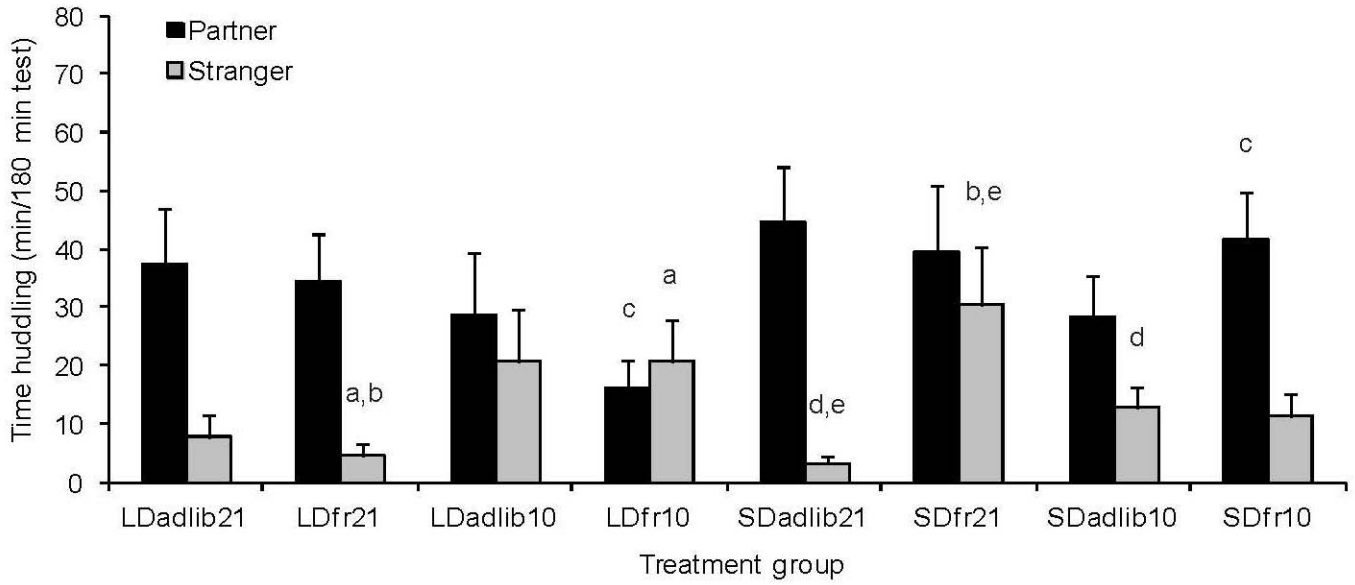

Fig. 3.1 Huddling times (min) for frequently-handled treatment groups. Shared letters indicate significant differences.

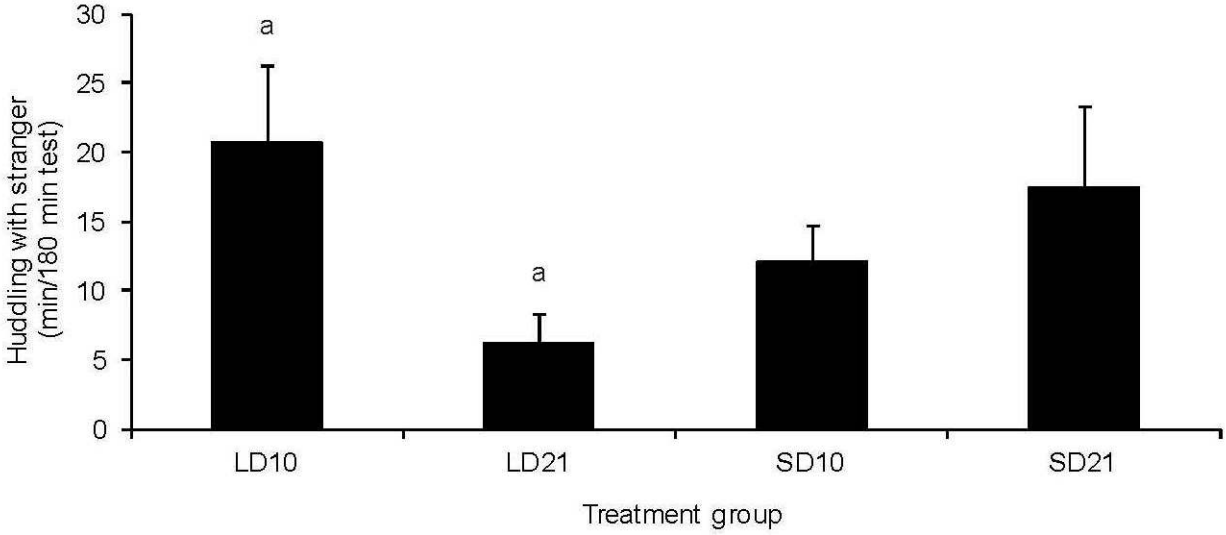

Fig. 3.2 Huddling times (min) for frequently-handled voles by day length/temperature treatment group. Shared letters indicate significant differences.

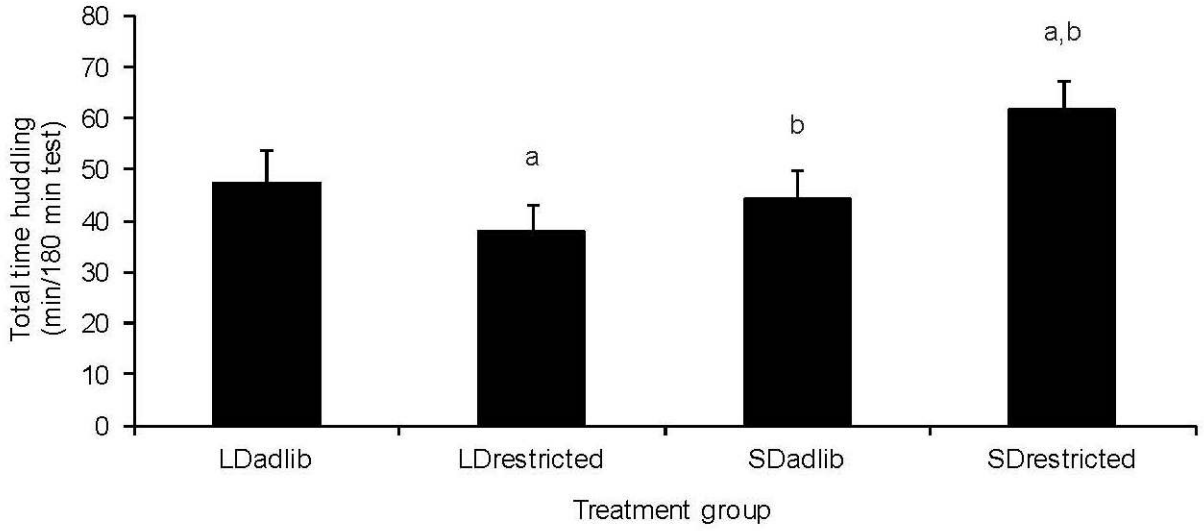

Fig. 3.3 Huddling times (min) for frequently-handled voles by day length/food availability treatment group. Shared letters indicate significant differences.

4

Environmental Modulation of Physiology

Since an organism is inseparable from its environment, any person who attempts to understand an organism's distribution must keep constantly in mind that the item being studied is neither a stuffed skin, a pickled specimen, nor a dot on a map. It is not even the live organism held in the hand, caged in a laboratory, or seen in the field. It is a complex interaction between a self-sustaining physicochemical system and the environment. An obvious corollary is that to know the organism it is necessary to know its environment.

-George A. Bartholomew (1958, quoted in Huey and Bennett 2008)

Perhaps more so than any other field of study, biological rhythms research has demonstrated that the physiological processes underlying animal behavior and survival are exquisitely sensitive to the external environment. Day length varies across the year with consistent predictability, making it particularly useful (when transduced into biologically-meaningful information) for measuring the passage of time and cueing advance preparation for the year's greatest environmental transitions (Gorman et al., 2001). As a result, day length is often considered the most important of all environmental cues. However, it would be surprisingly maladaptive if animals were incapable of responding to marked variations in environmental factors aside from day length. In fact, in at least some species, particularly those exposed to year-to-year temporal variations in seasonal events, non-photic signals appear to function as "fine-tuners" of seasonal rhythms that are otherwise chiefly directed by photoperiod (Lee and Gorman 2000; Paul et al., 2008; Wingfield et al., 2000). For instance, although the California vole (*M. californicus*) is considered a long day breeder (based upon patterns of gonadal regression and recrudescence observed in captive-housed voles), free-living voles commonly breed under short day lengths. Nelson et al. (1983) provided strong evidence that this apparent reversal in the seasonal timing of breeding occurs because of seasonal rhythms in rainfall (rainy winters, dry summers) and the availability of green vegetation that occur in the San Francisco Bay area.

Like many other rodents living in non-equatorial regions, meadow voles display physiological preparations for the onset of winter. These changes include increased pelage density, increased nest-building activity, decreased energy demand, and cessation of reproduction, all of which can be replicated in the laboratory by transferring animals from long to short day lengths (Dark and Zucker 1983; Dark and Zucker 1986; Gorman et al., 1993). Of all the SD-associated changes that rodents undergo, none has received more attention than reproductive quiescence. By temporarily shutting down reproduction and suspending participation in its associated activities (e.g., intra-sexual aggression, territory and offspring defense, lactation), rodents are able to conserve energy at a time of year when food availability is often low and thermoregulatory demands are high (Prendergast et al. 2001). Although day length is considered the primary environmental regulator for seasonality in voles, there is evidence that other factors, particularly ambient temperature, also contribute to temporal regulation of the seasonal rhythm in reproduction. For instance, although low ambient temperatures alone are ineffective for induction of reproductive regression in the prairie vole (a close relative of the meadow vole), in combination with SDs, low temperatures enhance gonadal regression (Nelson et al., 1989).

The significance of non-photic factors in the regulation of reproductive quiescence is also supported by findings garnered from investigations of photoperiodic nonresponders, individuals that undergo reproductive regression when exposed to SDs alone. Photoperiodic nonresponders constitute a small, but significant proportion (10-30%) of many seasonally-breeding rodent populations and may represent an alternative reproductive strategy, one in which individuals forgo the benefits of reproductive quiescence in exchange for an opportunity to breed during a less competitive time of year. This tactic is thought to offer maximum benefit, in terms of reproductive success, during relatively mild winters, when SDs coincide with moderate temperatures and readily available food supplies (Nelson et al., 1989; Prendergast et al., 2001). Although most investigations of photoperiodic nonresponders have focused on males, females are capable of employing the strategy as well. In one study, more than 80% of female prairie voles housed in SDs conceived litters (Nelson 1985).

Like the hypothalamic-pituitary-gonadal (HPG) axis, the hypothalamic-pituitary-adrenal (HPA) axis also may play a role in preparing individuals for seasonal changes in their environments. Glucocorticoids, a class of steroid hormones that constitute a major component of the HPA axis, are involved in mediating physiological and psychological responses to adverse environmental conditions (Romero et al., 2008). However, much of the work on glucocorticoids has focused on their involvement in stress-induced pathologies, while comparatively little is known about the role of these hormones in regulating seasonal adaptations to shifting environmental conditions. Seasonal variations in glucocorticoid concentrations have been described in a number of free-living species (mostly amphibians, reptiles, and birds), but comparable changes are poorly documented in laboratory animals. Non-mammalian vertebrates generally display a robust elevation of glucocorticoids during the breeding period, whereas seasonal rhythms in mammals are less clear, in part because relatively few species have been examined for seasonal variations in glucocorticoid secretion (Romero 2002; Romero et al., 2008).

This study examined the roles of day length, ambient temperature, and food availability in the induction of behavioral and physiological changes—namely decreased body mass, decreased food intake, and reproductive quiescence—commonly considered to be preparations for the onset of winter. The effects of these environmental factors on corticosterone were examined to determine if this hormone varies on a seasonal basis in female meadow voles and if its concentrations correlate with reproductive state. Given that both natural and laboratory populations of meadow voles display a range of reproductive responses to short day lengths, I hypothesized that temperature and food availability, in conjunction with day length, would influence the physiological traits measured.

Materials and Methods

Uterine Mass

Uterine mass has been used as a proxy for estradiol exposure (Beery et al., 2008). One day after behavior testing, focal voles were sacrificed and their uteri removed, trimmed just above the ovaries, defatted with forceps, and weighed (±0.001 g).

