UNIVERSITY OF CALIFORNIA
RIVERSIDE

The Physiological, Exercise Performance, and Morphological Consequences of Fatherhood in the Biparental California Mouse (*Peromyscus californicus*)

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Jacob Ryan Andrew

March 2018

Dissertation Committee:
Dr. Wendy Saltzman, Chairperson
Dr. Theodore Garland, Jr.
Dr. Mark Chappell
Dr. Christopher Clark

The Dissertation of Jacob Ryan Andrew is approved:

_____

_____

_____

_____

Committee Chairperson

University of California, Riverside

ACKNOWLEDGEMENTS

I would like to thank Dr. Wendy Saltzman for everything she has done as my advisor and as my friend. She has always been there to help me design and carry out my experiments, as well as help me with writing the manuscripts. She has always been there when I needed her most and this dissertation would not have been possible without her help and words of encouragement. I would like to thank Dr. Ted Garland for his near-constant help with planning, statistical analysis, and writing. I would also like to thank my other committee members, Dr. Mark Chappell and Dr. Chris Clark, for all of their technical knowledge and help with writing. Thank you to the graduate students of the Saltzman lab, Dr. Juan Pablo Perea-Rodriguez, Gemma Zhao, and Nathan Horrell, for all of their help and assistance. A huge thank you to all of the undergraduates from the Saltzman lab for all the time they spent helping with data collection and data entry. Thank you for the emotional and monetary support from my parents, Michael and Lynn Andrew. Finally, thank you to my girlfriend, Ashley Wong, for being an amazing, loving, caring person, this dissertation would not have been possible without you.

ABSTRACT OF THE DISSERTATION

The Physiological, Exercise Performance, and Morphological Consequences of Fatherhood in the Biparental California Mouse (*Peromyscus californicus*)

by

Jacob Ryan Andrew

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, March 2018
Dr. Wendy Saltzman, Chairperson

In biparental mammals, becoming a father can significantly alter males' neural and endocrine systems, but effects of fatherhood on physiology and morphology are less clear. This dissertation tested the hypothesis that fatherhood has significant physiological, morphological and exercise-performance costs, and are modulated by parity and environmental stress. Three studies were performed using the monogamous, biparental California mouse (*Peromyscus californicus*). In each study, physiology, exercise performance, and morphology were compared among breeding males, non-breeding males (with a tubally ligated female [Chapter One] or an ovariectomized, estrogen/progesterone-treated female [Chapters Two and Three]) and virgin males (with another male [Chapter One] or an ovariectomized, untreated female [Chapters Two and Three]).

Chapter One examined acute effects of fatherhood. New fathers had significantly larger hindlimb muscles than non-breeding males, but virgin males had heavier

subcutaneous fat pads than non-breeding or virgin males. No differences were found in physiology (basal metabolic rate [BMR], hematocrit), or locomotor performance (maximum sprint speed, treadmill endurance, maximal oxygen consumption [$\dot{V}O_2$max]).

Chapter Two characterized long-term effects of fatherhood across multiple reproductive bouts. Breeding males differed from non-breeding and/or virgin males in several physiological (resting metabolic rate [RMR]), exercise performance (sprint speed, predatory aggression), and morphological (lean, fat, and organ masses) measures at specific time points; however, differences among groups were inconsistent, and few differences were found overall. Moreover, the number of differences between breeding males and controls did not increase with parity.

Chapter Three examined effects of cold acclimation and determined how these effects were influenced by males' reproductive condition. Cold-acclimating virgins had lower body, fat, and lean masses, and higher RMR and $\dot{V}O_2$max, than virgins at room temperature. Among long-term cold-acclimated animals, breeding males had higher BMR, lower $\dot{V}O_2$max, longer feet, and larger bacula than non-breeding and virgin males. These results suggest that cold stress may reveal energetic costs of fatherhood.

The results from this dissertation suggest that fatherhood, under the conditions tested, has few pronounced or consistent effects on physiology, exercise performance, and morphology in male California mice. This dissertation has important implications for the understanding of evolution of biparental care and the life-history of this species.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

LIST OF TABLES

Introduction

Chapter 1

Chapter 2

INTRODUCTION

Life-history trade-offs have been an important theme in evolutionary biology for more than eight decades (Fisher, 1930; Reznick, 1992; Roff, 1992; Stearns, 1992; Williams, 1966). To maximize lifetime fitness, an organism must balance investment in itself and in reproductive output (Reznick et al., 1990; Roff, 1992; Zera and Harshman, 2001). Investing too heavily in one or the other, especially under challenging conditions (e.g. inadequate resources, extreme temperatures, high predation risks) could result in damage to the organism itself and/or damage to current or future reproductive efforts. Mammalian parenthood illustrates the importance of life-history trade-offs, as the heavy costs (e.g. nutritional, energetic, immunological) of raising young is often at odds with the parent's survival. Researchers have often focused on females when studying the costs of parenting but have largely overlooked species in which fathers also provide care. Paternal care occurs in only 5-10% of mammalian taxa (Kleiman and Malcom, 1981) and could be costly to fathers, similar to how reproduction is costly to mothers. Little is known about the costs of paternal care in fathers, yet fatherhood can be a vital life-history trait in biparental species because it increases offspring survival and paternal fitness.

The relatively few researchers who have studied the proximate basis of mammalian paternal care have focused primarily on the behavioral and hormonal changes that accompany fatherhood. In females, however, parenthood can also affect physiology, morphology, behavior, metabolic rates, and exercise performance in mothers. In particular, exercise performance, metabolic rates, and reproduction are thought to trade

off through competing demands on resources and endocrine regulation as well as through constraints on the response to natural and/or sexual selection (Careau and Garland, 2012; Harshman and Zera, 2007; Husak et al., 2009; John-Alder et al., 2009; Ketterson et al., 2009; Martin et al., 2008; Moore and Hopkins, 2009; Ricklefs and Wikelski, 2002). I assessed the impact of fatherhood on two ecologically relevant aspects of the phenotype in a biparental species, exercise performance and body composition, as well as on whole-animal energetics and behavior. This research is the first to directly characterize the physiological, exercise performance, and morphological (body composition) consequences of fatherhood in a biparental mammal and to evaluate possible influences of parity, offspring age, and environmental stress. All relevant measures of physiology, exercise performance, and morphology that were used in this work and their predicted effects in males that behave paternally can be found in Table 0.1.

Chapter 1

This chapter characterized the acute effects of fatherhood on maximal and basal metabolic rate (measured as oxygen consumption), body mass, organ masses, hematocrit, maximum endurance, and maximum sprint speed in the biparental California mouse (*Peromyscus californicus*).

Chapter 2

This experiment evaluated the long-term effects of fatherhood on resting metabolic rate, maximum oxygen consumption, body mass, organ masses, and body

composition (fat and lean mass), hematocrit, grip strength, maximum sprint speed, and predatory aggression across seven successive reproductive bouts in the California mouse.

Chapter 3

This chapter comprised two experiments that characterized short- and long-term effects of cold acclimation on resting metabolic rate, maximum oxygen consumption, summit metabolism, body mass, organ masses, and body composition (fat and lean mass), hematocrit, grip strength, predatory aggression, and maximum sprint speed in in male California mice, as well as effects of fatherhood on cold acclimation.

Background

Costs of Maternal Care

In female mammals, the costs of maternity can include the energetic and nutritional requirements as well as morphological, physiological, and behavioral changes that are necessary for gestation, lactation, and/or maternal behavior (Speakman, 2008). Maternal costs of reproduction can largely be split into two categories: direct and indirect costs (Speakman, 2008). Direct costs of reproduction can include energy requirements, nutrient requirements, morphological, physiological, and behavioral changes that are necessary for parturition and/or lactation, whereas indirect costs of reproduction can affect thermoregulation, hyperthermia, activity levels, bone density, immune function, disease risk, toxin neutralization, and tissue renewal (reviewed in Speakman, 2008).

Importantly, hormones can influence costs at nearly every physiological level (e.g., see Garland et al. 2016; Speakman, 2008).

Females that are pregnant and/or lactating often experience significantly increased energetic demands. To support the increase in energy demands, females often eat significantly more than they normally would (Hammond and Diamond, 1994; Zhao et al., 2013b). In northern elephant seals (*Mirounga angustirostris*), for example, suckling pups consume large quantities of high-energy milk for almost 27 days (Champagne et al., 2006). During this time, the mother fasts and may lose up to 40% of her body mass through lactation alone (Costa et al., 1986). Maternal species in which females do not fast during lactation must continue to forage throughout the offspring care period (Costa and Gales, 2000). In general, female rodents have some of the highest energetic output during lactation compared to other taxa, with reports of females increasing energetic output by 2 - 4 times (Canas et al., 1982; Havera, 1979; Konig et al., 1988; Liu et al., 2003; Millar, 1978; Randolph et al., 1977; Schierwater and Klingel, 1986; Zhao et al., 2010). The energetic demands and major behavioral changes in females during pregnancy and lactation have cascading effects on the metabolic rate and performance of the female.

Metabolic rates are higher throughout pregnancy and during the early stages of lactation in female house mice (*Mus musculus*) compared to virgin females (Krol, 2003; Sadowska et al., 2013; Speakman and McQueenie, 1996). Metabolic rate immediately after parturition significantly correlates with litter size but not total pup mass (Johnson et al., 2001). Additionally, pregnant female mice, when given access to running wheels, run

significantly less than virgin females (Zhao et al., 2013a). Although not every aspect of maternal care is fully known, far less attention has been given to consequences of paternal care in species where fathers invest heavily in reproduction and provide care for the young.

Costs of Paternal Care

Male mammals invest heavily in efforts to increase mating success (aspects of courtship and combat), but rarely invest in rearing their offspring. Consequently, male paternal care is relatively understudied (Kleiman and Malcom, 1981; Woodroffe and Vincent, 1994). In species that practice paternal care, however, fathers undergo systematic changes in circulating concentrations of numerous hormones (Table 0.2; Campbell et al., 2009; Saltzman and Ziegler, 2014; Wynne-Edwards and Timonin, 2007), some of which can have important effects on energy utilization, body composition, and exercise performance (Table 0.3).

Fatherhood has the potential to be very costly to male mammals. Males may spend much of their time protecting, transporting, huddling, grooming, nest building, and generally caring for the young alongside the female (Brown, 1985; Brown, 1993; Dewsbury, 1985). This leaves reduced time for the males to forage, defend their territories from conspecifics and predators, and prevent potential extra-pair copulations. Without the father present in these biparental species, pup survival decreases markedly under at least some environmental conditions (Gubernick and Teferi, 2000; Gubernick et al., 1993). New fathers in some species (e.g., *Microtus ochrogaster*, *Saguinis Oedipus*,

Callithrix jacchus, *P. californicus*) lose a significant amount of body mass after the birth of their first litter (Achenbach and Snowdon, 2002; Campbell et al., 2009; Harris et al., 2011; Ziegler et al., 2006) before regaining it as they become more experienced, suggesting that fatherhood is physiologically, morphologically, and/or behaviorally taxing.

Given the known hormonal and behavioral changes in fathers (Table 0.2), I predicted that fatherhood would influence measures of energy utilization, body composition, and exercise performance. The measures that I characterized in the proposed studies, as well as the predicted effects of fatherhood, are listed in Table 0.1. A novel aspect of this research is the inclusion of measures of exercise performance, which are seen as a crucial focal point for organismal biology (e.g., see Careau and Garland 2012; Fig. 1 in Storz et al. 2015).

The Genus Peromyscus

Deer mice (*Peromyscus* spp.) are found throughout most of North and South America. They live in a wide variety of environments (tropics, deserts, grasslands, temperate and boreal forests, and alpine tundra) from sea level to elevations over 4,500 m (King, 1968; Hill, 1983). Depending on the species, *Peromyscus* construct nests in trees, rock outcroppings, and underground burrows, and a single nest may house multiple individuals together during winter months (Wolff, 1993). *Peromyscus* species range from 10 to 30 cm long and 14 to 70 g in mass (Svihla, 1932; Hayward, 1965; Hill, 1983; Gubernick, 1988; Earle and Lavigne, 1990). In *Peromyscus* species that have been

studied, most survive less than one year under wild conditions, whereas they can live significantly longer in laboratory settings (up to eight years in some species; King, 1968). Their diets consist primarily of seeds and fruits but may also include arthropods (Jameson, 1952). Most *Peromyscus* breed seasonally, and litter sizes are highly variable among species, ranging from 1 to 9 offspring (King, 1968). *Peromyscus* are primarily polygamous, but some species are monogamous, such as the California mouse (*P. californicus*).

The California Mouse

These studies were conducted in the California mouse, a genetically and socially monogamous, biparental, and nocturnal rodent (Gubernick and Alberts, 1987a; Ribble, 1991). California mice breed well in captivity, and their physiology, morphology, and behavior have been fairly well characterized (Dudley, 1974b; Merritt, 1978; Ribble, 1992). Fathers in this species greatly enhance pup survival and development of their offspring in both natural and lab settings (Bredy et al., 2007; Dudley, 1974a; Gubernick and Alberts, 1987b; Gubernick and Nordby, 1992; Vieira and Brown, 2003). Fathers engage in all of the same parental behaviors as mothers, except lactation (Cantoni and Brown, 1997). Thus, this species provides a good model system for the study of the physiological costs of paternal care.

California mice mate for life and can produce multiple litters each year in favorable environmental conditions (Merritt, 1978). Females become reproductively active as early as 40 days of age (Gubernick, 1988), while males become reproductively

active at ~90 days (unpub. obs.). Females produce litters of 1-5 pups (mean = approximately 2 pups) at 31- to 40-day intervals and are almost continuously pregnant in lab environments due to postpartum estrus (Gubernick, 1988). Animals can live 9-18 months in the wild (Merritt, 1978) and longer than 5 years in captivity (unpub. obs.).

Significance

The biology of paternal care has received considerable interest over the past three decades (Kentner et al., 2010; Wynne-Edwards and Timonin, 2007). Previous work has focused primarily on the neural, hormonal, and experiential factors facilitating the expression of paternal behavior in biparental species, including some non-human primates, humans, and rodents (Brown, 1985; Dewsbury, 1985; Wynne-Edwards, 2001; Wynne-Edwards and Reburn, 2000). In contrast, almost no research has been conducted on the metabolic, exercise performance, and morphological consequences of fatherhood (Campbell et al., 2009; Andrew et al., 2016; Zhao et al., 2017). Given the potential for physiological, exercise performance, and morphological measures to influence fitness (Careau and Garland, 2012; Storz et al. 2015), such research can provide insight into the direct consequences of fatherhood, not only aiding future work in animal models, but also potentially providing clues about the effect of fatherhood on men's health in our own species. This dissertation provides novel insight into both the short- and long-term consequences of paternal care and, potentially, their evolutionary and ecological significance.

References

Achenbach, G. G. and Snowdon, C. T. (2002). Costs of caregiving: Weight loss in captive adult male cotton-top tamarins (*Saguinus oedipus*) following the birth of infants. *Int J Primatol* **23**, 179-189.

Andrew, J. R., Saltzman, W., Chappell, M. A. and Garland, T., Jr. (2016). Consequences of fatherhood in the biparental California mouse (*Peromyscus californicus*): Locomotor performance, metabolic rate, and organ masses. *Physiol Biochem Zool* **89**, 130-40.

Bates, S. H., Dundon, T. A., Seifert, M., Carlson, M., Maratos-Filer, E. and Myers, M. G. (2004). LRb-STAT3 signaling is required for the neuroendocrine regulation of energy expenditure by leptin. *Diabetes* **53**, 3067-3073.

Berg, S. J. and Wynne-Edwards, K. E. (2001). Changes in testosterone, cortisol, and estradiol levels in men becoming fathers. *Mayo Clin Proc* **76**, 582-92.

Blouin, K., Boivin, A. and Tchernof, A. (2008). Androgens and body fat distribution. *J Steroid Biochem Mol Biol* **108**, 272-80.

Bredy, T. W., Brown, R. E. and Meaney, M. J. (2007). Effect of resource availability on biparental care, and offspring neural and behavioral development in the California mouse (*Peromyscus californicus*). *Eur J Neurosci* **25**, 567-75.

Brown, R. E. (1985). Hormones and paternal behavior in vertebrates. *Am Zoo* **25**, 895-910.

Brown, R. E. (1993). Hormonal and experiential factors influencing parental behaviour in male rodents: an integrative approach *Behav Process* **30**, 1-28.

Campbell, J. C., Laugero, K. D., Van Westerhuyzen, J. A., Hostetler, C. M., Cohen, J. D. and Bales, K. L. (2009). Costs of pair-bonding and paternal care in male prairie voles (*Microtus ochrogaster*). *Physiol Behav* **98**, 367-73.

Canas, R., Romero, J. J. and Baldwin, R. L. (1982). Maintenance energy requirements during lactation in rats. *J Nutr* **112**, 1876-1880.

Cantoni, D. and Brown, R. E. (1997). Paternal investment and reproductive success in the California mouse, *Peromyscus californicus*. *Anim Behav* **54**, 377-86.

Careau, V. and Garland, T., Jr. (2012). Performance, personality, and energetics: Correlation, causation, and mechanism. *Physiol Biochem Zool* **85**, 543-71.

Champagne, C. D., Houser, D. S. and Crocker, D. E. (2006). Glucose metabolism during lactation in a fasting animal, the northern elephant seal. *Am J Physiol Regul Integr Comp Physiol* **291**, R1129-1137.

Chong, P. K. K., Jung, R. T., Scrimgeour, C. M. and Rennie, M. J. (1994). The effect of pharmacological dosages of glucocorticoids on free living total energy expenditure in man. *Clin Endocrinol* **40**, 577-581.

Choong, K., Lakshman, K. M. and Bhasin, S. (2008). The physiological and pharmacological basis for the ergogenic effects of androgens in elite sports. *Asian J Androl* **10**, 351-63.

Costa, D. P. and Gales, N. J. (2000). Foraging energetics and diving behavior of lactating New Zealand sea lions, *Phocarctos hookeri*. *J Exp Biol* **203**, 3655-3665.

Costa, D. P., Leboeuf, B. J., Huntley, A. C. and Ortiz, C. L. (1986). The energetics of lactation in the Northern elephant seal, *Mirounga angustirostris*. *J Zool* **209**, 21-33.

Cox, R. M., Stenquist, D. S., Henningsen, J. P. and Calsbeek, R. (2009). Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. *Physiol Biochem Zool* **82**, 686-98.

De Maddalena, C., Vodo, S., Petroni, A. and Aloisi, A. M. (2012). Impact of testosterone on body fat composition. *J Cell Physiol* **227**, 3744-8.

Dewsbury, D. A. (1985). Paternal behavior in rodents. *Amer Zool* **25**, 841-852.

Dudley, D. (1974a). Contributions of paternal care to the growth and development of the young in *Peromyscus californicus*. *Behav Biol* **11**, 155-66.

Dudley, D. (1974b). Paternal behavior in the California mouse, *Peromyscus californicus*. *Behav Bio* **11**, 247-252.

Earle, M. and Lavigne, D. M. (1990). Intrapecific variation in body size, metabolic rate, and reproduction of deer mice (*Peromyscus maniculatus*). *Can J Zool* **68**, 381-388.

Fisher, R. A. (1930). The Genetical Theory of Natural Selection. Oxford: Oxford University Press.

Garland, Jr., T., Zhao, M., and Saltzman, W. (2016). Hormones and the evolution of complex traits: Insights from artificial selection on behavior. *Int Comp Biol* **56**, 207–224.

Gettler, L. T., McDade, T. W., Feranil, A. B. and Kuzawa, C. W. (2011). Longitudinal evidence that fatherhood decreases testosterone in human males. *P Natl Acad Sci USA* **108**, 16194-16199.

Goncalves, A., Resende, E. S., Fernandes, M. L. and da Costa, A. M. (2006). Effect of thyroid hormones on cardiovascular and muscle systems and on exercise tolerance: A brief review. *Arq Bras Cardiol* **87**, e45-7.

Gray, P. B., Parkin, J. C. and Samms-Vaughan, M. E. (2007). Hormonal correlates of human paternal interactions: a hospital-based investigation in urban Jamaica. *Horm Behav* **52**, 499-507.

Gubernick, D. J. (1988). Reproduction in the California mouse, *Peromyscus californicus*. *J Mammal* **69**, 857-860.

Gubernick, D. J. and Alberts, J. R. (1987a). The biparental care system of the California mouse, *Peromyscus californicus*. *J Comp Psychol* **101**, 169.

Gubernick, D. J. and Alberts, J. R. (1987b). "Resource" exchange in the biparental California mouse (*Peromyscus californicus*): Water transfer from pups to parents. *J Comp Psychol* **101**, 328-34.

Gubernick, D. J. and Nordby, J. C. (1992). Parental influences on female puberty in the monogamous California mouse, *Peromyscus californicus*. *Anim Behav* **44**, 259-267.

Gubernick, D. J. and Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proc Biol Sci* **267**, 147-50.

Gubernick, D. J., Wright, S. L. and Brown, R. E. (1993). The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Anim Behav* **46**, 539-546.

Hammond, K. and Diamond, J. (1994). Limits to dietary nutrient intake and intestinal nutrient uptake in lactating mice. *Physiol Zool* **67**, 282-303.

Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2011). Acute effects of corticosterone injection on paternal behavior in California mouse (*Peromyscus californicus*) fathers. *Horm Behav* **60**, 666-75.

Harshman, L. G. and Zera, A. J. (2007). The cost of reproduction: The devil in the details. *Trends Ecol Evol* **22**, 80-86.

Havera, S. P. (1979). Energy and nutrient cost of lactation in fox squirrels. *J Wildlife Manage* **43**, 958-965.

Hayward, J. S. (1965). The gross body composition of six geographic races of *Peromyscus*. *Can J Zool* **43**, 297-308.

Hill, R. W. (1983). Thermal physiology and energetics of *Peromyscus*; Ontogeny, body temperature, metabolism, insulation, and microclimatology. *J Mammal* **64**, 19-37.

Hukshorn, C. J. and Saris, W. H. (2004). Leptin and energy expenditure. *Curr Opin Clin Nutr Metab Care* **7**, 629-33.

Husak, J. F. and Irschick, D. J. (2009). Steroid use and human performance: Lessons for integrative biologists. *Integr Comp Biol* **49**, 354-64.

Husak, J. F., Irschick, D. J., McCormick, S. D. and Moore, I. T. (2009). Hormonal regulation of whole-animal performance: Implications for selection. *Integr Comp Biol* **49**, 349-53.

Huyghe, K., Husak, J. F., Moore, I. T., Vanhooydonck, B., Van Damme, R., Molina-Borja, M. and Herrel, A. (2010). Effects of testosterone on morphology, performance and muscle mass in a lizard. *J Exp Zool A Ecol Genet Physiol* **313**, 9-16.

Jameson, E. W. J. (1952). Food of deer mice, *Peromyscus maniculatus* and *P. boylei*, in the northern Sierra Nevada, California. *J Mammal* **33**, 50-60.

John-Alder, H. B. (1984). Reduced aerobic capacity and locomotory endurance in thyroid-deficient lizards. *J Exp Biol* **109**, 175-89.

