Exposing the elusive: Franklin's ground squirrel (*Poliocitellus franklinii*)

demography, nest relocation, and dam response to ectoparasitism

by

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Abstract

Franklin's ground squirrels (*Poliocitellus franklinii;* FGS) are increasingly considered of conservation concern throughout much of their range, yet little is known about this species as compared to their congeners. The main objectives of this thesis were to (1) collate and summarize demographic and life-history data to present an up-to-date account of population characteristics for a FGS population near Delta Marsh, Manitoba, (2) document nest movements wherein female FGS relocate their nests and litter during the energetically demanding lactation period and to investigate proximate factors mediating movements, including intraspecific interaction, ectoparasite burden, and nest habitat associations, and, (3) investigate the influence of ectoparasite infestation on dam and litter attributes. Dams consistently relocated litters during lactation in response to conspecific nest discovery, ectoparasite pressure and habitat type. Relocations may compensate for major costs of ectoparasitism, as beyond the frequency of relocation, only litter sex ratio was influenced by ectoparasite burden in this study.

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Thesis Format

       This thesis is presented in a manuscript ("sandwich") style format. As such, Chapter 1 is an introductory chapter that serves to present overall themes, background information, and relevant literature. Chapters 2 – 4, however, are written in a manuscript style complete with their own title, abstract, introduction, methods, results, discussion, and literature cited sections. Chapter 5 serves as a concluding chapter summarizing major findings from the earlier chapters and identifies directions for further study. My contribution to all primary chapters (2-4) includes the collation and analysis of previously collected data and writing of manuscripts. In addition, I performed field manipulations and collected data for the 2014 field season summarized in Chapters 3 and 4.

Chapter 1: Thesis Introduction

Franklin's ground squirrels (*Poliocitellus franklinii*) are an obligate-hibernating species that typically occur at low densities throughout the American Midwest, and north through the aspen parkland region of southern Canada (Hall 1955). In reference to Franklin's ground squirrels, naturalist Ernest Thompson Seton (1929) stated that "less is known of its life history than of that of any if its kinsman". This relative paucity of information regarding Franklin's ground squirrels remained true nearly twenty years ago (Murie 1999), and persists to the present day (Huebschman 2007). This scarcity of information is likely attributable to difficulty in study resulting largely from the species' unique habitat preferences relative to its congeners for thick, savanna-like areas complete with grassy and woody components (Huebschman 2007), its secretive nature (Hall 1955; Krohne et al. 1972; Duggan 2011), limited time spent above ground (Sowls 1948), and high motility (Haberman and Fleharty 1971; Jones et al. 1983).

Much of what is known about Franklin's ground squirrels results from interest in the species as potential duck nest predators. Nest predation studies identified Franklin's ground squirrels as "major predators of duck eggs" in North Dakota (Sargeant et al. 1987; Sargeant et al.1993; Sovada et al. 2000), and even documented an adult Franklin's ground squirrel consuming a nearly full-grown mallard in Manitoba (Sowls 1948). Studies detail both the extent (Sargaent et al. 1987) and manner (Sowls 1948) of egg predation by Franklin's ground squirrels. In the context of investigating nest predation, one of the most comprehensive studies of Franklin's ground squirrel natural history was undertaken in Manitoba (Sowls 1948). Likewise, one of the few studies pertaining to Franklin's ground squirrel movements and home range (Choromanski-Norris et al. 1989) was carried out in conjunction with an investigation into duck nest predation.

The primary literature also focuses on presumptive Franklin's ground squirrel range reductions and population declines. Reports detail reductions in the eastern range limit of the species (Indiana: Johnson and Choromanski-Norris 1992; Illinois: Pergams and Nyberg 2001; Martin et al. 2003), and inspired attempted population reintroductions in Illinois (Gensburg-Markham Prairie: Panzer 1986, and Knox College Biological Field Station: Van Petten and Schramm 1972), though both attempts ultimately failed (Martin et al. 2003). Reports also prompted the International Union for Conservation of Nature (IUCN) to list the species as "Vulnerable" on the 2003 Red List (Pergams et al. 2008). While the IUCN currently lists the species as of "Least Concern" given "locally high population densities" (Pergams et al. 2008), Franklin's ground squirrels are considered vulnerable in Iowa (Iowa Department of Natural Resources 2012), threatened in Illinois (Illinois Endangered Species Board 2015), endangered in Indiana (Indiana Department of Natural Resources 2013), imperilled to vulnerable in Missouri (Missouri Department of Conservation 2015), and imperilled in Wisconsin (Wisconsin Department of Natural Resources 2014). Recent studies regard the species as rare (Duggan 2011) and threatened (Green et al. 2013), however, researchers affirm the need for more comprehensive research into the species' ecology and habitat use in order to properly assess population status of Franklin's ground squirrels (Van Petten and Schramm 1972; Lewis and Rongstad 1992; Johnson and Choromanski-Norris 1992; Martin et al. 2003; Huebschman 2007; Duggan 2011).

Some information gaps are more glaring than others for Franklin's ground squirrels. Basic demographic studies are few (Haggerty 1968; Iverson and Turner 1972; Murie 1973), and even amidst population status concerns, studies of population dynamics and regulation are virtually non-existent for Franklin's ground squirrels. Though knowledge of intraspecific interaction, attraction, and avoidance would inform population reintroductions and site

occupancy (Duggan 2011), surprisingly little is known about Franklin's ground squirrel sociality. While often characterized as asocial (Kivett et al. 1976; Armitage 1981; Michener 1983; Lindsay and Galloway 1997; Hare 2004; Green et al. 2013), such accounts rely on a single investigation of scent glands (Kivett et al. 1976) and a study of the level of social discrimination amongst juveniles in a wild population of Franklin's ground squirrels near Delta Marsh, Manitoba (Hare 2004). Spatial organization, which largely underpins sociality (Michener 1979), and adult behaviour remain largely uninvestigated.

Though there are many accounts of Franklin's ground squirrel preference for habitat that is both herbaceous and woody in the literature (Huebschman 2007 and references therein), many researchers characterize the species as prairie-obligates (Van Petten and Schramm 1972; Krohne et al. 1972; Pergams and Nyberg 2001). This "apparent misconception" (Huebschman 2007) among researchers highlights a knowledge gap and need for further classification. Researchers have pointed out that further knowledge of habitat associations and seasonal resource use are needed to improve census methods and the management of this species (Johnson and Choromanski-Norris 1992; Martin et al. 2003; Huebschman 2007; Duggan 2011). At a finer scale within their habitat, Sowls (1948) estimated that Franklin's ground squirrels may spend as much as 90% of their lives in burrows (Sowls 1948). Burrows serve a variety of functions for animals, including protection from environmental extremes, defence against predators, and refuges in which reproduction and rearing of young may occur (Reichman and Smith 1990; Kinlaw 1999). Despite the importance and heavy use of burrows, the only investigation into Franklin's ground squirrel burrow use has been Martin and Heske's study (2004), in which they explored the location of both male and female burrows in relation to vegetation, soil, and cover

objects within a single 12-ha study plot. Burrowing behaviour and activity in or near burrows remains uninvestigated.

While researchers reference Franklin's ground squirrel's motility as impeding study and potentially influencing population disappearances, little data are available in the primary literature reflecting the species' movements. To my knowledge, there has been just four published accounts pertaining to the species' movements that utilize telemetry data (Krohne et al. 1972; Choromanski-Norris et al. 1989; juveniles only, Martin and Heske 2005; gap-crossing movements following translocation, Duggan 2011), and all studies that examine home range (Krohne et al. 1972; Choromanski-Norris et al. 1989; Duggan 2011) employed the minimum convex polygon (MCP) estimation method and offer little data or insight into core range use or intraspecific overlap. Dispersal, though relevant to spatial organization, population dynamics, and site occupancy (Duggan 2011), is also little studied in Franklin's ground squirrels. Martin and Heske (2005) tracked just 5 individual juveniles (4 females and 1 male) to hibernation, while Duggan (2011) apparently radio-tracked juvenile males before immergence while investigating gap-crossing movements, but did not disclose the underlying data. In addition, while female Franklin's ground squirrels have a propensity to move their nest and litter during the energy-intensive period of lactation (J. Hare, unpublished data), these puzzling movements remain unexplained where they are mentioned in print (Hall 1955; Haggerty 1968).

In the chapters that follow, I attempt to fill some of these important knowledge gaps that persist pertaining to Franklin's ground squirrel ecology. Chapter 2 provides an overview of demographic and life history traits collated from four years' of trapping effort in a free-living Franklin's ground squirrel population at Delta Marsh, Manitoba in the years leading up to population crash. In Chapter 3, I present an investigation of local nest relocation movements

undertaken by lactating females, and explore the contributions of ectoparasite response, intraspecific interactions, and nesting habitat associations to these apparently costly movements. In Chapter 4, I report the findings of a field experiment in which I manipulated ectoparasite abundance to test for any effect of ectoparasite pressure on nest movements, dam mass, litter size, litter sex ratio, and juvenile mass. Studies manipulating ectoparasite load (discussed in subsequent chapters) are seldom undertaken in a field setting, and have not previously been attempted with Franklin's ground squirrels.

Literature Cited

Armitage, K.B. 1981. Sociality as a life-history tactic of ground squirrels. Oecologia 48:36-49.

Choromanski-Norris, J.F., K. Fritzell, and A.B. Sargeant. 1989. Movements and habitat use of Franklin's ground squirrels in duck-nesting habitat. Journal of Wildlife Management 53: 324-331.

Duggan, J.M. 2011. Occupancy dynamics, personality, and behavior of Franklin's ground squirrel in agricultural landscapes. PhD. Thesis, University of Illinois, Urbana-Champaign, USA.

Green, M.L., T.F. Ting, M.B. Manjerovic, N. Mateus-Pinilla, and J. Novakofski. 2013. Noninvasive alternatives for DNA collections from threatened rodents. Natural Science 5: 18-26.

Haberman, C.G., and E.D. Fleharty. 1971. Natural history notes on Franklin's ground squirrels in Boone County, Nebraska. Transactions of the Kansas Academy of Science 74: 76-80.

Haggerty, S.M. 1968. The ecology of the Franklin's ground squirrel (*Citellus franklinii*) at Itasca State Park, Minnesota. M.Sc. Thesis, University of Minnesota, Minneapolis, USA.

Hall, E.R. 1955. Handbook of the mammals of Kansas. University of Kansas Museum of Natural History, Miscellaneous Publication 7: 1-103.

Hare, J.F. 2004. Kin discrimination by asocial Franklin's ground squirrels (*Spermophilus franklinii)*: is there a relationship between kin discrimination and ground squirrel sociality? Ethology Ecology & Evolution 16: 157-169.

Huebschman, J.J. 2007. Distribution, abundance, and habitat associations of Franklin's ground squirrel (*Spermophilus franklinii* Sabine 1822). Illinois Natural History Survey Bulletin 38: 1–58.

Illinois Endangered Species Board. 2015. Checklist of endangered and threatened animals and

    plants of Illinois. http://www.dnr.illinois.gov/ESPB/Documents/2015_ChecklistFINAL_for_

    webpage_051915.pdf

    Accessed on 01 June, 2015.

Indiana Department of Natural Resources. 2013. Indiana's State endangered species.

    http://www.in.gov/dnr/fishwild/files/fw-Endangered_Species_List.pdf

    Accessed on 01 June, 2015.

Iowa Department of Natural Resources. 2012. Iowa wildlife action plan.

    http://www.iowadnr.gov/Environment/WildlifeStewardship/IowaWildlifeActionPlan.aspx

    Accessed on 01 June, 2015.

Iverson, S.L., and B.N. Turner. 1972. Natural history of a Manitoba population of Franklin's

    ground squirrels. Canadian Field Naturalist 86: 145-149.

Johnson, S.A., and J.F. Choromanski-Norris. 1992. Reduction in the eastern limit of the range of

    the Franklin ground squirrel (*Spermophilus franklinii)*. American Midland Naturalist 128:

    325-331.

Jones, J.K., Jr., D.M. Armstrong, R.S. Hoffmann, and C. Jones. 1983. Mammals of the Northern

    Great Plains. University of Nebraska Press, Lincoln, USA.

Kinlaw, A. 1999. A review of burrowing by semi-fossorial vertebrates in arid environments.

    Journal of Arid Environments 41: 127-145.

Kivett, V.K., J.O. Murie, and A.L. Steiner. 1976. A comparative study of scent-gland location

    and related behavior in some northwestern Nearctic ground squirrel species (Sciuridae): an

    evolutionary approach. Canadian Journal of Zoology 54:1294–1306.

Krohne, D.T., J. Hauffe, and P. Schramm. 1972. Radio-tracking the Franklin's ground squirrel in a restored prairie, pp. 84-88. In: L.C. Hulbert, editor. Third Midwest Prairie Conference Proceedings. Kansas State University, Manhattan, USA.

Lewis, T.L., and O.J. Rongstad. 1992. The distribution of Franklin's ground squirrel in Wisconsin and Illinois. Transactions of the Wisconsin Academy of Sciences Arts and Letters 80: 57−62.

Lindsay, L.R., and T.D. Galloway. 1997. Seasonal activity and temporal separation of four species of fleas (Insecta: Siphonaptera) infesting Richardson's ground squirrels, *Spermophilus richardsonii* (Rodentia: Sciuridae), in Manitoba, Canada. Canadian Journal of Zoology 75: 1310-1322.

Martin, J.M., and E.J. Heske. 2004. Cover and soil drainage influence burrow location of Franklin's ground squirrel (*Spermophilus franklinii)* in Champaign County, Illinois. Transactions of the Illinois State Academy of Science 97: 227-233.

Martin, J.M., and E.J. Heske. 2005. Juvenile dispersal of Franklin's ground squirrel (*Spermophilus franklinii)* from a prairie "island." American Midland Naturalist 153: 444-439.

Martin, J.M., E.J. Heske, and J.E. Hoffmann. 2003. Franklin's ground squirrel (*Spermophilus franklinii*) in Illinois: a declining prairie mammal? American Midland Naturalist 150: 130-138.

Michener, G.R. 1979. Spatial relationships and social organization of adult Richardson's ground squirrels. Canadian Journal of Zoology 57: 125-139.

Michener, G.R. 1983. Kin identification, matriarchies, and the evolution of sociality in ground-dwelling sciurids, pp. 528-572. In: J.F. Eisenberg and D.G. Kleiman, editors.

Advances in the Study of Mammalian Behavior. American Society of Mammalogists Special

Publication No. 7.

Missouri Department of Conservation. 2015. Missouri species and communities of conservation

concern checklist. http://mdc.mo.gov/sites/default/files/resources/2010/04/2015species

concern.pdf

Accessed on 01 June, 2015.

Murie, J.O. 1973. Population characteristics and phenology of a Franklin ground squirrel

(Spermophilus franklinii) colony in Alberta, Canada. American Midland Naturalist 90:

334-340.

Murie, J.O. 1999. Franklin's ground squirrel, *Spermophilus franklinii*, pp. 419–420. In: D.E.

Wilson and S. Ruff, editors. The Smithsonian Book of North American Mammals.

Smithsonian Institution Press, Washington, D.C., USA.

Panzer, R. 1986. Franklin's ground squirrel translocation to an Illinois prairie preserve.

Restoration and Management Notes 4:27.

Pergams, O., and D. Nyberg. 2001. Museum collections of mammals corroborate the exceptional

decline of prairie habitat in the Chicago region. Journal of Mammalogy 82: 984-992.

Pergams, O., Nyberg, D, and G. Hammerson. 2008. *Spermophilus franklinii* in IUCN 2013.

IUCN Red List of Threatened Species. Version 2013.1. <www.iucnredlist.org>.

Accessed on 01 June, 2015.

Reichman, O.J., and S.C. Smith. 1990. Burrows and burrowing behavior by mammals, pp.

197-244. In: H.H. Gennoways, editor. Current Mammalogy. Volume 2. Plenum Press, New

York, USA.

Sargeant, A.B., R.J. Greenwood, M.A. Sovada, and T.L. Shaffer. 1993. Distribution and

    abundance of predators that affect duck production - Prairie Pothole Region. United States

    Department of the Interior, Fish and Wildlife Service, Resource Publication 194.

    Washington, D.C. 96 pp.

Sargeant, A.B., M.A. Sovada, and R.J. Greenwood. 1987. Responses of three prairie ground

    squirrels species, *Spermophilus franklinii, S. richardsonii,* and *S. tridecemlineatus*, to duck

    eggs. Canadian Field-Naturalist 101: 95–97.

Seton, E.T. 1929. Lives of Game Animals. Volume 4. Doubleday, Doran and Co., Garden City,

    USA.

Sovada, M.A., M.C. Zicus, R.J. Greenwood, D.P. Rave, W.E. Newton, R.O. Woodward, and J.A.

    Beiser. 2000. Relationships of habitat patch size to predator community and survival of duck

    nests. Journal of Wildlife Management 64: 820-831.

Sowls, L.K. 1948. The Franklin ground squirrel, *Citellus franklinii* (Sabine), and its relationship

    to nesting ducks. Journal of Mammalogy 29: 113–137.

Van Petten, A., and P. Schramm. 1972. Introduction, dispersal, and population increase of the

    Franklin's ground squirrel, *Spermophilus franklinii*, in a restored prairie, pp. 166–173. In: J.

    H. Zimmerman, editor. Proceedings of the Second Midwest Prairie Conference, University of

    Wisconsin−Madison.

Wisconsin Department of Natural Resources. 2014. Franklin's ground squirrel *(Spermophilus

    franklinii)*. http://dnr.wi.gov/topic/endangeredresources/animals.asp?mode=detail&speccode

    =amafb05120

    Accessed: 01 June, 2015.

Chapter 2: Demography and life history of a population of Franklin's ground squirrels (*Poliocitellus franklinii*) near Delta Marsh, Manitoba

Abstract

Demographic and life history traits provide important basic knowledge about species and are necessary for determining population dynamics, viability and status. Concerns over population declines and disappearances persist for Franklin's ground squirrels (*Poliocitellus franklinii*) throughout the American Midwest, yet comparatively little is known about even basic aspects of this species' ecology. We live-trapped a free-living population of Franklin's ground squirrels near Delta Marsh, Manitoba over the course of four years, and here collate and analyze population demographics and life history traits, including adult density and sex ratio, adult survival, adult growth, and fecundity, as well as juvenile mass and litter sex ratio. The mass cycles observed for groups of maternal and non-maternal females and males in the Delta Marsh population were similar across seasons and largely consistent with those reported among other Franklin's ground squirrel populations. Adult females outnumbered adult males by roughly 2:1, while litter sex ratio at juvenile emergence was nearly 1:1 in this population. Maternal females at the Delta Marsh site had a lower average litter size (6.31) than reported elsewhere for Franklin ground squirrels, and juvenile males weighed more than females at emergence. Survival varied between sexes and among years in the Delta Marsh population. Low survival between 2000 and 2001 followed harmful agricultural practices and flooding, and subsequently led to a sharp population decline in 2001. The Delta Marsh population studied eventually crashed in 2004 but rebounded by 2014. While population disappearances fuel concern over Franklin's ground

squirrel population status in the Midwest, apparent losses may reflect periodic fluctuations typical of this species' demography, or population relocation rather than loss.

Introduction

As population dynamics are fundamentally determined by a population's vital rates, management and conservation decisions often rely upon information about an organism's life history (Saether et al. 1996b; Sibly and Hone 2002; Oli and Dobson 2003; Krebs 2009). Life history and demographic traits determine a population's finite growth rate, which is used widely by managers in population monitoring and modeling (Winemiller and Rose 1992; Saether et al. 1996a; Beston 2011), while life history evolution constitutes a field of biological research unto itself following the seminal theoretical works of Cole (1954) and Lack (1954). Knowledge of life history traits, as influenced by mechanisms of population regulation (Charlesworth 1980; Kawecki and Stearns 1993), is thus central to both evolutionary and population biology, and of interest to wildlife managers and ecological theorists alike.

Ground squirrel species generally make ideal subjects for demographic studies given the relative ease with which individuals are trapped, marked, and observed in open habitats (Smith and Johnson 1985; Dobson 1995; Hoffmann et al. 2003). The study of Franklin's ground squirrels (*Poliocitellus franklinii*), however, has been impeded by the species' preference for densely vegetated habitats (Hall 1955; Haberman and Fleharty 1971; Krohne et al. 1972; Huebschman 2007). Franklin's ground squirrels tend to occupy areas between woodland-field or woodland-marsh ecotones where woody scrub and tall grass are prevalent, thus obscuring direct observation and complicating trapping (Sowls 1948; Hall 1955; Haberman and Fleharty 1972; Jones et al. 1983; Benjamin 1991; Martin and Heske 2005). Field study is further impeded by the

species' "secretive" nature (Hall 1955; Krohne et al. 1972), "semi-nomadism" (Jones et al. 1983), and limited time spent above ground, even compared to other hibernating sciurid species (Sowls 1948). As such, while long-term demographic and life-history studies are readily available for many ground squirrel species, particularly North American species (Slade and Balph 1974; Dunford 1977; Michener and Michener 1977; Boag and Murie 1981; Sherman and Morton 1984; Smith and Johnson 1985; Sherman and Runge 2002), published accounts remain relatively scarce for Franklin's ground squirrels (Iverson and Turner 1972; Murie 1973).

Demographic and life history data are particularly important for Franklin's ground squirrels as concerns over the species' population status exist throughout the more southern and eastern reaches of the species' range (Pergams and Nyberg 2001). Franklin's ground squirrels typically occur at low densities throughout the American Midwest, and north through the aspen parkland region of southern Canada (Hall 1955). Though the species is reported to occur locally at high densities (Pergams et al. 2008), reports of population declines, crashes, and disappearances have warranted concern for the species throughout much of its range. Currently, Franklin's ground squirrels are considered vulnerable in Iowa (Iowa Department of Natural Resources 2012), threatened in Illinois (Illinois Endangered Species Board 2015), endangered in Indiana (Indiana Department of Natural Resources 2013), imperilled to vulnerable in Missouri (Missouri Department of Conservation 2015), and imperilled in Wisconsin (Wisconsin Department of Natural Resources 2014). While apparent declines and habitat loss drive concern in these areas, researchers admit that more complete knowledge of the species' life history and basic ecology is required for accurate population assessment and any future management plans (Van Petten and Schramm 1972; Lewis and Rongstad 1992; Johnson and Choromanski-Norris 1992; Martin et al. 2003; Huebschman 2007).

We conducted a demographic study of a Franklin's ground squirrel population located near Delta Marsh, Manitoba, less than 10 km southwest of the beach ridge utilized by Sowls (1948) during his seminal natural history investigation of the species over a half century ago. We here summarize and report population demographics and life history traits, including adult density and sex ratio, adult survival, adult growth, and fecundity, as well as juvenile mass and litter sex ratio. As this population was investigated over the years leading up to a population crash, the population parameters and life history traits compiled here lend insight into population declines that have led to concern over the species' status across its range.

Methods

Study area and animals

Research on Franklin's ground squirrels was conducted from the start of May through to the start of August in 1998-2000, and from 8 May through 26 May and 30 June through 15 July in 2001 at 50°9'N, 98°21'W near Delta Marsh, Manitoba, Canada. This population was later utilized in an investigation of nest relocation behaviour (Chapter 3) from 27 April through 30 July in 2014. The study site consisted of an approximately 1 km^2 area comprised of hayfield, crop fields, mixed deciduous forest, marsh edge and rural roadsides (for details of the geology, biogeography, and ecology of the area, see Love and Love 1954; Shay 1999).

Franklin's ground squirrels are obligate hibernators. In Manitoba, males emerge from hibernacula as early as the end of April, one to two weeks before females. Mating occurs from the time of female emergence in early May through early June, and yearlings can breed (Sowls 1948; Iverson and Turner 1972). Gestation lasts 28 d (Choromanski-Norris et al. 1986) and

lactation lasts up to 31 d (Turner et al. 1976). Adult males immerge into hibernacula as early as late July, followed by adult females in late August, and juveniles may remain active above ground as late as early October (Sowls 1948; Iverson and Turner 1972).

Trapping and handling of squirrels

We trapped Franklin's ground squirrels throughout May, June, July, and into August using National and Tomahawk (Tomahawk Live Trap, Tomahawk, WI) live traps baited with peanut butter and rolled oats. We covered all traps with corrugated plastic to protect squirrels from overheating in intense sun. Upon initial capture in the season, we marked all squirrels with a unique metal ear tag (Monel #1, National Band & Tag Co., Newport, KY) for permanent individual identification and with a unique dye mark on their dorsal pelage for visual identification (Clairol Hydrience™ Pearl Black #52, Procter and Gamble Co., Stamford, CT). We checked traps hourly to limit animal stress and re-applied dye marks as needed throughout the season. In the context of a concurrent study on nest relocation movements (Chapter 3), we attached radio transmitters (model PD-2C, Holohil Systems Ltd., Carp, ON) to all adult female squirrels. Upon each capture, we weighed squirrels to the nearest 5 g (Pesola™ spring scale, Baar, Switzerland) and assessed reproductive status.

We assessed squirrels for reproductive status and assumed breeding date based on observation of genitalia as described by Murie and Harris (1982). We estimated parturition based on a 28 d gestation period (Choromanski-Norris et al. 1986) and observed weight loss > 40 g over a 24 hr period. We thereafter estimated juvenile emergence based on a 31 d lactation period (Turner et al. 1976), and began more intensive monitoring of nests so as to trap young of the year as soon as possible. We sexed, marked, and weighed all juveniles upon capture, and typically completed full litter assessment within 5 d of initial emergence.