Plasma Estradiol and Corticosterone Measurement

On the day after behavior testing, between 13:00 and 14:00 PDT, blood was collected from the retro-orbital sinus of focal voles that were anaesthetized with isoflurane vapor. Samples were centrifuged at 4°C for 20 min at 3000 rpm and plasma was collected. Plasma was stored at −80°C until quantification with commercially available free plasma corticosterone (Enzo Life Sciences, Ann Arbor, MI, USA) and estradiol ELISA kits (Calbiotech, Spring Valley, CA, USA). All samples were processed in duplicate with appropriate inter- and intra-assay controls and standards. Because prairie voles are glucocorticoid resistant (Taymans et al., 1997), plasma for corticosterone assay was diluted at 1:1000. Dilutions were optimized such that results fell within the linear portion of the standard curve. Intra-assay coefficient of variation was less than 10% for both corticosterone and estradiol ELISA; inter-assay variation was 9.27% and 8.89% respectively. All other procedures were completed in accordance with manufacturer's instructions. Statistical analyses were conducted using the averages of duplicate measures and values are given in pg/ml. For each treatment group except SDfr21, n=12/assay. Sample sizes for SDfr21 were n=11 for the corticosterone assay and n=10 for the estradiol assay because blood could not be collected from one focal vole, and a sample from another female was misplaced prior to estradiol assay.

Data Analysis

Uterine mass and plasma hormone concentrations were examined using planned comparisons between groups differing in one treatment factor. Statistical analyses were conducted using both raw uterine mass data and values that were corrected for body size by calculating uterine mass as a percentage of body mass. In SD groups, voles with uterine mass values that exceeded the group mean by more than 2 standard deviations were classified as photoperiodic nonresponders.

Differences in average weekly food intake (n=12/treatment group) and body mass (*n*=24/treatment group) were analyzed by ANOVA for repeated measures to examine between-subject and within-subject effects (i.e., the effects of treatment over time). Factorial ANOVA was subsequently employed to identify specific weeks in which differences occurred and to compare the magnitude of change in body mass and food intake.

Results

Planned Between-Group Comparisons

Uterine Mass

Statistical analyses excluded data from photoperiodic non-responders in SD groups. The following groups contained photoperiodic nonresponders: SDadlib21*high* (2 nonresponders), SDadlib21*low* (1 nonresponder), SDfr21*high* (1 nonresponder). Inter-group comparisons of uterine masses and uterine masses adjusted to account for body mass yielded identical findings of significance; only statistical outcomes for analyses using uncorrected uterine mass values are presented here.

Uterine mass is presented for all frequently-handled groups in Fig. 4.1. Food-restricted females in 21˚C had significantly heavier uteri than voles fed ad lib under both LDs (t=2.27, P<0.05) and SDs (t=2.43, P<0.05). At 10˚C, females fed ad lib had heavier uteri when housed in LDs than SDs (Z=2.81, P<0.01). In SDs, voles in 21˚C had heavier uteri than those housed in 10˚C, whether they were food-restricted (t=3.46, P<0.01) or fed ad lib (t=2.20, P<0.05). In LDs, voles maintained in 21˚C had significantly heavier uteri than their 10˚C counterparts only when food-restricted (Z=3.53, P<0.001). In LDs, infrequently-handled females had larger uteri than frequently-handled voles (t=2.87, P<0.01). LD and SD voles housed in 21˚C did not differ in uterine mass unless they were infrequently-handled (Z=2.83, P<0.01) or food-restricted (t=2.36, P<0.05; Fig. 4.2); in both cases, LD females had heavier uteri than SD voles.

Hormone Assays

Estradiol

Food-restricted LD voles in 10˚C had higher estradiol concentrations than SD voles (Z=3.44, P<0.001). In SDs, voles in 10˚C had lower estradiol concentrations than females in 21˚C, whether fed ad lib (Z=2.17, P<0.05) or food-restricted (Z=2.60, P<0.01; Fig. 4.3). In both day lengths, estradiol values of low-handled females were lower than high-handled voles, an effect of borderline significance in SDs (Z=1.94, P=0.05) and non-significant in LDs (P>0.5; Fig. 4.4).

Corticosterone

In 21˚C, corticosterone concentrations in food-restricted voles were lower than in voles fed ad lib, an effect that was significant in LDs (t=2.09, P<0.05), but not in SDs (t=1.90, P=0.07). Females fed ad lib and housed in 10˚C had lower corticosterone concentrations in SDs than LDs (t=4.48, P<0.001). For SD females fed ad lib, housing in 21˚C was associated with higher corticosterone values than 10˚C (t=3.74, P<0.01; Fig. 4.5). In SDs, infrequently-handled voles had higher corticosterone concentrations than their frequently-handled counterparts (t=2.43, P<0.05; Fig. 4.6).

Main Treatment Effects, Treatment Interactions, and Correlations

Main treatment effects of day length (P<0.001), food intake (P<0.01), and temperature (P<0.001) significantly affected uterine mass. LDs were associated with heavier uteri than SDs (Z=2.89, P<0.01). Females in 10°C had lighter uteri than those in 21°C (Z=4.10, P<0.001). Food-restricted voles had heavier uteri than females fed ad lib (Z=2.48, P<0.05). Uterine mass was also affected by the interaction of food intake with temperature (P<0.01) and day length with handling (P<0.05). In 21°C, food-restricted voles had heavier uteri than females fed ad lib (Z=3.15, P<0.01). Food-restricted voles had heavier uteri in 21°C than 10°C (Z=4.60, P<0.001). In LDs, frequently-handled voles had lower uterine mass values than infrequently-handled females (t=2.87, P<0.01). Among infrequently-handled voles, uterine mass was reduced in SD compared to LD females (t=4.50, P<0.001). Uterine mass was positively correlated with body mass (simple linear regression, R^2=0.24, P<0.001), estradiol (R^2=0.09, P<0.01), and corticosterone (R^2=0.08, P<0.01).

Plasma estradiol concentration was significantly affected by interactions between day length and temperature (P<0.05) and food intake and temperature (P<0.05). At 10°C, females in LDs had significantly higher plasma estradiol than females in SDs (Z=3.25, P<0.01). In SDs, females housed in 10°C had significantly lower plasma estradiol than females housed in 21°C (Z=3.35, P<0.001). When voles were fed ad lib, those housed in 10°C had significantly lower plasma estradiol than voles maintained in 21°C (Z=2.08, P<0.05). Main treatment effects of temperature (P<0.05) on estradiol also were significant; females housed in 21°C had significantly higher estradiol than females housed in 10°C (Z=2.48, P<0.05).

Main treatment effects of day length (P<0.001), food availability (P<0.01), and temperature (P<0.01) on plasma corticosterone were significant. Females in LDs had higher corticosterone than females in SDs (t=3.54, P<0.001); females fed ad lib had higher corticosterone than voles fed a restricted diet (Z=2.23, P<0.05); and voles housed in 10°C had lower corticosterone than females housed in 21°C (Z=2.92, P<0.01). Correlations between corticosterone and estradiol were influenced by an interaction between day length and food availability (P<0.01).

Body Mass

Body mass was significantly affected by interactions between time and day length ($F_{9,180}$=2.42, P<0.05) and time and temperature ($F_{9,180}$=9,39, P<0.001). Temperature affected body mass during weeks 3 (P<0.05) and 4 (P<0.05) of treatment. Voles housed in 10˚C weighed significantly more than those in 21˚C after three (Z=2.90, P<0.01) and four (Z=3.07, P<0.01) weeks of treatment. After five weeks, this difference was no longer evident.

To facilitate further examination of treatment effects over time, between-subject differences in body mass were minimized by calculating percent of baseline mass (week 0) values (Fig. 4.7). Percent change in body mass over time was significantly influenced by day length ($F_{9,177}$=2.56, P<0.01) and temperature ($F_{9,177}$=10.72, P<0.001). The effects of temperature became apparent after two weeks of treatment and persisted until week 6 (P<0.05 for all weeks, except week 5, when P=0.06). During these weeks, females housed in 10°C were significantly heavier relative to baseline values than voles in 21°C. The influence of day length on percent body mass emerged after five weeks of treatment and continued for the duration of the experiment; during weeks 5 through 7, LD females were significantly heavier relative to their baseline mass than SD females (P<0.05 for all weeks).

The magnitude of body mass change after one week of treatment was influenced by temperature ($F_{6,185}$=6.28, P<0.001); females in 10°C gained more weight than those in 21°C (t=5.69, P<0.0001). On average, females gained 2.0±0.2 g after one week in 10°C, whereas voles in 21°C gained 0.7±0.1 g. This difference persisted through the second week of treatment; between weeks 1 and 2 (t=3.06, P<0.01), females in 10°C gained an average of 0.7±0.1 g and voles in 21°C gained 0.2±0.1 g.

The magnitude of body mass change from weeks 1-2 ($F_{6,185}$=2.79, P<0.05) and weeks 2-3 ($F_{6,185}$=3.25, P<0.01) also varied with day length. Females in LDs gained more weight than those in SDs. Between weeks 1 and 2 (t=2.10, P<0.05), LD females gained 0.6±0.1 g and SD females gained 0.2±0.1 g; between weeks 2 and 3 (t=2.74, P<0.01), voles in LDs gained 0.5±0.1 g, whereas voles in SDs lost 0.04±0.1 g. During the final week of treatment, the magnitude of body mass change was significantly influenced by food availability and temperature ($F_{6,185}$=17.26, P<0.001). Voles on a restricted diet lost more weight than voles fed ad lib (t=6.08, P<0.001); food-restricted females lost 1.9±0.2 g and females fed ad lib lost 0.6±0.1 g. Voles housed in 10°C also lost more weight than those housed in 21°C (t=4.71, P<0.001); females in 10°C lost 1.8±0.2 g, compared to a loss of 0.7±0.1 g in 21°C. After the week of food restriction, the magnitude of body mass lost was also affected by significant interaction between food availability and temperature (P<0.001). Consequently, all combinations of food

availability and temperature were analyzed by one-way ANOVA followed by the Tukey-Kramer test. Food-restricted females in 10°C lost more body mass than any other group (P<0.001; Table 4.1).

Food Intake

Temperature significantly affected between-subject differences in food intake ($F_{1,81}$=55.67, P<0.001), beginning after one week of treatment and persisting through week 7 (P<0.001 for all weeks). During weeks 1-7, females housed in 10°C ate significantly more than females in 21°C. Between weeks 1 and 7, voles in 10°C ate between 59.2±1.2 g and 65.3±1.4 g of food, whereas those in 21°C consumed between 45.4±0.8 g and 50.1±1.1 g of food.

Food intake also was influenced by interactions between day length and time ($F_{9,73}$=2.30, P<0.05) and temperature and time ($F_{9,73}$=21.55, P<0.001). Between-subject differences in food intake were minimized by calculating subsequent intake relative to baseline values at week 0 (Fig. 4.8). Percent food intake was affected by both day length ($F_{1,81}$=8.26, P<0.01) and temperature ($F_{1,81}$=58.93, P<0.001). Females in 10°C ate significantly more than those in 21°C during weeks 1-7 (P<0.001 for all weeks). Females also ate more relative to baseline intake in LDs versus SDs; this effect was significant for weeks 1 and 3 (P<0.05) and borderline significant for weeks 2 and 4 (P=0.05 and P=0.06, respectively).

The magnitude of change in food intake after one week of treatment was affected by both day length and temperature ($F_{6,88}$=17.80, P<0.001). Increases were greater after one week in LDs than SDs (P<0.05). Females in LDs and SDs increased their food intake by 9.2±1.5 g and 4.8±1.3 g, respectively. After one week of treatment, increases in intake were greater in 10°C (13.9±1.4 g) than 21°C (0.2±0.6 g; P<0.001).

Discussion

This study confirmed that body mass, food intake, corticosterone, and the reproductive axis of female meadow voles are influenced by day length, ambient temperature, and food availability. Beery et al. (2008) found that voles housed in 21°C and fed ad lib displayed a day length-dependent difference in uterine mass; females in LDs had significantly heavier uteri than SD females. In contrast, I found no significant difference in either uterine mass or estradiol between LD and SD females maintained in 21°C and fed ad lib. However, analysis across all treatment groups revealed that LD females had significantly heavier uteri than SD females. In combination, these outcomes suggest that although day length is an important regulator of uterine mass in meadow voles, other environmental factors are also influential. In the 10°C, ad lib treatment condition, LD voles had heavier uteri than SD females, indicating that ambient temperature likely constitutes one of these factors. In support of this conclusion, analysis across treatment groups demonstrated that voles in 21°C had heavier uteri than those in 10°C.