John-Alder, H. B., Cox, R. M., Haenel, G. J. and Smith, L. C. (2009). Hormones, performance and fitness: Natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). *Integr Comp Biol* **49**, 393-407.

Johnson, M. S., Thomson, S. C. and Speakman, J. R. (2001). Limits to sustained energy intake II. Inter-relationships between resting metabolic rate, life-history traits and morphology in *Mus musculus*. *J Exp Biol* **204**, 1937-1946.

Kentner, A. C., Abizaid, A. and Bielajew, C. (2010). Modeling dad: Animal models of paternal behavior. *Neurosci Biobehav Rev* **34**, 438-51.

Ketterson, E. D., Atwell, J. W. and McGlothlin, J. W. (2009). Phenotypic integration and independence: Hormones, performance, and response to environmental change. *Integr Comp Biol* **49**, 365-79.

King, J. A. (1968). Biology of *Peromyscus* (Rodentia). Pittsburgh, Pennsylvania, US: The American Society of Mammalogists.

Kleiman, D. G. and Malcom, J. R. (1981). The evolution of male parental investment in mammals. In *Parental Care in Mammals*, pp. 347-387. Springer US: Plenum Publishing Corp.

Konig, B., Riester, J. and Markl, H. (1988). Maternal care in house mice (*Mus musculus*): The energy cost of lactation as a function of litter size. *J Zool* **216**, 195-210.

Krol, E. (2003). Limits to sustained energy intake VIII. Resting metabolic rate and organ morphology of laboratory mice lactating at thermoneutrality. *J Exp Biol* **206**, 4283-4291.

Leshan, R. L., Greenwald-Yarnell, M., Patterson, C. M., Gonzalez, I. E. and Myers, M. G., Jr. (2012). Leptin action through hypothalamic nitric oxide synthase-1-expressing neurons controls energy balance. *Nat Med* **18**, 820-3.

Liu, H., Wang, D. H. and Wang, Z. (2003). Energy requirements during reproduction in female Brandt's voles (*Microtus Brandtii*). *J Mammol* **84**, 1410-1416.

Mantzoros, C. S., Magkos, F., Brinkoetter, M., Sienkiewicz, E., Dardeno, T. A., Kim, S. Y., Hamnvik, O. P. and Koniaris, A. (2011). Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* **301**, E567-84.

Martin, L. B., Weil, Z. M. and Nelson, R. J. (2008). Seasonal changes in vertebrate immune activity: Mediation by physiological trade-offs. *Philos Trans R Soc Lond B Biol Sci* **363**, 321-39.

Merritt, J. F. (1978). *Peromyscus californicus*. *Mamm Spec* **85**, 1-6.

Miles, D. B., Calsbeek, R. and Sinervo, B. (2007). Corticosterone, locomotor performance, and metabolism in side-blotched lizards (*Uta stansburiana*). *Horm Behav* **51**, 548-54.

Millar, J. S. (1978). Energetics of reproduction in *Peromyscus Leucopus*: The cost of lactation. *Ecol* **59**, 1055-1061.

Moore, I. T. and Hopkins, W. A. (2009). Interactions and trade-offs among physiological determinants of performance and reproductive success. *Integr Comp Biol* **49**, 441-51.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T. and Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **269**, 540-543.

Randolph, P. A., Randolph, J. C., Mattingly, D. K. and Mead Foster, M. (1977). Energy costs of reproduction in the cotton rat, *Sigmodon hispidus*. *Ecol* **58**, 31-45.

Reinehr, T. (2010). Obesity and thyroid function. *Mol Cell Endocrinol* **316**, 165-71.

Reznick, D. A. (1992). Measuring the costs of reproduction. *Trends Ecol Evol* **7**, 42-45.

Reznick, D. A., Bryga, H. and Endler, J. A. (1990). Experimentally induced life-history evolution in a natural population. *Nature* **346**, 357-359.

Ribble, D. O. (1991). The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behav Ecol Sociobiol* **29**, 161-166.

Ribble, D. O. (1992). Lifetime reproductive success and its correlates in the monogamous rodent, *Peromyscus californicus*. *J Anim Ecol* **61**, 457-468.

Ricklefs, R. E. and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol Evol* **17**, 462-468.

Roff, D. A. (1992). Evolution of Life Histories: Theory and Analysis. New York: Chapman and Hall.

Sadowska, J., Gebczynski, A. K. and Konarzewski, M. (2013). Basal metabolic rate is positively correlated with parental investment in laboratory mice. *Proc Biol Sci* **280**, 1-7.

Sainsbury, A. and Zhang, L. (2012). Role of the hypothalamus in the neuroendocrine regulation of body weight and composition during energy deficit. *Obes Rev* **13**, 234-57.

Saltzman, W. and Ziegler, T. E. (2014). Functional significance of hormonal changes in mammalian fathers. *J Neuroendocrinol* **26**, 685-96.

Schierwater, B. and Klingel, H. (1986). Energy costs of reproduction in the djungarian hamster *Phodopus sungorus* under laboratory and seminatural conditions. *Oecologia* **69**, 144-147.

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos Trans R Soc Lond B Biol Sci* **363**, 375-98.

Speakman, J. R. and McQueenie, J. (1996). Limits to sustained metabolic rate: The link between food intake, basal metabolic rate, and morphology in reproducing mice, *Mus musculus*. *Physiol Zool* **69**, 746-769.

Stearns, S. C. (1992). The Evolution of Life Histories. Oxford: Oxford University Press.

Storz, J. F., Bridgham, J. T., Kelly, S. A., and Garland, Jr., T. (2015). Genetic approaches in comparative and evolutionary physiology. *Am J Phys Reg Int Comp Phys* **309**, R197–R214.

Svihla, A. (1932). A comparative life history study of the mice of the genus *Peromyscus*. *Univ Mich Mus Zool Misc Publs* **24**, 1-39.

Tataranni, P. A., Larson, D. E., Snitker, S., Young, J. B., Flatt, J. P. and Ravussin, E. (1996). Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol-Endoc M* **271**, E317-E325.

Trainor, B. C., Bird, I. M., Alday, N. A., Schlinger, B. A. and Marler, C. A. (2003). Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. *Neuroendocrinol* **78**, 36-44.

Ueta, C. B., Olivares, E. L. and Bianco, A. C. (2011). Responsiveness to thyroid hormone and to ambient temperature underlies differences between brown adipose tissue and skeletal muscle thermogenesis in a mouse model of diet-induced obesity. *Endocrinol* **152**, 3571-81.

van Dijk, G. (2001). The role of leptin in the regulation of energy balance and adiposity. *J Neuroendocrinol* **13**, 913-21.

Vieira, M. L. and Brown, R. E. (2003). Effects of the presence of the father on pup development in California mice (*Peromyscus californicus*). *Dev Psychobiol* **42**, 246-51.

Williams, G. C. (1966). Adaptation and Natural Selection. Princeton, NJ: Princeton University Press.

Wolff, J. O. (1993). Reproductive success of solitarily and communally nesting white-footed mice and deer mice. *Behav Ecol* **5**, 206-209.

Woodroffe, R. and Vincent, A. (1994). Mother's little helpers: Patterns of male care in mammals. *Trends Ecol Evol* **9**, 294-7.

Wynne-Edwards, K. E. (2001). Hormonal changes in mammalian fathers. *Horm Behav* **40**, 139-45.

Wynne-Edwards, K. E. and Reburn, C. J. (2000). Behavioral endocrinology of mammalian fatherhood. *Trends Ecol Evol* **15**, 464-468.

Wynne-Edwards, K. E. and Timonin, M. E. (2007). Paternal care in rodents: Weakening support for hormonal regulation of the transition to behavioral fatherhood in rodent animal models of biparental care. *Horm Behav* **52**, 114-21.

Yadav, V. K., Oury, F., Suda, N., Liu, Z. W., Gao, X. B., Confavreux, C., Klemenhagen, K. C., Tanaka, K. F., Gingrich, J. A., Guo, X. E. et al. (2009). A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. *Cell* **138**, 976-89.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu Rev Ecol Syst* **32**, 95-126.

Zhao, Z. J., Chi, Q. S. and Cao, J. (2010). Milk energy output during peak lactation in shaved Swiss mice. *Physiol Behav* **101**, 59-66.

Zhao, Z. J., Krol, E., Moille, S., Gamo, Y. and Speakman, J. R. (2013a). Limits to sustained energy intake. XV. Effects of wheel running on the energy budget during lactation. *J Exp Biol* **216**, 2316-2327.

Zhao, Z. J., Song, D. G., Su, Z. C., Wei, W. B., Liu, X. B. and Speakman, J. R. (2013b). Limits to sustained energy intake. XVIII. Energy intake and reproductive output during lactation in Swiss mice raising small litters. *J Exp Biol* **216**, 2349-2358.

Zhao, M., Garland, T., Jr., Chappell, M. A., Andrew, J. R. and Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiol Behav* **177**, 57-67.

Ziegler, T. E., Prudom, S. L., Schultz-Darken, N. J., Kurian, A. V. and Snowdon, C. T. (2006). Pregnancy weight gain: Marmoset and tamarin dads show it too. *Biol Lett* **2**, 181-3.

Table 0.1. Measures of energy utilization, body composition, and exercise performance. Fatherhood is predicted to have significant effects on each measure; symbols: + = increase; - = decrease; ? = unknown.

	Relevance to Proposed Research	Predicted Effect in Fathers
Energy Utilization		
Resting Metabolic Rate (RMR)	Rate of energy expenditure while at rest in a thermoneutral environment	+
Basal Metabolic Rate (BMR)	Rate of energy expenditure while at rest and post-absorbative in a thermoneutral env.	+
Summit Metabolism (VO_2sum)	Maximum rate of resting metabolic thermogenesis	+
Body Composition		
Body Mass	Total mass of an organism	+
Fat Mass	Fraction of fat mass to total mass	-
Lean Mass	Fraction of skeletal muscle mass to total mass	-
Brain Mass	Brain mass of an organism	?
Heart Mass	Heart mass of an organism	-
Lung Mass	Lung mass of an organism	?
Liver Mass	Liver mass of an organism	-
Spleen Mass	Spleen mass of an organism	?
Pancreas Mass	Pancreas mass of an organism	?
Stomach Mass	Empty stomach mass of an organism	?
Intestine Mass	Empty intestinal mass of an organism	?
Kidney Mass	Kidney mass of an organism	-
Adrenal Mass	Adrenal mass of an organism	-
Testis Mass	Testis mass of an organism	-
Gastrocnemius Muscle Mass	Gastrocnemius muscle mass of an organism	-
Exercise Performance		
Maximum Sprint Speed	Highest attainable running velocity	-
Maximum Exercise Endurance	Longest attainable running time at increasing speeds	-
Maximal Metabolic Rate (VO_2max)	Highest aerobic metabolic rate of an endotherm during forced exercise	-
Predatory Aggression	Measure of aggression and ability to catch prey	-
Grip Strength	Measure of limb grip strength ~ muscle strength	-
Hematocrit	Blood oxygen-carrying capacity	-

Table 0.2. Effects of fatherhood on metabolically important hormones testosterone, glucocorticoids (corticosterone and cortisol), leptin, and thyroid hormones (triiodothyronine and thyroxine). Symbols: + = increase; - = decrease; 0 = no effect. Adapted from Saltzman, Chappell, and Garland NSF IOS-1256572.

Testosterone	Glucocorticoids	Leptin	Thyroid Hormones
- [1-4, 8]	+/0/- [3-8]	- [6]	No Data

[1]Gettler et al. (2011); [2]Trainor et al. (2003); [3]Wynne-Edwards (2001); [4]Wynne-Edwards and Timonin (2007); [5]Berg and Wynne-Edwards (2001); [6]Campbell et al. (2009); [7]Gray et al. (2007); [8]Saltzman and Ziegler (2014)

Table 0.3. Effects of testosterone, glucocorticoids, leptin, and thyroid hormones on measures of energy utilization, body composition, and exercise performance. Symbols: + = increase; - = decrease; 0 = no effect. Adapted from Saltzman, Chappell, and Garland NSF IOS-1256572.

	Testosterone	Glucocorticoids	Leptin	Thyroid Hormones
Energy Utilization				
Resting or Basal Metabolic Rate	+/0/-[1]	+/-[1]	+/-[4,7,8]	+[9,10]
Body Composition				
Fat Mass	-[11,12]	+[10]	-[13,14]	-[9]
Lean Mass	+[10]	-[10]	0[15]	+[10]
Exercise Performance				
Maximum Sprint Speed	+/0[16-18]	No Data	No Data	No Data
Maximum Exercise Endurance	+/0[17,19,20]	+[21]	No Data	+[22,23]
Maximal Metabolic Rate (VO_2max)	+/0[19]	0[21]	+[15]	+[23]
Predatory Aggression / Grip Strength	No Data	No Data	No Data	No Data

[1]Moore and Hopkins (2009); [2]Chong et al. (1994); [3]Tataranni et al. (1996); [4]Bates et al. (2004); [5]Yadav et al. (2009); [6]Ueta et al. (2011); [7]van Dijk (2001); [8]Hukshorn and Saris (2004); [9]Reinehr (2010); [10]Sainsbury and Zhang (2012); [11]Blouin et al. (2008); [12]De Maddalena et al. (2012); [13]Mantzoros et al. (2011); [14]Pelleymounter et al. (1995); [15]Leshan et al. (2012); [16]Cox et al. (2009); [17]Husak and Irschick (2009); [18]Huyghe et al. (2010); [19]Choong et al. (2008); [20]John-Alder et al. (2009); [21]Miles et al. (2007); [22]Goncalves et al. (2006); [23]John-Alder (1984).

CHAPTER 1

Consequences of Fatherhood in the Biparental California Mouse (*Peromyscus californicus*): Locomotor Performance, Metabolic Rate, and Organ Masses

Jacob R. Andrew[1], Wendy Saltzman[1], Mark A. Chappell[1], and Theodore Garland, Jr.[1]

[1]Department of Biology, University of California, Riverside, California 92521

Abstract

Although effects of motherhood on mothers have been well documented in mammals, the effects of fatherhood on fathers are not well known. We evaluated effects of being a father on key metabolic and performance measures in the California mouse, *Peromyscus californicus*. California mice are genetically monogamous in the wild, and fathers show similar parental behavior to mothers, with the exception of lactation. To investigate the impact of fatherhood on fathers, focal males were paired with an intact female (breeding males), a tubally ligated female (non-breeding males) or another male (virgins). Starting 3-5 days after the birth of each breeding pair's first litter, males were tested for locomotor performance (maximum sprint speed, treadmill endurance), basal metabolic rate (BMR), and maximum oxygen consumption ($\dot{V}O_2$max). At the end of the 11-day test period, mice were euthanized, hematocrit determined, and organs weighed. Speed, endurance, and $\dot{V}O_2$max were significantly repeatable between two replicate measurement days but did not differ among groups, nor did BMR. Breeding males had significantly larger hindlimb muscles than did non-breeding males, whereas virgin males had heavier subcutaneous fat pads than non-breeding and breeding males. Several correlations were observed at the level of individual variation (residuals from ANCOVA models), including positive correlations for endurance with $\dot{V}O_2$max, $\dot{V}O_2$max with testes mass, and some of the digestion-related organs with each other. These results indicate that fatherhood may not have pronounced performance, metabolic or morphological effects on fathers, at least under standard laboratory conditions and across a single breeding cycle.

Introduction

Life-history trade-offs have been an important theme in evolutionary biology for more than eight decades (Fisher, 1930; Reznick, 1992; Roff, 1992; Stearns, 1992; Williams, 1966). Organisms have limited resources, so to maximize Darwinian fitness an organism must balance investment in itself with investment in its offspring (Zera and Harshman, 2001). Investing too heavily in one or the other, especially under energetically challenging conditions, could result in damage to the organism itself and/or to current or future reproductive efforts. Mammalian parenthood illustrates the importance of life-history trade-offs, as the heavy cost of raising young is often at odds with the parent's own survival.

Mammalian motherhood engenders a variety of well-documented effects on morphology, physiology, and behavior, as well as substantial energetic costs (e.g. Gittleman and Thompson, 1988; Hammond, 1997; Speakman, 2008). What we know about the energetic costs comes primarily from work on rodents and human beings. Evidence from house mice suggests that metabolic rate is higher in pregnant and lactating females than in control counterparts (e.g. Speakman and McQueenie, 1996). Mothers' metabolic rate after parturition correlates significantly with litter size but not average pup mass at weaning (Hammond et al., 1994; Johnson et al., 2001). Additionally, pregnant females, when given voluntary access to running wheels, run significantly less than virgin and ovariectomized females (Zhao et al., 2013).

In contrast to females, virtually nothing is known about physiological, morphological, behavioral, or energetic effects of fatherhood in species that exhibit

paternal care. Male mammals often invest heavily in efforts to increase mating success (e.g. through territoriality, courtship, or combat), but rarely invest directly in rearing their offspring; paternal care occurs in only 5-10% of mammalian taxa (Kleiman and Malcom, 1981). Fatherhood in these taxa can potentially be quite costly. Depending on the species, males may care for young while females forage (Gubernick et al., 1993; Wright and Brown, 2000). Fathers may also spend much time protecting, transporting, huddling, grooming, nest building, and generally caring (maintaining health and thermoregulation) for the young in monogamous species (Brown, 1985; Dewsbury, 1985; Lonstein and De Vries, 1999). Without the father present, offspring survival can decrease significantly (Gubernick et al., 1993; Wright and Brown, 2000; Wynne-Edwards and Lisk, 1989).

Under field conditions, fathers are likely to have increased activity levels and therefore increased energetic costs. In several biparental species, fatherhood can affect body mass and body composition (Campbell et al., 2009; Ziegler et al., 2006). For example, in California mice (*Peromyscus californicus*), males housed with primigravid females did not gain mass over the course of their mates' pregnancy, whereas age-matched males housed with a nonbreeding (tubally ligated) female showed significant increases in mass (Saltzman et al., 2015). Moreover, relative masses of several organs (adrenal gland, thymus, spleen) differed between California mouse fathers and non-fathers (de Jong et al., 2013; see also Harris et al., 2013). In prairie voles (*Microtus ochrogaster*), reductions in fat pad mass were reported in first-time fathers compared to non-fathers (Campbell et al., 2009).

Fatherhood in biparental mammals is associated with hormonal changes that may influence energy balance. For example, testosterone concentrations decrease significantly during fatherhood (Bales and Saltzman, 2015; Saltzman and Ziegler, 2014; Wynne-Edwards, 2001). Testosterone plays a major role in development and maintenance of many aspects of male physiology and morphology, such as potentially increasing energy utilization (Moore and Hopkins, 2009), altering body composition (Blouin et al., 2008), and increasing physical activity (Ibebunjo et al., 2011). Few studies have characterized effects of fatherhood on glucocorticoid (cortisol and corticosterone) concentrations in males of biparental mammalian species; however, in several species, glucocorticoid levels increase during the mate's pregnancy and decrease back to baseline levels shortly after parturition (Saltzman and Ziegler, 2014; Wynne-Edwards, 2001). Glucocorticoids can increase energy mobilization (Sapolsky et al., 2000) and alter body composition (Sainsbury and Zhang, 2012), in addition to having possible effects on activity levels (Malisch et al., 2007). Leptin levels have received little attention with respect to fatherhood, but were significantly lower in fathers compared to unpaired male prairie voles (Campbell et al., 2009). Along with androgens and glucocorticoids, leptin is essential for energy balance. Much of the work on leptin has focused on its effects on increasing energy utilization (van Dijk, 2001), but body composition can also be affected (Pelleymounter et al., 1995), as can activity levels (Girard et al., 2007; Meek et al., 2012). Other metabolically important hormones (e.g. thyroid hormones, adiponectin, progesterone, prolactin) might also influence paternal energetics (Saltzman and Ziegler, 2014).

Changes in metabolically important hormones and/or in body mass might also affect locomotor performance, which in turn can be crucial for success in various behaviors (e.g. foraging, predator escape, territorial defense) under natural conditions (Careau and Garland, 2012). Some aspects of locomotor performance correlate with body mass. If fathers lose a significant portion of their body mass post-partum, then locomotor performance could be affected as well. It is not known if decreases in body mass in fathers of biparental mammals are completely accounted for by changes in body fat or if visceral organs and/or skeletal muscle are also altered (Saltzman et al., 2015). Changes in heart, skeletal muscle or lung mass and associated changes in cardiovascular or respiratory function might alter locomotor performance or maximal aerobic metabolic rate ($\dot{V}O_2$max). $\dot{V}O_2$max is a strong determinant of endurance capacity (Joyner and Coyle, 2008) and shows a weak positive correlation with home range size among species of mammals (Albuquerque et al., 2015), as well as a positive correlation with relative brain size (Raichlen and Gordon, 2011). Given that testosterone often correlates positively with locomotor performance and can increase $\dot{V}O_2$max (Caminiti et al., 2009), lower circulating testosterone levels in fathers might decrease performance.

The present experiment was designed to characterize the short-term effects of parenthood on performance, energetics, and morphology of fathers housed in a relatively benign environment. It was conducted in the California mouse, a genetically and socially monogamous, biparental, nocturnal rodent. This species' physiology, morphology, and behavior have been well characterized (Chauke et al., 2011; Dlugosz et al., 2012; Gubernick, 1988; Trainor and Marler, 2001). California mouse fathers enhance pup

survival and development of their offspring in both natural and lab settings (Dudley, 1974; Gubernick and Teferi, 2000; Wright and Brown, 2002), and engage in all of the same parental behaviors as mothers, except lactation (Gubernick and Alberts, 1987). Thus, this species provides a good model for studying the physiological effects of paternal care. We characterized ecologically relevant measures of locomotor performance (maximum sprint speed, treadmill endurance, $\dot{V}O_2$max), energetics (basal metabolic rate [BMR]), morphology (body and organ masses), and an indicator of blood oxygen carrying capacity (hematocrit) in first-time fathers during the early to mid-postpartum period, as compared with two types of non-reproductive males (housed with either a tubally ligated female or another male).

Methods

Subjects

All animals were healthy adults born and reared in a breeding colony at the University of California, Riverside. Mice were descended from animals purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia) (Harris and Saltzman, 2013). Animals were housed in 44 x 24 x 20 cm polycarbonate cages with aspen shavings. Food (Purina 5001 rodent diet) and water were available *ad libitum*. Animals were kept on a 14L:10D lighting cycle (lights on at 05:00 h) with humidity at approximately 60-70% and ambient temperature at approximately 20-25°C. At the time of weaning (27-31 days of age), animals were ear-punched for identification and housed in same-sex groups of 4 age-matched individuals.

After sexual maturity (age range = 83-126 days) males were placed into one of three social and reproductive conditions: paired with an intact female (breeding males; n = 18), paired with a tubally ligated female (non-breeding males; n = 12), and paired with an unrelated male from their original same-sex group (virgin males; n = 14). Non-breeding males and virgin males were used to control for cohabitation with pups and with a female, respectively. Pairs that engaged in persistent aggression and were removed from the study (1 breeding and 1 non-breeding).

All procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* and approved by the University of California, Riverside IACUC.

Experimental Design

Animals were weighed twice per week beginning when they were paired into their respective reproductive conditions. Intact females typically gave birth 31-45 days after pairing. Breeding males were given 3-5 days after parturition before the 11-day period of data collection commenced. Non-breeding and virgin males were tested in conjunction with matched breeding males. All animals remained housed with their respective cage mates throughout the period of data collection, except where indicated below. For logistical reasons, males were processed in five separate cohorts, and cohort was used as a cofactor in statistical analyses (see below). However, all cohorts followed an identical procedural sequence (Table 1.1).

Tubal Ligation

Females from the non-breeding male group were tubally ligated so that mating and ovarian hormone production would occur but pregnancy would not (Harris and Saltzman, 2013). Females were anesthetized using isoflurane, oviducts were ligated, and the incision was sealed with tissue glue. Females were then housed singly for two weeks to recover before being paired with a male.

Maximum Sprint Speed

Locomotor performance (e.g. speed and stamina) is thought to be an important determinant of an animal's survival and reproduction in the wild (e.g. in terms of foraging or escaping from predators; Careau and Garland, 2012). Maximum sprint speed is a common index of locomotor performance in terrestrial animals and is measured according to well-established procedures (Djawdan and Garland, 1988). Briefly, males were placed on a 'racetrack' (8 m long by 10 cm wide and with 30 cm high walls) equipped with 12 sets of aligned photocells at 50 cm intervals. A rough rubber floor provided traction and ease of cleaning. At the beginning of each test, the mouse was placed near the start of the racetrack and encouraged to walk or run down it 2-4 times to become familiar with the track. Once testing began, the mouse was returned to the starting area. The photocells were activated and calibrated with a computer. The mouse was chased down the track, triggering the photocells. This was performed on two consecutive days, 5 times per day, yielding a total of 10 trials per individual, from each of which we recorded the fastest 1.0-m interval. Trials were scored subjectively as poor,

fair, ok, good or excellent (see Swallow et al., 1998; trials scored less than "ok" were excluded from further consideration). The highest values from each day were used for analysis of repeatability. The single highest value for each mouse (assuming it had at least one trial scored as "ok" or better) was used as its maximum sprint speed (Djawdan and Garland, 1988).

Maximum Exercise Endurance

Maximum endurance, like maximum sprint speed, is a common measure of exercise physiology in terrestrial animals (e.g. Djawdan, 1993). Mice were placed in a clear Plexiglass chamber (6.5cm x 12.5cm x 44cm) with an open bottom over a treadmill set to an incline of 25 degrees (Kemi et al., 2002). An electrical grid with mild AC current (adjustable) at the back of the treadmill provided motivation to run. Males were trained over 3 consecutive days for 15 minutes, at 10, 14, and 18 m/min, respectively (Meek et al., 2009). On the two days of testing, males were placed on the treadmill as per the training regimen. The speed of the treadmill was brought up to 18 m/min for 15 minutes. From that point, the speed of the treadmill was increased by 4 m/min every 5 minutes until mice were exhausted, determined as when they could no longer maintain speed and remained on the electrical gird for more than 4 seconds (Lerman et al., 2002; Meek et al., 2009). Values for the two days were used to assess repeatability, and the higher value was used for analysis of maximum performance. Any non-cooperating trials (scored as less than "ok") were excluded from statistical analysis.

Maximal Aerobic Metabolic Rate

Maximal oxygen consumption ($\dot{V}O_2$max) is an index of the upper limit to the intensity of work that can be maintained aerobically for prolonged periods (Dlugosz et al., 2013; Dohm et al., 1994; Joyner and Coyle, 2008). It was measured in a small running-wheel respirometer (circumference: 51.8 cm; effective volume: 900 mL) (Chappell and Dlugosz, 2009). This device yields $\dot{V}O_2$max that do not differ significantly from values obtained with a motorized treadmill for California mice (see also Dlugosz et al., 2013; Dlugosz et al., 2012).

Mice were forced to run a short warm-up period, followed by a ramping up of speed approximately every 30 s until either the gas concentration did not change or the mouse could not keep up. Flow rates (2400 mL/min) and gas concentrations were measured every second using Warthog LabHelper software (www.warthog.ucr.edu). Measurements were taken at approximately room temperature (20-25°C). Excurrent air was subsampled (~150 mL/min), dried with soda lime and Drierite, and sent through an oxygen analyzer. Reference air was taken at the beginning and end of every trial, and a baseline was computed by linear regression.

Oxygen consumption was calculated with Warthog LabAnalyst. Instantaneous corrections were used to account for the mixing and washout characteristics of the chamber (Bartholomew et al., 1981). $\dot{V}O_2$max (highest $\dot{V}O_2$ averaged over one minute) was determined on each of two days to assess repeatability, and the higher of the two values for each animal was used for statistical analysis. No trials were scored as less than "ok" (Chappell et al., 2004).

Basal Metabolic Rate

Basal metabolic rate (BMR), metabolic rate of an individual when inactive, performing no thermoregulatory work and not digesting any food, was measured as previously described (Dlugosz et al., 2012). Eight hours before testing, food was removed from the male's cage. After fasting, males were separated from their cage mates and placed in one of two small Plexiglass metabolic chambers (525 mL) with a small amount of bedding. The metabolic chambers were placed in a controlled environmental chamber set to 28-30°C. BMR measurements were taken over an 8-hour period during the males' inactive period (lights-on). Subsampled excurrent air was dried (soda lime and Drierite) and sent through an oxygen analyzer as for $\dot{V}O_2$max (previous paragraph). Gas concentration, temperature, and flow rates (800 mL/min) were measured every 5 seconds, and 3-minute reference readings were taken every 42 minutes. BMR was computed as the lowest $\dot{V}O_2$ averaged over 10-minute intervals when $\dot{V}O_2$ was low and stable.

Organ Masses

Mice were euthanized with CO_2 for dissection according to previously used techniques (Chappell et al., 2007). The brain and all subcutaneous fat were removed and weighed. The following organs were removed, blotted dry, and weighed: ventricles of the heart, stomach, intestines, spleen, liver, kidneys, reproductive organs (including testes, epididymes, vas deferens), and right hind limb muscle (all muscles of the thigh and calf region). At a later date, stomach and intestines were thawed, cut open, cleaned

of internal contents, blotted, and weighed. In addition, reproductive organs were thawed and the testes were weighed. Heart, liver, kidneys, and gut were weighed because they are thought to correlate with BMR (e.g. Chappell et al., 1999; Ksiazek and Konarzewski, 2012); the heart and right hind limb muscle are expected to be related to locomotor performance; the spleen is linked to immune function (e.g. Ksiazek and Konarzewski, 2012; Yang et al., 2013). Summed organ and/or muscle masses have also been linked with metabolism and energetics (e.g. Konarzewski and Diamond, 1995; Russell and Chappell, 2007).

Blood Collection and Hematocrit

Males were anesthetized with isoflurane, and blood was collected using heparinized microhematocrit capillary tubes inserted into the retro-orbital sinus (e.g. Chauke et al., 2011; Harris et al., 2011). Tubes were centrifuged at 4°C and 1300 RPM for 12 minutes. Hematocrit was recorded and plasma was removed and stored at -80°C for later use. Hematocrit is an indicator of blood oxygen-carrying capacity and is expected to correlate positively with $\dot{V}O_2$max (Kolb et al., 2010; Schuler et al., 2010).

Statistical Analysis

Repeatability of whole-animal performance measures was gauged with Pearson's correlations and paired t-tests. Analysis of covariance (ANCOVA) in SPSS 22.0 was used to compare traits among reproductive conditions (breeding males, non-breeding males, and virgin males), with measurement cohort as a factor, and age and body mass as

covariates. Initially, we performed the overall F test for group differences, but eventually decided that it was somewhat more informative to compute all three a priori contrasts among the three groups. Although the three contrasts are not orthogonal (independent), each is of interest in one context or another, and as this is one of the first studies of its kind we wanted to adopt a somewhat statistically liberal approach to encourage further research.

Cohort and age were nuisance variables, so we do not report results for them (note that cohort effects may include seasonal variation as well as any unintended minor procedural differences that may have occurred among test sets). For analysis of BMR, chamber was included as a nuisance factor. Organ masses, BMR, and $\dot{V}O_2$max were $\log_{10}$-transformed prior to analysis, as was body mass when used as a covariate for analyses of $\log_{10}$-transformed measures. Residuals were checked for approximate normality and homogeneity among groups (reproductive conditions). We then used these residuals for analysis of correlations at the level of individual variation (e.g. Chappell et al., 1999; Chappell et al., 2007; Dlugosz et al., 2012; Konarzewski and Diamond, 1995; Russell and Chappell, 2007). Within the breeding males only, we also performed regression analysis to determine if litter size was a predictor of any trait, while controlling for age and body mass as covariates, and including cohort as a factor.

Nominal statistical significance was judged at $P < 0.05$ (2-tailed). In total, this study involved 204 P values (not including nuisance variables, and replacing the single P values from the ANCOVAs reported in Tables 1.2 and 1.3 with the three a priori contrasts for each trait), 30 of which had nominal P values < 0.05 (see Results). These

tests include a substantial amount of nonindependence from each other, both because the traits under study are in some cases correlated and because many of the tests are interrelated (e.g., the a priori contrasts computed for all three groups). To adjust for conducting multiple related tests, we used the Adaptive False Discovery Rate procedure as implemented in PROC MULTTEST in SAS 9.4. Based on this procedure, the 16 smallest P values would have adjusted P values < 0.05, the largest having a nominal P value of 0.0041. All P values reported in the text are raw values, not adjusted for multiple comparisons.

Results

To examine changes in body mass that occurred within individual males, we used all of the measures of body mass (about 10 per male) that were recorded between the time of pairing and the last mass recorded prior to parturition (mice were not weighed on the day of birth) and computed a least-squares linear regression of body mass on measurement day individually for each male. We then analyzed the slope of this regression as the dependent variable in an ANCOVA that included age at pairing as a covariate and cohort as a factor. We found no statistically significant differences among the three groups, whether considering the overall F test ($F_{2,36} = 0.84$, $P = 0.441$) or the three a priori contrasts (all $P > 0.229$). We also then analyzed the mean body mass for all values prior to parturition and again found no significant differences among groups ($F_{2,36} = 0.87$, $P = 0.429$) or for the three a priori contrasts (all $P > 0.202$). Finally, we analyzed the mean body mass during the measurement trials (excluding the one taken after fasting

for BMR) and again found no group differences ($F_{2,36} = 0.87$, P = 0.427; all P values for a priori contrasts > 0.194).

Using the highest value recorded on each of the two measurement days (Fig. 1.1), sprint speed was repeatable between days (r = 0.466, N = 35, 2-tailed P = 0.005) and did not differ on average between days (paired t = -0.196, P = 0.846). Maximum sprint speed (highest value ever recorded) did not differ significantly among groups and did not vary with body mass (Table 1.2). When mass was removed from the model, maximum sprint again did not differ among reproductive conditions ($F_{2,36} = 1.87$, P = 0.168).

Treadmill endurance running time (Fig. 1.1) was repeatable between days (r = 0.692, N = 40, 2-tailed P < 0.0001), but averaged significantly lower on trial 2 (paired t = -2.105, P = 0.042). Reproductive condition did not have a significant effect on males' maximum locomotor endurance and endurance was unrelated to body mass (Table 1.2). Endurance also did not differ significantly among reproductive conditions when mass was removed from the model ($F_{2,36} = 1.48$, P = 0.241).

The measurements of maximal aerobic capacity were highly repeatable between days (r = 0.947, N = 44, 2-tailed P << 0.0001) and did not differ between days (paired t = -0.501, P = 0.619). However, $\dot{V}O_2$max was correlated with body mass (r = 0.609 and 0.619 for the two trial days), and body mass itself was highly repeatable (r = 0.988). Therefore, following previous studies (e.g. Dohm et al., 2001; Hayes and Chappell, 1990), we also computed residuals from linear regressions of $\dot{V}O_2$max on body mass separately for the two days and found that residual values (Fig. 1.1) were still highly repeatable (r = 0.904, N = 44, 2-tailed P << 0.0001).

$\dot{V}O_2$max (Fig. 1.2, Table 1.2) covaried positively with body mass but did not differ significantly among reproductive conditions (Table 1.2). Similarly, BMR varied with body mass but did not differ among breeding, virgin, and non-breeding males (Table 1.2).

Breeding males (Fig. 1.3) had significantly larger hindlimb muscle masses (estimated marginal mean (grams) for $\log_{10}$ hindlimb muscle mass = 0.211, 95% confidence interval = 0.192-0.230) than did non-breeding males (0.185, 0.164-0.207) (a priori contrast P = 0.018), but not significantly larger than virgin males (0.192, 0.176-0.209) (P = 0.163). Virgin males (estimated marginal mean (grams) for $\log_{10}$ fat pad mass = 0.328, 95% confidence interval = 0.252-0.403) had significantly heavier subcutaneous fat pads than non-breeding males (0.140, 0.050-0.230) (P = 0.0028 for a priori contrast) or fathers (0.192, 0.105-0.278) (P = 0.0254 for a priori contrast) (Table 1.3).

No other organ masses differed among reproductive conditions when age- and mass-corrected (Table 1.3). Hematocrit did not differ significantly among reproductive conditions whether (Table 1.3) or not ($F_{2,33} = 1.12$, P = 0.337) body mass was included as a covariate.

At the level of individual variation (residuals from the ANCOVA models presented in Tables 1.2 and 1.3), positive correlations were observed for sprint speed with endurance, endurance with $\dot{V}O_2$max, $\dot{V}O_2$max with testes mass, and some of the digestion-related organs with each other (stomach with intestine, pancreas, and kidney).

Considering only the breeding males, litter size (which ranged from 2 to 4) was a significant positive predictor of stomach mass (P = 0.019) (adjusting for body mass), but not of any other trait.

Discussion

We examined the question of whether parenthood can influence morphological, physiological, and/or behavioral phenotypes of fathers. In this study, we tested whether fatherhood affected locomotor performance, metabolic rate, and morphology in the biparental California mouse. Contrary to our expectations, of all the performance and metabolic variables and organ masses measured, only hindlimb muscle mass and subcutaneous fat pad mass (both adjusted for variation in body mass) differed statistically among males from the three groups.

Performance of various types of locomotion is essential to almost all behaviors, including components of paternal care. For that reason, we investigated sprint speed and locomotor endurance as measures of locomotor performance, as well as maximal rate of oxygen consumption ($\dot{V}O_2$max) as a primary determinant of aerobic locomotor endurance (Dlugosz et al., 2012; Joyner and Coyle, 2008). For a small rodent like *Peromyscus californicus*, sprint speed is presumably important when avoiding or escaping from predators, whereas endurance may be necessary during such prolonged activities as foraging or patrolling the home range or territory (Djawdan, 1993; Djawdan and Garland, 1988). We expected that fathers would have reduced locomotor abilities and $\dot{V}O_2$max compared to non-breeding males and virgins for three reasons. First, if

males devoted energetic resources towards caring for offspring then they might have less energy available to maintain or repair muscle. Second, previous studies have shown breeding male California mice to have reduced circulating androgen levels (Trainor et al., 2003), which could adversely affect muscle performance. Third, a previous study found reduced body mass in males housed with breeding (primigravid) females as compared with non-breeding males (Saltzman et al., 2015), suggesting that reproductive status affects body condition in male California mice. Our predictions were not upheld by results of the present study, as we found no statistically significant differences in performance among males maintained in the three reproductive conditions (Table 1.2).

The absence of differences in speed, endurance, and $\dot{V}O_2$max in this study might be explained in several ways. First, unlike the previous study (Saltzman et al., 2015), we did not find differences among groups in body mass. Second, given how often males were tested (Table 1.1), it is possible that stress or some sort of carryover effects overshadowed any effect fatherhood may have had on speed, endurance or $\dot{V}O_2$max. In any case, the lack of differences in organismal performance abilities are consistent with our finding of few differences in the sizes (adjusted for body mass) of performance-relevant internal organs or hematocrit (Table 1.3), although fathers and non-breeding males had smaller fat pads than virgins, and fathers had larger hindlimb muscles than did non-breeding males (and virgins).

Basal metabolic rate reflects the sum of all processes that require energy in an endotherm under conditions of minimal energy use (e.g., resting, fasting, and thermoneutrality). Changes in body composition or in endocrine function are expected to

alter BMR (Ksiazek and Konarzewski, 2012; White and Seymour, 2005). Although we did find group differences for subcutaneous fat pad mass (Table 1.3, Fig. 1.3), we did not find a parallel difference in BMR, perhaps because these fat pads were a rather small fraction of total body mass.

At the level of individual variation (residuals from the ANCOVA models shown in Tables 1.2 and 1.3), the strongest correlation we found was between $\dot{V}O_2$max and mass of the testes (r = 0.461, P = 0.002). Although we did not measure circulating hormone levels in the present study, this result is consistent with experimental manipulations in humans showing that testosterone can increase $\dot{V}O_2$max (Caminiti et al., 2009).

Several caveats should be kept in mind when interpreting the results of this study. First, conditions of *ad lib* food and water, coupled with small cages that do not require any exercise per se, may ameliorate any energetic costs related to fatherhood. Second, it is possible that the time frame of this study was not long enough to allow differences to emerge among the reproductive groups. We are currently addressing this possibility in a longitudinal study examining possible effects of fatherhood across multiple successive reproductive bouts. Third, repeated handling of animals and brief separations from their cagemates might have influenced paternal behavior and/or pup development. This seems unlikely, however, based on previous work in this species (de Jong et al., 2013; Harris et al., 2013).

In summary, we found little evidence that first-time fathers in a biparental rodent species experience important alterations in their morphology, physiology or locomotor

abilities. In other words, fatherhood does not appear to be "costly" in California mice, at least in terms of running performance, aerobic metabolism, and organ morphology. However, we caution that these results may not hold for fathers after caring for multiple litters or under natural conditions that are likely much more energetically (or psychologically) stressful than lab housing, possibilities are being addressed in current studies.

References

Albuquerque, R. L., Sanchez, G. and Garland, T. (2015). Relationship between maximal oxygen consumption and home range area in mammals. *Physiol Biochem Zool*.

Bales, K. L. and Saltzman, W. (2015). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Horm Behav*.

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen-consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J Exp Biol* **90**, 17-32.

Blouin, K., Boivin, A. and Tchernof, A. (2008). Androgens and body fat distribution. *J Steroid Biochem Mol Biol* **108**, 272-80.

Brown, R. E. (1985). Hormones and paternal behavior in vertebrates. *Am Zoo* **25**, 895-910.

Caminiti, G., Volterrani, M., Iellamo, F., Marazzi, G., Massaro, R., Miceli, M., Mammi, C., Piepoli, M., Fini, M. and Rosano, G. M. (2009). Effect of long-acting testosterone treatment on functional exercise capacity, skeletal muscle performance, insulin resistance, and baroreflex sensitivity in elderly patients with chronic heart failure a double-blind, placebo-controlled, randomized study. *J Am Coll Cardiol* **54**, 919-27.

Campbell, J. C., Laugero, K. D., Van Westerhuyzen, J. A., Hostetler, C. M., Cohen, J. D. and Bales, K. L. (2009). Costs of pair-bonding and paternal care in male prairie voles (*Microtus ochrogaster*). *Physiol Behav* **98**, 367-73.

Careau, V. and Garland, T., Jr. (2012). Performance, personality, and energetics: Correlation, causation, and mechanism. *Physiol Biochem Zool* **85**, 543-71.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J Exp Biol* **202**, 2269-79.

Chappell, M. A. and Dlugosz, E. M. (2009). Aerobic capacity and running performance across a 1.6 km altitude difference in two sciurid rodents. *J Exp Biol* **212**, 610-9.

Chappell, M. A., Garland, T., Jr., Robertson, G. F. and Saltzman, W. (2007). Relationships among running performance, aerobic physiology and organ mass in male Mongolian gerbils. *J Exp Biol* **210**, 4179-97.

Chauke, M., Malisch, J. L., Robinson, C., de Jong, T. R. and Saltzman, W. (2011). Effects of reproductive status on behavioral and endocrine responses to acute stress in a biparental rodent, the California mouse (*Peromyscus californicus*). *Horm Behav* **60**, 128-38.

de Jong, T. R., Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2013). Physiological and neuroendocrine responses to chronic variable stress in male California mice (*Peromyscus californicus*): Influence of social environment and paternal state. *Psychoneuroendocrinol* **38**, 2023-33.

Dewsbury, D. A. (1985). Paternal behavior in rodents. *Amer Zool* **25**, 841-852.

Djawdan, M. (1993). Locomotor performance of bipedal and quadrupedal heteromyid rodents. *Funct Ecol* **7**, 195-202.

Djawdan, M. and Garland, T., Jr. (1988). Maximal running speeds of bipedal and quadrupedal rodents. *J Mamm* **69**, 765-772.

Dlugosz, E. M., Chappell, M. A., Meek, T. H., Szafrańska, P. A., Zub, K., Konarzewski, M., Jones, J. H., Bicudo, J. E. P. W., Nespolo, R. F. and Careau, V. (2013). Phylogenetic analysis of mammalian maximal oxygen consumption during exercise. *J Exp Biol* **216**, 4712-4721.

Dlugosz, E. M., Harris, B. N., Saltzman, W. and Chappell, M. A. (2012). Glucocorticoids, aerobic physiology, and locomotor behavior in California mice. *Physiol Biochem Zool* **85**, 671-83.

Dohm, M. R., Hayes, J. P. and Garland Jr, T. (2001). The quantitative genetics of maximal and basal rates of oxygen consumption in mice. *Genetics* **159**, 267-277.

Dohm, M. R., Richardson, C. S. and Garland, T. (1994). Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. *Am J Physiol-Reg I* **267**, R1098-R1108.

Dudley, D. (1974). Contributions of paternal care to the growth and development of the young in *Peromyscus californicus*. *Behav Biol* **11**, 155-66.

Fisher, R. A. (1930). The Genetical Theory of Natural Selection. Oxford: Oxford University Press.

Girard, I., Rezende, E. L. and Garland, T., Jr. (2007). Leptin levels and body composition of mice selectively bred for high voluntary locomotor activity. *Physiol Biochem Zool* **80**, 568-79.

Gittleman, J. L. and Thompson, S. D. (1988). Energy allocation in mammalian reproduction. *Am Zool* **28**, 863-875.

Gubernick, D. J. (1988). Reproduction in the California mouse, *Peromyscus californicus*. *J Mammal* **69**, 857-860.

Gubernick, D. J. and Alberts, J. R. (1987). "Resource" exchange in the biparental California mouse (*Peromyscus californicus*): Water transfer from pups to parents. *J Comp Psychol* **101**, 328-34.

Gubernick, D. J. and Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proc Biol Sci* **267**, 147-50.

Gubernick, D. J., Wright, S. L. and Brown, R. E. (1993). The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Anim Behav* **46**, 539-546.