Data summary and analyses

Bi-weekly mass measurements were summarized for adult (≥ 1 yr) males, maternal females (litter-producing), and non-maternal females (no evidence of lactation as judged by nipple status and failure to produce litter) across the trapping period within each year. Mean mass values for individuals were included in population-wide mass averages whenever more than one mass measurement was taken for a single individual over the bi-weekly period. We included only resident squirrels (considered as individuals trapped on site during at least 2 bi-weekly periods) in adult sex ratio, mass, and population density summaries as well as overwinter survival estimates. Only litters confidently assessed within 5 days of initial juvenile emergence were included in juvenile mass analyses, and all confidently assessed litters were included in summaries of litter size, litter sex ratio (proportion of males to total litter size), and dates of initial juvenile emergence. Breeding dates for maternal females were estimated by backdating from the date of litter emergence based on the aforementioned gestation and lactation period durations, and corroborated with observations of copulatory plugs and evidence of semen in the vaginal area of trapped females during the breeding season.

Adult sex ratio (F: M) was examined during each season as well as each bi-weekly trapping period for all resident squirrels. Adult overwinter survival was calculated between years (1998-1999, 1999-2000, and 2000-2001) as the proportion of resident adults that were trapped in the final three trapping periods (26 June – 6 August) and trapped again the following season. As losses may also represent emigration from the site, this calculation represents minimum adult survival.

Data were assessed for normality and homoscedasticity using Shapiro-Wilk normality tests and Levene's tests (respectively), as well as visual inspection of the data. We compared all

litter variables (litter emergence date, litter size, juvenile mass, litter sex ratio) among years using one-way ANOVAs, with the exception of average breeding dates as these values were largely determined from dates of litter emergence. We compared average adult mass values within each group (adult males, adult maternal females, and adult non-maternal females) among years for each bi-weekly period using one-way ANOVAs. We compared average male and female juvenile masses within litters using paired t-tests, and compared adult male and maternal female masses within each bi-weekly period using two-sample t-tests. Summary values are reported as mean $\pm$ 1 SE. All statistics were performed using R software (version 3.0.2, R Development Core Team, 2013). We report actual significance levels from statistical tests except where $p < 0.001$, where we simply report that range.

Results

Within adult male and maternal female groups, average mass values differed among years during only one trapping period (males: 12 June – 25 June, one-way ANOVA, $F_{2,19} = 4.267$, $p = 0.029$; maternal females: 26 June – 09 July, one-way ANOVA, $F_{3,40} = 2.971$, $p = 0.043$), and thus all seasons were ultimately pooled. Small sample sizes among seasons prohibited seasonal comparisons for non-maternal females. Excepting a decline between the periods comprising late lactation (29 June – 23 July), maternal females gradually gained weight between all bi-weekly periods encompassed in the trapping seasons (Table 2.1, Figure 2.1). Adult males lost weight only between the two initial trapping periods of the season (1 May – 28 May), and experienced the sharpest increase in mass leading up to the end of trapping late in the season (10 July – 6 August; Table 2.1, Figure 2.2). Non-maternal adult females experienced an average weight increase between all bi-weekly periods throughout the trapping season. While

non-maternal females tended to be lighter than maternal females upon spring emergence, they had a greater average mass than females that had reared a litter at the end of the season (Figure 2.3). Average mass for adult males differed from adult maternal females in only two trapping periods (1 May – 14 May and 10 July – 23 July; Table 2.1).

The average date of initial juvenile emergence occurred within the first 10 days of July for all years (Table 2.2), and thus estimated breeding dates for maternal females across all seasons occurred within the first two weeks of May, coinciding with observations of copulatory plugs and evidence of semen in the vaginal areas of trapped females (J. Hare, unpublished data). While litter size did not differ among years (one-way ANOVA, $F_{3,38} = 1.331$, $p = 0.278$), the lowest average litter size occurred in 1999, while the highest occurred in 2001 (Table 2.2). Average litter sex ratio at juvenile emergence was also similar across years (one-way ANOVA, $F_{3,38} = 1.137$, $p = 0.347$), and the pooled average approximated parity (Table 2.2). Average juvenile mass at emergence differed among years (one-way ANOVA, $F_{3,31} = 9.267$, $p < 0.001$); however, post-hoc comparisons using Tukey's HSD tests indicated that among all years, only the 1999 season differed from the 2000 season (1999 = 99.11 ± 5.403 g, 2000 = 66.60 ± 3.836 g, two-sample t-test: $t_{26} = 4.907$, $p < 0.001$), and thus years were ultimately pooled (average juvenile mass = 83.17 ± 3.349 g, n = 35 litters). Within litters, average juvenile male mass was greater than average juvenile female mass at emergence (paired t-test: males = 85.18 g, females = 81.26 g, $t_{28} = 1.724$, $p = 0.048$).

Adult females outnumbered adult males in this population by at least 2:1 for > 90% of the observed bi-weekly trapping periods throughout the study period, while the operational sex ratio (OSR) of breeding females to males during the breeding period ranged from 1.3:1 in 2001 to 2:1

in 1998 and 1999 (Table 2.3). Adult survival varied unpredictably between sexes by as much as 13% (Table 2.4), and survival of both sexes varied extensively among years (Table 2.4). The lowest overwinter survival for both sexes (males: 11%, females: 24%; Table 2.4) occurred between 2000-2001 following a high-speed disc harrow agricultural event during lactation in 2000 and early spring overland flooding in 2001 (J. Hare, personal observation).

Discussion

Reports of seasonal mass cycles are commonplace for populations of hibernating ground squirrel species (Morton 1975; Knopf and Balph 1977; Boag and Murie 1981; Fagerstone 1988; Michener and Locklear 1990; Buck and Barnes 1999). Franklin's ground squirrel maternal females gained the most weight during early gestation, while they gained the least weight or lost weight between periods encompassing parturition in early June and the end of lactation in late July, respectively. Females in this population experienced less prominent periods of weight loss following parturition and the energetically demanding period of lactation than among Franklin's ground squirrel populations observed elsewhere (Iverson and Turner 1972; Murie 1973; Choromanski-Norris et al. 1986; Benjamin 1991). This may reflect either ideal conditions and abundant resource availability during this study, which allowed females to quickly regain mass following parturition and partially compensate for increased energetic demands placed on females during lactation (Kenagy et al. 1989; Rogowitz 1996), or that smaller observed litter sizes in the Delta Marsh populations (discussed below) buffered females from possible high costs of reproduction that would have produced a more pronounced weight loss. Though the sample of confirmed non-maternal females on this site was small and necessitates cautious interpretation, the data garnered here indicate that non-maternal females are able to gain more

weight throughout the season than maternal females who are burdened with the energetic and nutritional costs of reproduction. Consistent with reports of Franklin's ground squirrel populations elsewhere (Iverson and Turner 1972; Murie 1973; Choromanski-Norris et al. 1986; Benjamin 1991), males within the Delta Marsh population lost mass only during the period coinciding with breeding early in the season, wherein males endure the costs of finding and competing for mates.

Across all years, average litter size observed here was less than that observed on Sowls' (1948) Delta site, or elsewhere in the species' range (Haggerty 1968; Iverson and Turner 1972; Murie 1973; Benjamin 1991). However, Sowls (1948) and Iverson and Turner (1972) occasionally used embryo counts and placental scars to assess litter size; thus, the lower litter sizes observed at the Delta Marsh population may reflect losses between parturition and juvenile emergence rather than differences in reproductive output. Though no statistically significant difference in litter size was detected among years, it is interesting to note that the greatest average litter sizes occurred in 2001 when the number of females reproducing within the population was at its lowest, and conversely that average litter size was least in 1999 when the population of reproducing females was at its peak. While we have no data to address whether this pattern is reflected at parturition, the observed disparity between litter sizes in low and high population years is consistent with some form of density-dependent population regulation (Karels and Boonstra 2000). At emergence, males weighed more than female littermates. Such sexual dimorphism at the time of juvenile emergence is also observed in Richardson's ground squirrels, where sexual size dimorphism continues through adulthood (Ryan et al. 2012; Gedir and Michener 2014), as observed for adult Franklin's ground squirrels in this study following spring emergence and at the close of trapping later in the active season.

Adult females outnumbered males in this population across most trapping periods by roughly 2:1, while primary litter sex ratio approximated parity. Adult female bias in sex ratio is characteristic of many ground squirrel species, which is largely attributed to increased male losses during male-biased dispersal typical among ground-dwelling squirrels (Schmutz et al. 1979), though some species exhibit a more pronounced sex ratio bias than that documented here (McCarley 1966; Dunford 1977; Michener and Michener 1977; Bronson 1979; Dobson 1979). Demographic investigations of Franklin's ground squirrels to date report a 1:1 adult sex ratio (Iverson and Tuner 1972; Murie 1973), leading some researchers to suggest distinctive differences in dispersion patterns and ultimately in the social organization of Franklin's ground squirrels as compared to their North American congeners (Murie 1973). The female-biased adult sex ratio observed here, however, fits with the typical ground squirrel pattern generated by male-biased dispersal and thus fails to support the contention that Franklin's ground squirrels exhibit a unique social structure based upon population sex ratio. Indeed, there is some evidence that Franklin's ground squirrels exhibit characteristic male-biased juvenile dispersal in the fall (Martin and Heske 2005). However, the sole investigation into Franklin's ground squirrel dispersal published to date relied on only 5 individuals that were followed to hibernation (1 male and 4 females, Martin and Heske 2005). Male-biased dispersal, though evidenced in Martin and Heske's (2005) limited study, was not pronounced, and both sexes sometimes traveled distances exceeding 1 km. Thus, further investigation is necessary to elucidate the nature of population sex ratio and any potential link to dispersal, as well as underlying social organization in Franklin's ground squirrels.

Overwinter survival and population density within this population varied extensively and unpredictably between sexes and among years. Male survival peaked at 83% between 1998 and

1999, while it fell to just 11% between 2000 and 2001. Though not as extreme as adult males, adult female survival likewise peaked between 1998 and 1999 at 69%, and fell to nearly 24% between 2000 and 2001. The high survival estimates observed between 1998 and 1999 for both sexes are higher than values reported in any other Franklin's ground squirrel population, corroborating the notion that ideal site conditions prevailed during those years. The low survival between 2000 and 2001 and subsequent population decline observed in 2001 following a catastrophic agricultural event and a high water table indicate that local conditions have a pronounced influence on population abundance at this site. While an ultimate crash in this population three years later (2004; J. Hare, unpublished data) remains uninvestigated, the population rebounded by 2014, enabling an investigation of nest relocation behaviours (Chapter 3). Sowls (1948) reported 4-6 yr cyclic population declines at Delta, and, in the absence of obvious variation in predation pressure, suggested climatic extremes as one of many possible mediating factors. Though spatially asynchronous, population cycles have also been purported to occur elsewhere across the range of Franklin's ground squirrels (Minnesota: 10 yr cycles, Erlien and Tester 1984). While multiannual periodic population fluctuations are rare for mammalian species (Sinclair 2003), cycles are characteristic and often pronounced in some small mammal species, particularly among microtines and lagomorphs (Elton 1942; Krebs and Myers 1974; Keith 1990; Norrdahl 1995; Stanseth et al. 1997).

There is some debate among population ecologists as to relevant categorizations of regulatory modes and the mechanisms that drive population cycles (Lidicker 1988; Sandell et al. 1991; Krebs 1996; Stenseth et al. 1996; Murray 1999). However, in addition to abiotic climatic effects (Merritt et al. 2001; Previtali et al. 2009), some major extrinsic and intrinsic biotic factors that may contribute to cyclic changes in population growth rates include food availability

(Boonstra et al. 1998), predation (Keith 1990), parasitism or disease (Boonstra et al. 1980), and various social interactions (Bondrup-Nielsen and Ims 1986; Ostfield et al. 1993; Wolff 1995). Though there have been claims of population cyclicity in Franklin's ground squirrels (Sowls 1948; Erlien and Tester 1984), factors mediating possible cycles remain uninvestigated. Climatic stochasticity and agricultural practices appear to drive declines in Manitoba (this study; Sowls 1948), while other extrinsic factors, including population motility (Martin et al. 2003), predation, and disease (Erlien and Tester 1984), have been hypothesized to drive Franklin's ground squirrel multiannual cycles elsewhere. No mention of intrinsic mechanisms of control appear in the literature (territoriality, infanticide, stress etc.) for Franklin's ground squirrel populations, likely because social and spatial organization are so poorly investigated in this species. The aforementioned factors remain merely speculative, highlighting the need for long-term population level studies to garner data that reflect the mode and mechanisms of population regulation in Franklin's ground squirrel populations.

The population decline observed during this single local investigation spanning a relatively short study period does not necessarily indicate a population trend and cannot address the question of population cyclicity. However, the observed decline here does mirror population crashes and disappearances prevalent among Franklin's ground squirrel populations in the Midwest region, many of which are not evidently associated with cycles (Johnson and Choromanski-Norris 1992; Martin et al. 2003; Huebschman 2007). Under the assumption that Franklin's ground squirrels are a prairie-obligate species, some researchers attribute population declines to loss of tall grass prairie habitat by mowing, grazing, or cultivation (Johnson and Choromanski-Norris 1992; Pergams and Nyberg 2001). However, categorizing Franklin's ground squirrels as a prairie-obligate species may represent a misconception (Huebschman 2007;

Duggan et al. 2011), and thus factors driving declines remain equivocal. In addition, measures of population viability and persistence (e.g. minimum population levels, Shaffer 1981) remain uninvestigated for Franklin's ground squirrel populations, yet are crucial for assessing accurate population status where declines are of concern.

In summary, the mass cycles observed for groups of maternal and non-maternal females and males in the Delta Marsh population were similar across seasons and largely consistent with other Franklin's ground squirrel populations. Adult females outnumbered adult males by roughly 2:1, while litter sex ratio at juvenile emergence was nearly 1:1 in this population. Maternal females at the Delta Marsh site had a lower average litter size (6.31) than reported elsewhere for Franklin ground squirrels, and juvenile males weighed more than females at emergence. Survival varied between sexes and among years, and low survival between 2000 and 2001 following harmful agricultural practices and flooding led to a sharp population decline in the Delta Marsh population in 2001. While population disappearances fuel concern over Franklin's ground squirrel population status in the Midwest, apparent losses may reflect periodic fluctuations typical of this species' demography, or population relocation rather than loss (see Chapter 3 discussion). Long-term data further elucidating population dynamics and viability remain sorely required for this species.

Literature Cited

Benjamin, P.M. 1991. The ecology of Franklin's ground squirrel in Indiana. M.Sc. Thesis, Indiana State University, Terre Haute, USA.

Beston, J.A. 2011. Variation in life history and demography of the American black bear. Journal of Wildlife Management 75: 1588-1596.

Boag, D.A., and J.O. Murie. 1981. Population ecology of Columbian ground squirrels in southwestern Alberta. Canadian Journal of Zoology 59: 2230-2240.

Bondrup-Nielsen, S., and R.A. Ims. 1986. Reproduction and spacing behaviour of females in a peak density population of *Clethrionomys glareolus*. Holarctic Ecology 9: 109-112.

Boonstra, R., C.J. Krebs, and T.D. Beacham. 1980. Impact of botfly parasitism on *Microtus townsendii* populations. Canadian Journal of Zoology 58: 1683-1692.

Boonstra, R., C.J. Krebs, and N.C. Stanseth. 1998. Population cycles in small mammals: the problem of explaining the low phase. Ecology 79: 1479-1488.

Bronson, M.T. 1979. Altitudinal variation in the life history of the golden-mantled ground squirrel *(Spermophilus lateralis)*. Ecology 60: 272-279.

Buck, C.L., and B.M. Barnes. 1999. Annual cycle of body composition and hibernation in free-living arctic ground squirrels. Journal of Mammalogy 80: 430-442.

Charlesworth, B. 1980. Evolution in Age Structured Populations. Cambridge University Press, Cambridge, UK.

Choromanski-Norris, J.F., K. Fritzell, and A.B. Sargeant. 1986. Seasonal activity cycle and
weight changes of the Franklin's ground squirrel. American Midland Naturalist 116:
101-107.

Cole, L.C. 1954. The population consequences of life history phenomena. Quarterly Review of
Biology 29: 103-137.

Dobson, F.S. 1979. An experimental study of dispersal in the California ground squirrel. Ecology
60: 1103-1109.

Dobson, F.S. 1995. Regulation of population size: evidence from Columbian ground squirrels.
Oecologia 102: 44-51.

Duggan, J.M., R.L. Schooley, and E.J. Heske. 2011. Modeling occupancy dynamics of a rare
species, Franklin's ground squirrel, with limited data: are simple connectivity metrics
adequate? Landscape Ecology 26: 1477-1490.

Dunford, C. 1977. Behavioral limitation of round-tailed ground squirrel density. Ecology 58:
1254-1268.

Elton, C. 1942. Voles, Mice and Lemmings. Clarendon Press, Oxford, UK.

Erlien, D.A., and J. R. Tester. 1984. Population ecology of sciurids in northwestern Minnesota.
Canadian Field Naturalist 98:1-6.

Fagerstone, K.A. 1988. The annual cycle of Wyoming ground squirrels in Colorado. Journal of
Mammalogy 69: 678-687.

Gedir, J.V., and G.R. Michener. 2014. Litter sex ratios in Richardson's ground squirrels:

long-term data support random sex allocation and homeostasis. Oecologia 174: 1225-1239.

Haberman, C.G., and E.D. Fleharty. 1971. Natural history notes on Franklin's ground squirrels in

Boone County, Nebraska. Transactions of the Kansas Academy of Science 74: 76-80.

Haggerty, S.M. 1968. The ecology of the Franklin's ground squirrel *(Citellus franklinii)* at Itasca

State Park, Minnesota. M.Sc. Thesis, University of Minnesota, Minneapolis, USA.

Hall, E.R. 1955. Handbook of the mammals of Kansas. University of Kansas Museum of Natural

History, Miscellaneous Publication 7: 1-103.

Hoffmann, I.E., E. Millesi, S. Huber, L.G. Everts, and J.P. Dittami. 2003. Population dynamics

of European ground squirrels *(Spermophilus citellus)* in a suburban area. Journal of

Mammalogy 84: 615-626.

Huebschman, J.J. 2007. Distribution, abundance, and habitat associations of Franklin's ground

squirrel (*Spermophilus franklinii* Sabine 1822). Illinois Natural History Survey Bulletin 38:

1-58.

Illinois Endangered Species Board. 2015. Checklist of endangered and threatened animals and

plants of Illinois. http://www.dnr.illinois.gov/ESPB/Documents/2015_ChecklistFINAL_for_

webpage_051915.pdf

Accessed on 01 June, 2015.

Indiana Department of Natural Resources. 2013. Indiana's State endangered species.

http://www.in.gov/dnr/fishwild/files/fw-Endangered_Species_List.pdf

Accessed on 01 June, 2015.

Iowa Department of Natural Resources. 2012. Iowa wildlife action plan.

    http://www.iowadnr.gov/Environment/WildlifeStewardship/IowaWildlifeActionPlan.aspx

    Accessed on 01 June, 2015.

Iverson, S.L., and B.N. Turner. 1972. Natural history of a Manitoba population of Franklin's

    ground squirrels. Canadian Field Naturalist 86: 145-149.

Johnson, S.A., and J. Choromanski-Norris. 1992. Reduction in the eastern limit of the range of

    the Franklin's ground squirrel *(Spermophilus franklinii)*. American Midland Naturalist 128:

    325-331.

Jones, J.K., Jr., D.M. Armstrong, R.S. Hoffmann, and C. Jones. 1983. Mammals of the Northern

    Great Plains. University of Nebraska Press, Lincoln, USA.

Karels, T.J., and R. Boonstra. 2000. Concurrent density dependence and independence in

    populations of arctic ground squirrels. Nature 408: 460-463.

Kawecki, T.J., and S.C. Stearns. 1993. The evolution of life histories in spatially heterogeneous

    environments: optimal reaction norms revisited. Evolutionary Ecology 7: 155-174.

Keith, L.B. 1990. Dynamics of snowshoe hare populations. Current Mammalogy 2: 119-195.

Kenagy, G.J., S.M. Sharbaugh, and K.A. Nagy. 1989. Annual cycle of energy and time

    expenditure in a golden-mantled ground squirrel population. Oecologia 78: 269-282.

Knopf, F.L., and D.F. Balph. 1977. Annual periodicity of Uinta ground squirrels. Southwestern

    Naturalist 22: 213-224.

Krebs, C.J. 1996. Population cycles revisited. Journal of Mammalogy 77: 8-24.

Krebs, C.J. 2009. Population dynamics of large and small mammals: Graeme Caughley's grand vision. Wildlife Research 36: 1-7.

Krebs, C.J., and J.H. Myers. 1974. Population cycles in small mammals. Advances in Ecological Research 8: 268-400.

Krohne, D.T., J. Hauffe, and P. Schramm. 1972. Radio-tracking the Franklin's ground squirrel in a restored prairie, pp. 84-88. In: L.C. Hulbert, editor. Third Midwest Prairie Conference Proceedings, Kansas State University, Manhattan, USA.

Lack, D. 1954. The natural regulation of animal numbers. Clarendon Press, Oxford, UK.

Lewis, T.L., and O.J. Rongstad. 1992. The distribution of Franklin's ground squirrel in Wisconsin and Illinois. Transactions of the Wisconsin Academy of Sciences Arts and Letters 80: 57–62.

Lidicker, W.Z., Jr. 1988. Solving the enigma of microtine "cycles". Journal of Mammalogy 69: 225-235.

Love, A., and D. Love. 1954. Vegetation of a prairie marsh. Bulletin of the Torrey Botanical Club 81: 16-34.

Martin, J.M., and E.J. Heske. 2005. Juvenile dispersal of Franklin's ground squirrel *(Spermophilus franklinii)* from a prairie "island." American Midland Naturalist 153: 444-449.

Martin, J.M., E J. Heske, and J.E. Hofmann. 2003. Franklin's ground squirrel *(Spermophilus franklinii)* in Illinois: a declining prairie mammal? American Midland Naturalist 150: 130-138.

McCarley, H. 1966. Annual cycle, population dynamics and adaptive behavior of *Citellus tridecemlineatus.* Journal of Mammalogy 47: 294-316.

Merritt, J.F., M. Lima, and F. Bozinovic. 2001. Seasonal regulation in fluctuating small mammal populations: feedback structures and climate. Oikos 94: 505-514.

Michener, G.R., and L. Locklear. 1990. Differential costs of reproductive effort in male and female Richardson's ground squirrels. Ecology 71: 855-868.

Michener, G.R., and D.R. Michener. 1977. Population structure and dispersal in Richardson's ground squirrels. Ecology 58: 359-368.

Missouri Department of Conservation. 2015. Missouri species and communities of conservation concern checklist. http://mdc.mo.gov/sites/default/files/resources/2010/04/2015species concern.pdf
Accessed on 01 June, 2015.

Morton, M.L. 1975. Seasonal cycles of body weights and lipids in Belding ground squirrels. Bulletin of the Southern California Academy of Science 74: 128-143.

Murie, J.O. 1973. Population characteristics and phenology of a Franklin ground squirrel *(Spermophilus franklinii)* colony in Alberta, Canada. American Midland Naturalist 90: 334-340.

Murie, J.O., and M.A. Harris. 1982. Annual variation of spring emergence and breeding in Columbian ground squirrels *(Spermophilus columbianus)*. Journal of Mammalogy 63: 431-439.

Murray, B.G., Jr. 1999. Can the population regulation controversy be buried and forgotten? Oikos 84: 148-152.

Norrdahl, K. 1995. Population cycles in northern small mammals. Biological Reviews of the Cambridge Philosophical Society 70: 621-637.

Oli, M.K., and F.S. Dobson. 2003. The relative importance of life-history variables to population growth rate in mammals: Cole's prediction revisited. The American Naturalist 161: 422-440.

Ostfield, R.S., C.D. Canham, and S.R. Pugh. 1993. Intrinsic density-dependent regulation of vole populations. Nature 366: 259-261.

Pergams, O., and D. Nyberg. 2001. Museum collections of mammals corroborate the exceptional decline of prairie habitat in the Chicago region. Journal of Mammalogy 82: 984-992.

Pergams, O., Nyberg, D, and G. Hammerson. 2008. *Spermophilus franklinii* in IUCN 2013. IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Accessed on 14 May 2015.

Previtali, M.A., M. Lima, P.L. Meserve, D.A. Kelt, and J.R. Gutierrez. 2009. Population dynamics of two sympatric rodents in a variable environment: rainfall, resource availability, and predation. Ecology 90: 1996-2006.

R Development Core Team. 2013. R: a language and environment for statistical computing. Vienna, Austria.

Rogowitz, G.L. 1996. Trade-offs in energy during lactation. American Zoologist 36: 197-204.

Ryan, C.P., W.G. Anderson, L.E. Gardiner, and J.F. Hare. 2012. Stress-induced sex ratios in ground squirrels: support for a mechanistic hypothesis. Behavioral Ecology 23: 160-167.

Saether, B.E., S. Engen, and R. Lande. 1996a. Density-dependence and optimal harvesting of fluctuating populations. Oikos 76: 40-46.

Saether, B.E., T.H. Ringsby, and E. Roskaft. 1996b. Life history variation, population processes and priorities in species conservation: towards a reunion of research paradigms. Oikos 77: 217-226.

Sandell, M., M. Åström, O. Atlegrim, K. Danell, L. Edenius, J. Hjältén, P. Lunberg, T. Palo, R. Pettersson, and G. Sjöberg. 1991. "Cyclic" and "non-cyclic" small mammal populations: an artificial dichotomy. Oikos 61: 281-284.

Schmutz, S.M., D.A. Boag, and J.K. Schmutz. 1979. Causes of unequal sex ratio in populations of adult Richardson's ground squirrels. Canadian Journal of Zoology 57: 1849-1855.

Shaffer, M.L. 1981. Minimum population sizes for species conservation. BioScience 31: 131-134.

Shay, J.M. 1999. Annotated vascular plant species list for the Delta Marsh, Manitoba and surrounding area. University of Manitoba Field Station (Delta Marsh) Occasional Publication No. 2.

Sherman, P.W., and M.L. Morton. 1984. Demography of Belding's ground squirrels. Ecology 65: 1617-1628.