Interestingly, all SD groups housed in 21°C contained photoperiodic nonresponders, whereas 10°C females were all responders. If photoperiodic nonresponsiveness is in fact a reproductive strategy adopted by some individuals to take advantage of mild winter conditions, one might predict that exposure to heightened environmental challenge during winter would decrease the proportion of nonresponders observed in the population. In support of this prediction, Kriegsfeld et al. (2000b) found that in male prairie voles, the combination of low temperature and SDs induced gonadal regression in all experimental animals, whereas voles housed under SDs and mild ambient temperature display a range of reproductive responses. Additionally, combined exposure to low temperatures and short days are required to induce the decline in the synthesis of gonadotropin-releasing hormone that precedes reproductive quiescence (Kriegsfeld et al. 2000a).

Like uterine mass, plasma estradiol concentration was influenced by day length and ambient temperature, although in this case, the two environmental factors had interactive effects. A day length-dependent difference in estradiol existed only when females were housed in 10°C, with values higher in LD than SD voles. A temperature-dependent difference in estradiol existed when females were housed in SDs; concentrations were elevated in 21°C compared to 10°C. Analysis across all groups also demonstrated that plasma estradiol was lower in 10°C than in 21°C.

Although uterine mass and estradiol were both influenced by day length and temperature, estradiol exposure alone only accounted for a relatively small fraction of the variability in uterine mass. In addition, the large uterine mass of voles in the 21°C food-restricted treatment group, which had the largest average uterine mass of all LD groups, did not correspond with an elevated concentration of plasma estradiol. Uterine mass has been used as a proxy for determining relative differences in estradiol exposure; in general, larger uteri are assumed to indicate higher estradiol concentration (Lundeen et al., 1997). My data suggest that, at least in female meadow voles, uterine mass is not an accurate predictor of relative differences in estradiol concentrations. A similar dissociation of uterine mass and estradiol occurs in Siberian hamsters (*Phodopus sungorus*), in which SD- and LD-reared females can display differences in uterine growth in the absence of differences in serum estradiol and uterine estrogen receptor levels (Phalen et al., 2010).

Several studies demonstrated a consistent link between decreased food availability and reproductive suppression (Bronson and Heideman 1994; Schneider and Wade 2000). In female meadow voles, food deprivation significantly decreases plasma estradiol and sexual receptivity; the latter is restored by re-feeding and estradiol treatment (Pierce and Ferkin 2005; Pierce et al., 2007). However, my data demonstrated that in LD voles housed in 21°C, food restriction significantly increased uterine mass, concurrent with a significant decline in plasma corticosterone. This outcome is even more surprising considering that food restriction at 21°C did not result in significant loss of body mass. Considered together, these results suggest that under LDs, female voles are capable of mounting a physiological response to relatively mild shortages in food availability. Uterine mass has been used as a means of evaluating the degree of reproductive activation (Steinman et al., 2012). In prairie voles, high concentrations of plasma corticosterone interfere with the development of opposite sex social preferences (DeVries et al., 1996). One speculative explanation for my findings is

that at moderate temperatures, summer phenotype females respond to mild decreases in food availability by enhancing their ability to engage with potential mates and reproduce. Aside from the effects of food restriction on corticosterone and uterine mass in LD females maintained in 21°C, food restriction did not elicit significant between-group differences in corticosterone, estradiol, or uterine mass.

Higher glucocorticoid concentrations during the breeding season have been documented in the majority of amphibian, reptile, and avian species studied to date. In mammals, similar investigations of seasonal variations in glucocorticoid secretion are less abundant; however, most of the species examined display differences in glucocorticoid levels between the breeding and nonbreeding season. Unlike non-mammalian vertebrates, the pattern of this seasonal difference in mammals appears to be species-specific, with some species displaying higher glucocorticoid concentrations during the breeding season and others during the non-breeding season (Romero et al., 2008). The present research places meadow voles in the former category. Plasma corticosterone varied by day length, food availability, and ambient temperature. Long day lengths, 21°C, and an ad lib diet were associated with higher corticosterone than SDs, 10°C, and a restricted diet, respectively. One interpretation is that corticosterone is highest under conditions favorable to breeding. If female meadow voles are like female prairie voles, in that high corticosterone levels interfere with the formation of social bonds, declines in corticosterone under winter-like day lengths, temperatures, and food availability may facilitate the increased social tolerance that has been noted in free-living, overwintering females.

One possible explanation for the absence of an SD-induced decrease in uterine mass in the ad lib, 21°C treatment is that the duration of SD treatment was too abbreviated. In Beery et al.'s investigation (2008), voles were housed in SDs for 8-10 weeks before uteri were collected, whereas my voles were in SDs for 7-8 weeks. However, comparison of infrequently-handled groups revealed a day length-dependent difference that does not support this conclusion. Rather, it appears that consistent disturbance by experimenters eliminated the photoperiodically-mediated difference in uterine mass by suppressing uterine mass in LD voles. In contrast, such disturbance appears to have had no effect on uterine mass in SD voles. The mechanism underlying this photoperiodic difference in reproductive response to handling is unclear. Plasma corticosterone and estradiol seem like plausible explanatory candidates; however, between-group differences in plasma estradiol and plasma corticosterone did not correspond with between-group differences in uterine mass. High levels of corticosterone are associated with handling stress and purportedly have suppressive effects on reproduction; thus, one might expect that frequently-handled LD females would display high corticosterone in conjunction with decreased uterine mass. However, although frequently-handled LD females did have significantly lighter uteri than infrequently-handled LD voles, these two groups did not differ in plasma corticosterone concentration.

Day length and ambient temperature had significant effects on body mass and food intake changes over time. Females housed in LDs increased food intake during the seven week treatment period, whereas food intake in SD females remained relatively stable throughout the treatment period. This suggests that SDs suppressed the gradual increase in body mass and food intake noted in LD females and decreased energy

requirements, as noted in other meadow vole studies (Dark and Zucker 1983; Dark et al., 1983; Dark et al., 1984). To cope with the increased energetic demand posed by low temperature, females gained weight and displayed significant increases in food intake that were apparent within one week of low temperature exposure and persisted throughout the treatment period.

Food restriction caused significant declines in body mass only in females housed in 10°C, suggesting that the combination of low temperature and decreased food availability was the most energetically challenging of all treatment conditions. Thus, it is surprising that this treatment condition was not associated with significant changes in uterine mass or plasma hormone concentrations. The present data suggest that day length and ambient temperature are the most salient environmental regulators of the examined physiological traits. The combination of low temperature and SDs was effective for inducing reproductive quiescence (low estradiol and uterine mass values), and SDs promoted maintenance of a lower body mass and decreased food intake.

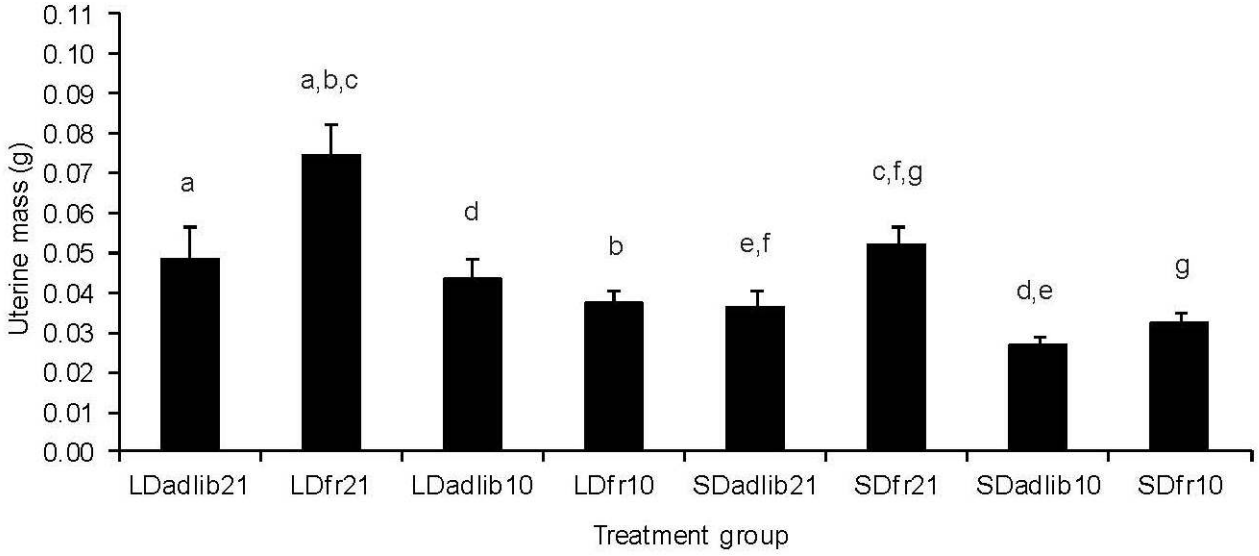

Fig. 4.1 Uterine mass in frequently-handled treatment groups. Shared letters indicate significant differences.

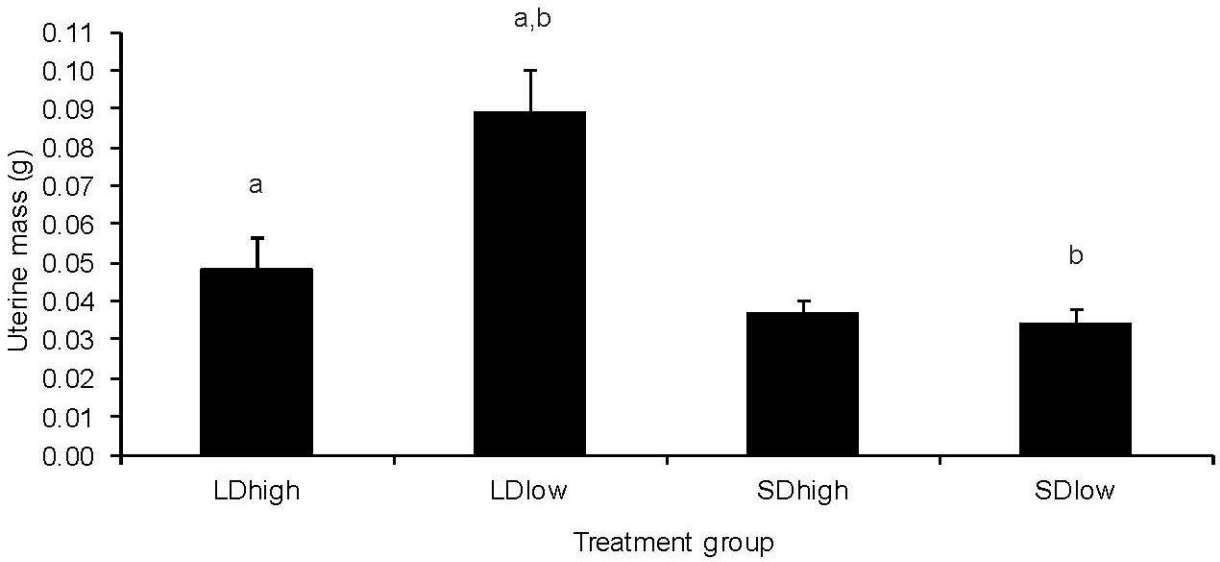

Fig. 4.2 Uterine mass by handling treatment in LDs versus SDs. “High” groups were handled frequently, while “low” groups were handled infrequently. Shared letters indicate significant differences.