Hammond, K. A. (1997). Adaptation of the maternal intestine during lactation. *J Mammary Gland Biol Neoplasia* **2**, 243-52.

Hammond, K. A., Konarzewski, M., Torres, R. M. and Diamond, J. (1994). Metabolic ceilings under a combination of peak energy demands. *Physiol Zool* **67**, 1479-1506.

Harris, B. N., de Jong, T. R., Yang, V. and Saltzman, W. (2013). Chronic variable stress in fathers alters paternal and social behavior but not pup development in the biparental California mouse (*Peromyscus californicus*). *Horm Behav* **64**, 799-811.

Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2011). Acute effects of corticosterone injection on paternal behavior in California mouse (*Peromyscus californicus*) fathers. *Horm Behav* **60**, 666-75.

Harris, B. N. and Saltzman, W. (2013). Effect of reproductive status on hypothalamic-pituitary-adrenal (HPA) activity and reactivity in male California mice (*Peromyscus californicus*). *Physiol Behav* **112-113**, 70-6.

Hayes, J. P. and Chappell, M. A. (1990). Individual consistency of maximal oxygen consumption in deer mice. *Funct Ecol* **4**, 495-503.

Ibebunjo, C., Eash, J. K., Li, C., Ma, Q. and Glass, D. J. (2011). Voluntary running, skeletal muscle gene expression, and signaling inversely regulated by orchidectomy and testosterone replacement. *Am J Physiol Endocrinol Metab* **300**, E327-40.

Johnson, M. S., Thomson, S. C. and Speakman, J. R. (2001). Limits to sustained energy intake II. Inter-relationships between resting metabolic rate, life-history traits and morphology in *Mus musculus*. *J Exp Biol* **204**, 1937-1946.

Joyner, M. J. and Coyle, E. F. (2008). Endurance exercise performance: The physiology of champions. *J Physiol* **586**, 35-44.

Kemi, O. J., Loennechen, J. P., Wisloff, U. and Ellingsen, O. (2002). Intensity-controlled treadmill running in mice: Cardiac and skeletal muscle hypertrophy. *J Appl Physiol* **93**, 1301-9.

Kleiman, D. G. and Malcom, J. R. (1981). The evolution of male parental investment in mammals. In *Parental Care in Mammals*, pp. 347-387. Springer US: Plenum Publishing Corp.

Kolb, E. M., Kelly, S. A., Middleton, K. M., Sermsakdi, L. S., Chappell, M. A. and Garland, T., Jr. (2010). Erythropoietin elevates VO2max but not voluntary wheel running in mice. *J Exp Biol* **213**, 510-9.

Konarzewski, M. and Diamond, J. (1995). Evolution of basal metabolic rate and organ masses in laboratory mice. *Evolution* **49**, 1239-1248.

Ksiazek, A. and Konarzewski, M. (2012). Effect of dietary restriction on immune response of laboratory mice divergently selected for basal metabolic rate. *Physiol Biochem Zool* **85**, 51-61.

Lerman, I., Harrison, B. C., Freeman, K., Hewett, T. E., Allen, D. L., Robbins, J. and Leinwand, L. A. (2002). Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains. *J Appl Physiol* **92**, 2245-55.

Lonstein, J. S. and De Vries, G. J. (1999). Comparison of the parental behavior of pair-bonded female and male prairie voles (*Microtus ochrogaster*). *Physiol Behav* **66**, 33-40.

Malisch, J. L., Saltzman, W., Gomes, F. R., Rezende, E. L., Jeske, D. R. and Garland Jr, T. (2007). Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol Biochem Zool* **80**, 146-156.

Meek, T. H., Dlugosz, E. M., Vu, K. T. and Garland Jr, T. (2012). Effects of leptin treatment and Western diet on wheel running in selectively bred high runner mice. *Physiol Behav* **106**, 252-258.

Meek, T. H., Lonquich, B. P., Hannon, R. M. and Garland, T., Jr. (2009). Endurance capacity of mice selectively bred for high voluntary wheel running. *J Exp Biol* **212**, 2908-17.

Moore, I. T. and Hopkins, W. A. (2009). Interactions and trade-offs among physiological determinants of performance and reproductive success. *Integr Comp Biol* **49**, 441-51.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T. and Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **269**, 540-543.

Raichlen, D. A. and Gordon, A. D. (2011). Relationship between exercise capacity and brain size in mammals. *PLoS One* **6**, e20601-e20601.

Reznick, D. (1992). Measuring the costs of reproduction *Trends Ecol Evol* **7**, 42-45.

Roff, D. A. (1992). Evolution of Life Histories: Theory and Analysis. New York: Chapman and Hall.

Russell, G. A. and Chappell, M. A. (2007). Is BMR repeatable in deer mice? Organ mass correlates and the effects of cold acclimation and natal altitude. *J Comp Physiol B* **177**, 75-87.

Sainsbury, A. and Zhang, L. (2012). Role of the hypothalamus in the neuroendocrine regulation of body weight and composition during energy deficit. *Obes Rev* **13**, 234-57.

Saltzman, W., Harris, B. N., de Jong, T. R., Nguyen, P. P., Cho, J. T., Hernandez, M. and Perea-Rodriguez, J. P. (2015). Effects of parental status on male body mass in the monogamous, biparental California mouse. *J Zool* **296**, 23-29.

Saltzman, W. and Ziegler, T. E. (2014). Functional significance of hormonal changes in mammalian fathers. *J Neuroendocrinol* **26**, 685-96.

Sapolsky, R. M., Romero, M. and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* **21**, 55-89.

Schuler, B., Arras, M., Keller, S., Rettich, A., Lundby, C., Vogel, J. and Gassmann, M. (2010). Optimal hematocrit for maximal exercise performance in acute and chronic erythropoietin-treated mice. *Proc Natl Acad Sci U S A* **107**, 419-23.

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos Trans R Soc Lond B Biol Sci* **363**, 375-98.

Speakman, J. R. and McQueenie, J. (1996). Limits to sustained metabolic rate: The link between food intake, basal metabolic rate, and morphology in reproducing mice, *Mus musculus*. *Physiol Zool* **69**, 746-769.

Stearns, S. C. (1992). The Evolution of Life Histories. Oxford: Oxford University Press.

Swallow, J. G., Garland, T., Carter, P. A., Zhan, W. Z. and Sieck, G. C. (1998). Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *J Appl Physiol* **84**, 69-76.

Trainor, B. C., Bird, I. M., Alday, N. A., Schlinger, B. A. and Marler, C. A. (2003). Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. *Neuroendocrinol* **78**, 36-44.

Trainor, B. C. and Marler, C. A. (2001). Testosterone, paternal behavior, and aggression in the monogamous California mouse (*Peromyscus californicus*). *Horm Behav* **40**, 32-42.

van Dijk, G. (2001). The role of leptin in the regulation of energy balance and adiposity. *J Neuroendocrinol* **13**, 913-21.

White, C. R. and Seymour, R. S. (2005). Allometric scaling of mammalian metabolism. *J Exp Biol* **208**, 1611-9.

Williams, G. C. (1966). Adaptation and Natural Selection. Princeton, NJ: Princeton University Press.

Wright, S. L. and Brown, R. E. (2000). Maternal behavior, paternal behavior, and pup survival in CD-1 albino mice (*Mus musculus*) in three different housing conditions. *J Comp Psychol* **114**, 183-192.

Wright, S. L. and Brown, R. E. (2002). The importance of paternal care on pup survival and pup growth in *Peromyscus californicus* when required to work for food. *Behav Process* **60**, 41-52.

Wynne-Edwards, K. E. (2001). Hormonal changes in mammalian fathers. *Horm Behav* **40**, 139-45.

Wynne-Edwards, K. E. and Lisk, R. D. (1989). Differential effects of paternal presence on pup survival in two species of dwarf hamster (*Phodopus sungorus* and *Phodopus cambelli*). *Physiol Behav* **45**, 465-469.

Yang, D. B., Xu, Y. C., Wang, D. H. and Speakman, J. R. (2013). Effects of reproduction on immuno-suppression and oxidative damage, and hence support or otherwise for their roles as mechanisms underpinning life history trade-offs, are tissue and assay dependent. *J Exp Biol* **216**, 4242-50.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu Rev Ecol Syst* **32**, 95-126.

Zhao, Z. J., Krol, E., Moille, S., Gamo, Y. and Speakman, J. R. (2013). Limits to sustained energy intake. XV. Effects of wheel running on the energy budget during lactation. *J Exp Biol* **216**, 2316-2327.

Ziegler, T. E., Prudom, S. L., Schultz-Darken, N. J., Kurian, A. V. and Snowdon, C. T. (2006). Pregnancy weight gain: Marmoset and tamarin dads show it too. *Biol Lett* **2**, 181-3.

Figure Legends

Figure 1.1. Statistically significant repeatability of sprint speed (r = 0.466), maximum endurance (r = 0.692), and residual maximal oxygen consumption (r = 0.904). See text for further statistical analyses.

Figure 1.2. Log_{10}-transformed maximal oxygen consumption during forced exercise ($\dot{V}O_2$max) was strongly related to log body mass (P < 0.001), but did not differ statistically among groups (P = 0.126).

Figure 1.3. Log_{10}-transformed right hindlimb muscle mass differed among groups (P = 0.051), adjusting for log body mass (P << 0.001), by analysis of covariance (age and cohort were also included in the statistical model). Breeding males had significantly larger hindlimb muscle masses than did non-breeding males, but not significantly larger than virgin males (see text).

Figure 1.1.

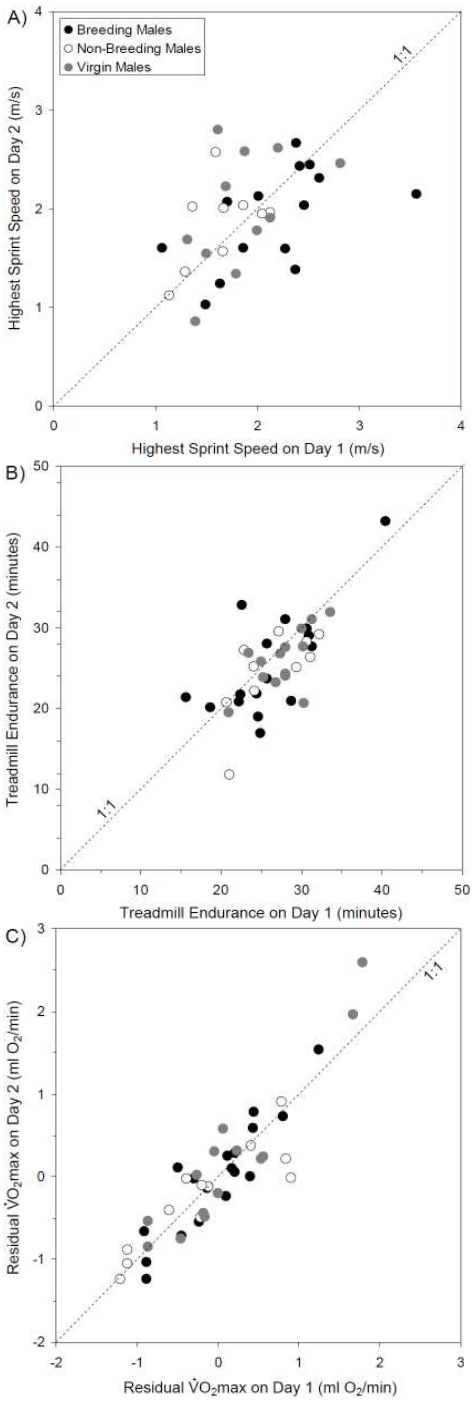

A)
Breeding Males
Non-Breeding Males
Virgin Males
1:1
Highest Sprint Speed on Day 2 (m/s)
Highest Sprint Speed on Day 1 (m/s)
B)
1:1
Treadmill Endurance on Day 2 (minutes)
Treadmill Endurance on Day 1 (minutes)
C)
1:1
Residual $\dot{V}O_2$max on Day 2 (ml O_2/min)
Residual $\dot{V}O_2$max on Day 1 (ml O_2/min)

Figure 1.2.

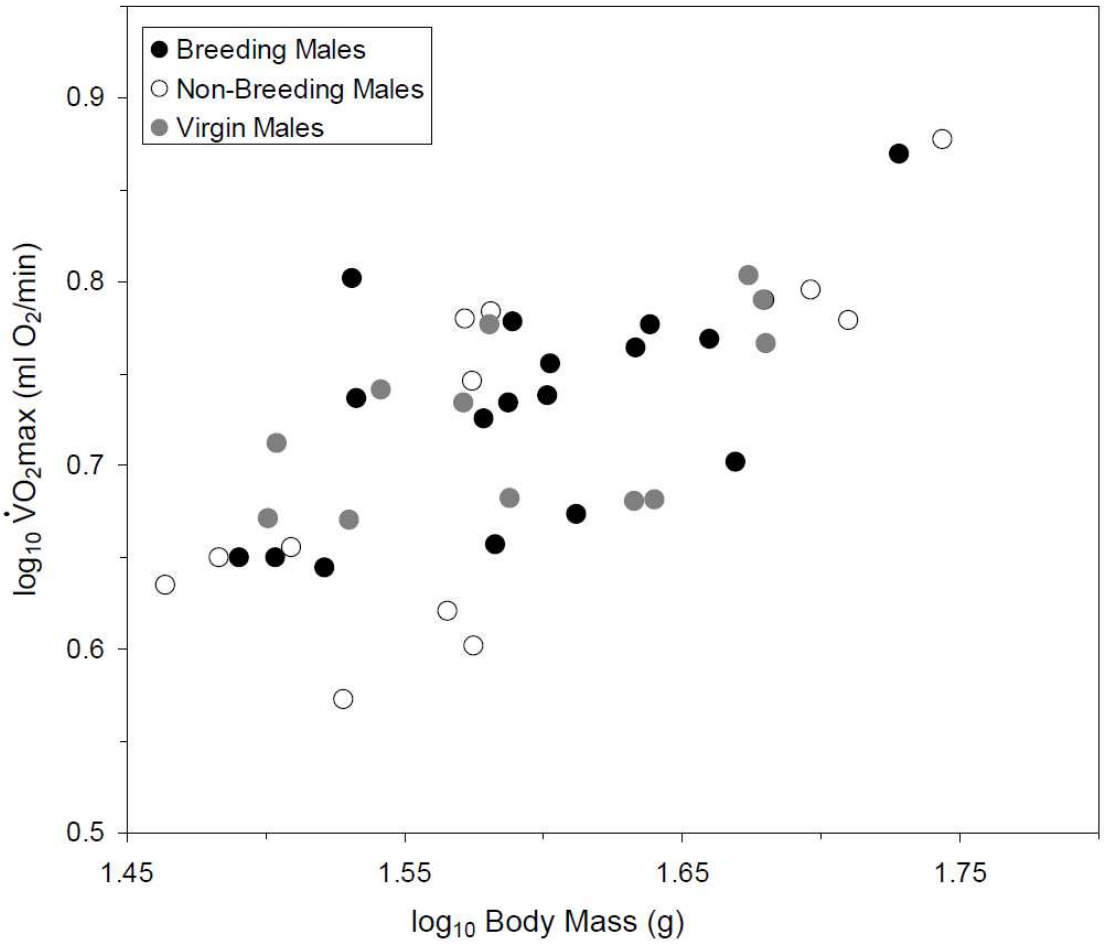

Breeding Males
Non-Breeding Males
Virgin Males
0.9
0.8
0.7
0.6
0.5
log10 V̇O2max (ml O2/min)
1.45
1.55
1.65
1.75
log10 Body Mass (g)

Figure 1.3.

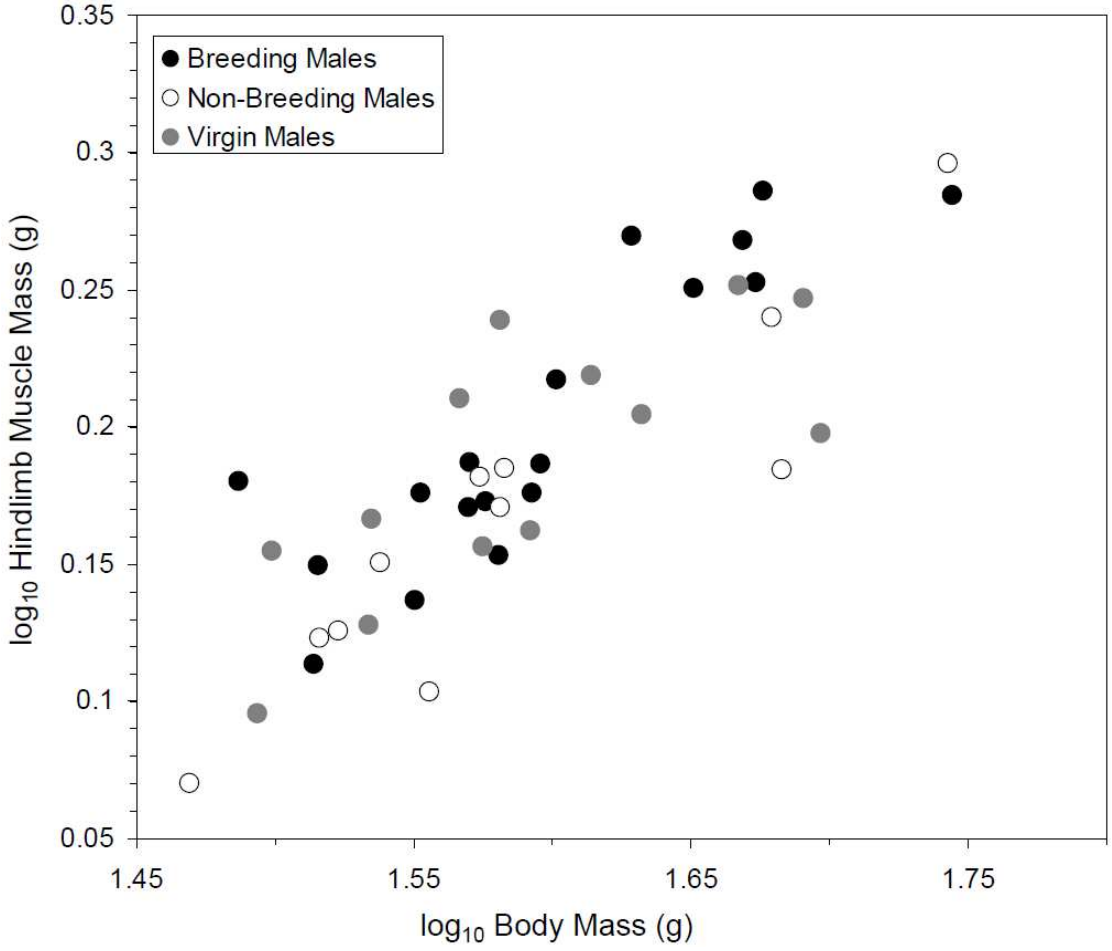

Breeding Males
Non-Breeding Males
Virgin Males
0.35
0.3
0.25
0.2
0.15
0.1
0.05
log10 Hindlimb Muscle Mass (g)
1.45
1.55
1.65
1.75
log10 Body Mass (g)

Table 1.1. Sequence of procedures over the 11 days of data collection in male California mice.

Time	Day 1	Day 2	Day 3	Day 4	Days 5,6,7	Day 8	Day 9	Day 10	Day 11
Morning	Sprint Speed	Sprint Speed	-----	BMR	Endurance Training	Endurance Test	Endurance Test	-----	Blood Sampling
Afternoon	VO_2max	VO_2max	-----	-----	-----	-----	-----	-----	Organ Masses

Experimental groups were pair-housed breeding males, non-breeding males (housed with a tubally ligated female), and virgin males (housed with another male). Timing of data collection in non-breeding males and virgin males was time-matched to the breeding males. BMR: basal metabolic rate, VO_2max: maximal aerobic metabolic rate.

Table 1.2. Results of analyses of covariance comparing locomotor performance and metabolic rate of male California mice housed in three experimental groups.

							Male Reproductive Condition					
							Breeding		Non-Breeding		Virgin	
Trait	**D.F.$_{group}$**	**F$_{group}$**	**P$_{group}$**	**D.F.$_{body\ mass}$**	**F$_{body\ mass}$**	**P$_{body\ mass}$**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Maximal Sprint Speed (m/s)	2,35	1.83	0.176	1,36	0.001	0.977	2.11	0.19	1.76	0.19	2.15	0.16
Treadmill Endurance (min)	2,35	1.45	0.248	1,36	0.04	0.844	26.76	2.06	24.20	1.98	28.31	1.70
$\log_{10}$ Maximal Oxygen Consumption (ml O_2/h)	2,33	2.20	0.126	1,34	38.52	$5*e^{-7}$	0.754	0.015	0.729	0.016	0.714	0.014
$\log_{10}$ Basal Metabolic Rate (ml O_2/h)	2,34	0.28	0.755	1,35	11.29	0.002	-0.266	0.023	-0.251	0.024	0.271	0.020

Values indicated for "mean" are estimated marginal means and associated standard errors. For analysis of VO_2max and BMR, body mass was $\log_{10}$-transformed. All analyses included cohort as a factor and age as a covariate. Results for cohort and age are not shown because these were considered nuisance variables. Breeding: n = 18, Non-Breeding: n = 12, Virgin: n = 12-14.

Table 1.3. Results of analysis of covariance comparing log_{10}-transformed organ masses (grams) and hematocrit (%, not logged) of male California mice housed in three experimental groups.

							Male Reproductive Condition					
							Breeding		Non-Breeding		Virgin	
Trait	**D.F.$_{group}$**	**F$_{group}$**	**P$_{group}$**	**D.F.$_{body\ mass}$**	**F$_{body\ mass}$**	**P$_{body\ mass}$**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Brain	2,33	1.44	0.252	1,34	0.00	0.983	-0.060	0.010	-0.068	0.010	-0.082	0.008
Stomach	2,34	0.46	0.634	1,35	9.47	0.004	-0.201	0.017	-0.213	0.019	-0.223	0.014
Intestines	2,33	0.46	0.635	1,34	14.62	0.001	0.344	0.032	0.310	0.036	0.324	0.026
Liver	2,35	1.17	0.321	1,36	113.31	$2*e^{-12}$	0.181	0.015	0.207	0.016	0.191	0.013
Right Hind Limb Muscle	2,33	3.27	0.051	1,34	98.86	$2*e^{-11}$	0.211	0.009	0.185	0.011	0.192	0.008
Subcutaneous Fat Pads	2,35	5.23	0.010	1,36	16.91	$2*e^{-4}$	0.192	0.043	0.140	0.044	0.328	0.037
Heart Ventricles	2,35	0.09	0.914	1,36	43.16	$1*e^{-7}$	-0.840	0.018	-0.831	0.019	-0.833	0.016
Pancreas	2,35	0.63	0.537	1,36	7.03	0.012	-1.167	0.036	-1.210	0.038	-1.203	0.031
Mean Kidney	2,34	0.39	0.680	1,35	52.05	$2*e^{-8}$	-0.670	0.023	-0.654	0.024	-0.648	0.021
Reproductive Organs	2,35	0.96	0.395	1,36	18.70	$1*e^{-4}$	-0.012	0.035	0.014	0.036	0.054	0.030
Mean Testis	2,35	0.13	0.881	1,36	4.57	0.040	-0.772	0.041	-0.787	0.043	-0.799	0.036
Hematocrit	2,32	1.33	0.278	1,32	1.17	0.288	43.57	1.65	46.24	1.87	46.42	1.50

Values indicated for "mean" are estimated marginal means and associated standard errors. For all organs (but not hematocrit), body mass was log-transformed. All analyses included cohort as a factor and age as a covariate. Results for cohort and age are not shown because they were considered nuisance variables. Breeding: n = 17-18, Non-Breeding: n = 11-12, Virgin: n = 12-14. "Reproductive organs" includes testes and all other male reproductive tissue except the baculum.