Sherman, P.W., and M.C. Runge. 2002. Demography of a population collapse: the northern Idaho ground squirrel *(Spermophilus brunneus brunneus)*. Ecology 83: 2816-2831.

Sibly, R.M., and J. Hone. 2002. Population growth rate and its determinants: an overview. Philosophical Transactions of the Royal Society B: Biological Sciences 357: 1153-1170.

Sinclair, A.R.E. 2003. Mammal population regulation, keystone processes and ecosystem dynamics. Philosophical Transactions of the Royal Society B: Biological Sciences 358: 1729-1740.

Slade, N.A., and D.F. Balph. 1974. Population ecology of Uinta ground squirrels. Ecology 55: 989-1003.

Smith, G.W., and D.R. Johnson. 1985. Demography of a Townsend ground squirrel population in southwestern Idaho. Ecology 66: 171-178.

Sowls, L.K. 1948. The Franklin ground squirrel, *Citellus franklinii* (Sabine), and its relationship to nesting ducks. Journal of Mammalogy 29: 113–137.

Stanseth, N.C., O.N. Bjørnstad, and W. Falck. 1996. Is spacing behaviour coupled with predation causing the microtine density cycle? A synthesis of current process-oriented and pattern-oriented studies. Proceedings of the Royal Society of London B: Biological Sciences 263: 1423-1435.

Stanseth, N.C., W. Falck, O.N. Bjørnstad, and C.J. Krebs. 1997. Population regulation in snowshoe hare and Canadian lynx: asymmetric food web configurations between hare and lynx. Proceedings of the National Academy of Science 94: 5147-5152.

Turner, B.N., S.L. Iverson, and K.L. Severson. 1976. Postnatal growth and development of captive Franklin's ground squirrels *(Spermophilus franklinii)*. American Midland Naturalist 95: 93-102.

Van Petten, A., and P. Schramm. 1972. Introduction, dispersal, and population increase of the

Franklin's ground squirrel, *Spermophilus franklinii*, in a restored prairie, pp. 166–173. In: J.

H. Zimmerman, editor. Proceedings of the Second Midwest Prairie Conference, University of

Wisconsin–Madison, Madison, USA.

Winemiller, K.O., and K.A. Rose. 1992. Patterns of life-history diversification in North America:

implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences

49: 2196-2218.

Wisconsin Department of Natural Resources. 2014. Franklin's ground squirrel *(Spermophilus

franklinii)*. http://dnr.wi.gov/topic/endangeredresources/animals.asp?mode=detail&speccode

=amafb05120

Accessed: 01 June, 2015.

Wolff, J.O. 1995. Friends and strangers in vole population cycles. Oikos 73: 411-414.

Tables and Figures

Table 2.1. Comparisons using two-sample *t*-tests of average mass for adult maternal female (litter producing) and adult male resident squirrels (present in at least 2 bi-weekly periods) within each bi-weekly period at the Delta Marsh site from 1998-2001. Values are reported as mean ± SE. Sample sizes were insufficient to compare between sexes in the 24 July-6 Aug. period.

Period	Reproductive Period	Females	Males	df	t	p
1 May- 14 May	Breeding	345.00 ± 11.658	389.93 ± 10.597	33	-2.755	0.010
15 May - 28 May	Gestation	397.36 ± 5.963	388.00 ± 8.484	62	0.929	0.357
29 May - 11 June	Gestation	400.14 ± 5.419	413.52 ± 8.988	57	-1.356	0.180
12 June - 25 June	Lactation	413.06 ± 5.118	425.05 ± 7.577	56	-1.359	0.180
26 June - 9 July	Lactation	429.80 ± 5.258	444.04 ± 11.420	65	-1.298	0.199
10 July - 23 July	Post Juv. Emergence	424.54 ± 7.303	491.64 ± 31.337	38	-3.640	< 0.001

Table 2.2. Summary of average litter emergence dates, litter sizes, and litter sex ratios for maternal (litter producing) female Franklin's ground squirrels that were resident (present in at least 2 bi-weekly periods) at the Delta Marsh site from 1998-2001. Only litters in which all juveniles were confidently assessed are included in the analyses. Mean emergence date differed among years (one-way ANOVA, $F_{3,38} = 20.150$, $p < 0.001$) and thus values were not pooled. Values are reported as mean ± SE, n = litters.

Year	n	Juvenile Emergence Date ± d	Litter Size	Sex Ratio
1998	5	5- July ± 0.812	6.60 ± 0.510	0.39 ± 0.082
1999	17	10- July ± 0.992	5.53 ± 0.637	0.53 ± 0.066
2000	14	1- July ± 0.520	6.64 ± 0.476	0.39 ± 0.070
2001	6	8- July ± 1.342	7.50 ± 1.176	0.58 ± 0.153
Pooled	42	---	6.31 ± 0.357	0.475 ± 0.042

Table 2.3. Total number and sex ratio (SR) of adult male and adult female resident squirrels (present in at least 2 bi-weekly periods) trapped within each bi-weekly period and entire trapping season from 1998-2001 at the Delta Marsh site. Figures in parentheses represent maternal (litter producing) females. SR is calculated as the proportion of females to males within each period.

Period	Repro. Period	1998			1999			2000			2001		
		F	M	SR	F	M	SR	F	M	SR	F	M	SR
1 May- 14 May	Breeding	8 (4)	2	4.0 (2.0) : 1	1 (1)	2	2.0 (2.0) : 1	18 (11)	8	2.3 (1.4) : 1	5 (4)	3	1.7 (1.3) : 1
15 May - 28 May	Gestation	12 (6)	5	2.4 (1.2) : 1	15 (13)	7	2.1 (1.9) : 1	21 (14)	10	2.1 (1.4) : 1	7 (6)	3	2.3 (2.0) : 1
29 May - 11 June	Gestation	12 (7)	4	3.0 (1.8) : 1	17 (14)	8	2.1 (1.8) : 1	22 (15)	11	2.0 (1.4) : 1	--	--	--
12 June - 25 June	Lactation	11 (8)	4	2.8 (2.0) : 1	16 (14)	8	2.0 (1.8) : 1	21 (14)	10	2.1 (1.4) : 1	--	--	--
26 June - 9 July	Lactation	13 (10)	6	2.2 (1.7) : 1	14 (13)	8	1.8 (1.6) : 1	21 (15)	7	3.0 (2.1) : 1	6 (6)	2	3.0 (3.0) : 1
10 July - 23 July	Post Juv. Emerge.	12 (10)	6	2.0 (1.7) : 1	2 (2)	1	2.0 (2.0) : 1	11 (9)	5	2.2 (1.8) : 1	5 (5)	2	2.5 (2.5) : 1
24 July - 6 Aug	Post Juv. Emerge.	2 (1)	2	1.0 (0.5): 1	--	--	--	--	--	--	--	--	--
Seasonal		18 (10)	7	2.6 (1.4) : 1	19 (16)	9	2.1 (1.8) : 1	26 (15)	12	2.2 (1.3) : 1	7 (6)	3	2.3 (2.0) : 1

Table 2.4. Minimum overwinter survival for adult male, adult female, maternal female (litter-producing), and adult non-maternal female (non-lactating and confirmed non-littering) Franklin's ground squirrels at the Delta Marsh site for 1998-2001. Only resident squirrels (trapped in at least 2 bi-weekly periods) and those individuals captured within the final three bi-weekly trapping periods (26 June-6 Aug.) are included in minimum survival estimates (see Methods for survival estimation procedure.

Year	Males		All Females		Maternal Females		Non-Maternal Females	
	Survival	n	Survival	n	Survival	n	Survival	n
1998-1999	83%	6	69%	13	70%	10	50%	2
1999-2000	36%	8	38%	16	33%	15	100%	1
2000-2001	11%	9	24%	21	14%	14	0%	2
Pooled	39%	23	40%	50	36%	39	40%	5
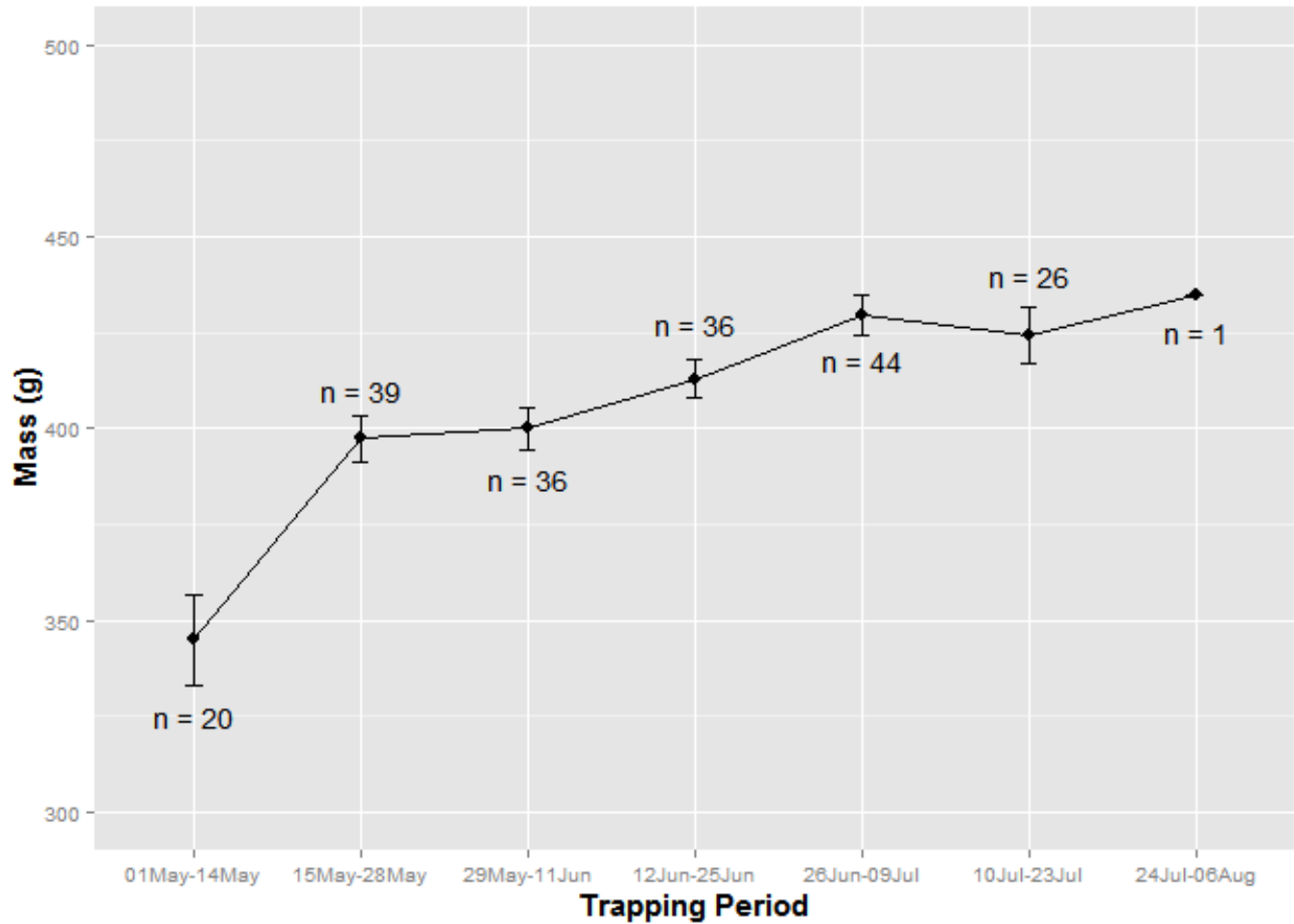
Figure 2.1. Bi-weekly average weights of maternal (litter producing) Franklin's ground squirrels that were resident (trapped in at least 2 bi-weekly periods) on the Delta Marsh site from 1998-2001. Points represent mean while error bars represent ± SE, sample size is shown for each mean value.
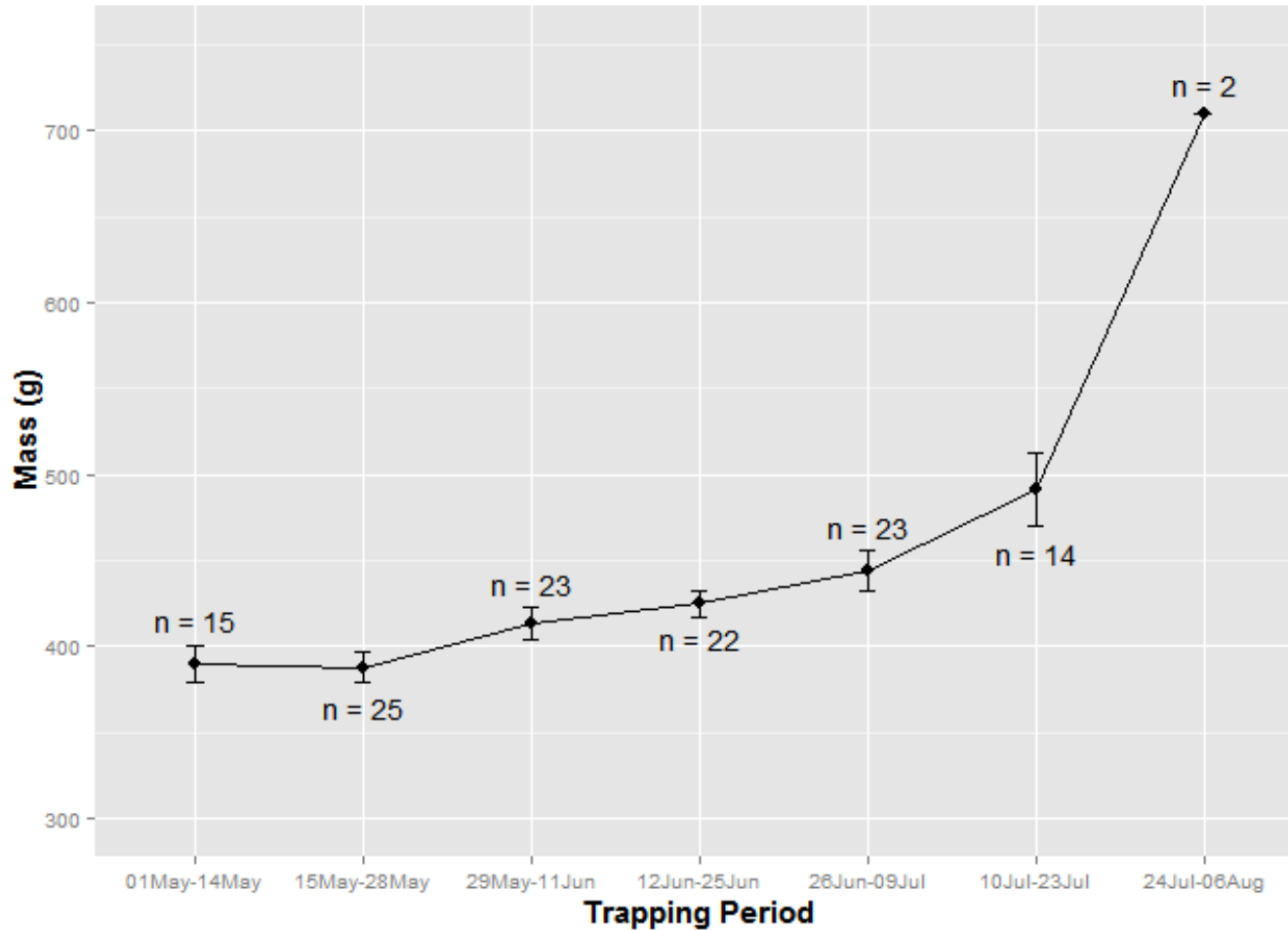
Figure 2.2. Bi-weekly average weights of adult male Franklin's ground squirrels that were resident (trapped in at least 2 bi-weekly periods) on the Delta Marsh site from 1998-2001. Points represent mean while error bars represent ± SE, sample size is shown for each mean value.
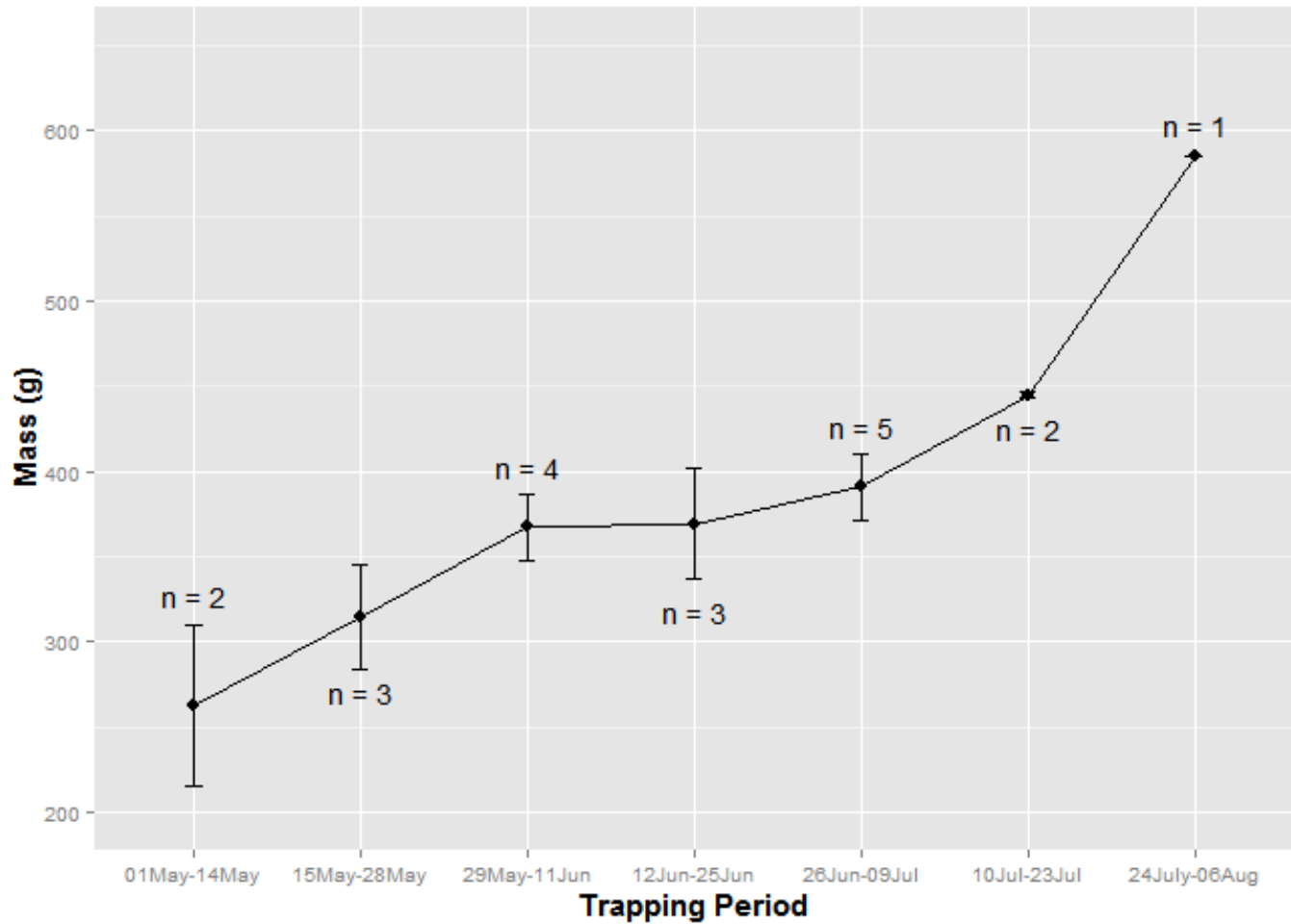
Figure 2.3. Bi-weekly average weights of adult non-maternal (non-lactating and confirmed non-littering) Franklin's ground squirrels that were resident (trapped in at least 2 bi-weekly periods) on the Delta Marsh site from 1998-2001. Points represent mean while error bars represent ± SE, sample size is shown for each mean value.

Chapter 3: Franklin's ground squirrel (*Poliocitellus franklinii*) local nest relocation

Abstract

Understanding movement patterns and habitat use are essential for designing effective research and management programs for any species. Franklin's ground squirrels are increasingly considered of conservation concern throughout portions of their range, while much about their basic ecology remains poorly understood. Field research on a Manitoba population of individually-marked, telemetry-collared Franklin's ground squirrels near Delta Marsh, Manitoba revealed that females relocate their nest and litter to alternative burrows following parturition, likely incurring significant energetic costs and enhanced predation risk with each move. Telemetry data and direct on-site observation revealed that in the context of these moves, females transport their pups one by one, above ground up to four times throughout the course of lactation, sometimes over distances approaching a kilometre. We investigated the role of conspecific nest visitation, flea infestation, and nest habitat type as proximal factors precipitating nest movements. As predicted, females treated with insecticide to reduce flea infestation tended to relocate fewer times than females receiving a control (water-only) treatment. Additionally, conspecific nest visits, registered with stationary Passive Integrated Transponder (PIT)-tag readers, increased the likelihood of movement within an observation day. Finally, nest habitat type was dependent on occupancy order, suggesting habitat associations also play a role in nest selection and relocation. Significant effects of all investigated factors suggest that nest movements evolved in response to myriad factors that impact female fitness. These findings offer novel insight into why lactating Franklin's ground squirrel females undertake what superficially appear to be costly nest movements, thus enhancing our understanding of this

under-studied and potentially vulnerable species. To what extent, if any, these nest movements lead to population-level location shifts and ultimately to observed population disappearances from areas of historical occupancy requires further investigation and long-term population monitoring.

Introduction

Establishing movement patterns is integral to understanding the ecology, life history, and behaviour of an animal and is a prerequisite for the effective development of management and research programs across taxa (Bulova 1994; Price et al. 1994; Hamer et al. 2008; Loarie et al. 2009; Kadlecova et al. 2012; Wattles and DeStefano 2013; Pauwels et al. 2014; Kays et al. 2015). A species' movements may influence landscape-scale patterns of habitat use, population structure and persistence, as well as metapopulation genetic structure (Greenwood 1980; Bowler and Benton 2005). Movements involving the relocation of "home" sites (e.g. nests, dens, roosts, setts) are of particular interest to ecologists. Leaving an established area may pose substantial costs to a relocating individual, such as the time and energetic demand of searching for, familiarizing with, or constructing a suitable site (Tanaka 1989), potential predation risk resulting from increased exposure to predators (Greenwood and Harvey 1982; Lubin et al. 1993; Van Vuren and Armitage 1994), agonistic interactions with unfamiliar conspecifics (Temeles 1994; McDougall and Kramer 2006), and the additional cost of carrying young if the individual moving is caring for dependent offspring (Tardif 1994; Sanchez et al. 1999).

Descriptions of ground squirrel nest relocation are generally focused on natal dispersal, one-way relocations from the natal area to a completely new home range (Lidicker 1975; Dobson 1982). Among ground squirrels, natal dispersal is typically male-biased and involves relatively

long distance movements (Michener and Michener 1977; Dobson 1979, 1982; Holekamp 1984, 1986; Waterman 1992; Byrom and Krebs 1999; Harris and Leitner 2005; Martin and Heske 2005). Arnaud et al. (2012), however, also considered within-colony dispersal movements, wherein adult females shift their home range so that their new range partially overlaps their original home range. These smaller-scale relocations include both natal dispersal and breeding dispersal, wherein females relocate between sites in any two successive breeding seasons (Greenwood 1980; Holekamp 1984). Both natal and breeding dispersal events described for female ground squirrels generally occur between reproductive seasons (Arnaud et al. 2012; Armitage et al. 2011; Holekamp 1984; Wiggett and Boag 1992), and are considered at least semi-permanent events.

Given the energetic demands placed on females during the reproductive season, within-season relocation among females is particularly infrequent. Relocations during the lactation period are especially puzzling as lactation is one of the most energetically taxing stages of a female's life history (Kenagy et al. 1989; Rogowitz 1996). In this study we (1) describe the pattern and extent of short-term within-season nest relocations among lactating Franklin's ground squirrel (*Poliocitellus franklinii*) females following a four-year observational study (1998-2001), and (2) investigate the potential influence of flea infestation, conspecific nest visitation, and habitat associations on nest movements. Franklin's ground squirrels are an ideal species to study nest relocations as they are seemingly unique among ground squirrels in their tendency to relocate multiple times within the reproductive season. To date, however, description and investigation of such behaviours are sparse and superficial (Hall 1955; Haggerty 1968).

We focus on flea infestation in this study because parasite infestation has been correlated with the frequency of site relocations across a wide range of taxa (European badgers *(Meles*

meles), Butler and Roper 1996; Brant's whistling rats *(Parotomys brantsii),* Roper et al. 2002; and Bechstein's bats *(Myotis bechsteinii),* Reckardt and Kerth 2007). Being mobile, mammals can relocate away from sites where flea eggs and larvae presumably accumulate as a way to behaviourally mitigate parasite infestation (Hart 1994; Moore 2002). In Manitoba, Franklin's ground squirrels are primarily associated with the flea *Opisocrostic bruneri* (Reichardt and Galloway 1994), but are also hosts to at least seven other flea species in addition to being accidental hosts to multiple flea species associated with other mammals (Galloway and Christie 1990). If nest relocations serve as an anti-parasite mechanism, we predicted that dams with lower relative flea abundances would relocate nests less often than dams with greater parasite loads.

Beyond flea infestation, we also focused on conspecific nest visitation as a potential factor causing nest movements. Franklin's ground squirrels are generally referred to as asocial or solitary (Kivett et al. 1976; Armitage 1981; Michener 1983; Lindsay and Galloway 1997; Hare 2004; Green et al. 2013), characterized by non-overlapping territories of males and females, distinct home ranges, and agonistic social interactions (Michener 1983; but see Hare 2004). Following breeding, we would thus expect individuals to avoid each other, particularly among maternal females protecting young from potentially infanticidal (Sherman 1981; Ebensperger 1998), or competing (Dobson 1982; Boag and Wigget 1994) conspecifics, and thus predicted that increasing nest visitation would increase the likelihood of nest relocation. As field research into social interaction and spatial organization of this species is limited, knowledge of the frequency of conspecific nest visitation will further our understanding of the social structure and both the extent and general nature of interactions among free-living Franklin's ground squirrels.