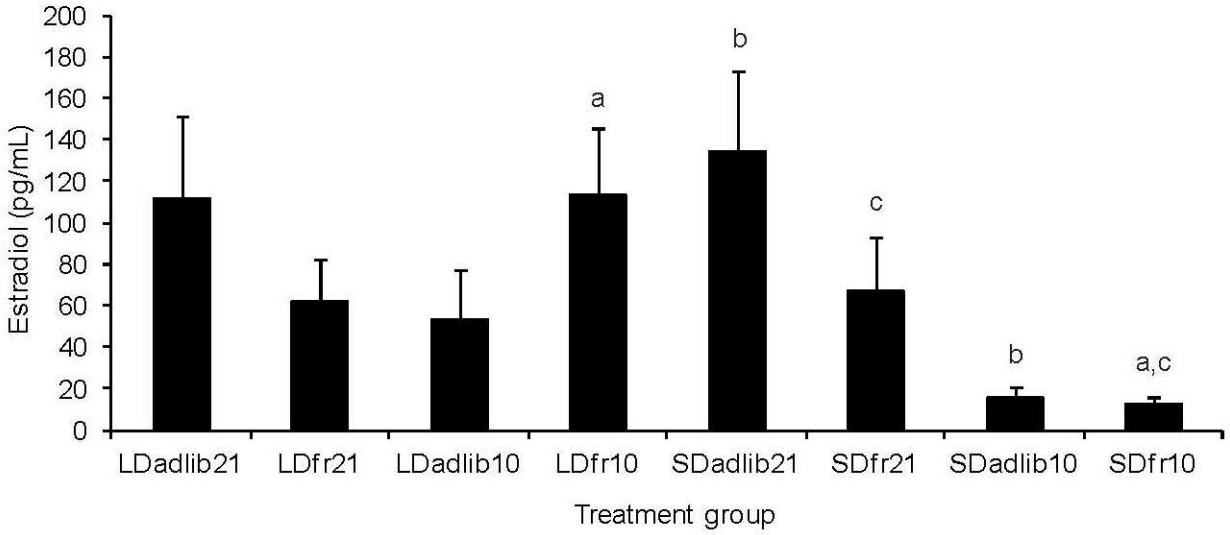

Fig. 4.3 Serum estradiol concentrations (pg/mL) in frequently-handled treatment groups. Shared letters indicate significant differences.

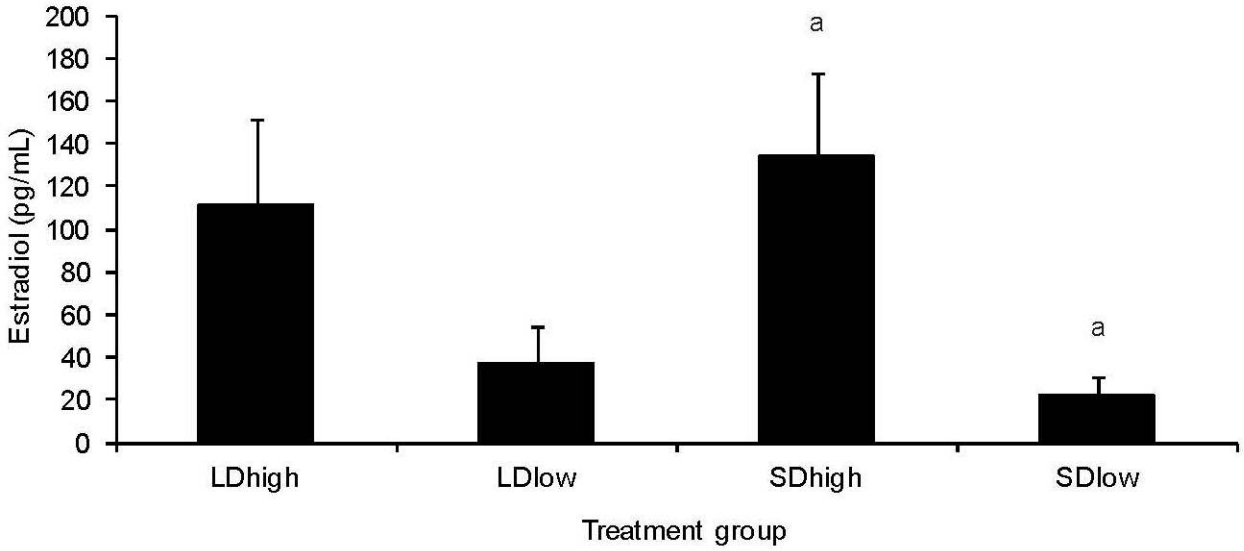

Fig. 4.4 Serum estradiol concentrations (pg/mL) by handling treatment in LDs versus SDs. “High” groups were handled frequently, while “low” groups were handled infrequently. The difference between SDhigh and SDlow females was of borderline significance (*P*=0.05).

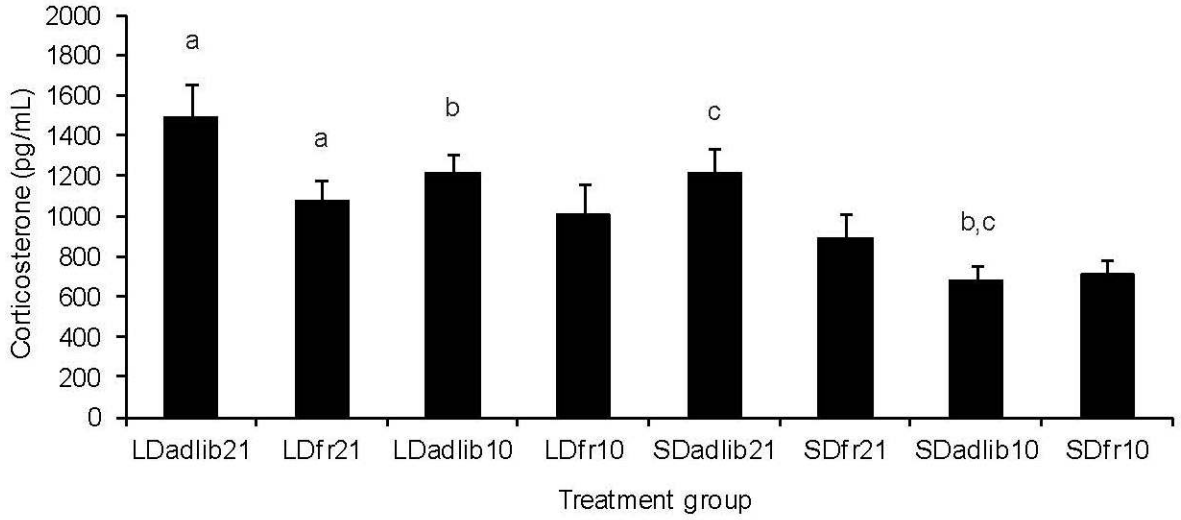

Fig. 4.5 Plasma corticosterone concentration (pg/mL) in frequently-handled treatment groups. Shared letters indicate significant differences.

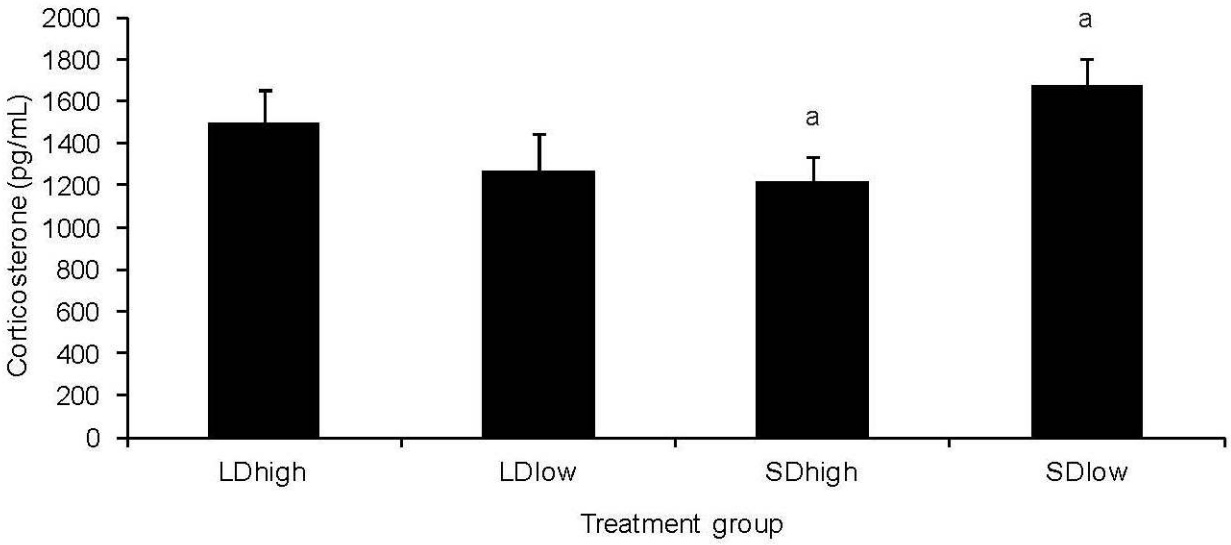

Fig. 4.6 Serum corticosterone concentrations (pg/mL) by handling treatment in LDs versus SDs. "High" groups were handled frequently, while "low" groups were handled infrequently. Shared letters indicate significant differences.

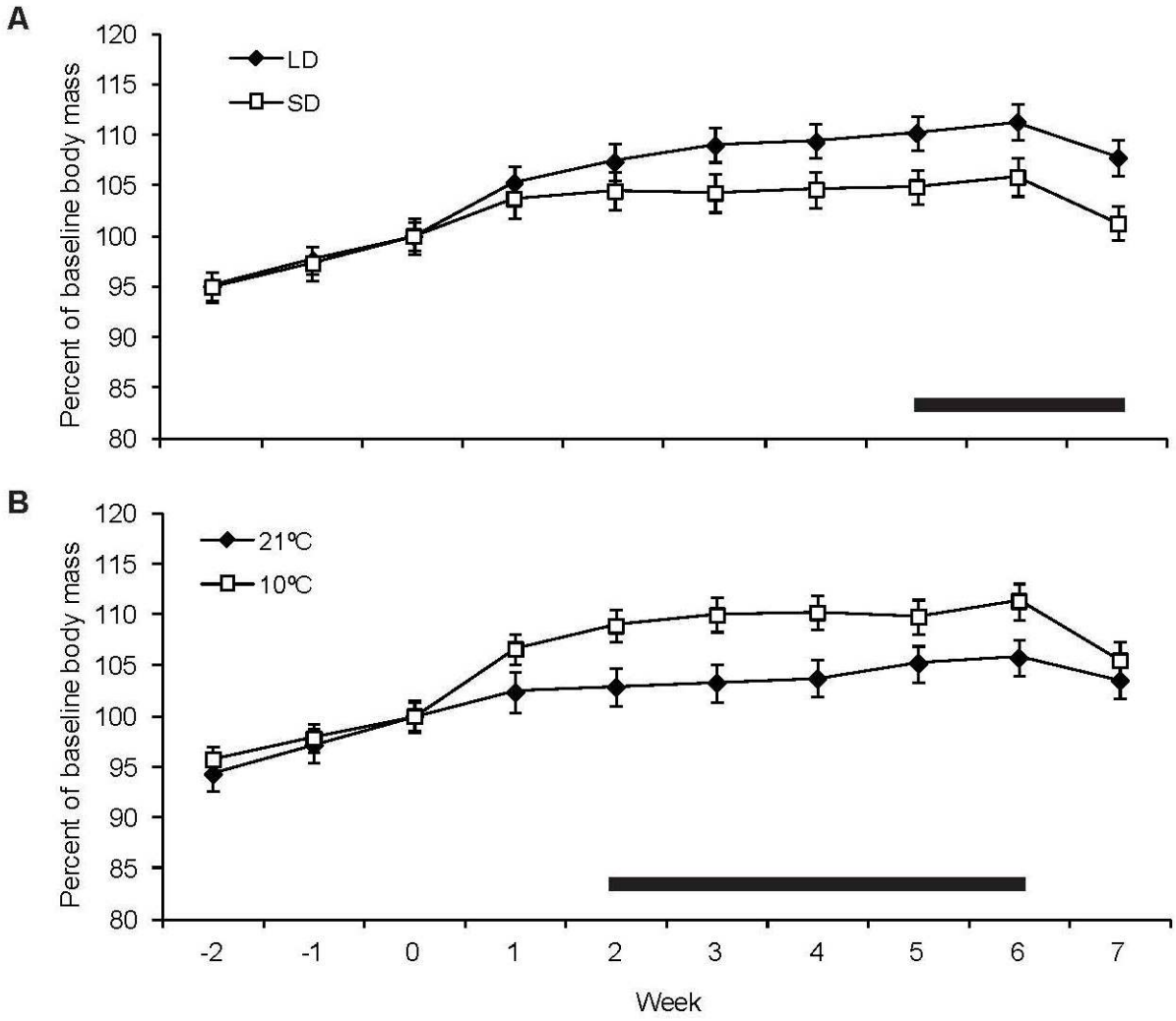

Fig. 4.7 Body mass expressed as a percent of baseline (week 0) body mass for frequently-handled voles housed in SDs or LDs (*A*) and 21°C or 10°C (*B).* Females were placed into their respective day length and temperature treatments immediately after body mass and food intake measurements taken at week 0. The horizontal bar above the abscissa indicates weeks during which treatment groups displayed significant differences in percent body mass (during week 5, the difference between 21°C and 10°C groups was of borderline significance, P=0.06).