CHAPTER 2

Long-Term Effects of Fatherhood on Morphology, Energetics, and Exercise Performance in California Mice (*Peromyscus californicus*)

Jacob R. Andrew[1], Theodore Garland, Jr.[1], Mark A. Chappell[1], Meng Zhao[1], Nathan D. Horrell[1], Wendy Saltzman[1]

[1]Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, CA 92521, USA

Abstract

In male mammals that provide care for their offspring, fatherhood can lead to changes in behavioral, morphological, and physiological traits. Relatively little is known about these changes, however, especially across multiple reproductive bouts, which are predicted to magnify differences between fathers and non-reproductive males. We evaluated the consequences of fatherhood in the monogamous, biparental California mouse (*Peromsycus californicus*) using a longitudinal approach across seven reproductive bouts. We compared three groups of adult males: breeding (housed with sham-ovariectomized females), non-breeding (housed with ovariectomized females treated with estrogen and progesterone to induce estrous behavior), and virgin males (housed with untreated ovariectomized females). At each of five time points (before pairing, early postpartum of the first litter, late postpartum of the second litter, early postpartum of the sixth litter, and late postpartum of the seventh litter) we measured fathers' body composition, hematocrit, predatory aggression, resting metabolic rate (RMR), maximal oxygen consumption ($\dot{V}O_2$max), grip strength, and sprint speed. We also determined organ masses at the final time point. We predicted that fathers would have lower body and fat mass, increased RMR, decreased $\dot{V}O_2$max and hematocrit, and reduced exercise performance, compared to both control groups, and that these effects would become more pronounced with increasing parity. Breeding and non-reproducing males differed in surprisingly few measures at any time point, and the number of differences did not increase with parity. Overall, these results are consistent with

previous studies suggesting that fatherhood has relatively few costs in this species under standard laboratory housing conditions.

Introduction

The behavioral, physiological, and morphological changes that occur in mammalian mothers have been well studied (e.g. Gittleman and Thompson, 1988; Hammond, 1997; Speakman, 2008); however, similar studies are largely lacking for mammalian fathers. Although biparental care (i.e. care of offspring by both parents) is relatively rare in mammals (estimated at around 5-10% of mammalian taxa (Kleiman and Malcom, 1981)), it can play a crucial role in biparental species. Direct care by fathers, such as huddling, grooming, protection, and transportation of offspring, can increase offspring survival and have lasting impacts on offspring development, including social, aggressive, and mating behaviors, neural and endocrine function, and cognitive ability (Braun and Champagne, 2014; Bales and Saltzman, 2016).

Although paternal care can offer clear benefits to offspring, it can have a variety of effects on fathers, some of which can be adverse (Achenbach and Snowdon, 2002). In humans for example, fatherhood is associated with increased rates of depression and anxiety (Bartlett, 2004) shortly after the birth of offspring and might increase the incidence of heart disease (Haynes et al., 1983). In the biparental common marmoset (*Callithrix jacchus*), cotton-top tamarin (*Saguinus oedipus*), and California mouse (*Peromyscus californicus*), lab studies have found that expectant fathers gain body mass during their mate's pregnancy, followed by loss of mass after parturition (Achenbach and Snowdon, 2002; Ziegler et al. 2006; Harris et al., 2011; Saltzman et al., 2015). Declines in body mass, potentially due to providing care for offspring, increased stress, or reduced

foraging, could potentially increase mortality rates in fathers, especially under adverse conditions such as extreme temperatures or low food availability.

In addition to the morphological changes fathers may experience before and after the birth of their offspring, fathers' hormonal profiles can change in accordance with mating or paternal experience (Ziegler et al., 2000; Nunes et al., 2001). In several biparental species, males' androgen concentrations decrease during their mate's pregnancy or after parturition, while glucocorticoid levels can rise throughout the mate's pregnancy and fall after parturition (Saltzman and Ziegler, 2014). Additionally, prolactin levels are higher in fathers than non-fathers in many biparental mammals (Saltzman and Ziegler, 2014). Androgens, glucocorticoids and prolactin have metabolic effects that can influence energy utilization (Moore and Hopkins, 2009), body composition (Blouin et al., 2008), and physical activity (Ibebunjo et al., 2011). Several additional hormones and neuropeptides, including estrogen, progesterone, oxytocin and vasopressin, can also change systematically in fathers, again potentially leading to changes in morphology, physiology and behavior (Saltzman and Ziegler, 2014; Zhao et al., 2017).

Such changes can potentially have short- and long-term effects on overall energetics and whole-organism performance that may impact Darwinian fitness (reproductive success). To date, few studies have examined the effects of paternal status on energetics and exercise capacity. In one study of California mice, first-time fathers showed few differences from non-breeding males in several measures of energy metabolism and exercise capacity under laboratory conditions (Andrew et al., 2016). Fathers did, however, have larger hind limb muscles and heavier subcutaneous fat pads.

A limitation of that study was that only first-time fathers, rather than experienced fathers, were evaluated; thus, it did not address the possibility that effects of fatherhood might become evident only after longer periods or with higher parity. Campbell et al. (2009) investigated long-term effects of fatherhood in prairie voles (*Microtus ochrogaster*) and found that body mass and circulating leptin concentrations decreased from before pairing until after the second litter of pups was born, whereas corticosterone and home-cage activity levels did not change significantly.

Our goal in the present study was to test the hypothesis that effects of fatherhood on energetics, morphology, and exercise performance increase with increasing parity. We used the California mouse, a socially and genetically monogamous, biparental rodent (Gubernick and Alberts, 1987b; Ribble and Salvioni, 1990; Ribble, 1991; Gubernick and Teferi, 2000). California mouse fathers engage in all the same parental behaviors as mothers, with the exception of nursing, and can enhance survival and development of their pups, especially under energetically demanding conditions (Dudley, 1974a; Dudley, 1974b; Cantoni and Brown, 1997; Gubernick et al., 1993; Gubernick and Teferi, 2000; Wright and Brown, 2002). To test for effects of high parity, males were followed from before pair formation until after the birth of their seventh litter. At each of five time points (before pairing, early postpartum of the first litter, late postpartum of the second litter, early postpartum of the sixth litter and late postpartum of the seventh litter), we measured body composition (body mass, fat mass, lean muscle mass, organ masses), hematocrit, predatory aggression, resting metabolic rate (RMR), maximal oxygen consumption ($\dot{V}O_2$max), grip strength, and sprint speed. We compared fathers with two

groups of non-reproducing control males housed with ovariectomized females. We predicted that fathers would have lower body mass and fat mass, increased RMR, decreased $\dot{V}O_2$max and hematocrit, and poorer exercise performance, compared to non-reproducing males. Finally, we predicted that effects of fatherhood would become more pronounced with increasing parity.

Methods

Animals

Mice were born and reared in a colony at the University of California, Riverside (UCR). They were descended from animals purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Animals were housed in polycarbonate cages (44 x 24 x 20 cm) with aspen shavings as bedding; food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN, USA) and water were available *ad libitum*. Lighting was on a 14:10 cycle (lights on at 05:00 h, off at 19:00 h), with humidity maintained at approximately 55% and ambient temperature at approximately 21°C. Mice were checked twice daily, and cages were changed weekly. At weaning (27 - 31 days of age; 27.5 ± 0.2 days, mean ± SEM), animals were ear-punched for identification and placed in same-sex groups of 3 - 4 related and/or unrelated, age-matched individuals.

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and approved by the UCR Institutional Animal Care and Use Committee.

Experimental Design

When each male reached 100 – 125 days of age (115.1 ± 1.1 days), it underwent a series of test procedures over a 7-day period (Fig. 2.1). Five to 10 days after testing concluded, the males were randomly paired with females, 111 - 148 days old (127.5 ± 3.2 days), in one of three conditions. Breeding males (BM, n = 21) were paired with a sham-ovariectomized female; non-breeding males (NB, n = 20) were paired with an ovariectomized female treated with estradiol benzoate and progesterone to induce estrous behavior (see below); and virgin males (VM, n = 20) were housed with an ovariectomized female not treated with hormones. NBs were used to control for mating, and VMs were used to control for cohabitation with an adult female. Female mates of BMs gave birth 26 - 54 days after pair formation (36.2 ± 4.0 days) and at ~35-day intervals thereafter (Table 2.1). For BMs, time point 2 occurred during the early postpartum period of the first litter, which corresponded to early gestation of the second litter; female California mice usually conceive shortly after parturition (Gubernick, 1988); thus, the lactation and pregnancy periods overlap substantially. Time point 3 was during the late postpartum period of litter 2 / late gestation of litter 3; time point 4 occurred during the early postpartum period of litter 6 / early gestation of litter 7; and time point 5 was during the late postpartum period of litter 7 / late gestation of litter 8. We selected these time points to allow us to assess the effects of fatherhood both in young, relatively inexperienced fathers (time points 2 and 3) and in older fathers with high parity (time points 4 and 5), as well as during both the early (time points 2 and 4) and late (time points 3 and 5) postpartum/gestation periods. Timing of data collection in

NBs and VMs was matched to that in BMs. For logistical reasons, pairs were processed in three separate cohorts, and cohort was used as a cofactor in statistical analysis (see below). However, all cohorts underwent an identical sequence of procedures (Fig. 2.1).

The methods and sequence of data-collection procedures were identical for each of the 5 time points, except that animals were euthanized on the last day of time point 5. All males and breeding females were weighed to the nearest 0.001 g twice per week at 13:00 - 15:00 h, at 3- to 4-day intervals, throughout the study, except during periods of data collection.

Ovariectomies and Estrogen/Progesterone Treatment

Females underwent bilateral ovariectomies prior to being paired with a VM or NB, or sham-ovariectomies prior to being paired with a BM. Animals were anesthetized with isoflurane, and surgeries were performed under aseptic conditions using standard procedures as previously described (Zhao et al., in press). They were then housed individually for 2 weeks before being paired.

The ovarian hormones estrogen and progesterone are necessary for activating sexual behavior in many female rodents (Beach, 1976), including California mice (unpub. data). Females from the NB group were injected subcutaneously with estradiol benzoate (0.072 mg, s.c.; suspended in sesame oil; Sigma-Aldrich, St. Louis, MO, USA) 48 h before injection with progesterone (0.48 mg, s.c.; suspended in sesame oil; Sigma-Aldrich, St. Louis, MO, USA) in the afternoon (adapted from Dewsbury, 1974). This study did not explicitly examine whether mating occurred after hormone treatment, but

previous work (Zhao et al., in press; unpublished data) has confirmed that this hormone regime induces mating behavior in female California mice. California mice typically ovulate and conceive immediately after parturition (Gubernick, 1988). Therefore, NB females were injected with estradiol benzoate and progesterone at the time of pairing and every 35 days thereafter to simulate mating cycles in the BM group.

At the end of the study, females from the non-breeding and virgin pairs were euthanized by CO_2 inhalation and dissected to check for the presence of fetuses in the uterine canal. No females from either control group had visible fetuses.

Body Mass

During each 7-day testing period (Fig. 2.1), males were weighed on days 1 (13:00 - 14:30 h), 4 (8:30 - 9:30 h), 5 (10:00 - 11:30 h), 6 (10:00 - 11:30 h), and 7 (9:00 - 10:30 h).

Body Composition

On test days 1 (13:00 - 14:30 h) and 7 (9:00 - 10:30 h), males were weighed and scanned with a magnetic resonance whole-body analyzer (EchoMRI-100; Echo Medical Systems, Houston, TX, USA) to assess body composition (fat mass, lean mass, free water mass, and total water mass; Zhao et al., 2017). Scans lasted ~90 s and did not require anesthesia or sedation. We report fat and lean mass both unaltered and as percentages of total body mass.

Hematocrit

Blood samples (~200 µl) were collected on test days 1 (9:00 - 10:30 h) and 7 (13:00 – 14:30 h) for measurement of hematocrit. Mice were anesthetized with isoflurane, and blood was collected into heparinized microhematocrit capillary tubes (Chauke et al., 2011; Harris et al., 2011). Blood was centrifuged at 4°C and 1300 RPM (~1,900 g) for 12 min (Sorvall Legend Micro 21R; Thermo Scientific, Waltham, MA, USA), hematocrit was recorded, and plasma was removed and stored at -80°C for future use.

Predatory Aggression

On test days 2 and 3 between 13:30 and 15:00 h, mice were tested for predatory aggression (Gammie et al., 2003; Zhao et al., 2017). Mice were placed singly in a clean cage with minimal aspen shavings to cover the cage bottom; no food or water was provided. After a 15-min habituation period, a live cricket of standard size (0.2–0.5 g) was dropped into the cage on the side opposite the mouse. Behavior was video recorded until the cricket was killed or 7 minutes had elapsed. Videos were scored for latency to attack and latency to kill the cricket. If the male did not kill the cricket within 7 minutes, the mouse was removed from further analysis. Predatory aggression was tested on two successive days to determine repeatability. Each animal's lowest latency from the two tests was used for comparisons among reproductive conditions.

Resting Metabolic Rate

Resting metabolic rate (RMR) was measured on test day 4 between 8:30 and 16:30 h. The procedure was identical to our previously described method for measuring basal metabolic rate (Andrew et al., 2016) except that animals were not fasted prior to testing. Males were separated from their cage mates and placed in a Plexiglass metabolic chamber (volume: 525 mL) with bedding, inside an environmental chamber maintained at 28-30°C. Two animals could be tested simultaneously in separate metabolic chambers at the same time; to account for this, we used the chamber number as a covariate in all statistical analyses. Oxygen consumption ($\dot{V}O_2$) was measured over 8 h during the inactive period (lights-on). Subsampled excurrent air was dried (soda lime and Drierite) and sent through an oxygen analyzer. Oxygen concentration, temperature, and flow rate were measured every 5 s, and 3-min reference readings were taken every 42 min. RMR was computed as the lowest 10-min average $\dot{V}O_2$ during the 8-h period.

Maximal Exercise-induced Oxygen Consumption

Maximal oxygen consumption during forced exercise ($\dot{V}O_2$max) was measured at 10:00 – 11:30 h on days 5 and 6 as previously described (Dlugosz et al., 2012; Andrew et al., 2016). Briefly, $\dot{V}O_2$max was measured on a small running-wheel respirometer (circumference: 51.8 cm; effective volume: 900 mL). Mice were given a ~2 min warm-up period, followed by gradually increasing the speed approximately every 30 s until either oxygen concentration did not change or mice could not maintain position. Flow

rates (2400 mL/min) and O_2 concentrations were measured every second using Warthog LabHelper software (www.warthog.ucr.edu). Measurements were taken at room temperature (20-25°C). Excurrent air was subsampled (~150 mL/min) and dried with soda lime and Drierite, and oxygen concentration was analyzed. Reference air was taken at the beginning and end of trials, and a baseline was computed by linear regression.

Oxygen consumption was calculated using Warthog LabAnalyst, and instantaneous corrections were used to account for the mixing and washout characteristics of the chamber. $\dot{V}O_2$max (the highest $\dot{V}O_2$ averaged over 1 min) was determined on each of the two days to assess repeatability, and the higher of the two values for each animal was used for further statistical analyses.

Grip Strength

Maximum grip strength was determined on test days 2 and 3 at 9:30 - 11:00 h. Males were suspended by their tail over a horizontal wire-mesh surface attached to a small force gauge (HF – 10N, M&A Instruments Inc. Arcadia, CA, USA). The male was lowered until both the forelimbs and hindlimbs were touching the mesh without pulling on the force gauge. Once the male was on the mesh and relaxed, which typically took less than 3 s, the end of its tail was gently pulled horizontally until it released its grip from the mesh. The peak force value was recorded and the test was repeated once more; the higher value was used for analysis. Maximum grip strength was measured on testing days 2 and 3 to assess repeatability, and the higher of the two values was used for analysis.

Sprint Speed

Maximum sprint speed was measured on test days 5 and 6 at 14:00 - 15:30 h. Males were placed on a 'racetrack' (8 m long by 10 cm wide, with 30 cm high walls) equipped with 12 sets of aligned photocells at 50-cm intervals (Andrew et al., 2016). A rough rubber floor provided traction and ease of cleaning. At the start of each test, a mouse was placed near the start of the track and encouraged to walk or run down the track 2-4 times to become familiar with it. The male was returned to the starting area, the photocells were activated, and the mouse was chased down the track, activating the photocells. Sprint speed was measured 5 times on each of the two days, yielding a total of 10 trials per individual, from each of which we took the fastest 1.0-m interval. Trials were scored subjectively as poor, fair, okay, good or excellent depending on mouse cooperation; trials in which cooperation was scored as poor or fair were excluded from further analysis. The highest values from each day were used for repeatability, and the single highest value for each individual was used as its maximum sprint speed.

Euthanasia and Organ Collection

On test day 7 of time point 5, between 13:00 and 15:00 h, males were anesthetized with isoflurane, blood (~1 ml) was collected from the retro-orbital sinus (see below), and animals were euthanized by CO_2 inhalation. Morphometric measurements were taken [snout-to-anus length, head length, head width, right hind foot length (tip of phalanges to tibia/fibula), and baculum length], and the brain and all subcutaneous fat were removed and weighed. Finally, remaining organs [heart ventricles,

lungs, spleen, pancreas, liver, stomach (emptied), small/large intestines (emptied), caecum (emptied), adrenals (left and right), kidneys (left and right), and testes (left and right)] and muscles (right hind leg, left hind thigh, and left hind gastrocnemius) were rapidly removed, blotted dry, weighed, and stored at −80 °C.

Statistical Analysis

For measures that derived values from two trials or for paired organs, repeatability was examined with Pearson correlations and paired *t*-tests. For comparisons of group means, we used single values, e.g., mean or maximum. We used analysis of covariance (ANCOVA) in SPSS 24.0 to compare traits among reproductive groups (BM, NB, and VM) within each time point. For all tests, we used cohort as a factor and age, days between pup birth and testing, and days since pairing as covariates. Cohort, age, days since pup birth, and days since pairing are considered nuisance variables, so we do not report results for them. Where appropriate, we also used body mass, lean mass, body length, RMR chamber, or cricket mass as covariates (noted in Tables 2.3 - 2.6). For each analysis, we checked standardized residuals for normality and homogeneity of variance using Levene's test, and data were $\log_{10}$- or rank-transformed prior to analysis where appropriate (noted in Tables 2.3 - 2.10). Results are presented in untransformed units (as estimated marginal means ± standard errors unless otherwise noted).

We computed residuals from ANCOVA results, and for time point 5 we regressed energetic and performance residuals on organ masses. We performed the overall *F*-test for group differences and all three *a priori* contrasts among the three groups.

Because we tested mice in both the early and late postpartum periods, we could not reasonably perform longitudinal comparisons across all five time points. Therefore, for time points 2 - 5 we calculated the change (Δ) from time point 1 to the time point in question for each individual mouse. We performed ANCOVAs on Δ values to compare mice before they were paired and at a specific time point. For breeding males only, we also performed multiple linear regressions of each trait on relevant covariates plus litter size to determine if litter size significantly affected any measure (e.g., Andrew et al., 2016; Zhao et al., 2017). Litter size was not significant for any trait at any time point, so these results are not shown.

Excluding such nuisance variables as age and results reported in Tables 2.11 – 2.13, this study includes 581 P values, 61 of which were < 0.05. These tests include a substantial amount of non-independence because the same individuals were measured for all traits, some traits were correlated, and many tests were interrelated. To compensate for non-independence in multiple related tests, we used the Adaptive False Discovery Rate procedure as implemented in PROC MULTTEST in SAS 9.4 (SAS Inc., Cary, NC, USA). Based on this procedure, the 23 smallest P values would have adjusted P values < 0.05 (the highest being 0.0021). All P values reported in the text and tables are raw values, not adjusted for multiple comparisons.

Results

Repeatability Within Time Points

Pearson correlations indicated that all traits were repeatable (Table 2.2), except for the two measures of predatory aggression (latency to attack and latency to kill the cricket at time points 1, 2, and 3). Additionally, the paired *t*-test was significant between test days for sprint speed at time point 1, indicating that animals ran more slowly during trial 2; grip strength at time points 3 and 4, indicating that grip strength was higher on trial 2 for both time points; and kidney mass, indicating that right kidneys were significantly heavier than left ones.

Time Point 2 (litter 1 early postpartum / litter 2 early gestation)

Breeding males (BM) had more lean mass than non-breeding males (NB) on day 1 ($P = 0.018$, Table 2.3) and day 7 ($P = 0.050$) of time point 2 (Table 2.3). On test day 7, all three groups tended to have higher body, lean, and fat mass, compared to time point 1, but the increase in lean mass (Δ) was smaller in BM than in both NB ($P = 0.013$, Table 2.7) and virgin males (VM, $P = 0.037$). BM also gained more fat mass (Δ) ($P = 0.049$) and percent fat mass (Δ) on day 1 ($P = 0.049$), as well as on day 7 (Δ fat mass: $P = 0.037$; Δ percent fat mass: $P = 0.011$), compared to NB.

BM, NB, and VM all tended to become faster at killing crickets from time point 1 to time point 2. However, the drop in latency to kill the cricket was greater in BM than in NB ($P = 0.015$, Table 2.7). All three groups showed mean increases in maximum grip strength from time point 1 to time point 2; however, BM had lower grip strength than NB

in time point 2 ($P = 0.030$, Table 2.3), and lower grip Δ strength than both NB ($P = 0.005$, Table 2.7) and VM ($P = 0.004$). Finally, VM had higher hematocrit on day 1 than NB ($P = 0.034$, Table 2.3) but showed a different Δ sprint speed from NB ($P = 0.015$, Table 2.7): sprint speed decreased from time point 1 to time point 2 in VM but increased in NB and BM.

Time Point 3 (litter 2 late postpartum / litter 3 late gestation)

At time point 3, as in time point 2, all three reproductive groups had higher lean mass than in time point 1, but BM had smaller gains than VM on day 1 ($P = 0.012$, Table 2.8). BM also had lower resting metabolic rate than both NB ($P = 0.048$, Table 2.4) and VM ($P = 0.031$). Changes from time point 1 to time point 3 in percentage fat mass (Δ) ($P = 0.038$, Table 2.8) and hematocrit (Δ) ($P = 0.033$) differed between NB and VM: NB showed a decrease in the percentage of fat mass and an increase in hematocrit, whereas VM showed the opposite pattern.