Where observations of Franklin's ground squirrel burrow relocations have been documented in the primary literature, researchers have speculated that relocations may serve to maintain ideal proximity to important, yet variable, resources that shift throughout the season (Haberman and Fleharty1971; Jones et al. 1983). We additionally examined habitat selection as a potential factor precipitating nest relocations. If dams relocate to maintain proximity to cover or forage resources, we predicted that the direction of relocation among different habitat types would be consistent with seasonal shifts in vegetation structure and floral characteristics associated with natal burrow sites.

Burrow use and relocation by Franklin's ground squirrels is of particular interest given the relative paucity of information published on this species' ecology (Seton 1929; Murie 1999; Huebschman 2007). Franklin's ground squirrels are considered to be of conservation concern throughout much of their range (Iowa Department of Natural Resources 2012; Indiana Department of Natural Resources 2013; Wisconsin Department of Natural Resources 2014; Illinois Endangered Species Board 2015; Missouri Department of Conservation 2015), yet researchers consistently preface concern with a call for further behavioural and ecological data on the species (Martin et al. 2003; Huebschman 2007). As obligate hibernators, Franklin's ground squirrels may spend up to 90% of their lifetimes in or near the burrow (Sowls 1948). This investigation centered on burrow use will thus provide insight into a significant portion of this species' behavioural repertoire and habitat use.

Methods

Study area and animals

Franklin's ground squirrels were studied from 1 May through to the start of August in 1998-2000, from 8 May through 26 May and 30 June through 15 July in 2001, and from 27 April through 30 July 2014 at 50°9'N, 98°21'W near Delta Marsh, Manitoba, Canada. The study site consisted of an approximately 1 km^2 area comprised of hayfield, crop fields, mixed deciduous forest, marsh edge and rural roadsides (for details of the geology, biogeography, and ecology of the area see Love and Love 1954; Shay 1999).

Franklin's ground squirrels are obligate hibernators. In Manitoba, males emerge from hibernacula as early as the end of April, one to two weeks before females. Mating occurs from the time of female emergence in early May through early June (Iverson and Turner 1972; Sowls 1948). Gestation lasts 28 d (Choromanski-Norris et al. 1986) and lactation lasts up to 31 d (Turner et al. 1976). Average litter size ranges from 6.3 (Chapter 2) to 9.4 (Iverson and Turner 1972). Adult males immerge into hibernation burrows as early as late July, followed by adult females in late August, and juveniles that remain active above ground as late as early October (Iverson and Turner 1972; Sowls 1948).

Trapping and radio-collaring of squirrels

We trapped Franklin's ground squirrels using National and Tomahawk (Tomahawk Live Trap, Tomahawk, WI) live traps baited with peanut butter and rolled oats. To protect squirrels from overheating in intense sun, we covered all traps with corrugated plastic. We marked all squirrels on initial capture with a unique metal ear tag (Monel #1, National Band & Tag Co., Newport, KY) for permanent individual identification and with a unique dye mark on their dorsal

pelage for visual identification (Pearl Black #52, Clairol Hydrience™, Procter and Gamble Co., Stamford, CT). We re-applied dye marks as needed throughout the season. We checked traps hourly to limit animal stress. Upon capture, we weighed squirrels to the nearest 5 g (Pesola™ spring scale, Baar, Switzerland) and assessed squirrels for reproductive status based on observation of genitalia as described by Murie and Harris (1982).

We attached radio transmitters (model PD-2C, Holohil Systems Ltd., Carp, ON) to all adult female squirrels using the transmitter antenna, and additional wire covered by flexible Tygon™ tubing (Saint-Gobain North America, Valley Forge, PA) to form a collar around the squirrels' necks. The Tygon™ tubing protected squirrels from abrasion and at least part of the transmitter antenna from being chewed through. In 2014, Tygon™ tubing also served to house individually coded Passive Integrated Transponder (PIT) tags (14 mm Avid DNA Identification System, Avid Identification Systems, Inc., Norco, CA). We additionally attached collars composed only of Tygon tubing, wire, and a PIT-tag to males in 2014. This mechanism of temporary PIT-tag deployment which capitalized on collar deployment allowed us to register conspecific burrow visitation (described below) while limiting obtrusive and potentially harmful manipulation associated with subcutaneous PIT-tag deployment (Tillmann et al. 1997; Elcock et al. 2001; Le Calvez et al. 2006; Siegal-Willott et al. 2007; Sura et al. 2011). Collars each weighed 5 g and never exceeded 2% of body mass.

Determining nest locations

We used a VHF telemetry receiver (TR-4 Receiver, Telonics Inc., Mesa, AZ) and 2-element Yagi antenna (RA-14K VHF Antenna, Telonics Inc., Mesa, AZ) to determine the precise location of squirrels in their burrows after sunset each night throughout the 31 d lactation period (time of sunset varied throughout the season). When the general area of the burrow the

squirrel was residing in on a given night was located, we removed the antenna from the receiver to pinpoint the nest chamber within the burrow system. We took a GPS location above the nest chamber using a WAAS-corrected handheld GPS receiver (Garmin GPS72, Garmin International, Inc., Olathe, KS). We located burrow entrances the following morning upon visual inspection of the area surrounding the point at which each female had been located the previous night.

Describing nest movements

We collated nest movement data from the years 1998-2001 and 2014. For this study, a shift in sleeping location (> 5 m) was considered to be a nest relocation event, unless on-site observation suggested otherwise. Nest distance is defined here as the one-way linear distance between successive sleeping locations. Given that females generally relocate their litter by carrying pups one by one in their mouths to new nests, we also calculated travel distance by multiplying nest distance by the minimum number of trips necessary to relocate each female's entire litter (based on litter sizes assessed at the time of juvenile emergence, and thus a conservative estimate). Seasonal distance was the sum of all travel distances performed by each female within one lactation season. Only female squirrels that were radio-tracked for more than half the duration of lactation (> 15 d) were included in description and analyses. Due to flooding events and agricultural practices (a high speed disc harrow eliminated multiple collared females), no female in 2001 was radio-tracked for a long enough period of time to be included in analyses.

Measurement and manipulation of flea infestation

In 2014, we systematically assigned telemetry-collared squirrels into control (n = 7 females) and insecticide-treatment (n = 7 females) groups so as to balance the assignment of

breeding females spatially across the study area, and to avoid any systematic bias in the mass of females comprising the two treatment groups. We treated squirrels with an experimental (anti-parasite product: FRONTLINE™ Spray, Merial Limited, Duluth, GA, fipronil 0.29%,) and control (water-only) spray early in the season prior to parturition (based on 28 d gestation period (Choromanski-Norris et al. 1986) and observed weight loss > 40 g), and again 21-30 d later.

Prior to applying the experimental or control treatment in 2014, we quantified relative flea abundance on all female squirrels. This quantification followed methods similar to those described by Patterson et al. (2013) with a search of the ventral pelage for 60 s using a metal flea comb, stroking the fur in a manner counter to the direction of hair growth. We then spent an additional 60 s searching for fleas in the dorsal pelage in the same manner, paying particular attention to the shoulder, groin, and "armpit" regions, as fleas often accumulated in these areas (Patterson et al. 2013).

We removed any fleas encountered during quantification from squirrels in the insecticide-treatment group by combing fleas into a petri dish held under the squirrel containing isopropyl alcohol (70%). We did not remove fleas from squirrels in the control-group. Though it is possible that the same flea could have been counted twice on control squirrels, this was likely a rare occurrence, given both the rapid and systematic nature of the search. A single and constant observer (EP) conducted flea counts to reduce bias in flea abundance quantification (except in one instance during the period in which the field assistant was being trained).

Directly following flea quantification, we applied a topical insecticide, fipronil, to squirrels in the insecticide-treatment group. Fipronil is classified as a broad-spectrum phenylpyrazole insecticide and is used to control a wide range of insects by blocking chloride channels in the central nervous system (Fipronil Technical Fact Sheet, Merial Limited). We

administered fipronil to squirrels by spraying 1.5 ml of FRONTLINE™ Spray (fipronil 0.29%) on the dorsal pelage of squirrels as described by Hillegass et al. (2010). To control for a possible response to the spray manipulation itself, we sprayed approximately 1.5 ml of ambient temperature tap water on squirrels in the control-treatment group, thus ensuring squirrels in both the insecticide-treated and control groups were handled in the same manner.

Determining conspecific nest visitations

We monitored conspecific nest visits with stationary PIT-tag readers (Avid Industrial Readers, Avid Identification Systems, Inc., Norco, CA) placed at nest burrow entrances in 2014. Following radio-collar and PIT-tag deployment, and prior to estimated parturition (based on the 28-day gestation period (Choromanski-Norris et al. 1986) and observed weight loss > 40 g), we distributed readers at nests by staking pass-through antennas (15 cm diameter) connected to reader systems over burrow entrances. We attached readers to 12 V batteries and placed both readers and batteries within toolboxes, camouflaged and protected from intense weather events with green plastic bags and vegetation. We covered exposed wiring with garden hosing and vegetation for protection against incidental chewing and to provide camouflage.

Readers registered and recorded PIT-tagged individuals passing < 5 cm of the antennae, as well as the time and date of the visit. We considered multiple nest visits by a single individual as distinct when they were separated by a period > 30 min. We downloaded reader data as necessary to confirm correct nest burrow entrances (every 24 h when establishing burrow entrances and every 72 h thereafter). When a nest relocation event was detected via the telemetric identification of a new nest burrow, we relocated reader systems to the newly occupied nest the next morning. In cases where nests appeared to possess two entrances (where entrances were within < 5 m and/or reader data suggested multiple entrances), we placed an

additional reader at nearby burrow entrances. In cases where nests appeared to have > 2 entrances, where we could not locate entrances due to high vegetation or burrow plugs, or where reader availability was not sufficient to cover all entrances, we abandoned reader placement and/or discarded data for the individual female in question from nest visitation analyses.

Assessing nest habitat characteristics

In 2014, we described and quantified habitat characteristics associated with all nest burrows. We visually estimated % cover of all major habitat types within a 10-m buffer surrounding nest burrow entrances within 2 wks of nest discovery (for 37 of 38 nests). Habitat types were collapsed into four primary categories: forest, marsh edge, cultivated field, or grass (live or dead herbaceous cover excepting cultivated areas). Alfalfa, row crop, and fallow field habitats were combined into 'cultivated field' as these habitat types shared similar cover and forage qualities and progression through the period of lactation. The total area of each habitat type within the field site was quantified from a land cover map produced in ArcMap 10 (ESRI 2011) from manual digitalization of a remotely sensed image (DigitalGlobe Incorporated, Longmont, CO). The image was captured on 12 Sept. 2006, and provided a 1 m resolution. Land use within the study area within at least the last decade has been consistent, and thus we deemed this image sufficient in representing the land cover that prevailed in the 2014 season. We digitized road, building, marsh, cultivated field, forest, grass, and pasture habitat types.

We measured the linear distance from nest burrow entrances to the nearest habitat edge (defined as the qualitative transition among the aforementioned habitat types) on site using either a measuring tape (where distances were approximately < 30 m) or a Laser range finder (Bushnell Yardage Pro Sport 450, Bushnell Outdoor Products, Overland Park, KS). We also estimated %

cover by vegetation type within a 1 m^2 quadrat surrounding nest burrow entrances, and recorded vegetation height 5 cm N of burrow entrances (to remove any potential bias in selecting a sampling location). Quadrat cover and vegetation height were excluded from analyses due to their seasonal variation over the lactation period (i.e. values at the time of measurement upon initial occupation were not necessarily reflective of values at the time of nest relocation).

Statistical analysis

For nest movement descriptions, we assessed relationships between nest movements (distances and frequencies) and female characteristics (age class defined as either yearling or adult, litter size and litter sex ratio) using Spearman's rank correlation coefficient and Mann-Whitney U tests. We compared relative flea abundances within and between insecticide and control-treatment groups before and after initial treatment with Mann-Whitney U tests. Likewise, we used the Mann-Whitney U test to compare frequency of nest movements between insecticide and control-treatment groups.

To test for any relationship between conspecific nest visits and the likelihood of relocation, as observation days belonging to a single focal female were not independent, we fit predictive models using generalized estimating equations (GEE) with the function geeglm in the geepack package (Halekoh et al. 2006) for R software (version 3.0.2, R Development Core 2013). We looked at models incorporating total nests visits, as well as only male and only female visits to explore any relationship between the sex of visiting conspecifics and likelihood of movement. We compared the models including frequency of nest visits against intercept-only models to gauge model fit according to the lowest quasi-likelihood information criterion (QIC) value (Pan 2001). We clustered by female, and selected the autoregressive correlation structure to account for the temporal progression of the lactation observation periods.

In addition, we used Fisher's exact tests to examine the relationship between initial or final nest occupation and habitat characteristics (habitat type and proximity to edge). We used chi-square goodness of fit tests to investigate whether the proportion of nests selected within a given habitat type differed from proportions expected based upon site wide habitat type availability.

All mean values are reported as mean ± 1 SE. All statistics were performed using R software (version 3.0.2, R Development Core 2013), and we report actual significance levels from statistical tests except where P < 0.001, where we simply report that range.

Results

Nest movement description

Between 1998 and 2000, 32 lactating Franklin's ground squirrel females were radio-tracked. All but one female relocated nests at least once during the 31 d lactation period (Table 3.1). The total number of relocations among collared females was greatest toward the end of lactation (Figure 3.1). Distance traveled between nests (nest distance) was greatest in 1998 (Table 3.1) as one female traveled over 21 km in total, relocating her litter approximately 1.2 km from her original nest (Table 3.2).

Total number of relocations decreased significantly with increasing litter size (Spearman's rank correlation coefficient, ρ = -0.34, df = 30, p = 0.054; Figure 3.2a), while average nest distance per female was not correlated with litter size (Spearman's rank correlation coefficient, ρ = 0.146, df = 29, p = 0.432; Figure 3.2b). Likewise, while frequency of nest relocation was significantly negatively correlated with litter sex ratio (Spearman's rank

correlation coefficient, ρ = -0.435, df = 30, p = 0.013), we observed no relationship between litter sex ratio and average distance between relocated nests (Spearman's rank correlation coefficient, ρ = -0.026, df = 29, p = 0.890). Adult females tended to relocate more frequently than yearling females (Mann-Whitney U test, $n_{yearling}$ = 11, n_{adult} = 21, W = 68.5, p = 0.047; Figure 3.3a), but age class did not affect the average distance moved between nests (Mann-Whitney U test, $n_{yearling}$ = 10, n_{adult} = 21, W = 140, p = 0.147; Figure 3.3b).

Flea infestation

Relative flea abundance on insecticide-treated squirrels was significantly reduced from the time of initial treatment to second treatment (Wilcoxon signed-rank test, n = 7, V = 21, p = 0.015; Figure 3.4), while no significant reduction in relative flea abundance was observed on squirrels receiving the control treatment (Wilcoxon signed-rank test, n = 7, V = 4.5, p = 0.498, Figure 3.4). Relative flea abundance was significantly greater on control squirrels than on insecticide-treated squirrels at the time of second treatment (Mann-Whitney U test, $n_{treatment}$ = 7, $n_{control}$ = 7, W = 44.5, p = 0.005; Figure 3.4), but there was no difference in relative flea abundance between groups prior to initial treatments (Mann-Whitney U test, $n_{treatment}$ = 7, $n_{control}$ = 7, W = 33, p = 0.272, Figure 3.4). Insecticide-treated squirrels tended to relocate fewer times than control squirrels over the course of lactation (Mann-Whitney U test, $n_{treatment}$ = 7, $n_{control}$ = 7, W = 37, p = 0.052, Figure 3.5).

Conspecific nest attendance

Throughout lactation in 2014, 9 females were monitored for 22.11 ± 2.37 observation days, and 67% of females received at least one conspecific nest visit during that time. Females received 2.89 ± 1.23 nest visits from conspecific females and 2.44 ± 1.25 visits from conspecific

males. The cumulative frequencies of both nest movements and nest visits were greatest later in lactation (Figure 3.6). When data were modeled with a GEE, we found that a one unit change in number of conspecific nest visits within an observation day significantly increased the likelihood of relocation (GEE logistic regression: odds ratio= 1.749, $X^2 = 7.21$, $p = 0.007$, repeated measure = female). Separate examination of visits by sex showed that visits from conspecific females increased likelihood of relocation (GEE logistic regression: odds ratio= 2.024, $X^2 = 7.22$, $p = 0.007$, repeated measure = female) little more than visits from conspecific males (GEE logistic regression: odds ratio= 2.252, $X^2 = 3.55$, $p = 0.069$, repeated measure = female).

Nest habitat characteristics

Over the 31 d lactation period in 2014, 14 radio-collared females used 38 nests throughout the study area. Dominant habitat types within a 10 m buffer of nest burrow entrances were tall grass (n = 25, 66%), forest (n = 4, 11%), and cultivated field (n = 9, 24%). Franklin's ground squirrel dams selected habitat types disproportionately to their site wide availability (chi-square goodness-of-fit (G.O.F.), X^2 (2, N = 38) = 68.74, p < 0.001), selecting nest sites in tall grass to a greater extent than the 11% availability of this habitat type, while they selected forested and cultivated field sites to a lesser extent than their proportional availability (forest = 19%, cultivated field = 53%). The proportions of dominant habitat types selected for initial nests departed significantly from those selected for final lactation nests (Fisher's exact test, p < 0.001, Table 3.3). Separate examination of habitat type selection for initial and final nest locations reveals that dams selected both initial and final habitat types disproportionately to their site wide availability (initial lactation nests: chi-square G.O.F., X^2 (2, N = 14) = 25.75, p < 0.001; final lactation nests: X^2 (2, N = 14) = 47.97, p < 0.001). Females selected a forested habitat type to a greater extent than expected based on its site wide availability (29% of initial nests vs. 11% site

availability), while dams selected a cultivated habitat type proportionately more than its site-wide availability for their final nest locations (64% of final nests vs. 53% site availability). While fewer females tended to be in proximity (< 10 m) of a habitat edge in their final as opposed to initial lactation nests (n=14, 14% and 50% respectively), proximity to habitat edge was not dependent on whether a nest was initially or finally occupied (Fisher's exact test, p = 0.103).

Discussion

Excepting one female in 1998, all radio-collared females on our study site through the years 1998-2000 and 2014 relocated their nest and litter at least once over the course of lactation. The near-ubiquity of such movements across females suggests that nest movements are characteristic of the Delta Marsh population of Franklin's ground squirrels. Observations of burrow movements throughout the literature span the species' range (Hall 1955; Haggerty 1968; Haberman and Fleharty 1971; Jones et al. 1983; Martin et al. 2003), suggesting nest relocation patterns may be characteristic of the species (though Choromanski-Norris et al. (1989) reportedly observed little female nest relocation over the course of lactation throughout their two-year study in North Dakota). In this study, frequency of relocation was greatest near the cessation of lactation, indicating a response to variable ecological factors.

Female Franklin's ground squirrels treated in this study against fleas relocated fewer times over the course of lactation than females receiving control-treatment. As nest-dwelling parasites, flea eggs and larvae develop in the host's nest and adult fleas, once developed, jump onto hosts to feed. As eggs and larvae remain stationary in the nest, relocating to a new nest presumably serves as a form of behavioural parasite avoidance (Hart 1994; Moore 2002).

Hillegass et al. (2010) observed a four-fold increase in reproductive output by individuals treated against parasites, directly demonstrating significant fitness costs associated with parasitism. More frequent movements among more heavily infested squirrels suggests that relocations evolved, at least in part, to combat costs imposed by ectoparasites. Elsewhere it has been suggested that nest relocations from sites of flea accumulation may slow or inhibit development of flea eggs and larvae (Butler and Roper 1996; Roper et al. 2002; Reckardt and Kerth 2006; but see Hillegass et al. 2010). However, the precise relationship between levels of flea infestation throughout lactation and relocations was not addressed in the present study.

Nest visits by conspecifics also significantly increased the likelihood of relocation by females. Isolating nests from conspecifics may serve as a means of defense against potential infanticidal events. Proximity to infanticidal females has been cited as a major factor contributing to cases of infanticide in ground squirrel species (Hoogland 1994; Trulio 1996). Elsewhere, females have been observed to abandon nests and relocate following incidents of infanticide, presumably to avoid their litter becoming victim to infanticide in the ensuing active season (McLean 1981; Sherman 1981; Wolff and Cicirello 1989). While infanticide is relatively widespread among ground squirrels (McLean 1983; Hoogland 1985; Hare 1991; Vestal 1991; Trulio 1996), it has never been directly observed in wild populations of Franklin's ground squirrels. While adult males may commit infanticide (McLean 1983), adult females are most commonly the perpetrators of infanticide within ground squirrel species (Hoogland 1985; Hare 1991; Trulio 1996). However, in this study the likelihood of nest relocation was only slightly greater in response to female as opposed to male visits. Like the factors that may influence larger-scale dispersal, nest relocation among lactating females may serve to reduce resource competition for themselves or for their young (Dobson 1982; McLean 1984).

By registering nest visits with PIT-tag readers, we were able to measure nest site visitation among tagged conspecifics where visibility limitations imposed by Franklin's ground squirrel preference for densely vegetated habitat would otherwise render such data impossible to accrue (Huebschman 2007). Franklin's ground squirrels are considered by most authors to be asocial (Kivett et al. 1976; Armitage 1981; Michener 1983; Lindsay and Galloway 1997; Hare 2004; Green et al. 2013; but see Hare 2004). Knowledge of the frequency and nature of conspecific interactions is necessary to assess social structure within a species (Michener 1979), yet little field study has been devoted to documenting conspecific interactions among free-living Franklin's ground squirrels. In asocial species, where adults are often highly dispersed, conspecific interactions among adults, particularly females, outside the breeding season are atypical events (Ferron 1985; Schwagmeyer and Woontner 1985). However, the vast majority (67%) of Franklin's ground squirrel females monitored in this study received nest visits from both male and female conspecifics throughout lactation. The frequency of conspecific nest visitation by both male and female Franklin's ground squirrels suggests that interactions among adults are more common than indicated by the species' asocial and solitary standing in the literature (Armitage 1981; Michener 1983). Further research into the nature of adult interactions and the spatial organization of this species is necessary to adequately assess Franklin's ground squirrel social structure. Indeed, daytime telemetric tracking of the Delta Marsh population uncovered multiple adult females sharing the same area and overlapping core ranges (J. Hare, unpublished data), indicating that further field investigation will prove worthwhile.

Few females (33%) in this study received no conspecific visits over the course of lactation. Interestingly, these females still relocated nests at least once over lactation. The persistence of movements by females in the absence of conspecific nest visitation, as well as in

the presence of insecticide treatment, suggests that nest movements are a composite strategy that has evolved in response to multiple selection pressures. In addition to flea infestation and conspecific nest discovery investigated here, multiple other biotic and abiotic factors may also influence nest movements.

Haberman and Fleharty (1971) and Jones et al. (1983) posited that nest movements may promote access to prime forage sources that vary in their location throughout the active season. In this study, early in the season when cultivated areas were not viable food or cover sources, nearly all squirrels nested in burrow sites dominated by either grass or forest habitat types. As cultivated areas became more capable of providing cover and forage toward the end of lactation, nest site selection shifted towards these, and tall grass, habitat types. Indeed, no squirrel occupied a forested nest by the end of lactation. Such shifts in nest habitat type might reflect relocations that increase proximity to superior cover and/or forage for adult females as well as their emergent young. However, as dams did select forested sites and other areas that provide little forage potential, cover is likely a more influential nest habitat attribute. Further research into seasonal resource use and forage preferences is required to empirically address this question. Beyond broad habitat associations, aspects of microclimate (insulative value, Guillemette et al. 2009) or predation threat (Kerr and Descamps 2008) may additionally influence nest relocations.

Even though energetic demands on maternal females are particularly high throughout lactation (Kenagy et al. 1989; Rogowitz 1996), females in this study averaged over two relocations of their nest and litter during each lactation season. It is clear that the fitness benefits accrued via relocations must outweigh the costs of movements at a time when energetic burden is great. While interesting in terms of individual fitness, movements may also have implications for population distribution and abundance.

Researchers have documented population abundance cycles and disappearances throughout Franklin's ground squirrel's range (Sowls 1948; Erlien and Tester 1984; Johnson and Choromanski-Norris 1992; Martin et al. 2003; Chapter 2). Factors mediating population cycles, 4-6 y fluctuations in Manitoba (Sowls 1948), and 10 y fluctuation periods in Minnesota (Erlien and Tester 1984), remain uninvestigated; however factors including the snowshoe hare cycle (Erlien and Tester 1984) and environmental stochasticity (Chapter 2; Sowls 1948) have been suggested. Indeed, the population at Delta Marsh suffered a decline in 2001 following damaging agricultural practices and exceptionally high water table levels (Chapter 2). Causes of apparent population disappearances in Indiana and Illinois, and failed population reintroductions in Illinois (Gensburg-Markham Prairie, Panzer 1986, and Knox College Biological Field Station, Van Petten and Schramm 1972) remain similarly unclear (Martin et al. 2003). In addition to local extirpation, Martin et al. (2003) suggested one possible cause of population disappearances throughout Illinois was population-level shifts in area use resulting from environmental disturbances. Our results indicate that Franklin's ground squirrels do make local movements in response to various ecological factors. Movements observed in this study may give way to broader, population-level relocations over multiple seasons if squirrels expand into more favorable habitat. To what extent, if any, the small-scale nest movements described here lead to population-level location shifts and ultimately to observed disappearances from areas of historical occupancy requires further investigation and long-term population monitoring.