Group abbrev.	Body mass lost after final week in treatment
adlib21	0.5±0.2
fr21	0.9±0.2
adlib10	0.6±0.2
fr10	2.9±0.2[a]

Table 4.1 Body mass (g) lost, by food availability and temperature treatment, after final week in treatment (week 7). During this week, food-restricted groups underwent restriction to 90% of daily ad lib intake. Designation with “a” indicates significant difference from all other groups.

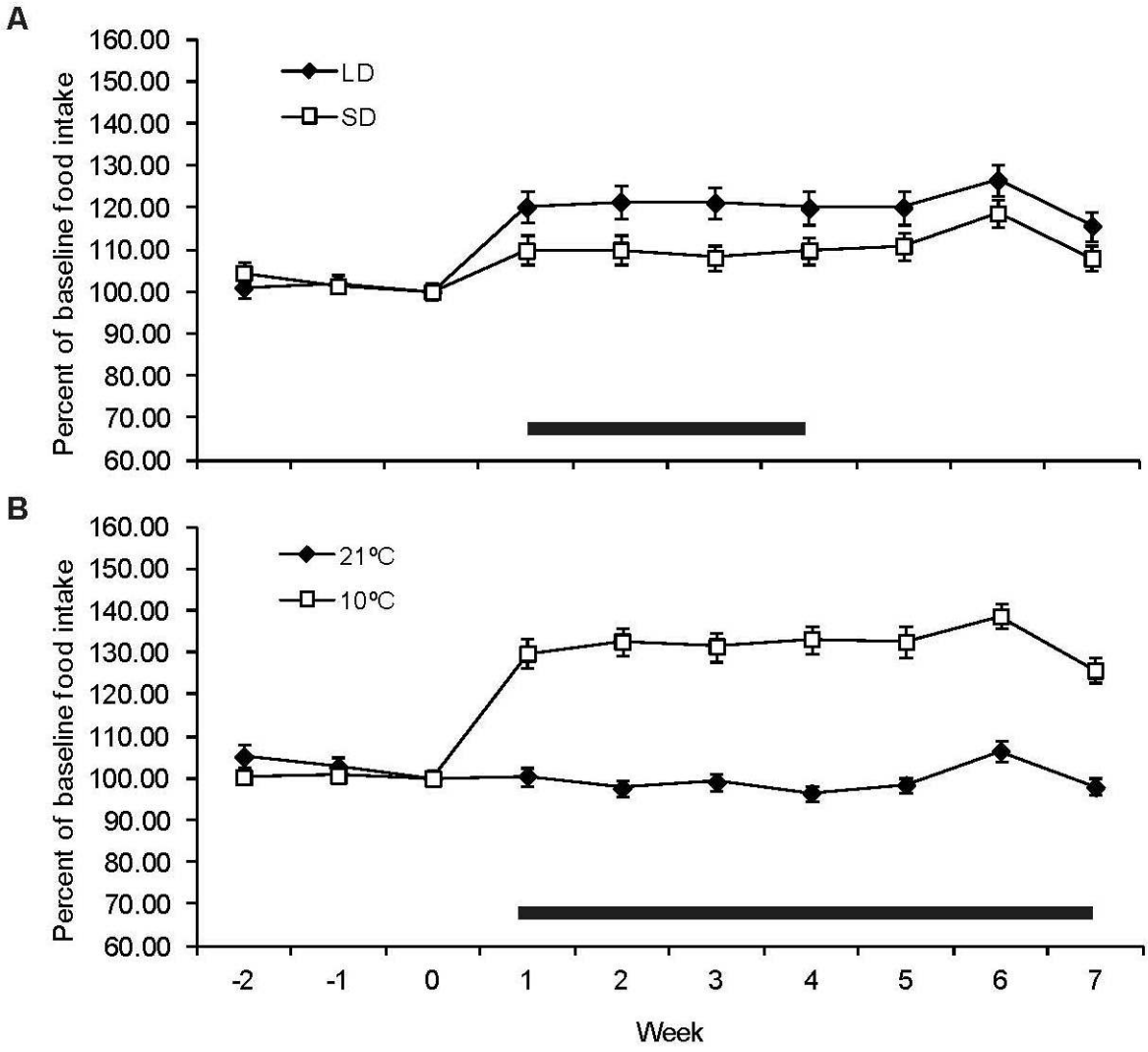

Fig. 4.8 Food intake expressed as a percent of baseline (week 0) food intake for frequently-handled voles housed in SDs or LDs (*A*) and 21°C or 10°C (*B).* Voles were placed into their respective day length and temperature treatments immediately after body mass and food intake measurements taken at week 0. The horizontal bar above the abscissa indicates weeks during which treatment groups displayed significant differences in percent food intake (during weeks 2 and 4, the difference between LD and SD groups was of borderline significance (P=0.05 and P=0.06, respectively).

5

Integrated Effects of Physiology and Environment on Same-Sex Affiliation

Remote from universal nature, and living by complicated artifice, man in civilization surveys the creature through the glass of his knowledge and sees thereby a feather magnified and the whole image in distortion.

-Henry Beston (1928)

For many organisms, the ability to respond behaviorally to environmental change plays a critical role in their survival. Deep-sea squid must time their daily ascent to the ocean's surface so that they arrive under cover of darkness, thereby minimizing risk of predation (Young and Mencher 1980). As the summer breeding season draws to an end, migrating birds must terminate reproductive activities and take flight towards their overwintering territories (Ball and Bentley 2000). In temperate climates, rodents survive extended periods of low temperatures and decreased food availability, in part by modifying their activity, food intake, or social behavior. Depending upon the environmental factor in question, cyclical variations can occur on the timescale of hours, days, or months. In order to respond appropriately to these changes, organisms must translate environmental signals into biological messages that promote adaptive modifications in behavior and physiology. The complex web of events that results in such modifications, and the manner in which multiple environmental signals interact to regulate these biological changes, is difficult to examine experimentally. As a result, for most species, comprehensive discussions of the integration of environmental and physiological factors with behavior are relatively uncommon; yet, such discussions are valuable if we are to understand how organisms function and survive.

An examination of winter sociality across several arvicoline rodent species led Madison (1984) to speculate that low temperatures may play a pivotal role in promoting the seasonal expression of social tolerance and grouping behavior. In light of what has been learned about affiliative behavior over the past twenty years, Madison's suggestion hits upon a novel idea—that thermoregulatory challenge can facilitate social affiliation by modulating the physiological factors that regulate social bonds. Such

physiological factors include sex steroids, glucocorticoids, the neurohormones vasopressin and oxytocin, and dopamine (Adkins-Regan 2009). The latter two categories of biological compounds were not included in my dissertation; however, I did examine possible interactions between environmental factors and endogenous concentrations of estradiol and corticosterone in the regulation of social affiliation. In particular, investigations of both free-living and captive-housed meadow voles suggest a potential role for sex steroids in the expression of winter-typical social behavior. In wild populations, reproductive quiescence appears necessary for displays of winter huddling behavior (Webster and Brooks 1981b; West and Dublin 1984). The minority of males that do not become reproductively inactive during winter display heightened aggression and a lack of huddling behavior when compared to males with regressed testes (McShea 1990). Interactive effects of day length and estradiol on the odor preference of female meadow voles were demonstrated by Ferkin and Zucker (1991), who showed that estradiol modifies preferences for conspecific odors in a day length-dependent manner. Under long days, the preference of female voles for the odors of males over other females is reversed by ovariectomy and restored by estradiol treatment. In contrast, neither ovariectomy nor estradiol affects the odor preference for other females displayed by short day females.

The goal of this chapter is to describe how interactions between physiological and environmental factors influenced huddling behavior. To accomplish this task, I will briefly revisit data from previous chapters and discuss the results of my efforts to model the effects of several variables on huddling behavior.

Materials and Methods

Data Analysis

Data from frequently-handled groups were examined for correlations of huddling behaviors to categorical variables (day length, temperature, food availability) and continuous variables (plasma estradiol, plasma corticosterone, and differences in body mass between focal and tethered voles) using *post hoc* 7-way ANCOVA. Models were constructed by selecting from this predetermined list of variables using stepwise regression. Automated selection of model factors used a *P*-value threshold stopping rule, which allowed effects to enter the model if they corresponded to $P<0.25$, and then subsequently excluded effects from the model if they corresponded to $P>0.1$. Significant effects were further investigated using simple linear regression.

Results

Huddling With a Partner

The following interactions were correlated with time spent huddling with a partner ($F_{15,77}=2.18$, $R^2=0.30$, $P<0.05$): day length, temperature, and the difference in body

mass between partners and focal voles (P<0.01). In 10°C, SD voles increased time spent huddling with partners as partner mass, relative to focal mass, increased (R^2=0.31, P<0.01); in contrast, LD females decreased huddling time with partners as partner mass, relative to focal mass, increased, although this effect was not significant (R^2=0.13, P=0.08; Fig. 5.1). Correlations between huddling time with a partner and the difference in mass between partners and focal voles were not apparent in voles housed in 21°C.

Huddling with a Stranger

The following interactions were correlated with time spent huddling with a stranger ($F_{20,72}$=3.24, R^2=0.47, P<0.001): day length, food availability, and plasma estradiol concentration (P<0.05); day length, food availability, and plasma corticosterone (P<0.01); and day length, temperature, and plasma corticosterone concentration (P<0.01). Among day length and food availability combinations, only food-restricted females housed in SDs displayed a significant correlation between time spent huddling with a stranger and estradiol (R^2=0.32, P<0.01). For voles exposed to these conditions, increasing estradiol was associated with an increase in time spent huddling with a stranger. SD voles fed ad lib displayed a negative correlation between time spent huddling with a stranger and plasma corticosterone (R^2=0.33, P<0.01), whereas those on a restricted diet displayed a positive correlation, although the latter was of borderline significance (R^2=0.17, P=0.05). Correlations between corticosterone and huddling with a stranger were not noted in any other combination of day length and food availability treatments. Among day length and temperature combinations, only voles housed in SDs and 10°C showed a correlation between corticosterone and time spent huddling with a stranger (R^2=0.21, P<0.05). Voles exposed to these conditions decreased huddling time with a stranger as plasma corticosterone increased.

Total Huddling Time

The following interactions were correlated with variations in total time spent huddling ($F_{13,79}$=2.92, R^2=0.32, P<0.01): temperature and plasma estradiol concentration (P<0.01); and day length, temperature, and the difference in mass between partners and focal voles (P<0.001). Females housed in 10°C displayed decreased total huddling time as plasma estradiol increased (R^2=0.09, P<0.05), whereas voles in 21°C showed no correlation between total huddling time and estradiol. In 10°C, LD females decreased total huddling time as partner body mass increased relative to the focal vole's mass (R^2=0.24, P<0.05). In contrast, SD voles in 10°C increased total huddling time as partner mass increased relative to focal mass (R^2=0.23, P<0.05; Fig. 5.1). Voles housed in 21°C did not display a correlation between total huddling time and partner mass relative to focal mass.

Discussion

The outcomes of this research indicate that all three examined environmental factors—food availability, day length, and ambient temperature—were capable of eliciting changes in the physiology and affiliative behavior of female meadow voles. Time spent huddling with a partner was surprisingly consistent across all treatment groups. When food-restricted and housed in 10°C, LD females spent less time huddling with partners than females in SDs. This day length-dependent difference may have occurred in part because LD and SD females housed in 10°C displayed opposing social responses to the relative body mass of their partners (Fig. 5.1). In LDs, focal voles spent *less* time huddling when their partners were heavier than them, whereas SD females spent *more* time huddling when their partners were heavier than them. This finding suggests that the loss of partner preference in LD10 may have occurred because females were actively discriminating against huddling with partners based upon relative differences in body mass. The behavioral difference between LD and SD females may reflect different strategies for dealing with thermoregulatory challenge. Given that long-term periods of low temperature are more likely to occur during winter than summer, overwintering voles may preferentially maintain social relationships with individuals that offer the most thermoregulatory benefit (i.e., individuals who produce more body heat or perhaps are better fed). In contrast, summer phenotype voles may prioritize access to resources over the maintenance of social bonds.