Time Point 4 (litter 6 early postpartum / litter 7 early gestation)

We found no significant contrasts among groups at time point 4 for absolute values of any traits, but (Δ) lean mass gains were greater in VM than NB ($P = 0.009$, Table 2.9). NB gained (Δ) sprint speed from time point 1 to 4 compared to VM ($P = 0.024$), which saw no gains, and to BM ($P = 0.021$), which decreased in sprint speed.

Time Point 5 (litter 7 late postpartum / litter 8 late gestation)

As in time points 2 and 3, day 1 Δ lean mass gains were lower in BM than in VM ($P = 0.004$, Table 2.10). BM also had less lean mass than NB on day 7 ($P = 0.036$, Table 2.6). Latency to attack crickets in the predatory-aggression test was longer in BM than in NB ($P = 0.004$) or VM ($P = 0.008$). NB had a greater percentage of fat than VM on day 1 ($P = 0.040$). VM also saw reductions in maximum sprint speed compared to NB ($P = 0.048$).

Masses of several organs differed among groups. BM had lower liver mass and caecum mass than both NB (liver: $P = 0.022$, caecum: $P = 0.005$; Table 2.6) and VM (liver: $P = 0.024$, caecum: $P = 0.001$). BM also had lower spleen mass ($P = 0.009$) and stomach mass (emptied of food contents) than VM ($P = 0.039$), and lower heart mass than NB ($P = 0.031$). No organ masses differed between NB and VM.

Correlations Between Organ Sizes and Energetic/Performance Measures

Correlational analyses using data from all three reproductive groups in time point 5 revealed numerous correlations between residuals of organ sizes and residuals of energetic or performance measures (Table 2.14). The highest voluntary $\dot{V}O_2$max correlated positively with heart mass ($r^2 = 0.436$, $P = 0.006$), spleen mass ($r^2 = 0.345$, $P = 0.031$), average adrenal mass ($r^2 = 0.346$, $P = 0.031$), and thigh mass ($r^2 = 0.404$, $P = 0.011$). Sprint speed correlated positively with day 7 hematocrit ($r^2 = 0.403$, $P = 0.011$) but negatively with average kidney mass ($r^2 = -0.510$, $P = 0.001$). Grip strength was positively correlated with both heart mass ($r^2 = 0.402$, $P = 0.011$) and liver mass ($r^2 =$

0.450, $P = 0.004$). Day 1 hematocrit correlated positively with day 7 hematocrit ($r^2 = 0.530$, $P = 0.001$) and testis mass ($r^2 = 0.317$, $P = 0.049$), but negatively with heart ($r^2 = -0.317$, $P = 0.049$), spleen ($r^2 = -0.481$, $P = 0.002$), kidney ($r^2 = -0.463$, $P = 0.003$), intestine ($r^2 = -0.342$, $P = 0.033$), caecum ($r^2 = -0.364$, $P = 0.023$), and thigh mass ($r^2 = -0.326$, $P = 0.043$). RMR did not correlate significantly with any measured organ mass.

Discussion

We tested the hypothesis that breeding males in a biparental rodent species experience energetic, morphological, and behavioral changes and declines in exercise performance, relative to non-reproductive males, that might represent costs of parenthood. We predicted that these effects would become more pronounced with increasing parity. Contrary to our expectations, we found little evidence that increasing parity exacerbated the energetic, performance, morphological, or behavioral effects of being a father. Although breeding males and non-reproducing males differed for some measures, we did not find enough consistent differences between reproductive conditions to suggest that fatherhood had substantial costs.

Previous work in *Mus musculus* and other small rodents has found that motherhood can have long-term effects on a female's longevity, physical activity, and immune function (Speakman, 2008; Jasienska, 2009). Although one study found evidence of similar long-term effects in fathers in the biparental prairie vole (Campbell et al., 2009), this possibility has received little attention in the scientific literature. We found no clear evidence that effects of fatherhood increased with increasing parity. Of

the 19 repeated measurements at each time point (*a priori* contrasts only), breeding males differed from non-breeders and/or virgins in only three measures at time point 2, one measure at time point 3, none at time point 4, and two measures at time point 5. For Δ values (i.e., differences compared to prior to pair formation), BM differed from one or both control groups in seven measures at time point 2 and one measure each at time points 3, 4 and 5. These results suggest that BM underwent more changes, relative to controls, from time point 1 to time point 2 than at any other time. Thus, their mate's first pregnancy and the birth of their first litter seem to affect males more strongly than subsequent reproductive bouts. Additionally, fatherhood is often associated with hormonal (Saltzman and Ziegler, 2014; Bales and Saltzman, 2016) and morphological (Sanchez et al., 2008) changes around their mate's first pregnancy, but it remains unclear to what extent such changes occur during later pregnancies.

California mouse fathers provide more offspring care (huddling, licking/grooming, pup retrieval) during the early postpartum period than late postpartum (Gubernick and Alberts, 1987a). To determine whether effects of fatherhood might differ between the early and late postpartum periods (which usually correspond to the mate's early and late gestation, as a consequence of postpartum conception), data collection from breeding males and age-matched control males started 5 – 10 days after parturition (early postpartum) at time points 2 and 4 (Fig. 2.1), and 15 – 20 days after parturition (late postpartum) at time points 3 and 5. The number of differences among reproductive groups did not differ between early and late postpartum tests: BM differed from control males in 3 of 38 (lean mass, grip strength, and RMR) total measures for early postpartum

testing (time points 2 and 4) as well as in 2 of 38 (lean mass and attack latency) measures for late postpartum testing (time points 3 and 5).

Although it is possible that the traits we measured do not change across the postpartum period, it is also conceivable that we did not leave enough time between our early and late postpartum testing periods. Also, specific differences among groups, not the total number of differences, could vary between early and late postpartum (i.e. not all group differences occur in the same part of the postpartum period). In previous studies, we found that California mouse fathers gain body mass during the second half of their mate's pregnancy, but only when this corresponds to a period of infant care (Harris et al., 2011; Saltzman et al., 2015). Consequently, in the present study we expected that breeding males would show pronounced changes in body mass during their mate's first pregnancy but would gain less body mass in the latter half of each subsequent pregnancy (in part because this species mates shortly after birth) during the late postpartum period. However, neither body mass nor patterns of change in body mass across the study differed between breeding males and control males. The lack of between-group differences in body mass might be an artifact of the abundant food and reduced metabolic demands in laboratory conditions, a lack of hormonal differences between reproductive conditions, or a combination of the two. Though endocrine differences have been found between males of different reproductive conditions (Gubernick and Nelson, 1989; Trainor et al., 2003), without measuring hormone levels, we cannot assume that the breeding males in this study had different endocrine profiles from the non-reproducing males.

In the present study, all reproductive groups gained absolute body and lean mass throughout the entire study. Although the breeding males had greater absolute lean mass at time point 2 compared to control males, breeding males actually gained less lean mass throughout the study from time point 1 onwards. In contrast, BM gained more fat mass than NB from time point 1 to time point 2, but not at subsequent time points. The smaller gains in lean mass and minimal changes in fat mass could indicate that BM allocated fewer resources to muscle growth and more to fat stores than did control males (Cinti, 2005; Pond, 2012). Previous work in California mice indicates that fathers have greater fat reserves than non-reproducing males, with larger subcutaneous fat pads under standard laboratory conditions (Andrew et al., 2016) and great fat mass under challenged conditions (Zhao et al., in press). Although we could not determine organ masses during time points 1 - 4, breeding males in this study had smaller stomachs and caecums at the end of time point 5, suggesting they could not process as much food as control males, which might have contributed to their lower lean mass.

Grip strength, sprint speed, and $\dot{V}O_2$max capture key aspects of an organism's total exercise performance abilities. Grip strength can be used as an index of muscular strength (Meyer et al., 1979; Maurissen et al., 2003), sprint speed is a common measure of burst exercise performance (Djawdan, 1993; Garland and Losos, 1994), and $\dot{V}O_2$max is a primary determinant of aerobic locomotor endurance (Joyner and Coyle, 2008; Dlugosz et al., 2012). Total exercise performance can be an essential component of behavior and survival because it can influence vulnerability to predation, foraging capacity, territorial defense, and other locomotor requirements (Djawdan and Garland,

1988; Garland and Albuquerque, 2017; Orr and Garland, 2017). Grip strength was lower in BM than in the control groups only at time point 2, and neither sprint speed nor $\dot{V}O_2$ max differed between BM and controls at any time point. This is surprising, given that BM had smaller hearts and livers than one or both control groups. Both of these organs are important for performance, especially $\dot{V}O_2$ max (although we found that $\dot{V}O_2$ max correlated significantly with heart mass but not liver mass). Hematocrit, an indicator of blood oxygen-carrying capacity (Kolb et al., 2010), is also expected to parallel changes in $\dot{V}O_2$ max. Additionally, hematocrit did not correlate with $\dot{V}O_2$ max (correlational analyses were conducted only at time point 5) but did correlate negatively with spleen mass [involved in blood filtration, immune function, and in some species, storage of red blood cells (Cesta, 2006)]. The paucity of differences in performance-related measures among reproductive groups is consistent with our previous studies, in which first-time fathers did not differ from non-reproductive control males in sprint speed, $\dot{V}O_2$ max, or exercise endurance (Andrew et al., 2016).

RMR reflects all processes that require energy in an endotherm under conditions of minimal energy use, except that the individual has not been fasted (Johnson et al., 2001), whereas predatory aggression indicates hunting/foraging/killing propensities and/or abilities (Gammie et al., 2003). Contrary to our predictions, we found that breeding males had lower RMR than controls at time point 3. The decrease in latency (i.e., increase in speed) to kill crickets from time point 1 to time point 2 was greater in

BM than NB, but BM took longer to attack crickets than both NB and VM in time point 5. These results suggest that fatherhood has minimal effects on these traits.

Our previous work on California mouse fathers has often used males housed with a tubally ligated female and males housed with another male as control groups (de Jong et al., 2013; Harris and Saltzman, 2013; Andrew et al., 2016; Zhao et al., 2017). In the present study, the control groups were males housed with an ovariectomized female treated with estradiol benzoate and progesterone to induce estrous behavior (non-breeding males) and males housed with an ovariectomized female untreated with hormones (virgin males). We chose these control groups to elucidate potential roles of cohabiting with a sexually receptive female (NB) or cohabitating with a (nonreceptive) female (VM) in mediating effects of fatherhood.

We found very few differences between non-breeding males and virgin males. Most notably, Δ sprint speed differed between the two groups at time points 2, 4 and 5, with non-breeding males showing greater increases in sprint speed relative to time point 1. Non-breeding males also had, at individual time points, lower Δ percent fat mass at time point 3 but higher Δ percent fat mass at time point 5, lower Δ lean mass (time point 4), and lower hematocrit (time point 2) than virgin males. Studies of other rodents have found that cohabitation with pregnant females and copulation can increase testosterone concentrations (Brown et al., 1995; Reburn and Wynne-Edwards, 1999) but testosterone concentrations have also been found to increase with copulation alone (Haltmeyer and Eik-Nes, 1969; Batty, 1978). Although we did not measure testosterone levels in the present study, these findings from other species suggest that testosterone was likely

higher in the non-breeding males than in the virgin males. Regardless, these findings indicate that cohabitation with a sexually receptive female can influence body composition and exercise performance to some extent, even in the absence of pups.

Some caveats should be kept in mind when interpreting our results. First, repeated handling of animals and brief separations from cage mates (especially during RMR tests) might have influenced behavior and/or pup development (Vieira and Brown, 2003). This seems unlikely, based on previous work in this species (de Jong et al., 2013; Harris et al., 2013). Second, standard laboratory housing conditions – e.g., *ad lib* food and water, thermoneutral environment, absence of predators and conspecific rivals, and small cages that do not require any additional exercise - could potentially diminish overall locomotor performance in all animals and might ameliorate any energetic stress related to fatherhood as experienced by free-living animals. Third, it is possible that the near-thermoneutral environment of this study did not allow differences to emerge among the reproductive groups: temperatures similar to what wild California mice naturally experience would make paternal care essential to offspring survival. We are currently addressing this possibility in a study examining possible effects of fatherhood in a cold environment. Finally, parity and age were highly correlated in our study because all males were paired at approximately the same age.

In summary, results from this study do not support our hypothesis that energetic, morphological, and exercise performance costs of fatherhood increase with increasing parity. We did find evidence that fatherhood can affect males' morphology (lower lean mass, higher fat mass, and smaller organs) and possibly exercise performance (lower grip

strength), but it seems to have little effect on RMR, hematocrit or predatory aggression. Moreover, the effects of reproductive condition do not seem to differ between the early and late postpartum periods, at least in a benign lab environment. We caution that these results may not hold for fathers under natural conditions, which are likely to be much more energetically (and perhaps psychologically) stressful than laboratory housing.

References

Achenbach, G. G. and Snowdon, C. T. (2002). Costs of caregiving: Weight loss in captive adult male cotton-top tamarins (*Saguinus oedipus*) following the birth of infants. *Int J Primatol* **23**, 179-189.

Andrew, J. R., Saltzman, W., Chappell, M. A. and Garland, T., Jr. (2016). Consequences of fatherhood in the biparental California mouse (*Peromyscus californicus*): Locomotor performance, metabolic rate, and organ masses. *Physiol Biochem Zool* **89**, 130-40.

Bales, K. L. and Saltzman, W. (2016). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Horm Behav* **77**, 249-59.

Bartlett, E. E. (2004). The effects of fatherhood on the health of men: A review of the literature. *J Men's Health Gender* **1**, 159-169.

Batty, J. (1978). Acute changes in plasma testosterone levels and their relation to measures of sexual behaviour in the male house mouse (*Mus musculus*). *Anim Behav* **26**, 349-357.

Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Horm Behav* **7**, 105-138.

Blouin, K., Boivin, A. and Tchernof, A. (2008). Androgens and body fat distribution. *J Steroid Biochem Mol Biol* **108**, 272-80.

Braun, K. and Champagne, F. A. (2014). Paternal influences on offspring development: Behavioural and epigenetic pathways. *J Neuroendocrinol* **26**, 697-706.

Brown, R. E., Murdoch, T., Murphy, P. R. and Moger, W. H. (1995). Hormonal responses of male gerbils to stimuli from their mate and pups. *Horm Behav* **29**, 474-491.

Campbell, J. C., Laugero, K. D., Van Westerhuyzen, J. A., Hostetler, C. M., Cohen, J. D. and Bales, K. L. (2009). Costs of pair-bonding and paternal care in male prairie voles (*Microtus ochrogaster*). *Physiol Behav* **98**, 367-73.

Cantoni, D. and Brown, R. E. (1997). Paternal investment and reproductive success in the California mouse, *Peromyscus californicus*. *Anim Behav* **54**, 377-86.

Cesta, M. F. (2006). Normal structure, function, and histology of the spleen. *Toxicol Pathol* **34**, 455-65.

Chauke, M., Malisch, J. L., Robinson, C., de Jong, T. R. and Saltzman, W. (2011). Effects of reproductive status on behavioral and endocrine responses to acute stress in a biparental rodent, the California mouse (*Peromyscus californicus*). *Horm Behav* **60**, 128-38.

Cinti, S. (2005). The adipose organ. *Prostaglandins Leukot Essent Fatty Acids* **73**, 9-15.

de Jong, T. R., Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2013). Physiological and neuroendocrine responses to chronic variable stress in male California mice (*Peromyscus californicus*): Influence of social environment and paternal state. *Psychoneuroendocrinol* **38**, 2023-33.

Dewsbury, D. A. (1974). Copulatory behavior of California mice (*Peromyscus californicus*). *Brain Behav Evol* **9**, 95-106.

Djawdan, M. (1993). Locomotor performance of bipedal and quadrupedal heteromyid rodents. *Funct Ecol* **7**, 195-202.

Djawdan, M. and Garland, T., Jr. (1988). Maximal running speeds of bipedal and quadrupedal rodents. *J Mamm* **69**, 765-772.

Dlugosz, E. M., Harris, B. N., Saltzman, W. and Chappell, M. A. (2012). Glucocorticoids, aerobic physiology, and locomotor behavior in California mice. *Physiol Biochem Zool* **85**, 671-83.

Dudley, D. (1974a). Contributions of paternal care to the growth and development of the young in *Peromyscus californicus*. *Behav Biol* **11**, 155-66.

Dudley, D. (1974b). Paternal behavior in the California mouse, *Peromyscus californicus*. *Behav Bio* **11**, 247-252.

Gammie, S. C., Hasen, N. S., Rhodes, J. S., Girard, I. and Garland, T., Jr. (2003). Predatory aggression, but not maternal or intermale aggression, is associated with high voluntary wheel-running behavior in mice. *Horm Behav* **44**, 209-221.

Garland, T., Jr. and Albuquerque, R. L. (2017). Locomotion, energetics, performance, and behavior: A mammalian perspective on lizards, and vice versa. *Integr Comp Biol* **57**, 252-266.

Garland, T., Jr. and Losos, J. B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In *Ecological morphology: integrative organismal biology*, pp. 240-302. Chicago: University of Chicago Press.

Gittleman, J. L. and Thompson, S. D. (1988). Energy allocation in mammalian reproduction. *Am Zool* **28**, 863-875.

Gubernick, D. J. (1988). Reproduction in the California mouse, *Peromyscus californicus*. *J Mammal* **69**, 857-860.

Gubernick, D. J. and Alberts, J. R. (1987a). The biparental care system of the California mouse, *Peromyscus californicus*. *J Comp Psychol* **101**, 169-177.

Gubernick, D. J. and Alberts, J. R. (1987b). "Resource" exchange in the biparental California mouse (*Peromyscus californicus*): Water transfer from pups to parents. *J Comp Psychol* **101**, 328-34.

Gubernick, D. J. and Nelson, R. J. (1989). Prolactin and paternal behavior in the biparental California mouse, *Peromyscus californicus*. *Horm Behav* **23**, 203-210.

Gubernick, D. J. and Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proc Biol Sci* **267**, 147-50.

Gubernick, D. J., Wright, S. L. and Brown, R. E. (1993). The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Anim Behav* **46**, 539-546.

Haltmeyer, G. C. and Eik-Nes, K. B. (1969). Plasma levels of testosterone in male rabbits following copulation. *J Reprod Fertil* **19**, 273-7.

Hammond, K. A. (1997). Adaptation of the maternal intestine during lactation. *J Mammary Gland Biol Neoplasia* **2**, 243-52.

Harris, B. N., de Jong, T. R., Yang, V. and Saltzman, W. (2013). Chronic variable stress in fathers alters paternal and social behavior but not pup development in the biparental California mouse (*Peromyscus californicus*). *Horm Behav* **64**, 799-811.

Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2011). Acute effects of corticosterone injection on paternal behavior in California mouse (*Peromyscus californicus*) fathers. *Horm Behav* **60**, 666-75.

Harris, B. N. and Saltzman, W. (2013). Effect of reproductive status on hypothalamic-pituitary-adrenal (HPA) activity and reactivity in male California mice (*Peromyscus californicus*). *Physiol Behav* **112-113**, 70-6.

Haynes, S. G., Eaker, E. D. and Feinleib, M. (1983). Spouse behavior and coronary heart disease in men: Prospective results from the Framingham heart study. *Am J Epidemiol* **118**, 1-22.

Ibebunjo, C., Eash, J. K., Li, C., Ma, Q. and Glass, D. J. (2011). Voluntary running, skeletal muscle gene expression, and signaling inversely regulated by orchidectomy and testosterone replacement. *Am J Physiol Endocrinol Metab* **300**, E327-40.

Jasienska, G. (2009). Reproduction and lifespan: Trade-offs, overall energy budgets, intergenerational costs, and costs neglected by research. *Am J Hum Biol* **21**, 524-32.

Johnson, M. S., Thomson, S. C. and Speakman, J. R. (2001). Limits to sustained energy intake II. Inter-relationships between resting metabolic rate, life-history traits and morphology in *Mus musculus*. *J Exp Biol* **204**, 1937-1946.

Joyner, M. J. and Coyle, E. F. (2008). Endurance exercise performance: The physiology of champions. *J Physiol* **586**, 35-44.

Kleiman, D. G. and Malcom, J. R. (1981). The evolution of male parental investment in mammals. In *Parental Care in Mammals*, pp. 347-387. Springer US: Plenum Publishing Corp.

Kolb, E. M., Kelly, S. A., Middleton, K. M., Sermsakdi, L. S., Chappell, M. A. and Garland, T., Jr. (2010). Erythropoietin elevates VO2max but not voluntary wheel running in mice. *J Exp Biol* **213**, 510-9.

Maurissen, J. P. J., Marable, B. R., Andrus, A. K. and Stebbins, K. E. (2003). Factors affecting grip strength testing. *Neurotoxicol Teratol* **25**, 543-553.

Meyer, O. A., Tilson, H. A., Byrd, W. C. and Riley, M. T. (1979). A method for the routine assessment of fore-and hindlimb grip strength of rats and mice. *Neurobehav Toxicol* **1**, 233.

Moore, I. T. and Hopkins, W. A. (2009). Interactions and trade-offs among physiological determinants of performance and reproductive success. *Integr Comp Biol* **49**, 441-51.

Nunes, S., Fite, J. E., Patera, K. J. and French, J. A. (2001). Interactions among paternal behavior, steroid hormones, and parental experience in male marmosets (*Callithrix kuhlii*). *Horm Behav* **39**, 70-82.

Orr, T. J. and Garland, T., Jr. (2017). Complex reproductive traits and whole-organism performance. *Integr Comp Biol* **57**, 407-422.

Pond, C. M. (2012). The evolution of mammalian adipose tissue. In *Adipose Tissue Biology*, pp. 227-269. New York: Springer Science.

Reburn, C. J. and Wynne-Edwards, K. E. (1999). Hormonal changes in males of a naturally biparental and a uniparental mammal. *Horm Behav* **35**, 163-76.

Ribble, D. O. (1991). The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behav Ecol Sociobiol* **29**, 161-166.

Ribble, D. O. and Salvioni, M. (1990). Social organization and nest co-occupancy in *Peromyscus californicus*, a monogamous rodent. *Behav Ecol Sociobiol* **26**, 9-15.

Saltzman, W., Harris, B. N., de Jong, T. R., Nguyen, P. P., Cho, J. T., Hernandez, M. and Perea-Rodriguez, J. P. (2015). Effects of parental status on male body mass in the monogamous, biparental California mouse. *J Zool* **296**, 23-29.

Saltzman, W. and Ziegler, T. E. (2014). Functional significance of hormonal changes in mammalian fathers. *J Neuroendocrinol* **26**, 685-96.

Sanchez, S., Pelaez, F., Fidalgo, A., Morcillo, A. and Caperos, J. M. (2008). Changes in body mass of expectant male cotton-top tamarins (*Saguinus oedipus*). *Folia Primatol* **79**, 458-62.

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos Trans R Soc Lond B Biol Sci* **363**, 375-98.

Trainor, B. C., Bird, I. M., Alday, N. A., Schlinger, B. A. and Marler, C. A. (2003). Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. *Neuroendocrinol* **78**, 36-44.