Overall, results of this multi-year investigation reveal that lactating Franklin's ground squirrel females routinely engage in nest relocation behaviour. Significant contributions of flea infestation, conspecific nest visitation, and nesting habitat to movement patterns and nest site selection suggest that nest relocations evolved in response to factors that impact female fitness.

These findings offer novel insight into why lactating Franklin's ground squirrel females undertake what superficially appear to be costly nest movements. Patterns of relocation should be taken into account by managers and researchers, particularly where census data are desired, in that the nest movements documented may help explain Franklin's ground squirrel population disappearances.

Literature Cited

Armitage, K.B. 1981. Sociality as a life-history tactic of ground squirrels. Oecologia 48:36-49.

Armitage, K.B., D.H. Van Vuren, A. Ozgul, and M.K. Oli. 2011. Proximate causes of natal dispersal in yellow-bellied marmots, *Marmota flaviventris*. Ecology 92: 218-227.

Arnaud, C.M., F.S. Dobson, and J.O Murie. 2012. Philopatry and within-colony movements in Columbian ground squirrels. Molecular Ecology 21: 493-504.

Boag, D.A., and D.R. Wigget. 1994. Food and space: resources defended by parous female Columbian ground squirrels. Canadian Journal of Zoology 72: 1908-1914.

Bowler, D.E., and T.G. Benton. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80: 205-225.

Bulova, S.J. 1994. Patterns of burrow use by desert tortoises: gender differences and seasonal trends. Herpetological Monographs 8: 133-143.

Butler, J.M., and T.J. Roper. 1996. Ectoparasites and sett use in European badgers. Animal Behaviour 52: 621-629.

Byrom, A.E., and C.J. Krebs. 1999. Natal dispersal of juvenile arctic ground squirrels in the boreal forest. Canadian Journal of Zoology 77: 1048-1059.

Choromanski-Norris, J.F., K. Fritzell, and A.B. Sargeant. 1986. Seasonal activity cycle and weight changes of the Franklin's ground squirrel. American Midland Naturalist 116: 101-107.

Choromanski-Norris, J.F., K. Fritzell, and A.B. Sargeant.1989. Movements and habitat use of

    Franklin's ground squirrels in duck-nesting habitat. Journal of Wildlife Management 53:

    324-331.

Dobson, F.S. 1979. An experimental study of dispersal in the California ground squirrel. Ecology

    60: 1103-1109.

Dobson, F.S. 1982. Competition for mates and predominant juvenile male dispersal in mammals.

    Animal Behaviour 30: 1183-1192.

Ebensperger, L.A. 1998. Strategies and counterstrategies to infanticide in mammals. Biological

    Reviews 73: 321-346.

Elcock, L.E., B.P. Stuart, B.S. Wahle, H.E. Hoss, K. Crabb, D.M. Millard, R.E. Mueller, T.F.

    Hastings, S.G. Lake. 2001. Tumors in long-term rat studies associated with microchip animal

    identification devices. Experimental and Toxicologic Pathology 52: 483-491.

Erlien, D.A., and J. R. Tester. 1984. Population ecology of sciurids in northwestern Minnesota.

    Canadian Field Naturalist 98:1–6.

ESRI (Environmental Systems Resource Institute). 2011. ArcMap 10. ESRI, Redlands,

    California.

Ferron, J. 1985. Social behavior of the golden-mantled ground squirrel (*Spermophilus lateralis).*

    Canadian Journal of Zoology 63: 2529-2533.

Galloway, T.D., and J.E. Christie. 1990. Fleas *(*Siphonaptera*)* associates with ground squirrels

    (*Spermophilus* spp.*)* in Manitoba, Canada. Canadian Entomologist 122: 449-458.

Green, M.L., T.F. Ting, M.B. Manjerovic, N. Mateus-Pinilla, and J. Novakofski. 2013.

    Noninvasive alternatives for DNA collections from threatened rodents. Natural Science 5:

    18-26.

Greenwood, P.J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. Animal

    Behaviour 28: 1140-1162.

Greenwood, P.J., and P.H. Harvey. 1982. The natal and breeding dispersal of birds. Annual

    Review of Ecology and Systematics 13: 1-21.

Guillemette, C.U., Q.E. Fletcher, S. Boutin, R.M. Hodges, A.G. McAdam, and M.M. Humphries.

    2009. Lactating red squirrels experiencing high heat load occupy less insulated nests.

    Biology Letters 5: 166-168.

Haberman, C.G., and E.D. Fleharty. 1971. Natural history notes on Franklin's ground squirrels in

    Boone County, Nebraska. Transactions of the Kansas Academy of Science 74: 76-80.

Haggerty, S.M. 1968. The ecology of the Franklin's ground squirrel (*Citellus franklinii*) at Itasca

    State Park, Minnesota. M.Sc. Thesis, University of Minnesota, Minneapolis, USA.

Halekoh, U., S. Højsgaard, and J. Yan. 2006. The R Package geepack for generalized estimating

    equations. Journal of Statistical Software 15: 1-11.

Hall, E.R. 1955. Handbook of the mammals of Kansas. University of Kansas Museum of Natural

    History, Miscellaneous Publication 7: 1-103.

Hamer, A.J., S.J. Lane, and M.J. Mahony. 2008. Movement patterns of adult Green and Golden

    Frogs *Litoria aurea* and the implications for conservation management. Journal of

    Herpetology 42: 397-407.

Hare, J.F. 1991. Intraspecific killing of preweaned young in the Columbian ground squirrel (*Spermophilus columbianus)*. Canadian Journal of Zoology 69: 797-800.

Hare, J.F. 2004. Kin discrimination by asocial Franklin's ground squirrels (*Spermophilus franklinii)*: is there a relationship between kin discrimination and ground squirrel sociality? Ethology Ecology & Evolution 16: 157-169.

Hart, B.L. 1994. Behavioural defense against parasites: interaction with parasite invasiveness. Parasitology 109: 139-151.

Harris, J.H., and P. Leitner. 2005. Long-distance movements of juvenile Mohave ground squirrels, *Spermophilus mohavensis*. The Southwestern Naturalist 50: 188-196.

Hillegass, M.A., J.M. Waterman, and J.D. Roth. 2010. Parasite removal increases reproductive success in a social African ground squirrel. Behavioral Ecology 21: 696-700.

Holekamp, K.E. 1984. Dispersal in ground-dwelling sciurids, pp. 295–320. In: J.O. Murie and G.R. Michener, editors. The Biology of Ground-Dwelling Squirrels. University of Nebraska Press, Lincoln, USA.

Holekamp, K.E. 1986. Proximal causes of natal dispersal in Belding's ground squirrels (*Spermophilus beldingii*). Ecological Monographs 56: 365-391.

Hoogland, J.L. 1985. Infanticide in prairie dogs: lactating females kill offspring of close kin. Science 230: 1037-1040.

Hoogland, J.L. 1994. Amicable and hostile treatment of juvenile prairie dogs by older conspecifics, pp. 321-337. In: S. Parmigiani, and F.S. vom Saal, editors. Infanticide and Parental Care. Harwood Academic Publishers, New York, USA.

Huebschman, J.J. 2007. Distribution, abundance, and habitat associations of Franklin's ground
squirrel (*Spermophilus franklinii* Sabine 1822). Illinois Natural History Survey Bulletin 38:
1–58.

Illinois Endangered Species Board. 2015. Checklist of endangered and threatened animals and
plants of Illinois. http://www.dnr.illinois.gov/ESPB/Documents/2015_ChecklistFINAL_
for_webpage_051915.pdf
Accessed on 01 June, 2015.

Indiana Department of Natural Resources. 2013. Indiana's State endangered species.
http://www.in.gov/dnr/fishwild/files/fw-Endangered_Species_List.pdf
Accessed on 01 June, 2015.

Iowa Department of Natural Resources. 2012. Iowa wild- life action plan.
http://www.iowadnr.gov/Environment/WildlifeStewardship/IowaWildlifeActionPlan.aspx
Accessed on 01 June, 2015.

Iverson, S.L., and B.N. Turner. 1972. Natural history of a Manitoba population of Franklin's
ground squirrels. Canadian Field Naturalist 86: 145-149.

Johnson, S.A., and J.F. Choromanski-Norris. 1992. Reduction in the eastern limit of the range of
the Franklin ground-squirrel (*Spermophilus franklinii)*. American Midland Naturalist 128:
325-331.

Jones, J.K., JR., D.M. Armstrong, R.S. Hoffmann, and C. Jones. 1983. Mammals of the Northern
Great Plains. University of Nebraska Press, Lincoln, USA.

Kadlecova, K., M. Bily, and M. Maciak. 2012. Movement patterns of the co-occurring species *Astacus astacus* (noble crayfish) and *Austropotamobius torrentium* (stone crayfish). Fundamental and Applied Limnology 180: 351-360.

Kays, R., M.C. Crofoot, W. Jetz, and M. Wikelski. 2015. Terrestrial animal tracking as an eye on life and planet. Science 348: aaa2478.

Kerr, T., and S. Descamps. 2008. Why do North American red squirrel, *Tamiasciurus hudsonicus*, mothers relocate their young? A prediction-based hypothesis. Canadian Field Naturalist 122: 65-66.

Kenagy, G.J., S.M. Sharbaugh, and K.A. Nagy. 1989. Annual cycle of energy and time expenditure in a golden-mantled ground squirrel population. Oecologia 78: 269-282.

Kivett, V.K., J.O. Murie, and A.L. Steiner. 1976. A comparative study of scent-gland location and related behavior in some northwestern Nearctic ground squirrel species (Sciuridae): an evolutionary approach. Canadian Journal of Zoology 54: 1294–1306.

Le Calvez, S., M.F. Perron-Lepage, and R. Burnett. 2006. Subcutaneous microchip-associated tumours in B6C3F1 mice: a retrospective study to attempt to determine their histogenesis. Experimental and Toxicologic Pathology 57: 255-265.

Lidicker, W.Z., Jr. 1975. The role of dispersal in the demography of small mammals, pp. 103-128. In: F.B. Golley, K. Petrusewicz, and L. Ryskowski, editors. Small Mammals: Their Productivity and Population Dynamics. Cambridge University Press, Cambridge, UK.

Lindsay, L.R., and T.D. Galloway. 1997. Seasonal activity and temporal separation of four species of fleas (Insecta: Siphonaptera) infesting Richardson's ground squirrels,

Spermophilus richardsonii (Rodentia: Sciuridae), in Manitoba, Canada. Canadian Journal of Zoology 75: 1310-1322.

Loarie, S.R., R.J. Van Aarde, and S.L. Pimm. 2009. Fences and artificial water affect African savannah elephant movement patterns. Biological Conservation 142: 3086-3098.

Love, A., and D. Love. 1954. Vegetation of a prairie marsh. Bulletin of the Torrey Botanical Club 81: 16-34.

Lubin, Y., S. Ellner, and M. Kotzman. 1993. Web relocation and habitat selection in a desert widow spider. Ecology 74: 1915-1928.

Martin, J.M., and E.J. Heske. 2005. Juvenile dispersal of Franklin's ground squirrel (*Spermophilus franklinii)* from a prairie "island." American Midland Naturalist 153: 444-449.

Martin, J.M., E.J. Heske, and J.E. Hoffmann. 2003. Franklin's ground squirrel (*Spermophilus franklinii)* in Illinois: a declining prairie mammal? American Midland Naturalist 150: 130-138.

McDougall, P.T., and D.L. Kramer. 2006. Short term behavioral consequences of territory relocation in a Caribbean damselfish, *Stegastes diencaeus.* Behavioral Ecology 18: 53-61.

McLean, I.G. 1981.Social ecology of the arctic ground squirrel. PhD Thesis, University of Alberta, Edmonton, Canada.

McLean, I.G. 1983. Paternal behavior and the killing of young in Arctic ground squirrels. Animal Behaviour 31: 32-44.

McLean, I.G. 1984. Spacing behavior and aggression in female ground squirrels, pp. 321-325.
In: J.O. Murie and G.R. Michener, editors. The Biology of Ground-Dwelling Squirrels.
University of Nebraska Press, Lincoln, USA.

Michener, G.R. 1979. Spatial relationships and social organization of adult Richardson's ground
squirrels. Canadian Journal of Zoology 57: 125-139.

Michener, G.R. 1983. Kin identification, matriarchies, and the evolution of sociality in
ground-dwelling sciurids, pp. 528-572. In: J.F. Eisenberg and D.G. Kleiman, editors.
Advances in the Study of Mammalian Behavior. American Society of Mammalogists Special
Publication No. 7.

Michener, G.R., and D.R. Michener. 1977. Population-structure and dispersal in Richardson's
ground squirrels. Ecology 58: 359-368.

Missouri Department of Conservation. 2015. Missouri species and communities of conservation
concern checklist. http://mdc.mo.gov/sites/default/files/resources/2010/04/2015species
concern.pdf
Accessed on 01 June, 2015.

Moore, J. 2002. Parasites and the Behavior of Animals. Oxford University Press, Oxford, UK.

Murie, J.O. 1999. Franklin's ground squirrel, *Spermophilus franklinii*, pp. 419–420. In: D.E.
Wilson and S. Ruff, eds. The Smithsonian Book of North American Mammals. Smithsonian
Institution Press, Washington, D.C., USA.

Murie, J.O., and M.A. Harris. 1982. Annual variation of spring emergence and breeding in
Columbian ground squirrels (*Spermophilus columbianus*). Journal of Mammalogy 63:
431-439.

Pan, W. 2001. Akaike's information criterion in generalized estimating equations. Biometrics 57:
120-125.

Panzer, R. 1986. Franklin's ground squirrel translocation to an Illinois prairie preserve.
Restoration and Management Notes 4:27.

Patterson, J.E., P. Nuehaus, S.J. Kutz, and K.E. Ruckstuhl. 2013. Parasite removal improves
reproductive success of female North American red squirrels (*Tamiasciurus hudsonicus*).
PLoS ONE 8: e55779.

Pauwels, I.S., P.L.M. Goethals, J. Coeck, and A.M. Mouton. 2014. Movement patterns of adult
pike (*Esox lucius* L.) in a Belgian lowland river. Ecology of Freshwater Fish 23: 373-382.

Price, M.V., P.A. Kelly, and R.L. Goldingay. 1994. Distances moved by Stephens' kangaroo rat
(*Dipodomys stephensi* Merriam) and implications for conservation. Journal of Mammalogy
75: 929-939.

R Development Core Team. 2013. R: a language and environment for statistical computing.
Vienna, Austria.

Reckardt K., and G. Kerth. 2006. The reproductive success of the parasitic bat fly *Basilia
nana* (Diptera: Nyctreibiidae) is affected by the low roost fidelity of its host, the
Bechstein's bat (*Myotis bechsteinii).* Parasitology Research 98: 237-243.

Reckardt K., and G. Kerth. 2007. Roost selection and roost switching of female Bechstein's bats (*Myotis bechsteinii)* as a strategy of parasite avoidance. Oecologia 154: 581-588.

Reichardt, T.R., and T.D. Galloway. 1994. Seasonal occurrence and reproductive status of *Opisocrostis bruneri* (Siphonaptera: Ceratophyllidae), a flea on Franklin's ground squirrel, *Spermophilus franklinii* (Rodentia: Sciuridae) near Birds Hill Park, Manitoba. Journal of Medical Entomology 31: 105-113.

Rogowitz, G.L. 1996. Trade-offs in energy during lactation. American Zoologist 36: 197-204.

Roper, T.J., T.P. Jackson, L. Conradt, and N.C. Bennet. 2002. Burrow use and the influence of ectoparasites in Brant's whistling rat *Parotomys brantsii.* Ethology 108: 557-564.

Sanchez, S., F. Pelaez, C. Gil-Burmann, and W. Kaumanns. 1999. Costs of infant-carrying in the cotton-top tamarin (*Saguinus oedipus*). American Journal of Primatology 48: 99-111.

Schwagmeyer, P.L., and S.J. Woontner. 1985. Mating competition in an asocial ground squirrel, *Spermophilus tridecemlineatus*. Behavioral Ecology and Sociobiology 17: 291-296.

Seton, E.T. 1929. Lives of Game Animals. Volume 4. Doubleday, Doran and Co., Garden City, USA.

Shay, J.M. 1999. Annotated vascular plant species list for the Delta Marsh, Manitoba and surrounding area. University of Manitoba Field Station (Delta Marsh) Occasional Publication No. 2.

Sherman, P.W. 1981. Reproductive competition and infanticide in Belding's ground squirrels and other animals. In: R.D. Alexander, editor. Natural Selection and Social Behavior. Chiron, New York, USA.

Siegal-Willott, J., D. Heard, N. Sliess, D. Naydan, and J. Roberts. 2007. Microchip-associated leiomyosarcoma in an Egyptian fruit bat (*Rousettus aegyptiacus).* Journal of Zoo and Wildlife Medicine 38: 352-356.

Sowls, L.K. 1948. The Franklin ground squirrel, *Citellus franklinii* (Sabine), and its relationship to nesting ducks. Journal of Mammalogy 29: 113-137.

Sura, R., R.A. French, B.D. Goldman, and D.R. Schwartz. 2011. Neoplasia and granulomas surrounding microchip transponders in Damaraland mole rats (*Cryptomys damarensis).* Veterinary Pathology 48: 896-902.

Tanaka, K. 1989. Energetic cost of web construction and its effect on web relocation in the web-building spider *Agelena limbata.* Oecologia 81: 459-464.

Tardif, S.D. 1994. Relative energetic cost of infant care in small-bodied neotropical primates and its relation to infant-care patterns. American Journal of Primatology 34: 133-143.

Temeles, E.J. 1994. The role of neighbours in territorial systems: when are they 'dear enemies'? Animal Behaviour 47: 339-350.

Tillmann, T., K. Kamino, C. Dasenbrock, H. Ernst, M. Kohler, G. Morawietz, E. Campo, A. Cardesa, L. Tomatis, and U. Mohr. 1997. Subcutaneous soft tissue tumours at the site of implanted microchips in mice. Experimental and Toxicologic Pathology 49: 197-200.

Trulio, L.A. 1996. The functional significance of infanticide of California ground squirrels (*Spermophilus beecheyi*). Behavioral Ecology and Sociobiology 38: 97-103.

Turner, B.N., S.L. Iverson, and K.L. Severson. 1976. Postnatal growth and development of captive Franklin's ground squirrels *(Spermophilus franklinii)*. American Midland Naturalist 95: 93-102.

Van Petten, A., and P. Schramm. 1972. Introduction, dispersal, and population increase of the Franklin's ground squirrel, *Spermophilus franklinii*, in a restored prairie, pp. 166–173. In: J. H. Zimmerman, editor. Proceedings of the Second Midwest Prairie Conference, University of Wisconsin–Madison, Madison, USA.

Van Vuren, D., and K.B. Armitage. 1994. Survival of dispersing and philopatric yellow-bellied marmots: what is the cost of dispersal? Oikos 69: 179-181.

Vestal, B. M. 1991. Infanticide and cannibalism by male thirteen-lined ground squirrels. Animal Behaviour 41: 1103- 1104.

Waterman, J.M. 1992. The use of space by yearling Columbian ground squirrels before male dispersal. Canadian Journal of Zoology 70: 2490-2493.

Wattles, D.W., and S. DeStefano. 2013. Space use and movements of moose in Massachusetts: implications for conservation of large mammals in a fragmented environment. Alces 49: 65-81.

Wiggett, D., and D.A. Boag. 1992. The resident fitness hypothesis and dispersal by yearling female Columbian ground squirrels. Canadian Journal of Zoology 70: 1984-1994.

Wisconsin Department of Natural Resources. 2014. Franklin's ground squirrel *(Spermophilus franklinii)*. http://dnr.wi.gov/topic/endangeredresources/animals.asp?mode=detail&speccode =amafb05120. Accessed: 01 June, 2015.

Wolff, J.O., and D.M. Cicirello. 1989. Field evidence for sexual selection and resource

competition infanticide in white-footed mice. Animal Behaviour 38: 637-642.

Tables and Figures

Table 3.1. Summary of average seasonal distance (see details in Methods) and average, maximum, and minimum frequencies of nest relocations for all radio-collared lactating female Franklin's ground squirrels throughout the 31 d lactation period in 1998-2000. Values are presented as mean ± SE, n = number of individuals.

Year	n	Lactation Moves	Max. Moves	Min. Moves	n	Seasonal Distance (m)
1998	7	2.1 ± 0.3	4	1	7	5870.8 ± 3052.3
1999	13	1.8 ± 0.3	3	0	12	1513.6 ± 329.2
2000	12	1.5 ± 0.2	3	1	12	2077.3 ± 537.9

Table 3.2. Summary of mean ± SE, maximum, and minimum nest distances for all relocations performed by radio-collared lactating female Franklin's ground squirrels throughout the 31 d lactation period in 1998-2000. Values are presented as mean ± SE, n = number of nest relocations.

Year	n	Nest Distance (m)	Max. Nest Distance (m)	Min. Nest Distance (m)
1998	15	153.8 ± 64.8	1020	6
1999	22	96.1 ± 19.2	406	23
2000	18	122.5 ± 28.0	376	28

Table 3.3. Frequency of dominant nest habitat types associated with initial and final nests occupied by radio-collared lactating female Franklin's ground squirrels throughout the 31 d lactation period in 2014. Fisher's exact test (p < 0.001) reveals that occupancy order is not independent of habitat type.

	Habitat Type		
	Forest	Tall Grass	Cultivated
First Nest	4	10	0
Final Nest	0	5	9
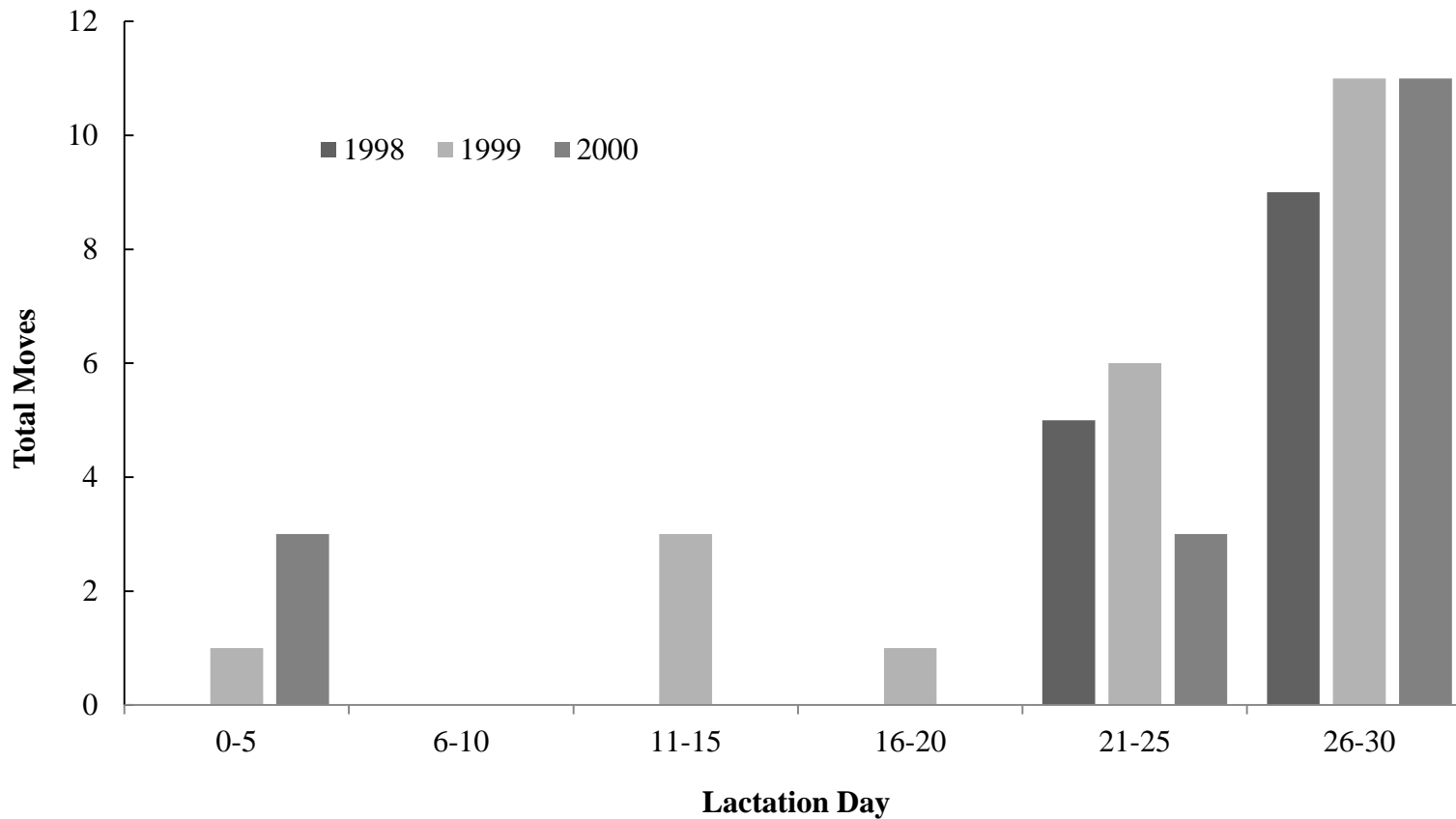
Figure 3.1. Temporal distribution of cumulative nest relocations performed by all radio-collared lactating female Franklin's ground squirrels within 5-day periods throughout the 31 d of lactation in 1998-2000 (1998, n = 7; 1999, n = 13; 2000, n = 12 females).
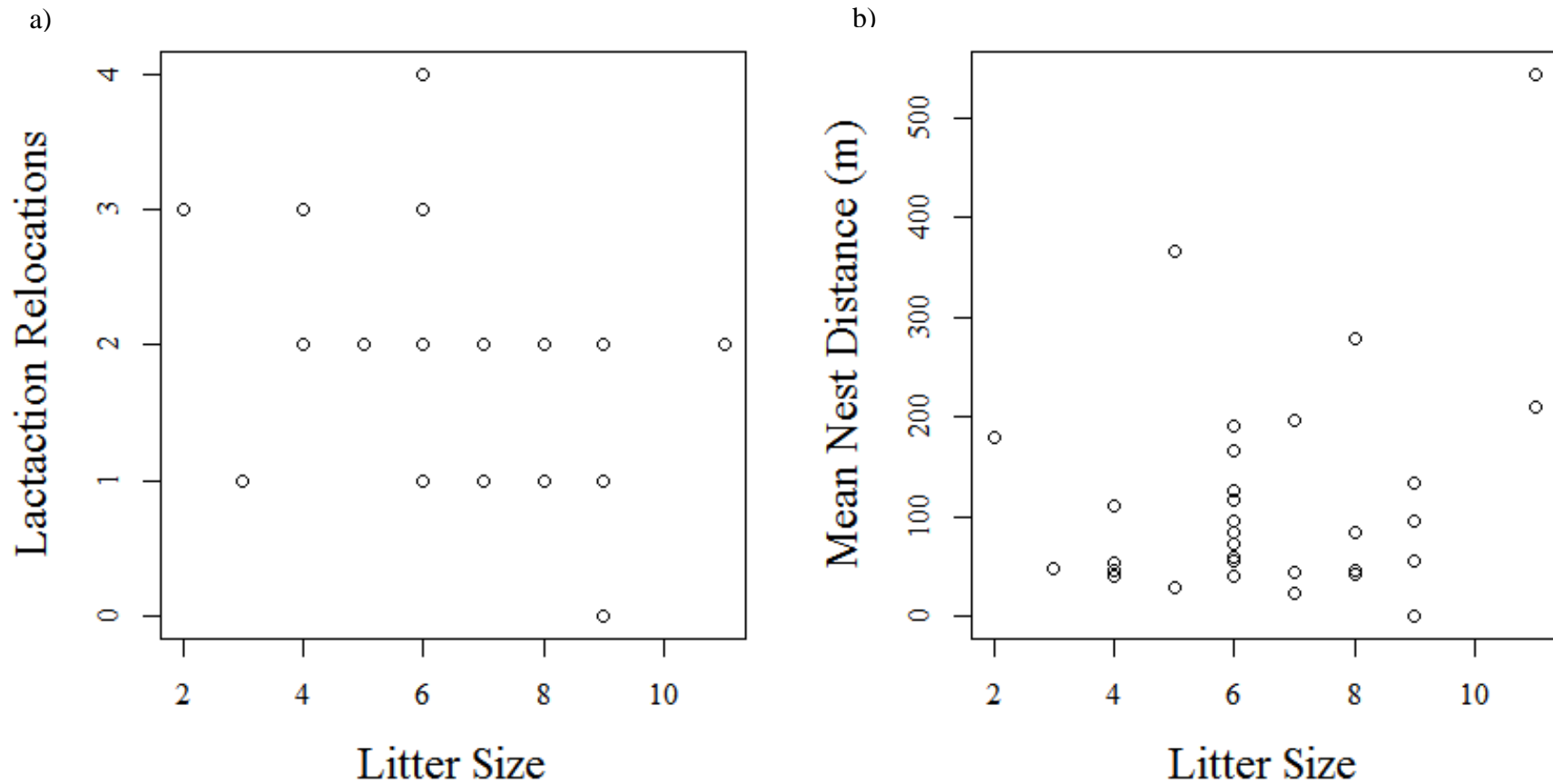
Figure 3.2. Relationship between litter size and a) frequency of nest relocation (n = 32 females) and b) average distance between