The only instance in which food restriction affected huddling behavior occurred in SDfr21 voles, which displayed a significant increase in time spent huddling with a stranger and total huddling time. This behavioral effect was not associated with significant perturbations of uterine mass, estradiol, or corticosterone; thus, the marked increase in affiliative behavior observed in this group is attributed to some presently unspecified physiological mediator. That food-restricted SD females in 21°C displayed such marked changes in social preference and huddling behavior, even in the absence of noticeable declines in body mass, suggests that the physiological systems responsible for regulating affiliative behavior are quite responsive to decreased food availability and capable of undergoing modification well before the onset of serious health consequences.

Time spent huddling with a stranger was correlated with plasma concentrations of estradiol and corticosterone only when voles were housed in SDs. As their estradiol levels increased, food-restricted voles spent increasing amounts of time huddling with strangers. Females fed ad lib or housed in 10°C spent less time huddling with strangers as corticosterone concentrations increased. These results suggest that estradiol and corticosterone may contribute to variations in social tolerance under SDs, but not LDs. Beery et al. (2008) similarly found that estradiol manipulations modified the affiliative behavior of SD, but not LD females, and concluded that differences in ovarian hormone secretions could not fully account for day length-dependent differences in affiliative behavior.

Beery et al. (2008) also reported that total huddling time was inversely correlated with uterine mass; females with smaller uterine masses spent the most time huddling. My data revealed no correlation between huddling behavior and uterine mass. If uterine mass is indeed an accurate measure of reproductive activation, then my data suggest

that: 1) factors other than plasma estradiol contribute to variations in reproductive status in female meadow voles, and 2) maintenance of reproductive activity does not prohibit same-sex affiliation in female meadow voles. Regarding the latter, it is of interest to note that Madison and McShea (1987) reported considerable overlap between the breeding period and the onset of group huddling behavior in a population of free-living meadow voles in Virginia and speculated that the purported temperature-dependent mechanism responsible for regulating seasonal changes in social tolerance is separate from the mechanism that governs seasonal breeding activity.

Only SD voles held at 10°C displayed significant concurrent modifications of huddling behavior, uterine mass, plasma estradiol, and plasma corticosterone concentration. These females increased the proportion of time spent huddling with strangers without losing partner preference, as occurred in LD10, and manifested significant decreases in uterine mass, estradiol, and corticosterone. In light of my findings, I suggest that, of all treatment groups, the behavior of SD10 meadow voles most closely resembles that of free-living, overwintering females. During fall and winter, wild females display increased social tolerance by permitting immigrants to join their territories. However, the persistence of same-sex dyads immediately after winter suggests that females are capable of maintaining social bonds in winter social groups.

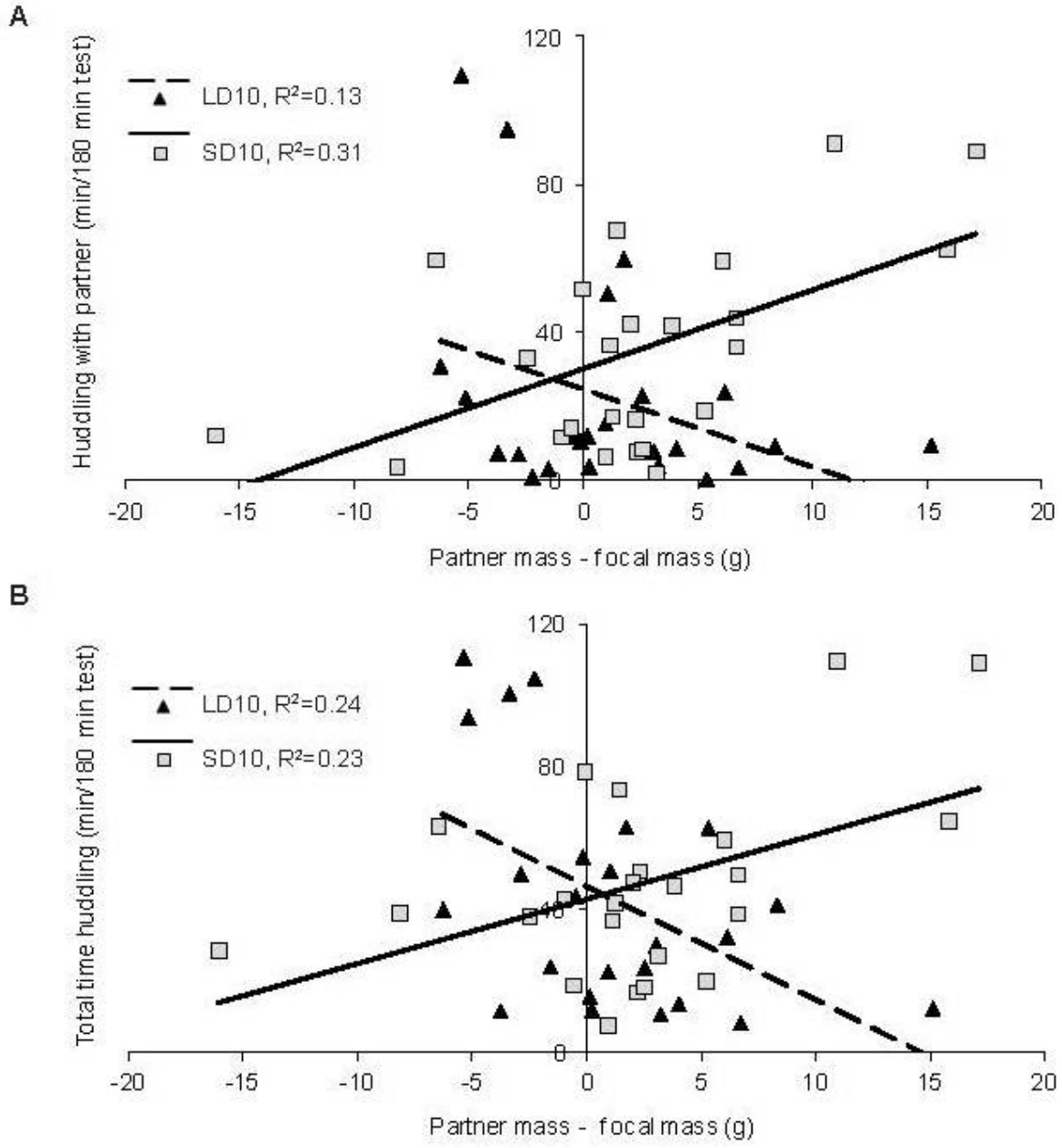

Fig. 5.1 Time spent huddling with a partner (A) and total time spent huddling (B) relative to differences in mass between partner and focal voles (data from frequently-handled groups only). Both graphs suggest that when exposed to low temperature, SD and LD females employed different huddling strategies. SD females spent more time total time huddling and time huddling with partners when their partners exceeded them in body mass, whereas LD females spent more time huddling when they exceeded their partners in body mass.

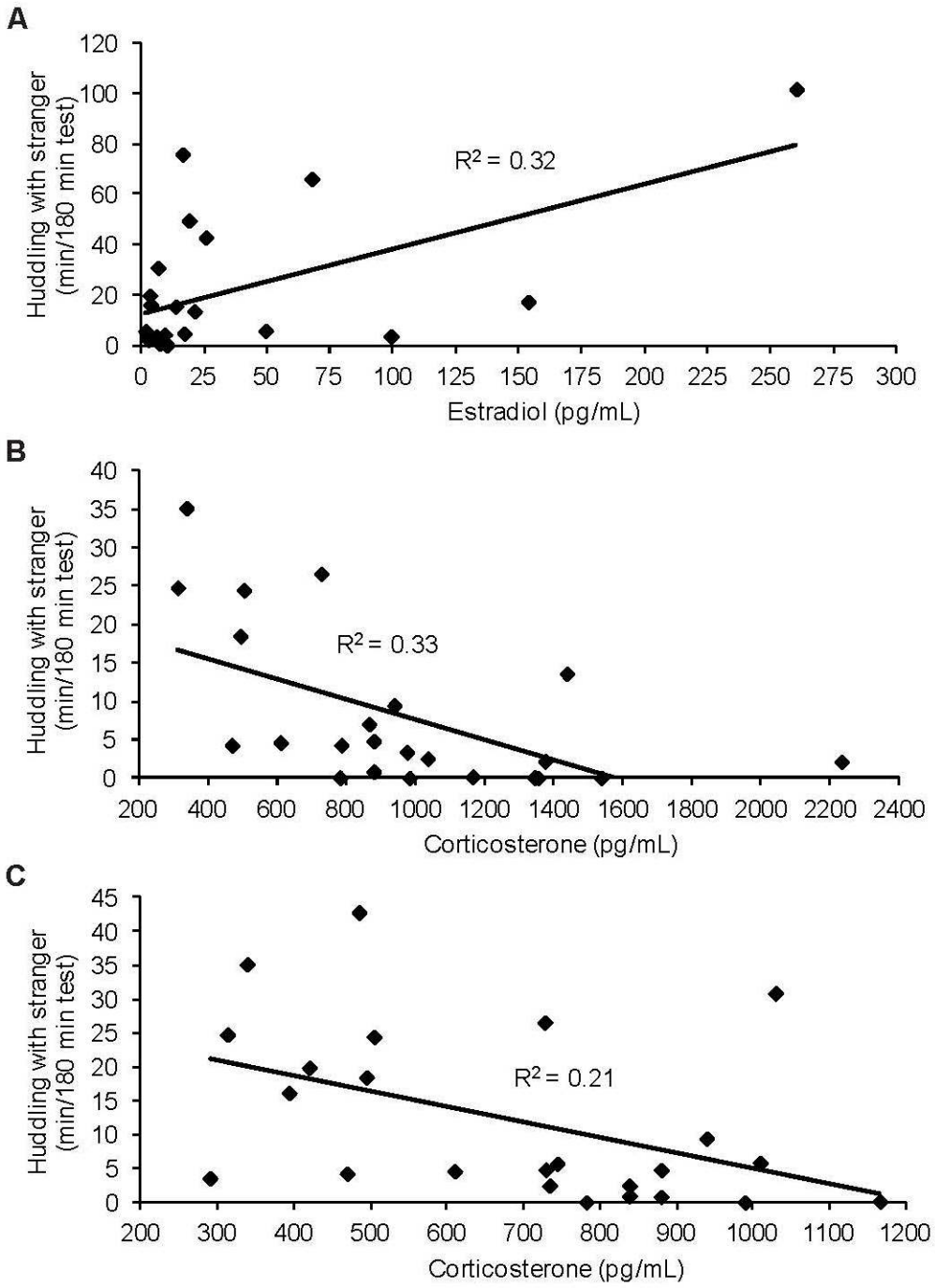

Fig. 5.2 Time spent huddling with a stranger relative to plasma hormone concentrations for frequently-handled SD voles that were (A) food-restricted, (B) fed ad lib, and (C) housed in 10°C. Significant correlations between huddling behavior and hormone concentrations were not evident in LD treatment groups.

6

Group Size and Social Preferences

In animals as diverse as African elephants and barnyard mice, blue monkeys of Kenya and feral horses of New Zealand, affiliative, longlasting and mutually beneficial relationships between females turn out to be the basic unit of social life, the force that not only binds existing groups together but explains why the animals' ancestors bothered going herd in the first place.