Vieira, M. L. and Brown, R. E. (2003). Effects of the presence of the father on pup development in California mice (*Peromyscus californicus*). *Dev Psychobiol* **42**, 246-51.

Wright, S. L. and Brown, R. E. (2002). The importance of paternal care on pup survival and pup growth in *Peromyscus californicus* when required to work for food. *Behav Process* **60**, 41-52.

Zhao, M., Garland Jr, T., Chappell, M. A., Andrew, J. R., Harris, B. N. and Saltzman, W. (in press). Effects of a physical and energetic challenge on male California mice (*Peromyscus californicus*): Modulation by reproductive condition. *J Exp Biol*.

Zhao, M., Garland, T., Jr., Chappell, M. A., Andrew, J. R. and Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiol Behav* **177**, 57-67.

Ziegler, T. E., Wegner, F. H., Carlson, A. A., Lazaro-Perea, C. and Snowdon, C. T. (2000). Prolactin levels during the periparturitional period in the biparental cotton-top tamarin (*Saguinus oedipus*): Interactions with gender, androgen levels, and parenting. *Horm Behav* **38**, 111-22.

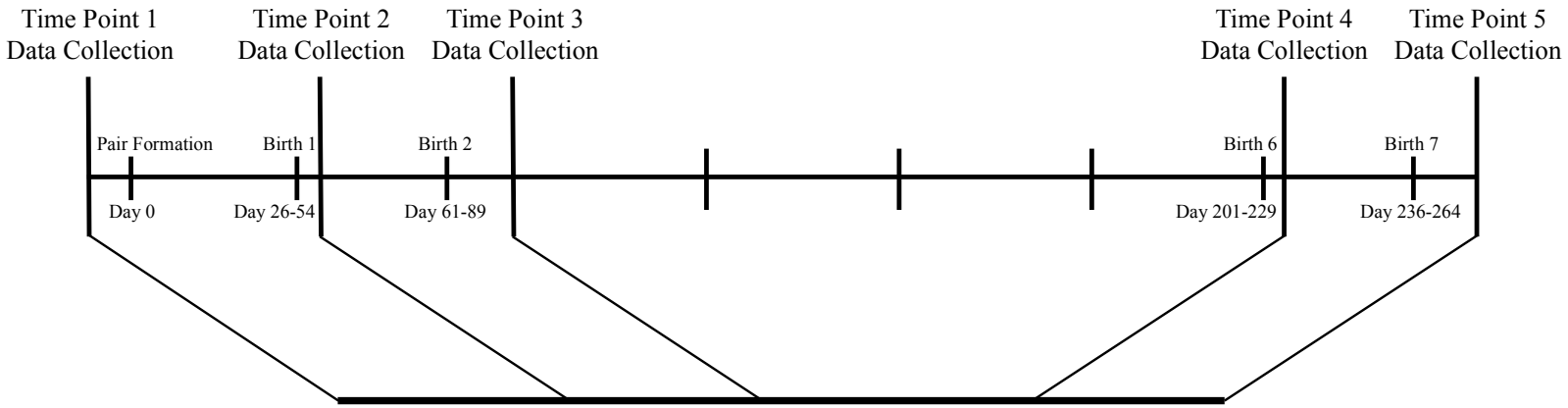
Figure Legends

Figure 2.1. Study timeline and design. The schedule of data collection at time points 2-5 was based on births of litters to breeding pairs, with non-breeding and virgin males time-matched to breeding males.

Figure 2.2. Mean number of pups born to breeding pairs at time points 2 – 5. Sample sizes were 21, 19, 17, and 14, respectively. Error bars are standard errors.

Figure 2.3. Number of pups born to breeding pairs versus number of days since pairing. Time point 2 = light grey triangles, time point 3 = white squares, time point 4 = black diamonds, time point 5 = dark grey circles.

Figure 2.1.



Test Day	Measures	Measures
1	9:00-10:30	Hematocrit
1	13:00-14:30	Body Mass/Fat Mass/Lean Mass
2/3	9:30-11:00	Maximum Grip Strength
2/3	13:30-15:00	Predatory Aggression
4	8:30-16:30	Body Mass/Resting Metabolic Rate
5/6	10:00-11:30	Body Mass/Maximal Oxygen Consumption
5/6	14:00-15:30	Maximum Sprint Speed
7	9:00-10:30	Body Mass/Fat Mass/Lean Mass
7	13:00-14:30	Hematocrit
7	13:00-15:00	Organ Masses (Time Point 5 Only)

Figure 2.2.

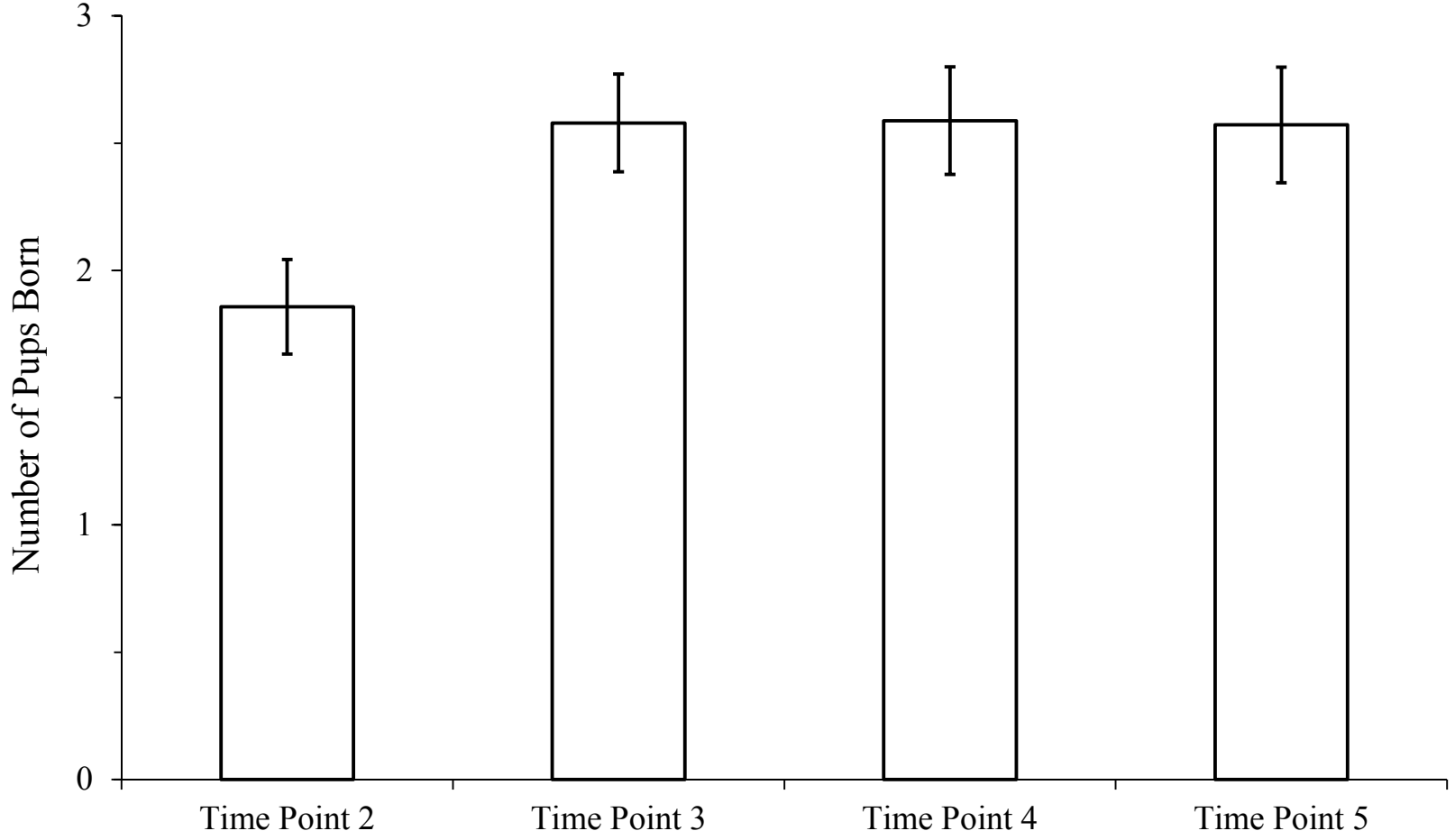

3
2
1
0
Number of Pups Born
Time Point 2
Time Point 3
Time Point 4
Time Point 5

Figure 2.3.

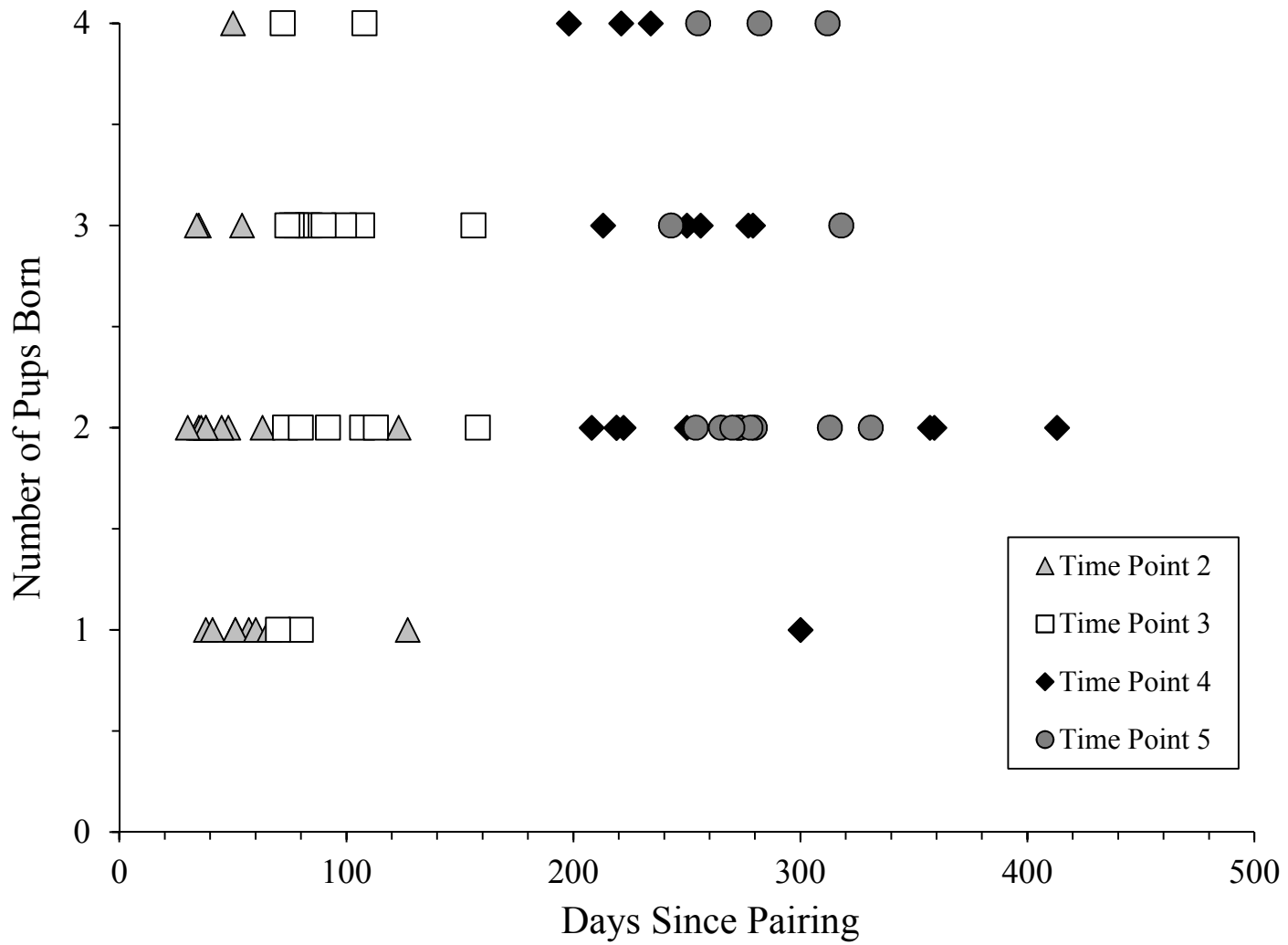

Number of Pups Born
0
1
2
3
4
0
100
200
300
400
500
Days Since Pairing
Time Point 2
Time Point 3
Time Point 4
Time Point 5

Table 2.1. Timeline of the experiment.

Groups	Time Point 1	Time Point 2	Time Point 3	Time Point 4	Time Point 5
Breeding Males (BM)	5-11 days before pairing with female	5-10 days after birth 1	15-20 days after birth 2	5-10 days after birth 6	15-20 days after birth 7
Nonbreeding Males (NB)	5-11 days before pairing with female	Matched to BM	Matched to BM	Matched to BM	Matched to BM
Virgin Males (VM)	5-11 days before pairing with female	Matched to BM	Matched to BM	Matched to BM	Matched to BM

Table 2.2. Results of Pearson correlations and paired t-tests comparing values from the two trials for tests conducted on two successive days, and for paired organ masses.

Trait	Unit	N of Paired Observations	Pearson Correlation	P of Pearson Correlation	t of Paired *t*-Test	P of Paired *t*-Test
Time Point 1						
Predatory Aggression: Latency to First Attack Cricket	Seconds	49	0.268	0.062	0.315	0.754
Predatory Aggression: Latency to Kill Cricket	Seconds	48	0.260	0.074	0.723	0.473
Maximal Oxygen Consumption	ml O_2/h	60	0.883	**9.45E-21**	-0.426	0.672
Maximum Grip Strength	Newtons	54	0.469	**3.42E-04**	1.618	0.112
Maximum Sprint Speed	m/s	38	0.608	**5.14E-05**	-5.773	**1.27E-06**
Time Point 2						
Predatory Aggression: Latency to First Attack Cricket	Seconds	50	0.269	0.059	-1.004	0.320
Predatory Aggression: Latency to Kill Cricket	Seconds	44	0.489	**0.001**	-1.915	0.062
Maximal Oxygen Consumption	ml O_2/h	57	0.887	**4.38E-20**	0.958	0.342
Maximum Grip Strength	Newtons	60	0.372	**0.003**	1.613	0.112
Maximum Sprint Speed	m/s	45	0.669	**5.20E-07**	0.591	0.558
Time Point 3						
Predatory Aggression: Latency to First Attack Cricket	Seconds	44	0.282	0.084	-0.583	0.563
Predatory Aggression: Latency to Kill Cricket	Seconds	35	0.329	0.054	-0.914	0.367
Maximal Oxygen Consumption	ml O_2/h	55	0.926	**4.89E-24**	0.324	0.747
Maximum Grip Strength	Newtons	54	0.458	**4.93E-04**	2.360	**0.022**
Maximum Sprint Speed	m/s	51	0.877	**3.27E-17**	-0.270	0.788
Time Point 4						
Predatory Aggression: Latency to First Attack Cricket	Seconds	42	0.410	**0.007**	-1.216	0.231
Predatory Aggression: Latency to Kill Cricket	Seconds	35	0.525	**0.001**	0.343	0.734
Maximal Oxygen Consumption	ml O_2/h	46	0.964	**4.53E-27**	1.966	0.056
Maximum Grip Strength	Newtons	46	0.667	**4.11E-07**	2.406	**0.020**
Maximum Sprint Speed	m/s	38	0.904	**7.94E-15**	-0.433	0.667

Table 2.2. Continued.

Trait	Unit	N of Paired Observations	Pearson Correlation	P of Pearson Correlation	t of Paired *t*-Test	P of Paired *t*-Test
Time Point 5						
Predatory Aggression: Latency to First Attack Cricket	Seconds	42	0.410	**0.007**	-1.184	0.245
Predatory Aggression: Latency to Kill Cricket	Seconds	35	0.525	**0.001**	-0.261	0.796
Maximal Oxygen Consumption	ml O_2/h	46	0.964	**4.53E-27**	-0.645	0.523
Maximum Grip Strength	Newtons	46	0.667	**4.11E-07**	-0.051	0.959
Maximum Sprint Speed	m/s	38	0.904	**7.94E-15**	0.289	0.774
Kidney Mass	Grams	39	0.984	**1.72E-29**	3.307	**0.002**
Adrenal Mass	Grams	39	0.936	**2.06E-18**	0.492	0.626
Testis Mass	Grams	39	0.985	**1.34E-29**	0.027	0.978

P values ≤ 0.05 are bolded and underlined.

Table 2.3. Comparisons among breeding males, non-breeding males, and virgin males in time point 2.

				P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**Covariates**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Mass (Day 1)	Grams	None	None	0.571	0.481	0.852	20	46.81	2.14	20	45.12	1.73	20	44.69	1.76
Body Mass (Day 4)	Grams	None	None	0.505	0.352	0.722	20	47.26	2.16	20	45.25	1.75	20	44.42	1.78
Body Mass (Day 7)	Grams	None	None	0.677	0.610	0.898	20	45.82	2.14	20	44.58	1.73	20	44.29	1.76
Fat Mass (Day 1)	Grams	None	None	0.898	0.790	0.610	20	8.35	1.05	20	8.54	0.85	20	7.96	0.86
Percent Fat Mass (Day 1)	%	None	None	0.586	0.930	0.557	20	16.98	1.69	20	18.27	1.37	20	17.19	1.39
Fat Mass (Day 7)	Grams	None	None	0.855	0.884	0.670	20	7.95	1.01	20	8.21	0.82	20	7.74	0.83
Percent Fat Mass (Day 7)	%	Log_{10}	None	0.441	0.744	0.568	20	16.50	1.64	20	17.89	1.33	20	16.90	1.35
Lean Mass (Day 1)	Grams	Log_{10}	None	**0.018**	0.116	0.266	20	36.48	1.12	19	32.71	0.92	19	34.04	0.94
Percent Lean Mass (Day 1)	%	Log_{10}	None	0.241	0.933	0.159	20	77.06	1.56	19	74.61	1.28	19	76.95	1.30
Lean Mass (Day 7)	Grams	None	None	**0.050**	0.175	0.396	20	35.57	1.12	19	32.42	0.91	20	33.44	0.90
Percent Lean Mass (Day 7)	%	None	None	0.445	0.760	0.541	20	76.62	1.59	19	74.90	1.30	20	75.94	1.28
Hematocrit (Day 1)	%	None	None	0.982	0.096	**0.034**	16	47.87	0.83	16	47.85	0.65	15	49.66	0.69
Hematocrit (Day 7)	%	Log_{10}	None	0.646	0.436	0.680	16	45.10	1.16	16	45.62	0.91	15	46.17	0.96
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	C	0.856	0.694	0.782	19	9.56	3.30	18	10.39	2.77	18	11.42	2.80
Predatory Aggression: Latency to Kill Cricket	Seconds	None	C	0.738	0.511	0.205	17	52.53	10.17	17	47.87	8.27	18	62.00	8.22
Resting Metabolic Rate	ml O_2/h	Log_{10}	B, R	0.860	0.753	0.516	18	0.89	0.03	20	1.27	0.03	20	1.19	0.06
Maximal Oxygen Consumption	ml O_2/h	None	B	0.126	0.370	0.412	19	6.08	0.13	19	5.79	0.11	19	5.91	0.11
Maximum Grip Strength	Newtons	None	B	**0.030**	0.084	0.569	20	4.68	0.19	20	5.25	0.15	20	5.14	0.15
Maximum Sprint Speed	m/s	Log_{10}	B	0.145	0.505	0.327	19	1.93	0.16	19	2.19	0.13	17	1.91	0.14

B = Body Mass, R = RMR Chamber, C = Cricket Mass

P values ≤ 0.05 are bolded and underlined.

Table 2.4. Comparisons among breeding males, non-breeding males, and virgin males in time point 3.

				P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**Covariates**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Mass (Day 1)	Grams	None	None	0.574	0.444	0.821	19	46.70	2.15	18	48.48	2.10	18	49.12	2.07
Body Mass (Day 4)	Grams	None	None	0.672	0.566	0.867	18	47.10	2.27	18	48.50	2.14	18	48.98	2.11
Body Mass (Day 7)	Grams	None	None	0.790	0.652	0.834	18	46.91	2.36	18	47.82	2.23	18	48.45	2.19
Fat Mass (Day 1)	Grams	Log_{10}	None	0.432	0.237	0.656	19	8.30	0.91	18	9.32	0.89	18	9.78	0.88
Percent Fat Mass (Day 1)	%	Log_{10}	None	0.372	0.184	0.623	19	17.07	1.31	18	18.68	1.28	18	19.52	1.27
Fat Mass (Day 7)	Grams	None	None	0.658	0.450	0.724	18	8.47	0.96	18	9.09	0.91	18	9.53	0.89
Percent Fat Mass (Day 7)	%	Log_{10}	None	0.686	0.333	0.521	18	17.40	1.41	18	18.30	1.34	18	19.24	1.31
Lean Mass (Day 1)	Grams	None	None	0.849	0.651	0.766	19	35.64	1.28	18	36.00	1.26	16	36.51	1.32
Percent Lean Mass (Day 1)	%	Log_{10}	None	0.233	0.352	0.796	19	76.86	1.23	18	74.73	1.21	16	75.08	1.27
Lean Mass (Day 7)	Grams	Log_{10}	None	0.736	0.855	0.553	18	35.43	1.38	17	34.90	1.34	18	35.90	1.29
Percent Lean Mass (Day 7)	%	Log_{10}	None	0.593	0.356	0.668	18	75.74	1.25	17	74.85	1.21	18	74.07	1.17
Hematocrit (Day 1)	%	Log_{10}	None	0.525	0.668	0.237	19	47.43	0.64	18	48.66	0.53	18	48.51	0.95
Hematocrit (Day 7)	%	Log_{10}	None	0.997	0.742	0.709	17	45.45	0.75	18	45.45	0.68	18	45.13	0.67
Predatory Aggression: Latency to First Attack Cricket	Seconds	Rank	C	0.277	0.628	0.080	17	7.92	3.42	15	10.18	3.48	17	9.93	3.22
Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	C	0.898	0.636	0.504	14	45.27	8.84	15	52.45	8.43	13	56.61	8.89
Resting Metabolic Rate	ml O_2/h	None	B, R	**0.048**	**0.031**	0.810	18	1.13	0.05	18	1.27	0.04	17	1.28	0.04
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	B	0.228	0.646	0.382	18	5.96	0.11	18	5.79	0.10	18	5.91	0.10
Maximum Grip Strength	Newtons	None	B	0.665	0.663	0.999	18	5.09	0.18	18	5.20	0.17	18	5.20	0.17
Maximum Sprint Speed	m/s	None	None	0.634	0.938	0.523	18	1.90	0.14	18	2.00	0.13	18	1.89	0.13

B = Body Mass, R = RMR Chamber, C = Cricket Mass

P values ≤ 0.05 are bolded and underlined.

Table 2.5. Comparisons among breeding males, non-breeding males, and virgin males in time point 4.