relocated nests (n = 31 females) performed and travelled by all radio-collared Franklin's ground squirrel females throughout the 31 d

of lactation in 1998-2000. Significance tests for Spearman's rank correlation coefficients reveal frequency of relocation significantly

decreases with increasing litter size (ρ = -0.34, df = 30, p = 0.054), while average distance between nests does not significantly

correlate with litter size (ρ = 0.146, df = 29, p = 0.432).
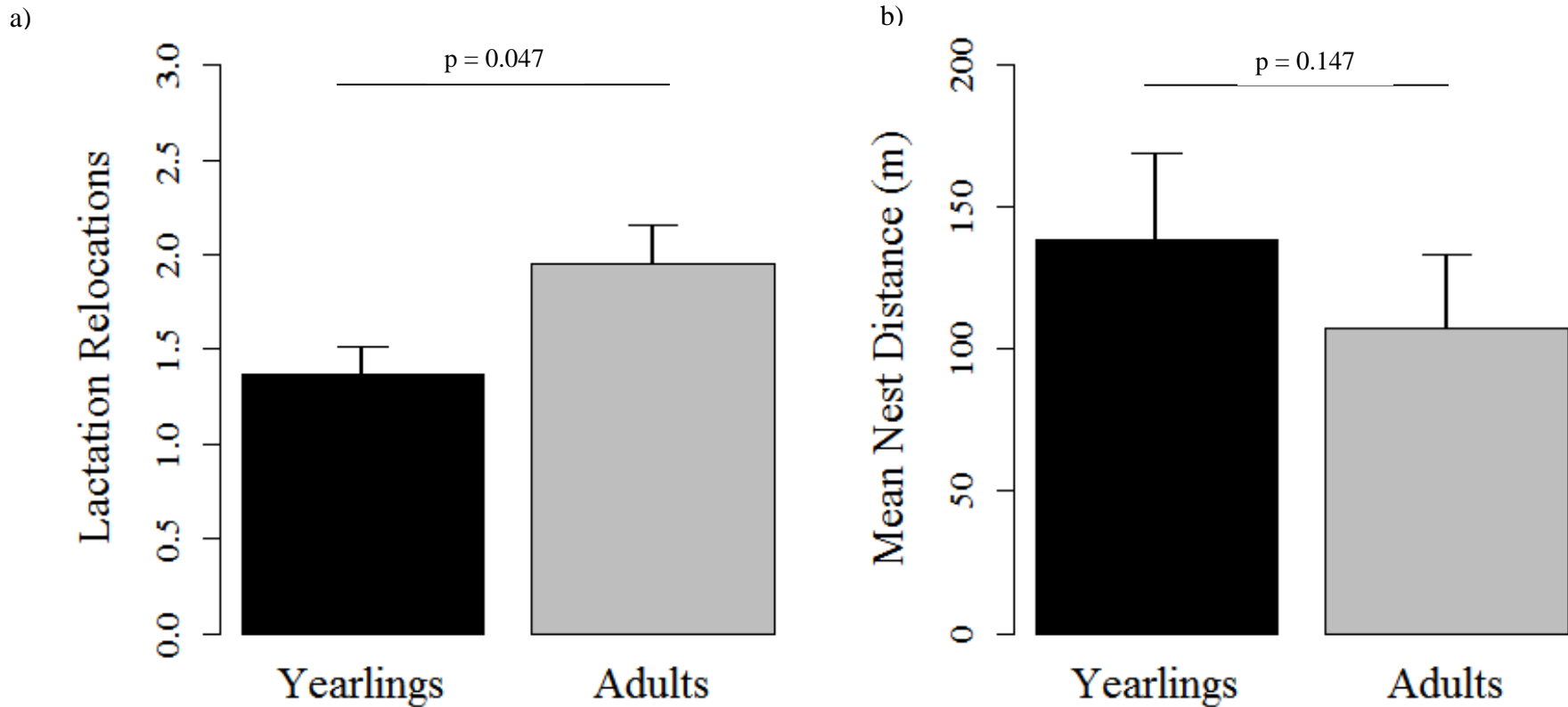
Figure 3.3. Comparison of yearling and adult squirrels with regards to a) average number of relocations (n = 32) and b) average distance between relocated nests (n = 31) performed and travelled by all radio-collared Franklin's ground squirrel females throughout the 31 d of lactation in 1998-2000. Error bars represent SE. Mann-Whitney U tests reveal a significant difference between yearlings and adults in the frequency of relocation ($n_{yearling}$ = 11, n_{adult} = 21, W = 68.5, p = 0.047), but not in the average distance between relocated nests ($n_{yearling}$ = 10, n_{adult} = 21, W = 140, p = 0.147).
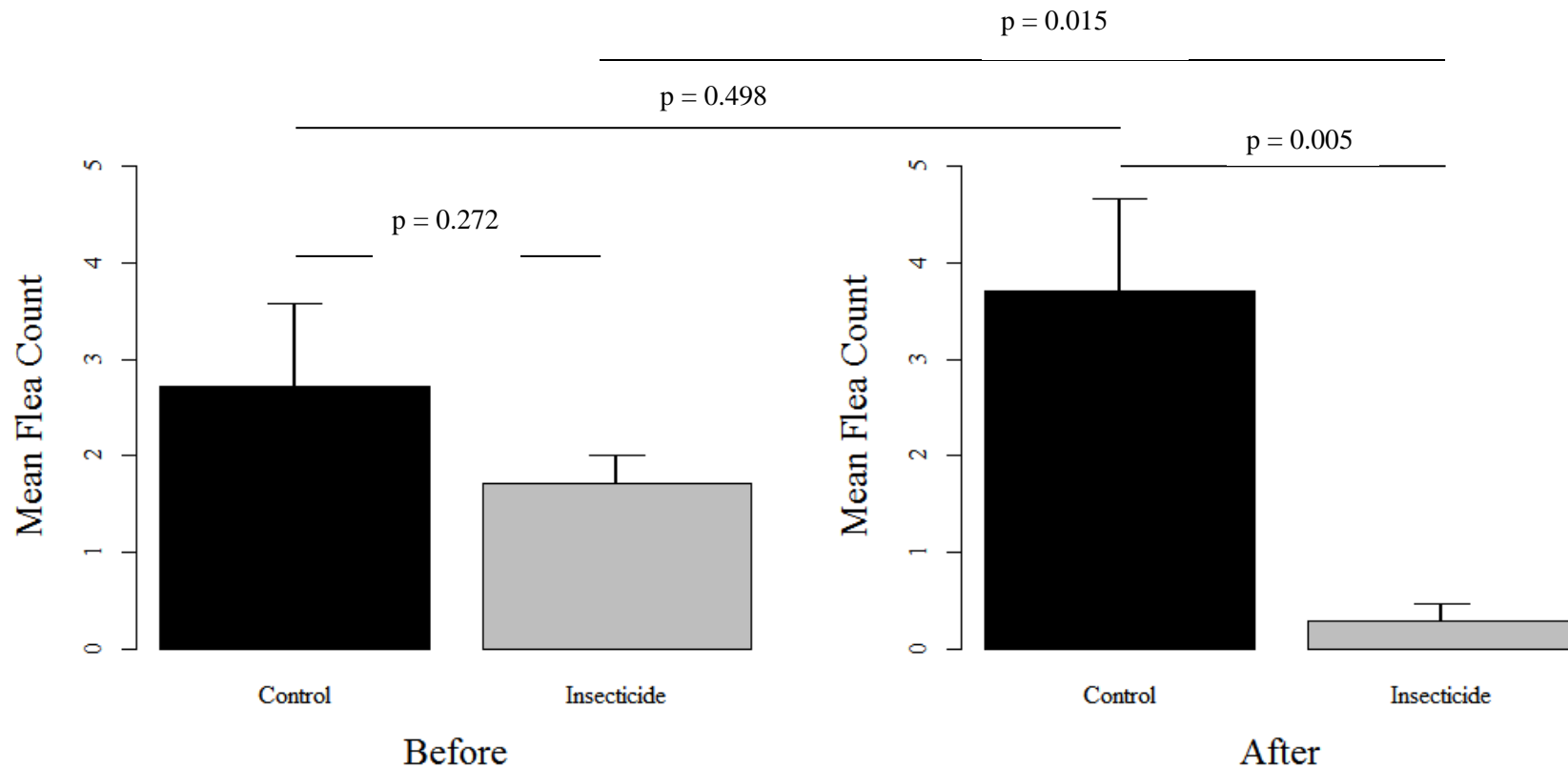
Figure 3.4. Comparison of control (n = 7) and insecticide-treated (n = 7) Franklin's ground squirrel females with regards to mean relative flea abundance before initial treatment and after initial treatment (21-30 d later) in 2014. Error bars represent SE. Mann-Whitney U tests reveal no difference in mean flea counts between control and insecticide groups before treatment, ($n_{treatment}$ = 7, $n_{control}$ = 7, W = 33, p = 0.272), but do reveal a difference after initial treatment ($n_{treatment}$ = 7, $n_{control}$ = 7, W = 44.5, p = 0.005). A Wilcoxon signed-rank test reveals no change in mean relative flea abundance within control dams before and after initial treatment (n = 7, V = 4.5, p = 0.498), but does reveal a decrease in relative flea abundance within insecticide-treated dams (n = 7, V = 21, p = 0.015).
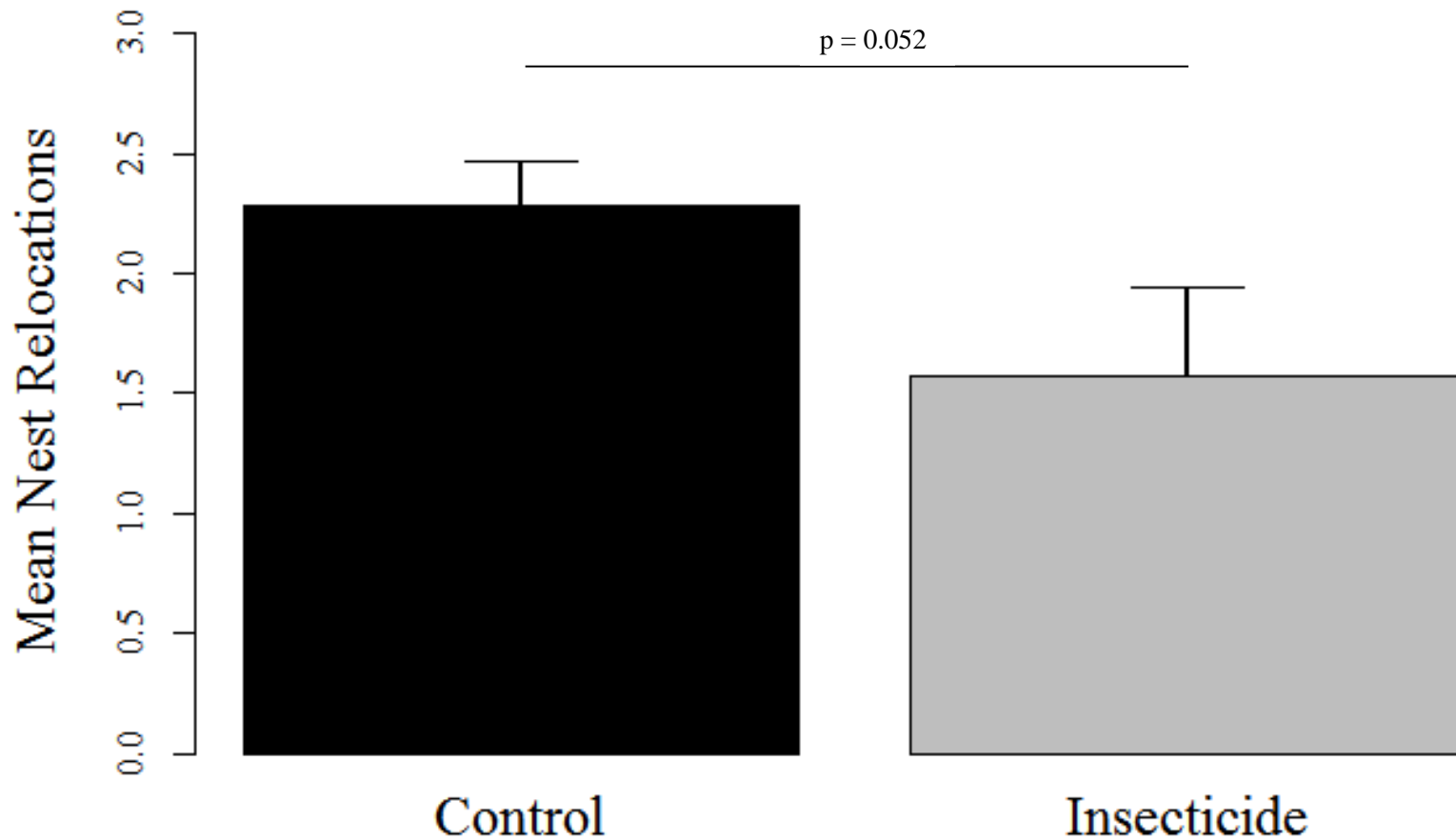
Figure 3.5. Comparison of mean number of nest relocations performed by control (n = 7) and insecticide-treated (n = 7) Franklin's ground squirrel females during the 31 d lactation period in 2014. Error bars represent SE. Mann-Whitney U test reveals a significant difference between groups ($n_{treatment}$ = 7, $n_{control}$ = 7, W = 37, p = 0.052).
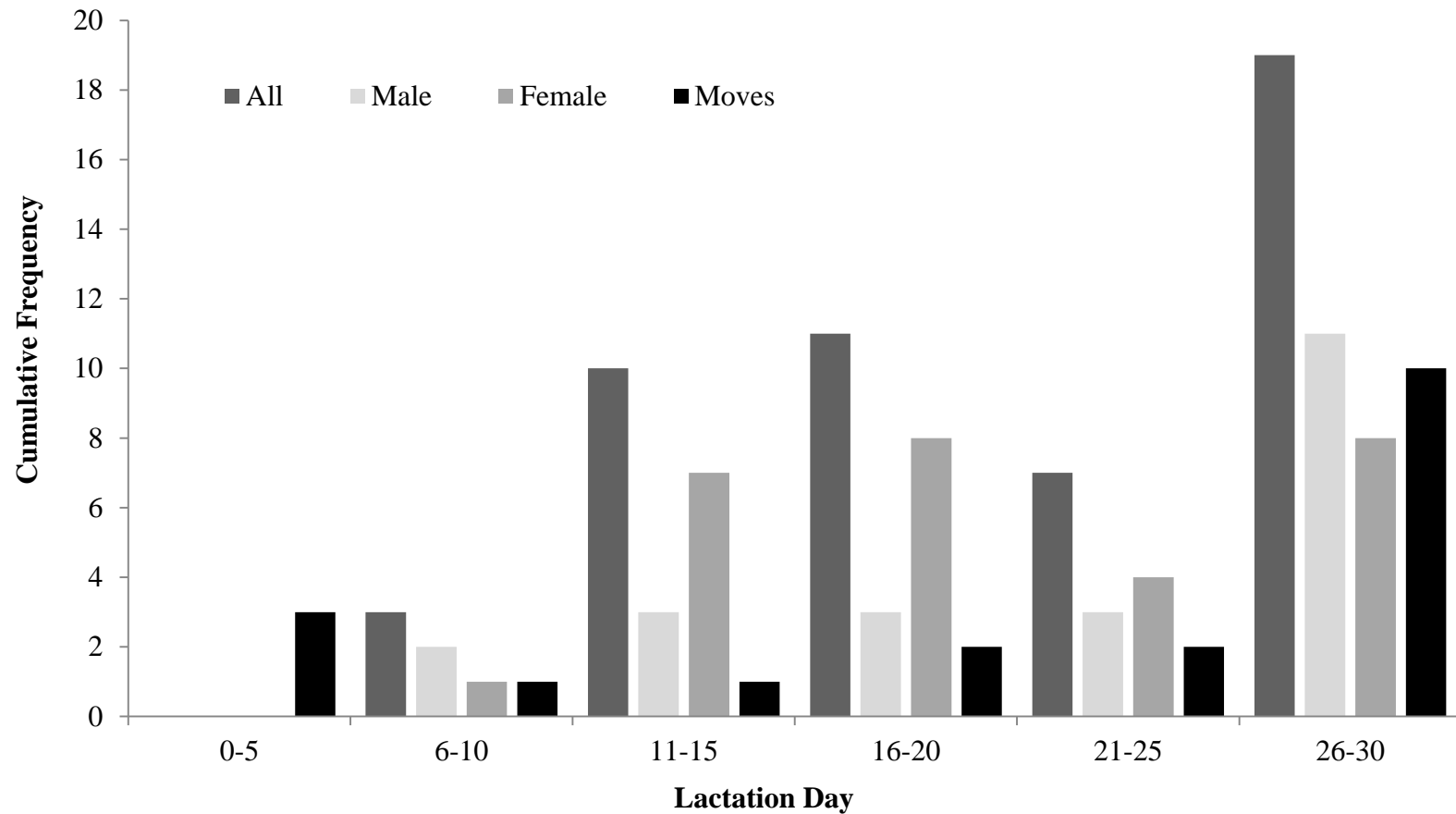
Figure 3.6. Temporal distribution of cumulative nest relocations performed by 9 radio-collared lactating Franklin's ground squirrel females together with cumulative nest visits females incurred within 5-day periods throughout the 31 d of lactation 2014. Nest moves are represented by black bars, while nest visits are represented by grey bars (sexes are distinguished by lighter grey shades).

Chapter 4: Franklin's ground squirrel (*Poliocitellus franklinii*) dam and litter response to ectoparasitism

Abstract

Parasite infection and infestation is widespread among animals and may impose indirect costs of increased immune defence and direct costs of blood, nutrient, and energy depletion. Given an animal's finite energetic and nutritional reserves, we investigated how ectoparasitism trades off with reproduction and body mass in a free-living population of Franklin's ground squirrels (*Poliocitellus franklinii*) located near Delta Marsh, Manitoba. We experimentally reduced ectoparasite burden by administering an insecticide product to the pelage of a group of reproductive females following breeding, and contrasted body mass and reproductive performance (litter size, mass and sex ratio of juveniles produced) of those individuals to sham-treated control females. Insecticide-treated dams did not differ from sham-treated dams in body mass, litter size, or juvenile mass, and thus results of the present study do not support the hypothesis that dam growth and reproduction trade-off with ectoparasite defence at the infestation levels that prevailed among subjects during this investigation. Taken at face value, the present findings suggest that dams compensate for any costs of immune response with elevated resource intake, or adequately manage infestation levels through non-immune defence (i.e. behavioural parasite avoidance). However, a statistically significant difference in litter sex ratio between insecticide-treated and control females suggests that female Franklin's ground squirrels manipulate litter sex ratio in response to costs imposed by ectoparasite pressure in accord with the predictions of the Trivers-Willard model for adaptive sex allocation. These findings broaden the comparative knowledge on the potential costs of ectoparasite infestations to include the least

social ground squirrel species investigated to date. Further and more comprehensive investigation into the spatial and social organization of Franklin's ground squirrels is necessary to empirically address how sociality may influence the expression of adaptive sex allocation.

Introduction

Life-history theory posits that organisms allocate finite resources among sometimes competing physiological systems to maximize fitness (Stearns 1976). Trade-offs between reproduction and immune function arise across taxa, evidenced by negative associations between reproductive output and various immune responses (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000). Decreased reproductive success sometimes follows elevated immune response (Rivera et al. 1998; Ilmonen et al. 2000; Cox et al. 2010; but see Williams et al. 1999), while depressed immune response may accompany increased reproduction (Deerenberg et al. 1997; Nordling et al. 1998; Ilmonen et al. 2002; Cox et al. 2010; but see Scantlebury et al. 2010). Similar negative associations have been identified between immune defence and growth (Nestor et al. 1996; Bayyari et al. 1997; Ots et al. 2001), suggesting that investment in immune function comes at the expense of other life history components.

Increased immune activity in the face of parasitic infection, and the associated energetic, protein, and nutritional demands, is considered an indirect cost of parasitism (Zuk and Stoehr 2002; Degen 2006; Bize et al. 2010). Costs associated with immune defence may include the costs of maintenance (i.e. keeping the system in a state ready to be employed), costs of use (i.e. mounting biochemical and physiological responses underlying recognition and attack), and associated nutritional constraints (i.e. anorexia; Lochmiller and Deerenberg 2000; Zuk and

Stoerh 2002; Schmid-Hempel 2003; Viney et al. 2005). Beyond the indirect cost of resource allocation to elevated immune response, parasites may directly influence life-history traits by depleting an organism's blood, nutrient, and energy stores (Lehmann 1993; Nelson et al. 1977; Sheldon and Verhulst 1996; Degen 2006). While endoparasites may damage internal systems (Lafferty and Kuris 2009; Coulson et al. 2010; Beani et al. 2011) or compete for nutrients (Giusti et al. 2007), ectoparasites impose these costs and may inflict wounds on the integument (Easton and Krantz 1973; Wikel 1996), inject toxins (Gregson 1973; Ribeiro 1989), and spread infection or disease (Hoogstraal 1967; Wikel 1996). Both direct and indirect costs of parasitism may diminish energy that otherwise could be allocated to growth, maintenance, or reproduction.

Investigations of parasitic influences on sciurid species generally hold that parasitism negatively impacts reproduction, dam condition, or both. Correlative studies indicate that higher parasite loads are associated with reduced reproductive effort (Van Vuren 1996; Gooderham and Schulte-Hostedde 2011), juvenile survival (Arnold and Lichtenstein 1993), and adult growth (Arnold and Lichtenstein 1993; Van Vuren 1996). Manipulative investigations reducing parasite load, though scarce, also indicate that parasitism may decrease reproductive success (Neuhaus 2003; Hillegass et al. 2010; Patterson et al. 2013) and adult condition (Neuhaus 2003). However, negative impacts of parasitism on reproduction or growth are not ubiquitous across sciurid species and populations (see Scantlebury et al. 2010; Raveh et al. 2015).