-Natalie Angier (2012)

Social groups, diverse in composition, size, and structure, exist across the animal kingdom. Some groups consist primarily of kin engaged in close social bonds, while others contain large numbers of individuals bound only loosely by a shared need for common resources. Since the 1990s, one of the central foci of affiliation studies has been the identification of factors that facilitate social bonding. These investigations often rely on partner preference tests that indicate the presence of social bonds between individuals. Such studies have proven invaluable in revealing the regulatory roles of neurohormones (including vasopressin and oxytocin), corticosteroids, dopamine, and social cues in the maintenance of close social bonds. However, the current body of research leaves unanswered some intriguing questions about the proximate determinants of group living. Is group cohesion maintained solely by strong social bonds between individuals? In gregarious species characterized by the aggregation of hundreds, or even millions, of individuals, maintenance of the group solely through social bonds is unlikely. Due to fitness benefits, strong social bonds are thought to occur primarily between mates or kin; yet aggregations of non-related, unmated animals are not uncommon in nature. A wide variety of species, including raccoons, deer mice, golden-brown mouse lemurs, Thomson's gazelles, emperor penguins, elephant seals, and many flock-forming passerine species form groups containing unrelated individuals; in some cases, as in the latter four examples, group members may number in the hundreds, thousands, or millions (Gilbert et al., 2010; Lacey and Sherman 2007). Even in species characterized by smaller group sizes, individuals engage in varying levels of affiliation with different members of their group. For instance, female baboons living in a troop of 70 individuals form particularly strong associations with a small number of their female peers, whom they preferentially spend time with and allogroom (Wittig et al.,

2008). The question raised by such observations is: are there proximate factors that promote a preference (or a tolerance) for group living, even in the absence of strong social bonds? As one author states, it is ”important to fight the temptation to equate the mechanisms governing social grouping with those that mediate social bonding" (Ophir 2011).

Evidence garnered from avian research suggests that the neurophysiological regulators of individual social bonds also may influence the propensity to live in groups of varying size (Goodson and Kingsbury 2011). However, similar studies have not been performed in non-avian species; thus, very little is known about the factors that regulate group formation, or aggregative behavior, in mammals. Meadow voles present an intriguing opportunity for the investigation of group size preferences in mammals. Field studies demonstrate that females undergo a marked transition from a summer-aggressive to a winter-affiliative phenotype, a change that can be replicated under laboratory conditions using environmental manipulations. Although numerous studies refer to meadow voles as "asocial," this classification is generally based on intersexual interactions and relies on behavioral testing done only under summer-like long day lengths. However, the seasonal variation in group living and gregariousness displayed by free-living meadow voles suggests that this species can be quite social, even in comparison to prairie voles, which maintain aggressively defended territories and small, kin-based social units throughout the year (Ophir 2011). Data generated in this dissertation and by the work of others (Beery et al., 2008; Parker et al., 2001; Parker and Lee 2003) document the ability of female meadow voles to develop partner preferences for both opposite- and same-sex conspecifics, supporting the notion that female meadow voles are not strictly asocial. If long day (LD) and short day (SD) voles differ in group size preference, comparisons of these phenotypes may prove useful for examining the neurophysiological mechanisms that underlie group living. Given that females display seasonal variations in group living, I examined the effect of day length on female group size and social preferences. I hypothesized that LD, but not SD, females would retain their partner preference and spend less time with a group of strangers than SD females.

Grouping preferences in meadow voles are also of particular interest because the seasonal transition in social behavior differs between males and females. Additionally, in prairie voles, the underlying neural and physiological mechanisms involved in the regulation of affiliative behavior often vary according to sex. Given the existing evidence for sex differences in social behavior, I hypothesized that SD females would spend more time, relative to SD males, huddling with a group of strangers.

Materials and Methods

Animals

At 18-20 days of age, male and female offspring were weaned and placed into same-sex trios. When animals from two or more litters were weaned concurrently, mixed trios consisting of non-siblings were assembled; otherwise, trios consisted of

siblings. Animals were housed in clear plastic cages (48 x 25 x 15 cm) containing pine bedding, two Nestlets, one paper nest tent, and two opaque plastic refuge tubes.

Experimental Design and Timeline

At weaning, female trios were either transferred to short day lengths (SDs; 10h of light/day) or remained in LDs for the duration of the experiment (*n*=8 trios/group). All male trios were transferred into SDs at weaning (*n*=8 trios). At 70-100 days of age, trios underwent behavioral testing in a social preference apparatus, as described below.

Behavioral Testing

Social preference was assessed as described in Chapter 2, with some modifications. Rather than tethering one partner and one stranger in opposing fore-chambers, one cage-mate and three strangers were tethered in opposing fore-chambers. The three strangers were a trio of cage-mates from the same treatment condition as the focal vole. Huddling was defined as side-by-side contact between the focal and tethered voles; side-by-side contact between a focal and at least one member of a trio of strangers was recorded as huddling with the trio. This design was used to evaluate a focal vole's preference for group assembly versus social bonds. The placement of strangers and partners into either the left or right chamber was randomized to avoid bias.

Data Analysis

Total time spent huddling with the partner versus the trio of strangers was compared within each treatment group using paired *t*-tests or a comparable non-parametric statistic (see Chapter 2 for more details). Social preference was inferred when focal voles spent at least twice as much time huddling with either the partner or the trio of strangers.

Analysis Between Groups

Groups differing in one treatment factor were compared using either unpaired t-tests or a comparable nonparametric statistic.

Results

Social Preference (Table 6.1)

Females housed in LDs and males housed in SDs spent significantly more time huddling with the partner than with the trio of strangers (females, $S=18.00$, $p<0.01$; males, $S=16.00$, $p<0.05$). Females housed in SDs displayed no significant difference in time spent huddling with the partner versus the trio of strangers ($S=2.00$, $p=0.8$).

Females housed in LDs and males housed in SDs displayed a partner preference (i.e., spent at least twice as much time with the partner than a trio of strangers), whereas females housed under SDs displayed no social preference.

Between-Group Comparisons

Groups did not differ in total huddling time or time spent huddling with the partner. Females housed in LDs and males housed in SDs did not differ in time spent huddling with the trio of strangers; however, females housed in SDs spent more time huddling with the trio of strangers than did males in SDs ($Z=-2.26$, $P<0.05$) and females in LDs; the latter outcome was of marginal significance ($Z=1.95$, $P=0.05$; Fig. 6.1).

Data for one male were excluded from the activity analyses because his activity count was more than 2x standard deviations from the SD male group mean. Females housed in SDs displayed significantly higher levels of investigatory activity during behavior tests than females maintained in LDs ($t=2.92$, $P<0.05$) and males housed in SDs ($t=-2.53$, $P<0.05$).

Observations

Only two instances of persistent aggression occurred throughout testing. Review of behavior videos revealed that one focal female from LDs directed persistent aggressive behavior (lunging, boxing, and vocalizing) towards the trio of strangers. One male trio had to be separated and removed from the experiment prior to behavior testing due to persistent aggression between trio members. Another male trio was added to maintain sample size. In neither instance did animals suffer serious or permanent injury. No instances of aggression amongst SD females were observed.

Four females from SDs and one from LDs engaged in stable group huddles, lasting for several minutes, with two or more members of the trio of strangers (Fig. 6.2). This behavior was not observed in the SD male group.

Discussion

The outcomes of this study strongly corroborate data regarding the behavior of both free-living and captive meadow voles and support my hypotheses that grouping behavior in this species varies by both day length and sex. First, females housed in SDs displayed no social preference and spent more time huddling with the trio of strangers than LD females, which displayed a strong preference for the partner. In free-living meadow voles, group living occurs on a seasonal basis. Females are highly territorial and do not cohabitate with other adults during the summer months; in contrast, in winter, females permit immigration, share nest sites, sleep in clusters, and engage in group territorial defense (Madison 1985; Madison et al., 1984; Madison and McShea 1987; McShea 1990; Webster and Brooks 1981b). Second, unlike SD females, males housed in SDs displayed a strong partner preference and spent little time huddling with the trio of strangers. Unlike female meadow voles, males do not display seasonal differences in partner preference (Beery et al., 2009). In addition, Ferkin and Seamon

(1987) showed that nonbreeding females prefer the odors of other females over male odors, whereas nonbreeding males show no preference. Of the two sexes, nonbreeding males also display more aggression towards conspecifics, while nonbreeding females are more amicable with other females and less so with males (Ferkin and Seamon 1987). Based on these findings, Ferkin and Seamon (1987) suggested that overwintering groups of meadow voles may be female-biased and that males are solitary during part of the nonbreeding season.

Also of interest are the following observations: 1) that incidents of persistent aggression occurred in LD females and SD males, but none were observed in SD females (anecdotally, I can report that a review of my colony records, maintained over a three-year period, reveals several instances of aggression between co-housed adult males maintained in both SDs and LDs, but none between co-housed females in either day length), and 2) during behavior tests, SD females engaged in stable group huddles with the trio of strangers more frequently than did any other group. Considered together, these observations and the data generated in this experiment offer provocative evidence that meadow voles display grouping behavior variations, mediated by photoperiod and sex-based physiological differences. More specifically, the results suggest that exposure to short day lengths may prime female, but not male voles for interactions with unfamiliar individuals and participation in larger social groups, perhaps by modifying the same mechanisms known to be involved in social bonding.

In addition to spending more time with the trio of strangers, SD females also displayed more investigatory activity than other treatment groups, which may correspond with the dispersal activity of free-living meadow voles. Winter dispersal in meadow voles is contact-seeking in nature and occurs following declines in group size, suggesting that voles prefer some optimal group size during this time of year (Madison and McShea 1987; McShea 1990). Behavioral studies in birds indicate that individuals are capable of weighing the costs and benefits of participating in groups of varying sizes relative to resource availability (Elgar 1986). The outcomes of this study, along with the other work in this dissertation, suggest that for meadow voles, the decision to interact with other individuals is more complex than a simple question of familiarity. Environmental factors, including the number of strangers, can have striking effects on the time that voles, specifically females, spend with unfamiliar individuals.

Although this study was of limited scope, it produced surprisingly clear results that offer guidance for future investigations of grouping behavior in meadow voles. Goodson and colleagues, who investigated gregariousness (i.e., group size) in estrildid finches, demonstrated that group size preferences are likely regulated by the same neurohormones that modulate social bonds. Goodson et al. (2009) presented female zebra finches with the option of consorting with large versus small groups of novel same-sex conspecifics and discovered that central infusions of oxytocin receptor antagonist (OTA) reduced the amount of time animals spent in close proximity to large groups, whereas intracerebroventricular infusions of mesotocin (the avian homologue of oxytocin) increased the time females spent with large groups. Interspecies comparisons within the estrildid family further demonstrated that the distribution of oxytocin-like receptors reflects varying levels of gregariousness, with highly gregarious species displaying higher receptor density in the dorsal lateral septum (LS) and lower binding in the ventral LS (reviewed in Goodson and Kingsbury 2011). Beery et al. (2010) found

that oxytocin is not necessary for the expression of baseline levels of same-sex huddling in female meadow voles, leading them to suggest that the neurohormone may not be necessary for maintenance of same-sex bonds in this species. However, the authors did find that oxytocin receptor densities differ significantly in two brain regions between LD and SD females (Beery and Zucker 2010), indicating that oxytocin may facilitate a seasonal change in some facet of affiliative behavior other than social bonding. One fascinating follow-up study to the work described in this chapter would involve giving females centrally-administered oxytocin or OTA and determining the impact that these treatments have on group size (smaller versus larger groups of strangers) and social preferences (groups of novel versus familiar conspecifics). Such studies could elucidate the neurophysiological bases of social tolerance and gregariousness in rodents.

Group abbrev.	Huddling with partner (min)	Huddling with trio (min)	Huddling with partner vs. trio	*P* value
LDfemale	48.6±15.5	2.2±1.8	**	0.008
SDfemale	23.7±8.2	16.8±8.6		0.8
SDmale	41.2±10.8	0.99±0.57	*	0.02

Table 6.1 Huddling times by treatment group. "**" and "*" denote $P<0.01$ and $P<0.05$, respectively.

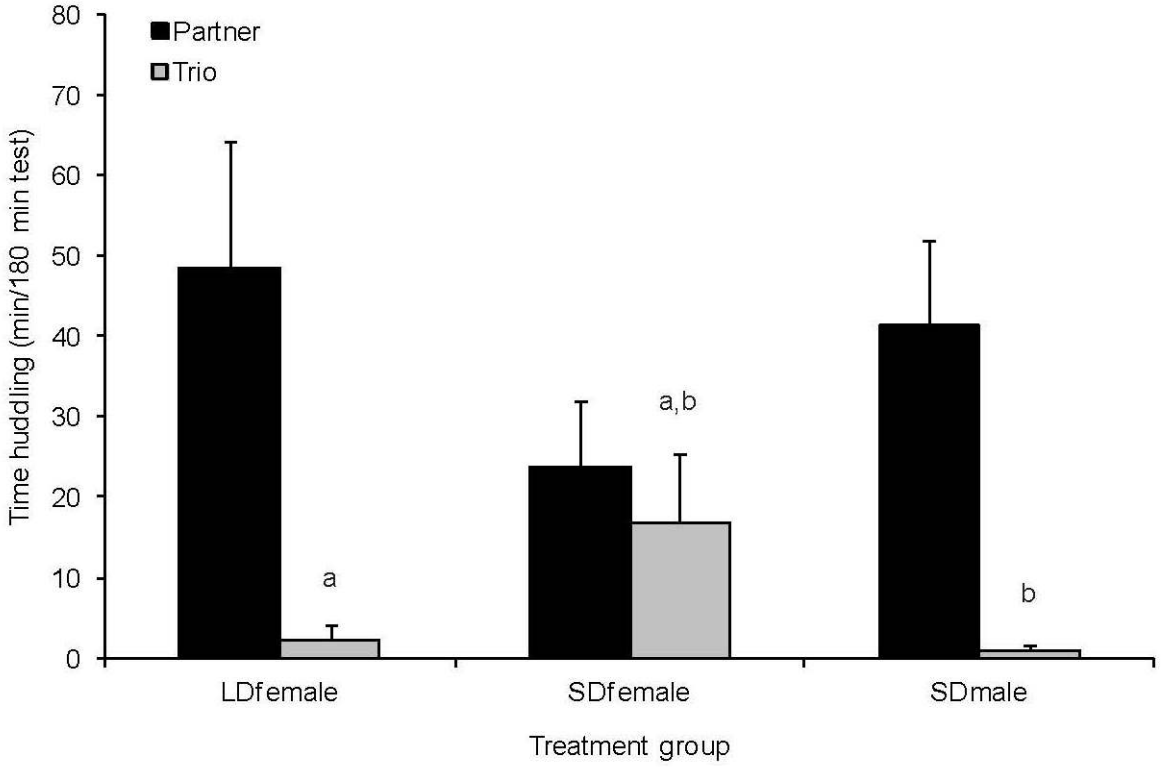

Fig. 6.1 Huddling times by treatment group. Bars sharing the same letter represent significant or near significant differences (a: P=0.05; b: P<0.05).

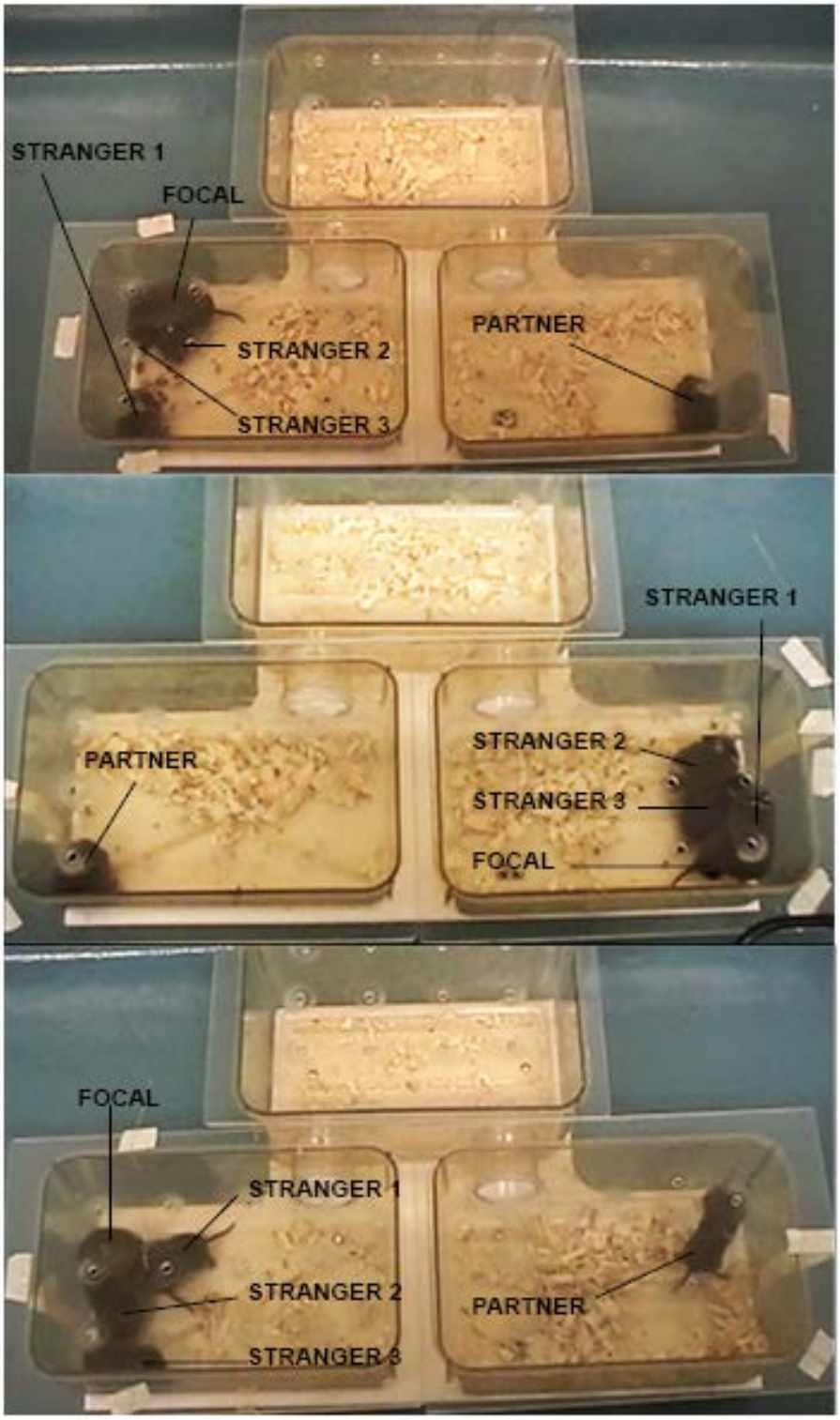

Fig. 6.2 Screen shots from videos of three behavior tests, each involving a different focal female from the short day length housing condition. Focal voles are all engaged in stable (lasting for several minutes) group huddles with two or more members from the trio of strangers; this behavior was almost exclusively displayed by SD females (one LD female also engaged in group huddling).

7

Conclusions

Natural selection is real but at the same time it is a shifting chimera, less a "law" than making its own law from age to age…The potential hidden in nature has flowered into a [great] variety of behavior. Thus what we call natural selection, "the war of nature," can either enclose living creatures in specialized prisons or, on occasion, open amazing doorways into unsuspected worlds.

-Loren Eiseley (1978)

My findings support the hypothesis that day length, in conjunction with food availability and ambient temperature, regulates the expression of same-sex affiliative behavior. Females in SDs and LDs differed behaviorally and physiologically in their responses to variations in food availability and ambient temperature. Low temperature conditions clearly constituted a thermoregulatory challenge to females in both day lengths, as both LD and SD voles increased body mass and food intake when housed in 10°C. However, SD females maintained lower body mass and food intake than their LD counterparts. This suggests that short day lengths promote energy conservation, a phenomenon previously described in several rodent species. My work is novel in that it demonstrates a possible link between thermoregulatory challenge and social bonds. Low temperatures prompted both LD and SD females to modify their affiliative behavior by spending more time with strangers. However, housing in 10°C appeared to interfere with the maintenance of social bonds in LDs, but not SDs. In addition, females in SDs, but not LDs, modified their affiliative behavior in response to food restriction and displayed correlations between two blood plasma hormones, corticosterone and estradiol, and huddling. Both hormones have been implicated in the regulation of opposite-sex pair bonds in prairie voles. These findings suggest that the physiological regulators of affiliative behavior may be more responsive to low temperatures and food scarcity during winter than during summer. However, it must be noted that in many cases, variations in plasma corticosterone, plasma estradiol, and uterine mass did not correspond with variations in huddling behavior. For example, food restriction of SD females housed in 21°C produced significant increases in affiliative behavior; however, the same group did not display significant changes in uterine mass or hormone concentrations. Thus, current exposure to these hormones cannot fully account for the

effects of day length, temperature, and food availability on same-sex affiliation in female meadow voles.

Females in short day lengths appeared more inclined to join new social groups than females in LDs or males in SDs. Females in SDs engaged in stable group huddles more frequently and spent more time huddling with a trio of strangers than did LD females or SD males. In addition, SD females displayed no social preference for either their familiar partners or a group of strangers, whereas LD females and SD males strongly preferred partners. Although males in LDs were not examined, I suspect that the behavioral outcomes for this group would look much like that of SD males because meadow voles are long day breeders and intraspecific aggression is more common during the breeding season. These outcomes lend credence to the conjecture that same-sex relationships between females likely play a significant role in the maintenance of social groups in some mammalian species. The marked differences in group size and social preferences between LD and SD females recommend female meadow voles as an excellent potential model species for future investigations of the neuroendocrine bases of mammalian gregariousness.

Collectively, the results described in this dissertation suggest that conditions associated with winter (food scarcity, lower temperatures, and short day lengths) increase social tolerance and promote aggregation of females into groups. A planned study of the distribution of oxytocin and corticotropin-releasing hormone receptors in the brains of voles used in this dissertation will more definitively investigate how environmental factors influence neuroendocrine regulators of social behavior, and how these effects relate to same-sex affiliation.

Contemplating the Evolutionary Origins of Social Groups

Although Alexander (1974) proposed a multitude of plausible causes for group living, the most thoroughly investigated explanations for the evolution of social groups fixate on the role of reproduction. It is easy to conceive how brief reproductive interactions could eventually lead to the evolution of increasingly complex social behaviors and different mating systems. However, non-reproductive factors may also facilitate the evolution of group living simply by bringing animals together in the same physical spaces. Proximity creates opportunities for increasingly complex interactions. This principle is applicable at every level of biological organization. Consider the appearance of life on Earth: although there are several hypotheses about how the first macromolecules and cells appeared, each proposal bears the common assumption that organizing increasingly more complex molecular assemblages required first that the basic building blocks coexist in physical proximity. Cells too may have aggregated because of the need for shared access to common resources (e.g., light, macromolecules), thereby creating opportunities for interaction and, eventually, grouping into the communities that we know today as multicellular organisms.

Likewise, in at least some instances the evolutionary precursors to social groups may have formed because animals needed to satisfy basic, non-social, or even non-reproductive requirements (for example, access to common resources, such as shelter or food, or the need for warmth). Freshwater planarians, which mostly reproduce asexually, aggregate into groups in response to their physical environments. Individuals

converge in regions protected from light and group around food sources; regarding the latter, worms feeding in groups display increased predation success (Cash et al., 1993; Reynierse et al., 1969). Such aggregations, formed initially to satisfy basic physical needs and not because of the reproductive benefits offered by social interactions, may have set the stage for the evolution of sociality in some animal lineages (Madison 1984; Shah 2003).

Relevance to Humans

Regardless of their specific endeavors, every biologist inevitably faces the anthropocentric question: what is the relevance of your work to humans? For those studying issues of biomedical import, with the ultimate goal of curing diseases or devising new treatments, the answer is clear. However, many scientists investigate the biological world because of its intrinsic interest. For them, the case for relevance to humans is perhaps less self-evident, but I would argue no less significant.

The theory of evolution through natural selection, proposed by Charles Darwin, emphasized the common features and relatedness of all life on Earth. The reality of this shared heritage means that studies conducted on one species can constitute a useful guide for investigations of similar phenomena in other species. In the case of affiliative behavior, this has proven to be true. During the early 1990s, C.S. Carter and her colleagues conducted the first neuroendocrine investigations of social affiliation using prairie voles (Tang-Martinez 2003). Today, although more is known about the mechanisms underlying social bonding in prairie voles than in any other mammalian species, the number of similar investigations in other species, including humans, is growing. For instance, the regulatory role of oxytocin in social bonding was first discovered in prairie voles, but has since been studied in humans. In addition, biologists have found that brain structure and the neuroendocrine mechanisms underlying social behavior are highly conserved across vertebrate taxa (Goodson 2008). Thus, what we have learned from voles—small, nondescript rodents that are often regarded as little more than agricultural pests—about the neuroendocrine factors governing social affiliation may help us understand one of the most basic aspects of human nature: our sociality.

8

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People learn and forget, they die, and even the strongest institutions they erect deteriorate, but knowledge continues to expand globally while passing from one generation to the next.

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