Beyond litter size and mass, the influence of parasitism on dam quality and reproduction may appear in the context of litter sex ratio. Significant deviations from equal investment into male and female offspring (Fisher 1930) are expected when fitness returns differ between sexes as influenced by local mate competition (Hamilton 1967), broader resource competition and enhancement (Clark 1978; Gowaty and Lennartz 1985), or maternal quality (Trivers and Willard

1973). Seminal empirical field-investigation involving food-supplementation in Virginia opossums demonstrated that females are capable of manipulating offspring sex ratio in response to experimentally increased body condition (Austad and Sunquist 1986). Early mammalian laboratory and field studies corroborated the finding that females may bias the allocation of resources to offspring sex according to dam quality (Labov et al. 1986; Meikle et al. 1993; Moses et al. 1995). Recently, Ryan et al. (2012, 2014) demonstrated that maternal condition, garnered from glucocorticoid-evidenced stress levels, influences litter sex ratio in Richardson's ground squirrels. These findings also highlight the complexity introduced to maternal condition-based adaptive sex allocation theory by trade-offs that may occur between litter size and litter sex ratio in polytocous species. Consideration of litter sex ratio in investigations that manipulate maternal quality through parasite load remain rare (Kankova et al. 2007; Hillegass et al. 2010), yet prove insightful.

We performed a manipulative field experiment exploring the influence of ectoparasites on life history traits of Franklin's ground squirrels (*Poliocitellus franklinii*). We experimentally reduced ectoparasite burdens on reproductive females following breeding, and contrasted body mass and reproductive performance (size, mass and sex ratio of juveniles produced) of those individuals to sham-treated control females. In that manipulative investigations of ectoparasite impacts on ground squirrels are rare, this study broadens the comparative picture on the potential costs of such infection to include the least social ground squirrel species investigated to date.

Methods

Study area and animals

Research on Franklin's ground squirrels was conducted from 27 April through 30 July 2014 at 50°9'N, 98°21'W near Delta Marsh, Manitoba, Canada. The study site consisted of an approximately 1 km^2 area comprised of hayfield, crop fields, mixed deciduous forest, marsh edge and rural roadsides (for details of the geology, biogeography, and ecology of the area see Love and Love 1954; Shay 1999).

Franklin's ground squirrels are obligate hibernators. In Manitoba, males emerge from hibernacula as early as the end of April, one to two weeks before females. Mating occurs from the time of female emergence in early May through early June (Iverson and Turner 1972; Sowls 1948; Chapter 2). Gestation lasts 28 d (Choromanski-Norris et al. 1986) and lactation lasts up to 31 d (Turner et al. 1976). Average litter size ranges from 6.3 (Chapter 2) to 9.4 (Iverson and Turner 1972), and juvenile males are roughly 5 g heavier than juvenile females at emergence (Chapter 2). Adult males immerge into hibernation burrows as early as late July, followed by adult females in late August, and juveniles who remain active above ground as late as early October (Iverson and Turner 1972; Sowls 1948).

Franklin's ground squirrels are hosts to numerous ectoparasite species (Galloway and Christie 1990). The flea *Opisocrostic bruneri* is primarily associated with Franklin's ground squirrels in Manitoba, with peak density of this species in early May around the time of adult emergence, and again in late August to early September (Reichardt and Galloway 1994). Franklin's ground squirrels are also hosts to at least seven other flea species, as well as accidental hosts to multiple flea species associated with other mammalian species (Galloway and Christie 1990).

Trapping and handling of squirrels

We trapped Franklin's ground squirrels using National and Tomahawk (Tomahawk Live Trap, Tomahawk, WI) live traps baited with peanut butter and rolled oats. To protect squirrels from overheating in intense sun, we covered all traps with corrugated plastic. Upon initial capture we marked all squirrels with a unique metal ear tag (Monel #1, National Band & Tag Co., Newport, KY) for permanent individual identification and with a unique dye mark on their dorsal pelage for visual identification (Pearl Black #52, Clairol Hydrience™, Procter and Gamble Co., Stamford, CT). We checked traps hourly to limit animal stress and re-applied dye marks as needed throughout the season. In the context of a concurrent study on nest relocation movements (Chapter 3), we attached radio transmitters (model PD-2C, Holohil Systems Ltd., Carp, ON), which housed Passive Integrated Transponder (PIT) tags (14 mm Avid DNA Identification System, Avid Identification Systems, Inc., Norco, CA), to all adult female squirrels. Upon each capture, we weighed squirrels to the nearest 5 g (Pesola™ spring scale, Baar, Switzerland). Any ticks we encountered on squirrels were removed upon discovery during handling, as they accumulated disproportionally on and around traps and were not the focus of our ectoparasite quantification.

We assessed squirrels for reproductive status and assumed breeding date based on observation of genitalia as described by Murie and Harris (1982). We estimated parturition based on a 28 d gestation period (Choromanski-Norris et al. 1986) and observed weight loss > 40 g over a 24 hr period. We thereafter estimated juvenile emergence based on a 31 d lactation period (Turner et al. 1976), and began daily monitoring of nests within at least 2 d of estimated juvenile emergence so as to trap young of the year as soon as possible. We sexed, marked, and weighed

all juveniles upon capture, and completed full litter assessment within 5 d of initial emergence for all litters.

Measurement and manipulation of flea infestation

We systematically assigned female squirrels into control (n = 7 females) and insecticide-treatment (n = 7 females) groups so as to balance the assignment of breeding females spatially across the study area, and to avoid any systematic bias in the mass of females comprising the two treatment groups. As outlined in Chapter 3, we treated squirrels with an experimental (anti-parasite product: FRONTLINE™ Spray, Merial Limited, Duluth, GA, fipronil 0.29%,) or a sham-control (water-only) spray early in the season prior to estimated parturition (as estimated above), and again 21-30 d later.

Prior to applying the experimental or control treatment, we quantified relative flea abundance on all female squirrels. This quantification followed methods similar to those described by Patterson et al. (2013) with a search of the ventral pelage for 60 s using a metal flea comb, stroking the fur opposite to the direction of hair growth. We then spent an additional 60 s searching for fleas in the dorsal pelage in the same manner, paying particular attention to the shoulder, groin, and "armpit" regions, as fleas often accumulate in these areas (Patterson et al. 2013).

We removed any fleas encountered during quantification from squirrels in the insecticide-treatment group by combing fleas into a petri dish held under the squirrel containing isopropyl alcohol (70%). We did not remove fleas from squirrels in the control group. Though it is possible that the same flea could have been counted twice on control squirrels, this was likely a rare occurrence, given both the rapid and systematic nature of the search. The same observer

(EP) conducted flea counts to reduce bias in flea abundance quantification (except in one instance during the field assistant training period).

Directly following flea quantification, we applied a topical insecticide, fipronil (FRONTLINE™ Spray), to squirrels in the insecticide-treatment group. Fipronil is classified as a broad-spectrum phenylpyrazole insecticide and is used to control a wide range of insects by blocking chloride channels in the central nervous system (Fipronil Technical Fact Sheet, Merial Limited, Duluth, GA). We administered fipronil to squirrels by spraying 1.5 ml of FRONTLINE™ Spray on the dorsal pelage of squirrels as described by Hillegass et al. (2010). To control for a possible response to the spray manipulation itself, we sprayed control females with approximately 1.5 ml of ambient temperature tap water, thus ensuring squirrels in both the insecticide-treated and control groups were handled in the same manner.

Statistical analysis

We used the nonparametric Wilcoxon signed-rank test to compare relative flea abundance before and after initial treatment within the control and insecticide-treated groups, and the Mann-Whitney U test to compare relative flea densities after initial treatment between groups. Comparisons between control and insecticide treatment groups for dam mass gain, average juvenile mass, total litter mass, and litter sex ratio (calculated as the proportion of males to total litter size at juvenile emergence) were tested using two-sample *t*-tests in that the parametric assumptions of normality and homoscedasticity were met for those variables. We used the Mann-Whitney U test to analyze comparisons of litter size, average male juvenile mass, and average female juvenile mass between groups in that those data failed to meet the parametric assumptions.

Dam reproductive mass gain was calculated by subtracting dam breeding mass from dam mass at the time of juvenile emergence. Breeding mass was considered the mass measurement taken within 5 d of and closest to, but not before, the estimated breeding date. We considered mass at juvenile emergence as the mass measurement taken within 7 d of and closest to, but not after, juvenile emergence.

Data were assessed for normality and homoscedasticity using Shapiro-Wilk normality tests and Levene's tests (respectively), as well as visual inspection of the data. Summary values are reported as mean $\pm$ 1 SE. All statistics were performed using R software (version 3.0.2, R Development Core Team, 2013). We report actual significance levels from statistical tests except where $P < 0.001$, where we simply report that range.

Results

Flea manipulation and quantification

Relative flea abundance on insecticide-treated squirrels was successfully reduced from the time of initial treatment to second treatment (Wilcoxon signed-rank test, $n = 7$, $V = 21$, $p = 0.015$; Figure 3.4). No significant reduction in relative flea abundance was observed among squirrels receiving the control treatment (Wilcoxon signed-rank test, $n = 7$, $V = 4.5$, $p = 0.829$; Figure 3.4). Relative flea abundance was significantly greater on control squirrels than on insecticide-treated squirrels at the time of second treatment (Mann-Whitney U test, $n_{treatment} = 7$, $n_{control} = 7$, $W = 44.5$, $p = 0.005$; Figure 3.4), but there was no difference in relative flea abundance between groups prior to initial treatments (Mann-Whitney U test, $n_{treatment} = 7$, $n_{control} = 7$, $W = 33$, $p = 0.272$, Figure 3.4).

Maternal mass and litter attributes

No difference in mass gain was detected between the insecticide-treated and control groups over the reproductive period, despite a trend tending toward greater mass gain among the insecticide-treated females (two-sample t-test, $t_9 = 1.066$, p = 0.157; Figure 4.1). Litters belonging to control and insecticide-treated females did not differ in average juvenile mass (two-sample t-test, $t_{12} = 0.420$, p = 0.341) or total litter mass at the time of juvenile emergence (Figure 4.1b; two-sample t-test, $t_{12} = -0.124$, p = 0.548; Figure 4.2). Likewise, there was no difference in litter size between females of insecticide-treated and control groups (Mann-Whitney U test, $n_{treatment}=7$, $n_{control} = 7$, $W = 18$, p = 0.822; Figure 4.3). Litter sex ratio was significantly greater among litters belonging to insecticide-treated females reflecting litters with a proportionally greater male bias (two-sample t test, $t_{12} = 3.974$, p = 0.002; Figure 4.4); however, neither male nor female juveniles of insecticide-treated females weighed more than juveniles of control-treated females at emergence from the natal burrow (males: Mann-Whitney U test, $n_{treatment} = 7$, $n_{control} = 7$, $W = 31.5$, p = 0.203; females: Mann-Whitney U test, $n_{treatment} = 7$, $n_{control} = 7$, $W = 32.5$, p = 0.169).

Discussion

Increased ectoparasite abundance did not negatively affect maternal mass gain or current reproductive effort in this study; however, sample sizes were small, and thus trends should be interpreted cautiously. Among sciurid species studied to date, female body mass is sometimes influenced by parasitism (Van Vuren 1996; Nuehaus 2003; Scantlebury et al. 2010), though such a trend is not ubiquitous (Hillegass et al. 2010; Raveh et al. 2015). Where dams maintain optimal

body condition under increased parasite abundance, we might expect a decrease in offspring investment (Patterson et al. 2013). However, we observed no difference in litter size or juvenile mass (either total litter mass or average juvenile mass) at emergence between litters belonging to control and insecticide-treated dams.

Lack of effect on maternal mass or current reproductive output may reflect the fact that the costs from ectoparasitism are minor, or only significant at parasite levels higher than those observed on this population during the reproductive season. Franklin's ground squirrels may be able to compensate for any slight costs of ectoparasitism during lactation and gestation with an influx of resources available during the active season (Schmid-Hempel 2003; Raveh et al. 2015), or sufficiently combat encountered ectoparasite burdens behaviourally (behavioural parasite avoidance through nest relocation, Hart 1994; Chapter 3). Reichardt (1989) found that flea densities on adult Franklin's ground squirrel females were lowest during the period corresponding to gestation and lactation, which comprised the bulk of this investigation, suggesting that ectoparasites may impose greater costs following spring emergence and preceding late summer immergence when flea densities are greatest. Additionally, Møller et al. (2001) conducted a comparative analysis on bird species in the family Hirundinidae and found that social species invested more heavily into immune function. As Franklin's ground squirrels are relatively asocial as compared to the ground squirrel species studied to date in which parasite effects were apparent (Arnold and Lichtenstein 1993; Van Vuren 1996; Nuehaus 2003; Hillegass et al. 2010), it is possible that Franklin's ground squirrels have evolved fewer mechanisms for allocating resources to immune function. Thus, any life history trade-offs with immune function, as evidenced by mass gain or reproductive effort, may not be as great.

Alternatively, it is possible that negative effects on Franklin's ground squirrels were present in this study even though they were not measurably manifested within-season. While investigating reproductive costs in female Columbian's ground squirrels (*Urocitellus columbians*), Skibiel et al. (2013) found no mass differences between females of litter augmented and control groups at the time of weaning, but did note emergent mass differences between groups the following season. While Skibiel et al. (2013) investigated costs of reproduction rather than costs of parasitism, the delayed mass differences they, and others (Hare and Murie 1992), observed indicate that body condition may be impacted even when effects are not observed within-season. Though body mass has been found to be an acceptable indicator of body fat, and thus body condition, in sciurid species (Becker 1992; Humphries and Boutin 1999), it is possible that costs of parasitism are simply not discernible by external metrics given potential disparate effects on different internal systems, or are detectable only as they accumulate over the long term (Zuk and Stoehr 2002; Kristan 2004; Scantlebury et al. 2010). As mass is not the sole measure of juvenile quality, juvenile survival might be a more informative measure in assessing the influence of parasitism on dam fitness (Arnold and Lichtenstein 1993; Van Vuren 1996; Patterson et al. 2013), and as Franklin's ground squirrels are an iteroparous species, we cannot rule out the possibility that effects of parasitism are observed in subsequent litters (Kristan 2002).

The significant difference in litter sex ratio between control and insecticide-treated females indicates that Franklin's ground squirrel reproduction is influenced by parasitism. Dams in the insecticide-treated group biased investment in offspring towards males, as predicted under the Trivers-Willard model of adaptive sex allocation (TWM, Trivers and Willard 1973). Trivers and Willard (1973) posit that in polygynous species, where male offspring are generally more

costly than female offspring, where males typically have more variable reproductive success than females, and thus where fitness returns of producing high-quality male offspring likely exceed those of producing high-quality female offspring, dams in good condition will bias investment towards male offspring, while dams in poorer condition will bias investment towards female offspring (Trivers and Willard 1973; Cameron and Linklater 2002). Though litters of both control and insecticide-treated groups fall within the range of what can be considered litter sex ratio parity (Gedir and Michener 2014), the significant difference between groups nonetheless supports the notion of adaptive sex ratio allocation.

Empirical support in favor of adaptive sex ratio allocation, particularly the TWM, has been extensively reviewed and investigated in a broad range of taxa (Charnov 1982; Clutton-Brock and Iason 1986; Frank 1990; Hardy 1997). There has been mixed support for the TWM in ground squirrels (Ryan et al. 2012, 2014; Gedir and Michener 2014). Compared to more social Marmotini, relatively asocial Franklin's ground squirrels are more likely to conform to the predictions of the TWM. More social species tend to be associated with tighter matrilineal units (Armitage 1981; Michener 1983, 1984; Rayor and Armitage 1991; Hare and Murie 2007), wherein maternal transmission of territory or rank may occur (Harris and Murie 1984). Within these species, if high quality dams pass on higher quality territory or rank to daughters, these daughters may then produce higher quality progeny ("advantaged matriline", Leimar 1996; Hewison and Gaillard 1999; Ryan et al. 2012). That is, investment in high quality daughters may actually lead to equal or greater fitness rewards as investing in high quality males, thus failing an assumption of the TWM in its cardinal form. This interpretation is consistent with the findings of Ryan et al. (2012; 2014) who reported that stressed dams produced smaller, more male-biased litters than dams that were subject to lower stress levels, as measured by fecal glucocorticoid

concentrations (Hare et al. 2014). Further, Michener (1980) found that just two females were responsible for 67% of female Richardson's ground squirrels (*Urocitellus richardsonii*) on her study site, highlighting exceptional variability in reproductive success among females. In a study comparing reproductive success of male and female Columbian ground squirrels (*Urocitellus columbianus*), Jones et al. (2012) further demonstrated that variability in reproductive success was generally balanced between sexes, failing another cardinal TWM assumption. Finally, in more social species where dams and daughters demonstrate significant space-use overlap (Rayor and Armitage 1991), post-weaning investment in daughters may be great enough to balance the higher pre-weaning investment required for sons (Gedir and Michener 2014), again failing cardinal TWM assumptions.

In less social species, where maternal transmission of rank or territory is not as prevalent or complete, the fitness benefits of investing in high quality males may indeed outweigh those of investing in high quality females, as the TWM assumes. While the per capita litter sex ratio of a more social ground squirrel species is evidently unaffected by parasitism (Cape ground squirrel, *Xerus inauris*: Hillegass et al. 2010), the more male-biased sex ratio of insecticide-treated Franklin's ground squirrels in this study is consistent with the original formulation of the Trivers-Willard hypothesis. Kankova et al. (2007) likewise provided evidence for a more female-biased litter sex ratio in parasitized laboratory mice as compared to unparasitized controls. Further and more comprehensive investigation into the spatial and social organization of Franklin's ground squirrels and other comparable species are necessary to empirically address how the extent of sociality may influence the expression of adaptive sex allocation.

Potential limitations of the present study include the limited scope of parasite diversity. Previous studies have shown parasite species richness as well as parasite intensity are important

when assessing effects of parasitism (Gooderham and Shulte-Hostedde 2011); however, the anti-parasite treatment used in this study removed only ectoparasites from treated squirrels, and my quantification of ectoparasite load focused solely on fleas. The parasite-host system examined is more complex than reported here as type and intensity of immune response, and ensuing costs, may differ among species of parasites (Degen 2006; Hillegass et al. 2008; Manjerovic and Waterman 2012; Waterman et al. 2014). Little is known about what endoparasites Franklin's ground squirrels harbour (Tobon et al. 1976; Durham et al. 1988; Jardine et al. 2005), let alone their possible influence on the immune system and other life-history traits, thus presenting a promising avenue for future study.

While small samples are prone to stochastic variation and spurious results, the number of females used in this study was necessarily small given the limited number of squirrels comprising the study population, and finite limits on the number of individuals that could reliably be tracked throughout the above-ground season. Cautious interpretation is thus warranted; however, the results of the present study do not support the hypothesis that dam growth and reproduction trade-off with ectoparasite defence at the infestation levels that prevailed among subjects in the summer of 2014. Taken at face value, the present findings suggest that dams compensate for any costs of immune response with elevated resource intake, or adequately manage infestation levels through non-immune defence (i.e. behavioural parasite avoidance; Chapter 3). A statistically significant difference in litter sex ratio between insecticide-treated and control females suggests, however, that female Franklin's ground squirrels manipulate litter sex ratio in response to costs imposed by ectoparasite pressure in accord with the predictions of the Trivers-Willard model for adaptive sex allocation.

Literature Cited

Arnold, W., and A.V. Lichtenstein. 1993. Ectoparasite loads decrease the fitness of alpine marmots (*Marmota marmota*) but are not a cost of sociality. Behavioral Ecology 4: 36–39.

Armitage, K.B. 1981. Sociality as a life-history tactic of ground squirrels. Oecologia 48:36-49.

Austad, S.N., and M.E. Sunquist. 1986. Sex-ratio manipulation in the common opossum. Nature 324: 58-60.

Bayyari, G.R., W.E. Huff, N.C. Rath, J.M. Balog, L.A. Newberry, J.D. Villines, J.K. Skeeles, N.B. Anthony, and K.E. Nestor. 1997. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poultry Science 76: 289-296.

Beani, L., R. Dallai, D. Mercati, F. Cappa, F. Giusti, and F. Manfredini. 2011. When a parasite breaks all the rules of a colony: morphology and fate of wasps infected by a strepsipteran endoparasite. Animal Behaviour 82: 1305-1312.

Becker, C.D. 1992. Proximate factors influencing the timing and occurrence of reproduction in red squirrels *Tamiasciurus hudsonicus*. Ph.D. Thesis, University of Alberta, Edmonton, Canada.

Bize, P., R. Piault, J. Gasparini, and A. Roulin. 2010. Indirect costs of parasitism are shaped by variation in the type of immune challenge and food availability. Evolutionary Biology 37: 169-176.

Cameron, E.Z., and W.L. Linklater. 2002. Sex bias in studies of sex bias: the value of daughters to mothers in poor condition. Animal Behaviour 63: F5-F8.

Charnov, E.L. 1982. The Theory of Sex Allocation. Princeton University Press, Princeton, New Jersey, USA.

Choromanski-Norris, J.F., K. Fritzell, and A.B. Sargeant. 1986. Seasonal activity cycle and weight changes of the Franklin's ground squirrel. American Midland Naturalist 116: 101-107.

Clark, A.B. 1978. Sex ratio and local resource competition in a prosimian primate. Science 201: 163-165.

Clutton-Brock, T.H., and G.R. Iason. 1986. Sex ratio variation in mammals. Quarterly Review of Biology 61: 339-374.

Coulson, J.O., S.J. Taft, and T.D. Coulson. 2010. Gastrointestinal parasites of the swallow-tailed kite (*Elanoides forficatus*), including a report of lesions associated with the nematode *Dispharynx* sp. Journal of Raptor Research 44: 208-214.

Cox, R.M., E.U. Parker, D.M. Cheney, A.L. Liebl, L.B. Martin, and R. Calsbeek. 2010. Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. Functional Ecology 6: 1262-1269.

Deerenberg, C., V. Apanius, S. Daan, and N. Bos. 1997. Reproductive effort decreases antibody responsiveness. Proceedings of the Royal Society B: Biological Sciences 264: 1021-1029.

Degen, A.A. 2006. Effect of macroparasites on the energy budget of small mammals, pp. 371-399. In: S. Morand, B.R. Krasnov, and R. Poulin, editors. Micromammals and Macroparasites: From Evolutionary Ecology to Management. Springer, Tokyo, Japan.

Durham, P.J.K., F.A. Leighton, and G.A. Wobeser. 1988. An adenovirus infection of the kidney of Franklin's ground squirrels (*Spermophilus franklinii*) in Saskatchewan, Canada. Journal of Wildlife Diseases 24: 636-641.

Easton, E.R., and G.W. Krantz. 1973. A *Euschoengastia* species (Acari: Trombiculidae) of possible medical and veterinary importance in Oregon. Journal of Medical Entomology 10: 225-226.

Fisher, R.A. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford, UK.

Frank, S.A. 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13-55.

Galloway, T.D., and J.E. Christie. 1990. Fleas *(*Siphonaptera*)* associated with ground squirrels (*Spermophilus* spp.*)* in Manitoba, Canada. Canadian Entomologist 122: 449-458.

Gedir, J.V., and G.R. Michener. 2014. Litter sex ratios in Richardson's ground squirrels: long-term data support random sex allocation and homeostasis. Oecologia 174: 1225-1239.

Giusti, F., L. Dallai, L. Beani, F. Manfredini, and R. Dallai. 2007. The midgut ultrastructure of the endoparasite *Xenos vesparum* (Rossi) (Insecta, Strepsiptera) during post-embryonic development and stable carbon isotopic analyses of the nutrient uptake. Arthropod Structure and Development 36: 183-197.

Gooderham, K., and A. Schulte-Hostedde. 2011. Macroparasitism influences reproductive success in red squirrels (*Tamiasciurus hudsonicus*). Behavioral Ecology 22: 1195-1200.

Gowaty, P.A., and M.R. Lennartz. 1985. Sex ratios of nestling and fledgling red-cockaded woodpeckers (*Picoides borealis*) favor males. American Naturalist 126: 347-353.

Gregson, J.D. 1973. Tick paralysis: an appraisal of natural and experimental data. Canadian

    Department of Agriculture Monograph 9: 1-109.

Hamilton, W.D. 1967. Extraordinary sex ratios. Science 156: 477-488.

Hardy, I.C.W. 1997. Possible factors influencing vertebrate sex ratios: an introductory overview.

    Applied Animal Behaviour Science 51: 217 241.

Hare, J.F., and J.O. Murie. 1992. Manipulation of litter size reveals no cost of reproduction in

    Columbian ground squirrels. Journal of Mammalogy 73: 449-454.

Hare, J. F., and J.O. Murie. 2007. Ecology, kinship, and ground-squirrel sociality: insights from

    comparative analyses, pp. 345-355. In: J.O. Wolff, and P.W. Sherman, editors. Rodent

    Societies: An Ecological and Evolutionary Perspective. Oxford University Press,

    Oxford, UK.

Hare, J.F., C.P. Ryan, C. Enright, L.E. Gardiner, L.J. Skyner, C.N. Berkvens, and W.G.

    Anderson. 2014. Validation of a radioimmunoassay-based fecal corticosteroid assay for

    Richardson's ground squirrels (*Urocitellus richardsonii*) and behavioural correlates of stress.

    Current Zoology 60: 591-601.

Harris, M.A., and J.O. Murie. 1984. Inheritance of nest sites in female Columbian ground

    squirrels. Behavioral Ecology and Sociobiology 15: 97-102.

Hart, B.L. 1994. Behavioural defense against parasites: interaction with parasite invasiveness.

    Parasitology 109: 139-151.

Hewison, A.J.M., and J.M. Gaillard. 1999. Successful sons or advantaged daughters? The Trivers-Willard model and sex-biased maternal investment in ungulates. Trends in Ecology and Evolution 14: 229-234.

Hillegass, M.A., J.M. Waterman, and J.D. Roth. 2008. The influence of sex and sociality on parasite loads in an African ground squirrel. Behavioral Ecology 19: 1006-1011.

Hillegass, M.A., J.M. Waterman, and J.D. Roth. 2010. Parasite removal increases reproductive success in a social African ground squirrel. Behavioral Ecology 21: 696-700.

Hoogstraal, H. 1967. Ticks in relation to human diseases caused by *Rickettsia* species. Annual Review of Entomology 12: 377-420.

Humphries, M.M., and S. Boutin. 1999. Mass-dependent reproduction or reproduction-dependent mass? A comment on body mass and first-time reproduction in female sciurids. Canadian Journal of Zoology 77: 171-173.

Ilmonen, P., T. Taarna, and D. Hasselquist. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proceedings of the Royal Society B: Biological Sciences 267: 663-670.

Ilmonen, P., T. Taarna, and D. Hasselquist. 2002. Are incubation costs in female pied flycatchers expressed in humoral immune responsiveness or breeding success? Oecologia 130: 199-204.

Iverson, S.L., and B.N. Turner. 1972. Natural history of a Manitoba population of Franklin's ground squirrels. Canadian Field Naturalist 86: 145-149.

Jardine, C., G. Appleyard, M.Y. Kosoy, D. McColl, M. Chirino-Trejo, G. Wobeser, and F.A. Leighton. 2005. Rodent-associated Bartonella in Saskatchewan, Canada. Vector-Borne and Zoonotic Diseases 5: 402-409.

Jones, P.H., J.L. Van Zant, and S.F. Dobson. 2012. Variation in reproductive success of male and female Columbian ground squirrels (*Urocitellus columbianus*). Canadian Journal of Zoology 90: 736-743.

Kankova, S., P. Kodymn, D. Frynta, R. Vavrinova, A. Kubena, and J. Flegr. 2007. Influence of latent toxoplasmosis on the secondary sex ratio in mice. Parasitology 134: 1709-1717.

Kristan, D.M. 2002. Effects of intestinal nematodes during lactation: consequences for host morphology, physiology and offspring mass. Journal of Experimental Biology 205: 3955-3965.

Kristan, D.M. 2004. Intestinal nematode infection affects host life history and offspring susceptibility to parasitism. Functional Ecology 73: 227-238.

Labov, J.B., U.W. Huck, P. Vaswani, and R.D. Lisk. 1986. Sex ratio manipulation and decreased growth of male offspring of undernourished golden hamsters (*Mesocricetus auratus*). Behavioral Ecology and Sociobiology 18: 241-249.

Lafferty, K.D., and A.M. Kuris. 2009. Parasitic castration: the evolution of body snatchers. Trends in Parasitology 25: 564-572.

Lehmann, T. 1993. Ectoparasites: direct impact of host fitness. Parasitology Today 9: 8-13.

Leimar, O. 1996. Life-history analysis of the Trivers and Willard sex-ratio problem. Behavioral Ecology 7: 316-325.

Lochmiller, R.L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88: 87-98.

Love, A., and D. Love. 1954. Vegetation of a prairie marsh. Bulletin of the Torrey Botanical Club 81: 16-34.

Manjerovic, M.B., and J.M. Waterman. 2012. Immunological sex differences in socially promiscuous African ground squirrels. PLoS ONE 7: e38524.

Meikle, D.B., L.C. Drickamer, S.H. Vessey, T.L. Rosenthal, and K.S. Fitzgerald. 1993. Maternal dominance rank and secondary sex ratio in domestic swine. Animal Behaviour 46: 79-85.

Michener, G.R. 1980. Differential reproduction among female Richardson's ground squirrels and its relation to sex ratio. Behavioral Ecology and Sociobiology 7: 173-178.

Michener, G.R. 1983. Kin identification, matriarchies, and the evolution of sociality in ground-dwelling sciurids, pp. 528-572. In: J.F. Eisenberg, and D.G. Kleiman, editors. Advances in the Study of Mammalian Behavior. American Society of Mammalogists Special Publication No. 7.

Michener, G.R. 1984. Age, sex, and species differences in the annual cycles of ground-dwelling sciurids: implications for sociality, pp. 81-107. In: J.O. Murie and G.R. Michener, editors. The Biology of Ground-Dwelling Squirrels. University of Nebraska Press, Lincoln, USA.

Møller, A.P., S. Merino, C.R. Brown, and R.J. Robertson. 2001. Immune defense and host sociality: a comparative study of swallows and martins. The American Naturalist 158: 136-145.

Moses, R.A., G.J. Hickling, and J.S. Millar. 1995. Variation in sex ratios of offspring in wild bushy-tailed woodrats. Journal of Mammalogy 76: 1047-1055.

Murie, J.O., and M.A. Harris. 1982. Annual variation of spring emergence and breeding in Columbian ground squirrels (*Spermophilus columbianus*). Journal of Mammalogy 63: 431-439.

Nelson, W.A., J.F. Bell, C.M. Clifford, and J.E. Keirans. 1977. Interaction of ectoparasites and their hosts. Journal of Medical Entomology 13: 389-428.

Nestor, K.E., D.O. Noble, J. Zhu, and Y. Moritsu. 1996. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. Poultry Science 75: 1180-1191.

Neuhaus, P. 2003. Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (Spermophilus columbianus). Biology Letters 270: 213-215.

Nordling, D., M. Andersson, S. Zohari, and L. Gustafsson. 1998. Reproductive effort reduces specific immune response and parasite resistance. Proceedings of the Royal Society B: Biological Sciences 265: 1291-1298.

Ots, I., A.B. Kerimov, E.V. Ivankina, T.A. Ilyina, and P. Horak. 2001. Immune challenge affects basal metabolic activity in wintering great tits. Proceedings of the Royal Society B: Biological Sciences 268: 1175-1181.

Patterson, J.E., P. Nuehaus, S.J. Kutz, and K.E. Ruckstuhl. 2013. Parasite removal improves reproductive success of female North American red squirrels (*Tamiasciurus hudsonicus*). PLoS ONE 8: e55779.

R Development Core Team. 2013. R: a language and environment for statistical computing. Vienna, Austria.

Raveh, S., P. Nuehaus, and F.S. Dobson. 2015. Ectoparasites and fitness of female Columbian ground squirrels. Philosophical Transactions of the Royal Society B: Biological Sciences 370: 20140113.

Rayor, L.S., and K.B. Armitage. 1991. Social behavior and space-use of young of ground-dwelling squirrel species with different levels of sociality. Ethology Ecology and Evolution 3: 185-205.

Reichardt, T.R. 1989. The seasonal dynamics and host-parasite relationship of *Opisocrostis bruneri* (Baker), a flea on Franklin's ground squirrel (*Spermophilus franklinii)* (Sabine) near Birds Hill Park, Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, Canada.

Reichardt, T.R., and T.D. Galloway. 1994. Seasonal occurrence and reproductive status of *Opisocrostis bruneri* (Siphonaptera: Ceratophyllidae), a flea on Franklin's ground squirrel, *Spermophilus franklinii* (Rodentia: Sciuridae) near Birds Hill Park, Manitoba. Journal of Medical Entomology 31: 105-113.

Ribeiro, J.M.C. 1989. Role of saliva in tick host interactions. Experimental and Applied Acarology 7: 15-20.

Rivera, D. L., S.M. Olister, X. Liu, J.H. Thompson, X.J. Zhang, K. Pennline, R. Azuero, D.A. Clark, and M.J.S. Miller. 1998. Interleukin-10 attenuates experimental fetal growth restriction and demise. FASEB Journal 12: 189-197.

Ryan, C.P., W.G. Anderson, L.E. Gardiner, and J.F. Hare. 2012. Stress-induced sex ratios in ground squirrels: support for a mechanistic hypothesis. Behavioral Ecology 23: 160-167.

Ryan, C.P., W.G. Anderson, C.N. Berkvens, and J.F. Hare. 2014. Maternal gestational cortisol and testosterone are associated with trade-offs in offspring sex and number in a free-living rodent (*Urocitellus richardsonii*). PLoS ONE 9: e111052.

Scantlebury, M., M. Maher McWilliams, N.J. Marks, J.T.A. Dick, H. Edgar, and H. Lutermann. 2010. Effects of life-history traits on parasite load in grey squirrels. Journal of Zoology 282: 246-255.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society B: Biological Sciences 270: 357-366.

Shay, J.M. 1999. Annotated vascular plant species list for the Delta Marsh, Manitoba and surrounding area. University of Manitoba Field Station (Delta Marsh) Occasional Publication No. 2.

Sheldon, B.C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution 11: 317-321.

Skibiel, A.L., J.R. Speakman, and W.R. Hood. 2013. Testing the predictions of energy allocation decisions in the evolution of life-history trade-offs. Functional Ecology 27: 1382-1391.

Sowls, L.K. 1948. The Franklin ground squirrel, *Citellus franklinii* (Sabine), and its relationship to nesting ducks. Journal of Mammalogy 29: 113-137.

Stearns, S.C. 1976. Life-history tactics: a review of the ideas. Quarterly Review of Biology 51: 3-47.

Tobon, J.L., T.M. Yuill, and W.M. Samuel. 1976. Adiaspiromycosis in the Franklin's ground squirrel, *Spermophilus franklinii*, and pika, *Ochotona princeps*, from Alberta, Canada. Journal of Wildlife Diseases 12: 97-100.

Turner, B.N., S.L. Iverson, and K.L. Severson. 1976. Postnatal growth and development of captive Franklin's ground squirrels *(Spermophilus franklinii)*. American Midland Naturalist 95: 93-102.

Trivers, R.L., and D.E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90-92.

Van Vuren, D. 1996. Ectoparasites, fitness, and social behaviour of yellow-bellied marmots. Ethology 102: 686-694.

Viney, M.E., E.M. Riley, and K.L. Buchanan. 2005. Optimal immune responses: immunocompetence revisited. Trends in Ecology and Evolution 20: 665-669.

Waterman, J.M., G.F. Macklin, and C. Enright. 2014. Sex-biased parasitism in Richardson's ground squirrels (*Urocitellus richardsonii*) depends on the parasite examined. Canadian Journal of Zoology 92: 73-79.

Wikel, S.K. 1996. Host immunity to ticks. Annual Review of Entomology 41: 1-22.

Williams, T.D., J.K. Christians, J.J. Aiken, and M. Evanson. 1999. Enhanced immune function does not depress reproductive output. Proceedings of the Royal Society B: Biological Sciences 266: 753-757.

Zuk, M., and A.M. Stoehr. 2002. Immune defense and host life history. American Naturalist 160: S9-S22.

Tables and Figures
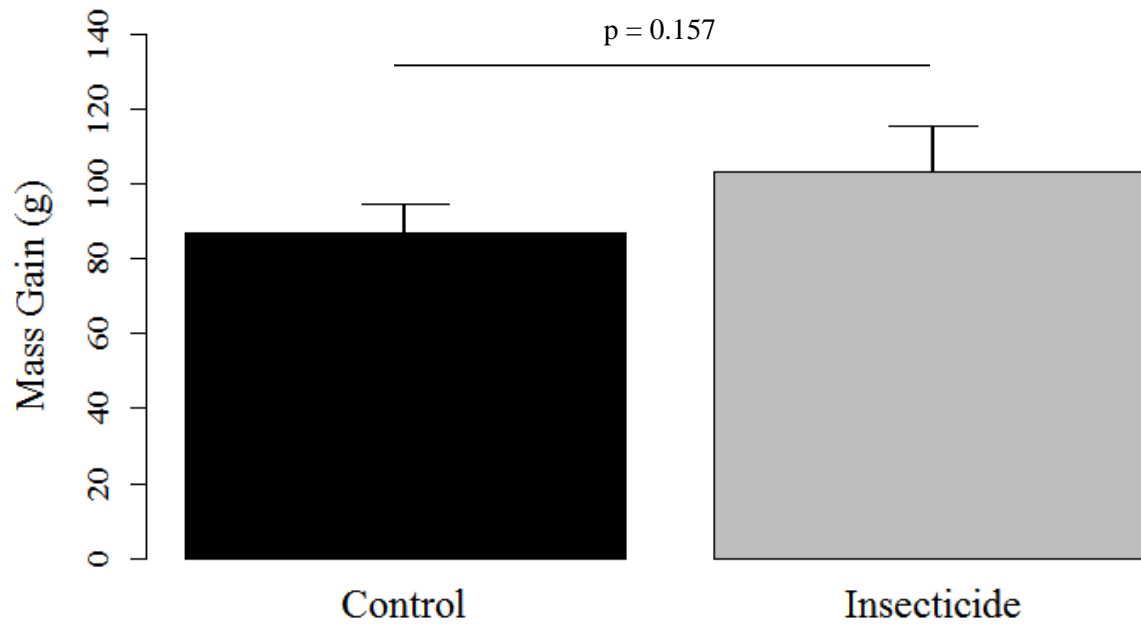
Figure 4.1. Comparison of control (n = 5) and insecticide-treated (n = 6) Franklin's ground squirrel females with regards to mass gain

(g) over the course of reproduction (see Methods for calculation) in 2014. Error bars represent SE. Student's two-sample t-test reveals

no difference between groups (t_9 = 1.066, p = 0.157).
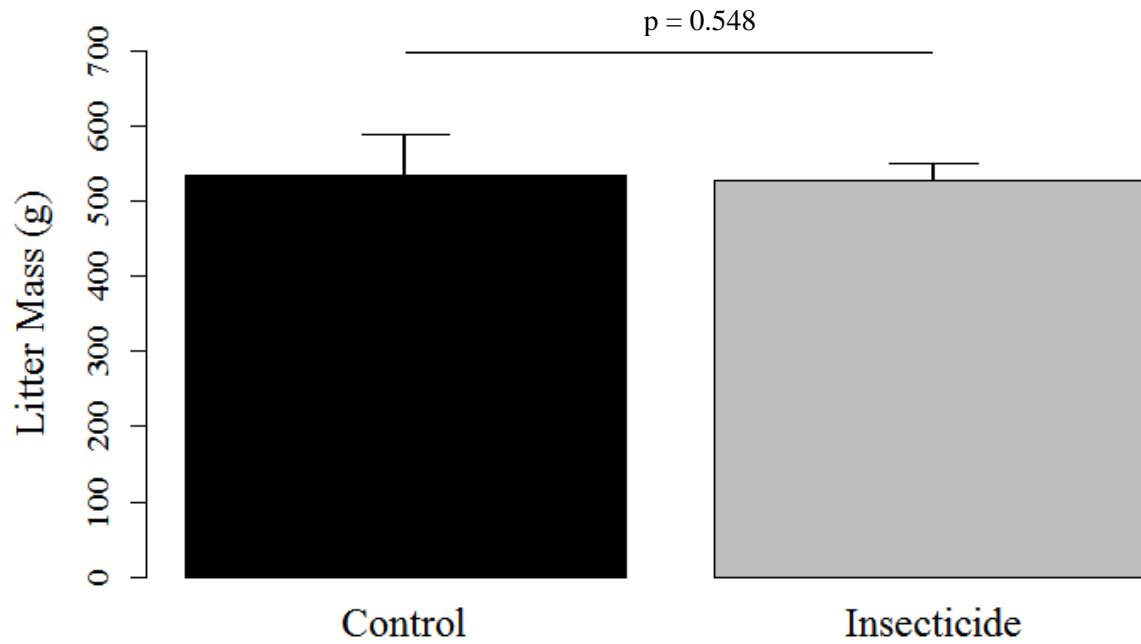
Figure 4.2. Comparison of control (n = 7) and insecticide-treated (n = 7) Franklin's ground squirrel females with regards to average total litter mass (g) in 2014. Error bars represent SE. Student's two-sample *t*-test reveals no difference between groups (t_{12} = -0.124, p = 0.548).
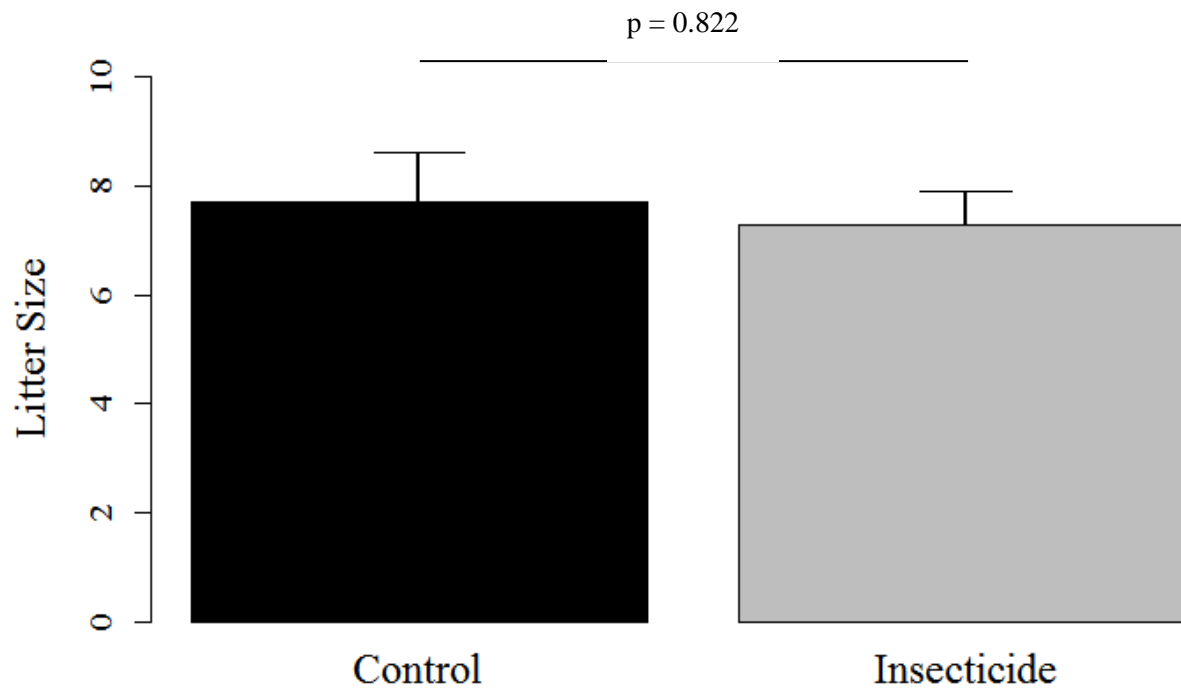
Figure 4.3. Comparison of control (n = 7) and insecticide-treated (n = 7) Franklin's ground squirrel females with regards to average total litter size at juvenile emergence in 2014. Error bars represent SE. Mann-Whitney U test reveals no difference between groups ($n_{treatment}$=7, $n_{control}$ = 7, W = 18, p = 0.822).
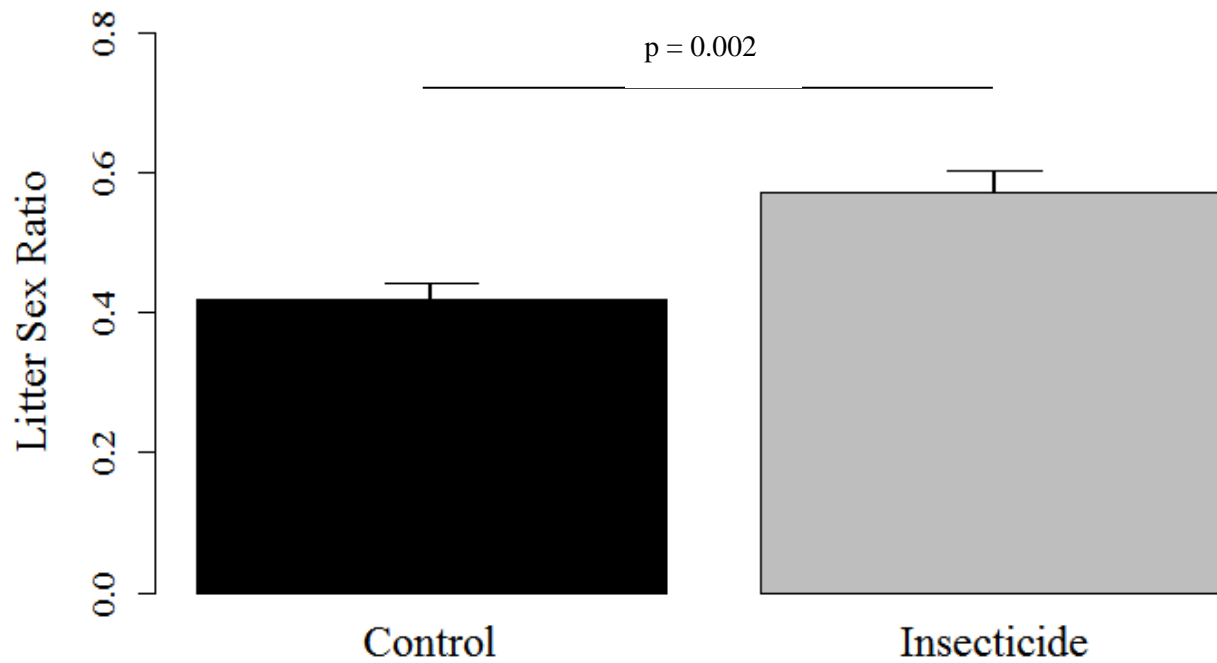
Figure 4.4. Comparison of control (n = 7) and insecticide-treated (n = 7) Franklin's ground squirrel females with regards to average litter sex ratio at juvenile emergence in 2014. Error bars represent SE. Student's two-sample *t*-test reveals that insecticide treated females produced litters that were significantly more male-biased than control females ($t_{12} = 3.974$, p = 0.002).

Chapter 5: Conclusions

The preceding chapters represent an initial step toward the work required in order to fill in information gaps pertaining to one of North America's least known ground squirrel species. In Chapter 2, I collated and analyzed four years' worth of demographic and life history data for a population of Franklin's ground squirrels located near Delta Marsh, Manitoba collected though the dedicated trapping efforts of Dr. James Hare and his students. The mass cycles we observed for adult males and females followed patterns similar to those reported for other populations of Franklin's ground squirrels, and for other hibernating ground squirrel species. While female-bias in adult sex ratio is common among ground squirrel species, adult sex ratio for populations of Franklin's ground squirrels is apparently variable. While researchers investigating populations elsewhere in Manitoba and in Alberta have reported nearly equal ratios of adult males and females, adult females outnumbered males in the Delta Marsh population by roughly 2:1, while litter sex ratios approximated parity.

Survival varied between sexes and among years in the Delta Marsh population, and low survival between 2000 and 2001 followed harmful agricultural practices and flooding, subsequently leading to a sharp population decline in 2001. The Delta Marsh population eventually crashed in 2004 but rebounded by 2014. The extreme population fluctuations observed for this Delta Marsh population reflect population variability observed elsewhere for this species. Some researchers report regular population cycles, while others report consistent declining trends. Knowledge of factors driving fluctuations and cycles awaits long-term data that will elucidate mechanisms of population regulation and metrics of population viability and persistence.

Franklin's ground squirrel motility may contribute to the difficulty experienced in studying this species and to population disappearances. In Chapter 3, I investigated nest relocation movements by lactating female Franklin's ground squirrels, and reported the results of an investigation designed to elucidate factors mediating these movements. With the exception of one lactating dam, females relocated their nest and litter to an alternative nest at least once throughout the energetically demanding period of lactation. In this context I investigated the behavioural movement response to ectoparasitism, conspecific interaction, and nesting habitat associations as possible influences on nest movements. Significant effects of all investigated factors suggest that nest relocations evolved in response to myriad factors that impact female fitness; however, further empirical investigation is required to tease apart causal versus temporally correlated factors that influence nest movement decisions by lactating Franklin's ground squirrels. To what extent, if any, these nest movements lead to population-level location shifts and ultimately to observed population disappearances from areas of historical occupancy requires long-term population monitoring.

Allocation of PIT-tags to all known adult squirrels and tag readers at nest entrances permitted the observation and quantification of conspecific burrow visitation. In addition to informing nest relocation behaviour, these data provide novel insight into intraspecific interactions among Franklin's ground squirrels. Females relocate partially in response to conspecific visitation, supporting the notion that Franklin's ground squirrels' are relatively asocial in comparison to their congeners. However, the near ubiquity and quantity of nest visits by both male and female conspecifics suggests that the characterization of Franklin's ground squirrels as solitary may, in fact, be overstated.

Females also demonstrated a difference in selection of nest sites according to habitat type between early and late season nests. These preferences appear to reflect the influence of seasonal resource use on nest selection and movements. Females appeared to base habitat selection on cover attributes, as nest sites were split between grass and forest early in the season when cultivated areas provided little cover, while late season nest selection was split between grass and cultivated field habitat types. While these assessments offer novel insight into nesting habitat associations, the habitat associations examined here are coarse. Further investigation into fine scale habitat characteristics, as well as aspects of microclimate, will provide useful information into seasonal resource use and habitat requirements, as well as further insight into nest site selection and relocation.

Finally, in context of investigating nest relocations, we successfully manipulated the relative flea abundance on a group of lactating female Franklin's ground squirrels. Such manipulations are rarely conducted on free-living animal populations and provided further insight into nest relocations and other dam responses to ectoparasitism, which I explored in Chapter 4. Despite small sample sizes, females treated with insecticide that served to lower relative ectoparasite levels tended to relocate nests less frequently. Even with relatively fewer fleas, however, all insecticide-treated individuals still relocated nests at least once throughout lactation, affirming the existence of additional factors influencing relocation decisions.

We observed no difference in dam mass, juvenile mass, or litter size between insecticide and control-treated females. These results could indicate either that ectoparasite levels experienced by females during this study were not high enough to influence body mass or litter size, that dams were able to compensate for any costs associated with ectoparasitism with elevated resource intake, or that dams adequately managed infestation levels through nest

movements. We did, however, detect a statistically significant difference in litter sex ratio between insecticide and control-treated females, with the former exhibiting a more male-biased sex ratio than the latter control-treatment group. This difference in litter sex ratios is consistent with the predictions of the Trivers-Willard model for adaptive sex allocation and suggests female Franklin's ground squirrels manipulate litter sex ratio in response to costs imposed by ectoparasite pressure. Further and more comprehensive investigation into the spatial and social organization of Franklin's ground squirrels is necessary to empirically address how the extent of sociality may influence the expression of adaptive sex allocation, as well as to address other important questions that remain for this understudied species, particularly in regards to dispersal, space use, population dynamics, and site occupancy.