				P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**Covariates**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Mass (Day 1)	Grams	Log_{10}	None	0.688	0.315	0.439	17	52.06	3.10	13	53.17	2.85	15	55.94	2.63
Body Mass (Day 4)	Grams	Log_{10}	None	0.695	0.559	0.813	17	52.16	3.36	13	53.27	3.04	16	54.43	2.73
Body Mass (Day 7)	Grams	Log_{10}	None	0.864	0.660	0.728	17	52.26	3.36	13	51.98	3.04	16	53.51	2.74
Fat Mass (Day 1)	Grams	None	E	0.413	0.175	0.485	17	11.43	0.83	13	10.38	0.75	15	9.71	0.71
Percent Fat Mass (Day 1)	%	None	None	0.568	0.186	0.332	17	20.83	1.25	13	19.72	1.15	15	18.33	1.06
Fat Mass (Day 7)	Grams	None	E	0.332	0.071	0.271	17	11.17	0.82	13	9.93	0.74	16	8.91	0.68
Percent Fat Mass (Day 7)	%	Log_{10}	None	0.656	0.117	0.140	17	20.45	1.28	13	19.24	1.15	16	17.32	1.04
Lean Mass (Day 1)	Grams	Log_{10}	None	0.601	0.183	0.296	17	37.33	2.13	13	38.69	1.96	15	41.16	1.80
Percent Lean Mass (Day 1)	%	Log_{10}	None	0.771	0.409	0.491	17	72.20	1.20	13	72.66	1.11	15	73.64	1.02
Lean Mass (Day 7)	Grams	Log_{10}	None	0.744	0.376	0.466	17	37.57	2.33	13	38.21	2.10	16	40.09	1.90
Percent Lean Mass (Day 7)	%	Log_{10}	None	0.605	0.137	0.201	17	72.47	1.21	13	73.31	1.09	16	75.09	0.98
Hematocrit (Day 1)	%	None	None	0.423	0.575	0.712	17	47.27	0.91	12	48.42	0.86	16	48.04	0.75
Hematocrit (Day 7)	%	Log_{10}	None	0.385	0.206	0.618	16	43.11	1.06	13	44.54	0.92	16	45.06	0.82
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	C	0.610	0.605	0.986	16	15.13	4.46	12	11.58	4.14	15	11.67	3.72
Predatory Aggression: Latency to Kill Cricket	Seconds	Rank	C	0.323	0.556	0.493	14	53.06	12.88	11	43.47	11.53	14	44.29	9.81
Resting Metabolic Rate	ml O_2/h	None	B, R	0.166	0.365	0.470	16	1.58	0.20	13	1.16	0.17	16	1.32	0.16
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	B	0.733	0.198	0.213	17	6.11	0.24	13	6.18	0.22	16	6.53	0.20
Maximum Grip Strength	Newtons	Log_{10}	B	0.301	0.096	0.415	17	5.22	0.27	13	5.63	0.25	15	5.91	0.23
Maximum Sprint Speed	m/s	None	None	0.116	0.575	0.148	17	1.71	0.19	13	2.18	0.17	16	1.87	0.16

B = Body Mass, E = Lean Mass, R = RMR Chamber, C = Cricket Mass

P values ≤ 0.05 are bolded and underlined.

Table 2.6. Comparisons among breeding males, non-breeding males, and virgin males in time point 5.

				P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**Covariates**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Mass (Day 1)	Grams	Log_{10}	None	0.086	0.225	0.475	14	51.00	2.57	12	57.09	2.52	14	55.40	2.31
Body Mass (Day 4)	Grams	Log_{10}	None	0.152	0.388	0.394	13	51.62	2.66	12	56.75	2.45	14	54.75	2.25
Body Mass (Day 7)	Grams	Log_{10}	L	0.288	0.592	0.446	13	51.24	2.70	12	56.34	2.48	14	54.91	2.28
Fat Mass (Day 1)	Grams	Log_{10}	None	0.136	0.936	0.054	14	10.12	0.84	12	11.70	0.82	14	9.99	0.75
Percent Fat Mass (Day 1)	%	Log_{10}	None	0.450	0.332	**0.040**	14	19.55	1.26	12	20.86	1.23	14	17.74	1.13
Fat Mass (Day 7)	Grams	Log_{10}	E	0.781	0.450	0.167	13	10.56	0.93	12	10.71	0.85	14	9.42	0.77
Percent Fat Mass (Day 7)	%	None	None	0.777	0.442	0.168	13	19.27	1.45	12	19.90	1.34	14	17.62	1.21
Lean Mass (Day 1)	Grams	Log_{10}	None	0.060	0.102	0.689	14	36.96	1.87	12	42.14	1.84	14	41.34	1.68
Percent Lean Mass (Day 1)	%	Log_{10}	None	0.754	0.317	0.412	14	72.85	1.45	12	73.69	1.43	14	75.01	1.31
Lean Mass (Day 7)	Grams	Log_{10}	None	0.427	**0.036**	0.088	13	37.21	2.13	12	40.66	1.96	14	40.83	1.78
Percent Lean Mass (Day 7)	%	Log_{10}	None	0.593	0.053	0.068	13	72.02	1.23	12	72.92	1.13	14	75.51	1.03
Body Length	Millimeters	Log_{10}	B	0.645	0.926	0.606	13	113.84	1.33	12	112.98	1.23	14	113.76	1.10
Head Length	Millimeters	None	B	0.121	0.065	0.759	13	35.02	0.59	12	33.58	0.55	14	33.38	0.49
Head Width	Millimeters	None	L	0.279	0.755	0.281	13	16.31	0.73	12	17.54	0.68	14	16.64	0.62
Right Hind Foot Length	Millimeters	None	L	0.224	0.752	0.210	13	24.20	0.30	12	24.77	0.28	14	24.34	0.25
Baculum Length	Millimeters	Log_{10}	B	0.520	0.483	0.975	13	15.12	0.27	12	14.85	0.25	14	14.84	0.22
Brain Mass	Grams	None	B	0.525	0.284	0.592	13	0.87	0.02	12	0.85	0.02	14	0.84	0.02
Subcutaneous Fat Mass	Grams	None	B, L	0.743	0.549	0.223	13	3.29	0.40	12	3.49	0.37	14	2.93	0.34
Heart Mass	Grams	Log_{10}	B	**0.031**	0.085	0.435	13	0.16	0.01	12	0.18	0.01	14	0.18	0.01
Lung Mass	Grams	None	B	0.351	0.146	0.524	13	0.31	0.02	12	0.28	0.02	14	0.26	0.02

Table 2.6. Continued.

				P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**Covariates**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Liver Mass	Grams	Log_{10}	B	**0.022**	**0.024**	0.832	13	2.56	0.32	12	3.58	0.29	14	3.51	0.26
Spleen Mass	Grams	Log_{10}	B	0.053	**0.009**	0.386	13	0.08	0.01	12	0.09	0.01	14	0.10	0.01
Pancreas Mass	Grams	None	B	0.421	0.923	0.325	13	0.18	0.02	12	0.16	0.01	14	0.18	0.01
Kidney Mass	Grams	None	B	0.096	0.389	0.235	13	0.33	0.04	12	0.42	0.03	14	0.37	0.03
Adrenal Mass	Grams	None	B	0.461	0.735	0.149	13	0.01	0.00	12	0.01	0.00	14	0.01	0.00
Stomach Mass	Grams	Log_{10}	B	0.277	**0.039**	0.204	13	0.72	0.03	12	0.75	0.03	14	0.80	0.03
Small + Large Intestine Mass	Grams	Log_{10}	B	0.211	0.063	0.452	13	1.67	0.12	12	1.91	0.11	14	1.96	0.10
Caecum Mass	Grams	Log_{10}	B	**0.005**	**0.001**	0.691	13	0.42	0.03	12	0.53	0.02	14	0.54	0.02
Testis Mass	Grams	Log_{10}	B	0.681	0.440	0.654	13	0.29	0.02	12	0.28	0.02	14	0.27	0.02
Baculum Mass	Grams	Log_{10}	B	0.391	0.387	0.960	13	0.01	0.00	12	0.01	0.00	14	0.01	0.00
Right Hind Leg Muscle Mass	Grams	None	B	0.719	0.767	0.379	13	1.94	0.06	12	1.91	0.05	14	1.97	0.05
Left Hind Thigh Muscle Mass	Grams	None	B	0.248	0.534	0.429	13	1.12	0.06	12	1.02	0.05	14	1.07	0.05
Left Hind Gastrocnemius Mass	Grams	None	B	0.127	0.068	0.751	13	0.29	0.02	12	0.35	0.02	14	0.35	0.02
Hematocrit (Day 1)	%	Log_{10}	None	0.212	0.595	0.353	14	48.65	0.83	12	47.07	0.81	14	48.05	0.74
Hematocrit (Day 7)	%	Log_{10}	None	0.603	0.437	0.760	13	45.77	1.59	12	44.64	1.47	14	44.23	1.33

Table 2.6. Continued.

				P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**Covariates**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	C	**0.004**	**0.008**	0.537	12	15.99	5.26	12	10.83	4.51	14	14.74	3.94
Predatory Aggression: Latency to Kill Cricket	Seconds	Rank	C	0.303	0.438	0.676	12	49.02	11.57	10	40.10	10.93	12	45.15	10.02
Resting Metabolic Rate	ml O_2/h	Log_{10}	F, R	0.243	0.195	0.950	13	1.21	0.15	12	1.39	0.14	13	1.43	0.13
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	B	0.475	0.946	0.368	13	6.30	0.24	12	6.07	0.22	14	6.27	0.20
Maximum Grip Strength	Newtons	Log_{10}	B	0.256	0.441	0.561	13	5.38	0.29	12	5.78	0.27	14	5.65	0.24
Maximum Sprint Speed	m/s	Log_{10}	None	0.877	0.613	0.388	13	1.86	0.21	12	2.02	0.19	14	1.81	0.17

B = Body Mass, L = Body Length, E = Lean Mass, F = Fat Mass, R = RMR Chamber, C = Cricket Mass

P values ≤ 0.05 are bolded and underlined.

Table 2.7. Comparisons among breeding males, non-breeding males, and virgin males for time point 2 Δ values (time point 2 minus time point 1).

			P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.239	0.461	0.563	20	5.19	1.17	20	3.27	0.93	20	3.99	0.95
Δ Body Mass (Day 4)	Grams	None	0.089	0.102	0.938	20	6.57	1.30	20	3.48	1.03	20	3.59	1.05
Δ Body Mass (Day 7)	Grams	None	0.945	0.998	0.926	20	3.64	1.19	20	3.75	0.94	20	3.64	0.96
Δ Fat Mass (Day 1)	Grams	Log_{10}	**0.049**	0.190	0.373	20	2.30	1.00	20	-0.47	0.79	20	0.48	0.80
Δ Percent Fat Mass (Day 1)	%	Log_{10}	**0.034**	0.255	0.185	20	3.05	1.99	20	-2.91	1.58	20	-0.10	1.60
Δ Fat Mass (Day 7)	Grams	Log_{10}	**0.037**	0.122	0.463	20	1.95	0.82	20	-0.48	0.65	20	0.16	0.66
Δ Percent Fat Mass (Day 7)	%	None	**0.011**	0.102	0.208	20	2.89	1.54	20	-2.71	1.22	20	-0.64	1.24
Δ Lean Mass (Day 1)	Grams	None	0.796	0.181	0.165	20	2.65	0.62	19	2.88	0.49	19	3.79	0.50
Δ Percent Lean Mass (Day 1)	%	Log_{10}	0.280	0.131	0.590	20	-2.83	2.31	19	0.68	1.84	19	2.00	1.87
Δ Lean Mass (Day 7)	Grams	None	**0.013**	**0.037**	0.596	20	1.42	0.59	19	3.51	0.47	20	3.17	0.48
Δ Percent Lean Mass (Day 7)	%	Log_{10}	0.186	0.273	0.729	20	-2.32	1.76	19	0.97	1.40	20	0.33	1.38
Δ Hematocrit (Day 1)	%	Log_{10}	0.160	0.122	0.839	16	-1.34	1.19	16	0.80	0.94	15	1.04	0.99
Δ Hematocrit (Day 7)	%	Log_{10}	0.754	0.903	0.821	16	-0.45	1.49	16	0.11	1.23	15	-0.23	1.32
Δ Predatory Aggression: Latency to First Attack Cricket	Seconds	Log_{10}	0.677	0.737	0.930	19	-17.93	15.10	18	-9.16	12.56	18	-10.66	12.89
Δ Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	**0.015**	0.260	0.079	17	-44.96	16.73	17	12.46	13.53	18	-19.06	13.18
Δ Resting Metabolic Rate	ml O_2/h	None	0.708	0.756	0.928	18	0.00	0.26	20	0.13	0.20	20	0.11	0.20
Δ Maximal Oxygen Consumption	ml O_2/h	None	0.861	0.505	0.265	19	0.15	0.14	19	0.18	0.11	19	0.02	0.11
Δ Maximum Grip Strength	Newtons	Log_{10}	**0.005**	**0.004**	0.945	20	-0.52	0.25	20	0.50	0.20	20	0.51	0.20
Δ Maximum Sprint Speed	m/s	None	0.206	0.495	**0.015**	19	0.10	0.17	19	0.40	0.13	17	-0.06	0.14

P values ≤ 0.05 are bolded and underlined.

Table 2.8. Comparisons among breeding males, non-breeding males, and virgin males for time point 3 Δ values (time point 3 minus time point 1).

			P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.478	0.113	0.320	19	4.97	1.41	18	6.42	1.34	18	8.22	1.33
Δ Body Mass (Day 4)	Grams	None	0.393	0.174	0.565	18	5.19	1.60	18	7.18	1.49	18	8.32	1.46
Δ Body Mass (Day 7)	Grams	None	0.491	0.346	0.777	18	5.16	1.43	18	6.59	1.33	18	7.10	1.31
Δ Fat Mass (Day 1)	Grams	None	0.649	0.292	0.093	19	1.08	1.01	18	0.41	0.96	18	2.62	0.95
Δ Percent Fat Mass (Day 1)	%	Log_{10}	0.441	0.268	**0.038**	19	0.20	1.87	18	-1.90	1.78	18	3.20	1.76
Δ Fat Mass (Day 7)	Grams	None	0.698	0.554	0.261	18	1.34	1.00	18	0.78	0.93	18	2.19	0.92
Δ Percent Fat Mass (Day 7)	%	None	0.511	0.453	0.109	18	0.72	1.81	18	-1.01	1.69	18	2.67	1.66
Δ Lean Mass (Day 1)	Grams	None	0.122	**0.012**	0.228	19	3.37	0.62	18	4.77	0.59	16	5.76	0.62
Δ Percent Lean Mass (Day 1)	%	Log_{10}	0.966	0.830	0.847	19	-0.73	2.16	18	-0.86	2.07	16	-1.42	2.18
Δ Lean Mass (Day 7)	Grams	None	0.139	0.158	0.905	18	3.37	0.67	17	4.84	0.64	18	4.74	0.62
Δ Percent Lean Mass (Day 7)	%	Log_{10}	0.677	0.746	0.398	18	-1.30	1.94	17	-0.11	1.85	18	-2.20	1.79
Δ Hematocrit (Day 1)	%	Log_{10}	0.662	0.648	0.984	19	0.10	0.92	18	-0.48	0.87	18	-0.50	0.86
Δ Hematocrit (Day 7)	%	None	0.882	0.077	**0.033**	17	0.07	0.81	18	0.24	0.74	18	-1.99	0.75
Δ Predatory Aggression: Latency to First Attack Cricket	Seconds	Log_{10}	0.263	0.676	0.403	17	-22.44	10.71	15	-4.20	10.63	17	-15.91	10.12
Δ Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	0.182	0.806	0.076	14	-31.96	16.36	15	-0.90	15.56	13	-37.52	15.62
Δ Resting Metabolic Rate	ml O_2/h	None	0.873	0.898	0.739	18	0.16	0.27	18	0.22	0.25	17	0.11	0.26
Δ Maximal Oxygen Consumption	ml O_2/h	None	0.378	0.906	0.373	18	0.07	0.12	18	0.22	0.11	18	0.09	0.11
Δ Maximum Grip Strength	Newtons	None	0.749	0.510	0.690	18	0.23	0.23	18	0.33	0.21	18	0.45	0.21
Δ Maximum Sprint Speed	m/s	None	0.595	0.779	0.345	18	0.14	0.11	18	0.22	0.10	18	0.09	0.10

P values ≤ 0.05 are bolded and underlined.

Table 2.9. Comparisons among breeding males, non-breeding males, and virgin males for time point 4 Δ values (time point 4 minus time point 1).

			P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.631	0.541	0.150	17	11.65	2.08	13	10.10	1.91	15	13.55	1.76
Δ Body Mass (Day 4)	Grams	None	0.327	0.768	0.334	17	13.38	2.37	13	9.80	2.15	16	12.35	1.93
Δ Body Mass (Day 7)	Grams	None	0.421	0.972	0.287	17	11.81	2.09	13	9.21	1.90	16	11.70	1.71
Δ Fat Mass (Day 1)	Grams	None	0.208	0.691	0.229	17	3.69	1.40	13	0.92	1.29	15	2.85	1.18
Δ Percent Fat Mass (Day 1)	%	Log_{10}	0.159	0.632	0.194	17	3.44	2.59	13	-2.29	2.38	15	1.59	2.19
Δ Fat Mass (Day 7)	Grams	None	0.185	0.346	0.551	17	3.89	1.38	13	1.04	1.25	16	1.96	1.13
Δ Percent Fat Mass (Day 7)	%	Log_{10}	0.170	0.334	0.527	17	4.05	2.48	13	-1.24	2.25	16	0.50	2.02
Δ Lean Mass (Day 1)	Grams	None	0.865	0.062	**0.009**	17	6.81	0.98	13	6.55	0.91	15	9.62	0.83
Δ Percent Lean Mass (Day 1)	%	Log_{10}	0.810	0.584	0.696	17	-4.12	2.89	13	-3.03	2.67	15	-1.74	2.45
Δ Lean Mass (Day 7)	Grams	None	0.946	0.367	0.199	17	6.84	1.15	13	6.72	1.05	16	8.39	0.94
Δ Percent Lean Mass (Day 7)	%	Log_{10}	0.306	0.395	0.771	17	-4.48	2.36	13	-0.75	2.14	16	-1.51	1.92
Δ Hematocrit (Day 1)	%	Log_{10}	0.774	0.608	0.291	17	-0.66	1.01	12	-0.21	0.96	16	-1.43	0.83
Δ Hematocrit (Day 7)	%	Log_{10}	0.388	0.950	0.269	16	-2.06	1.21	13	-0.55	0.99	16	-1.95	0.92
Δ Predatory Aggression: Latency to First Attack Cricket	Seconds	Log_{10}	0.056	0.308	0.198	16	-21.52	8.85	12	5.41	8.14	15	-7.70	7.59
Δ Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	0.721	0.815	0.387	14	-26.34	27.37	11	-11.43	23.50	14	-35.46	20.34
Δ Resting Metabolic Rate	ml O_2/h	None	0.691	0.390	0.561	16	0.39	0.24	13	0.54	0.22	16	0.70	0.20
Δ Maximal Oxygen Consumption	ml O_2/h	None	0.623	0.305	0.499	17	0.33	0.24	13	0.52	0.22	16	0.70	0.19
Δ Maximum Grip Strength	Newtons	None	0.523	0.121	0.247	17	0.32	0.35	13	0.67	0.33	15	1.15	0.30
Δ Maximum Sprint Speed	m/s	Log_{10}	**0.021**	0.462	**0.024**	17	-0.17	0.17	13	0.45	0.15	16	0.02	0.14

P values ≤ 0.05 are bolded and underlined.

Table 2.10. Comparisons among breeding males, non-breeding males, and virgin males for time point 5 Δ values (time point 5 minus time point 1).

			P Value			Breeding (BM)			Non-Breeding (NB)			Virgin (VM)		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.505	0.208	0.494	14	10.70	2.08	12	12.86	2.09	14	14.65	1.87
Δ Body Mass (Day 4)	Grams	None	0.491	0.252	0.565	13	11.24	2.55	12	13.92	2.33	14	15.56	2.10
Δ Body Mass (Day 7)	Grams	None	0.904	0.591	0.588	13	11.25	2.54	12	11.71	2.32	14	13.25	2.09
Δ Fat Mass (Day 1)	Grams	None	0.831	0.987	0.806	14	3.09	1.40	12	2.62	1.41	14	3.05	1.26
Δ Percent Fat Mass (Day 1)	%	None	0.508	0.779	0.624	14	2.86	2.61	12	0.17	2.62	14	1.77	2.34
Δ Fat Mass (Day 7)	Grams	None	0.815	0.903	0.873	13	2.99	1.64	12	2.41	1.49	14	2.70	1.35
Δ Percent Fat Mass (Day 7)	%	None	0.851	0.991	0.787	13	1.67	2.83	12	0.86	2.58	14	1.72	2.33
Δ Lean Mass (Day 1)	Grams	None	0.097	**0.004**	0.135	14	6.04	0.99	12	8.64	0.99	14	10.52	0.89
Δ Percent Lean Mass (Day 1)	%	Log_{10}	0.794	0.549	0.695	14	-4.51	3.15	12	-3.24	3.16	14	-1.70	2.83
Δ Lean Mass (Day 7)	Grams	None	0.429	0.115	0.306	13	6.21	1.45	12	7.97	1.33	14	9.64	1.20
Δ Percent Lean Mass (Day 7)	%	Log_{10}	0.570	0.471	0.860	13	-4.43	2.83	12	-1.98	2.59	14	-1.42	2.33
Δ Hematocrit (Day 1)	%	Log_{10}	0.353	0.291	0.916	14	0.49	1.31	12	-1.42	1.32	14	-1.59	1.18
Δ Hematocrit (Day 7)	%	Log_{10}	0.916	0.209	0.135	13	0.25	1.77	12	-0.03	1.62	14	-3.05	1.46
Δ Predatory Aggression: Latency to First Attack Cricket	Seconds	Log_{10}	0.572	0.945	0.440	12	-6.82	23.22	12	-26.70	19.06	14	-9.16	17.70
Δ Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	0.295	0.076	0.304	12	7.61	21.96	10	-30.78	22.76	12	-57.73	21.21
Δ Resting Metabolic Rate	ml O_2/h	None	0.739	0.947	0.589	13	0.42	0.30	12	0.27	0.27	13	0.45	0.25
Δ Maximal Oxygen Consumption	ml O_2/h	None	0.940	0.797	0.812	13	0.32	0.30	12	0.35	0.27	14	0.43	0.25
Δ Maximum Grip Strength	Newtons	None	0.685	0.886	0.719	13	0.62	0.39	12	0.86	0.35	14	0.71	0.32
Δ Maximum Sprint Speed	m/s	Log_{10}	0.463	0.422	**0.048**	13	-0.01	0.18	12	0.19	0.16	14	-0.22	0.15

P values ≤ 0.05 are bolded and underlined.

Table 2.11. Comparisons among breeding males, non-breeding males, and virgin males in time point 5. Shown are transformations (where applicable), degrees of freedom, F values, and significance levels from ANCOVAs.

		Time Point 2				Time Point 3				Time Point 4			
Trait	**Unit**	**Transform**	**DF**	**F**	**Group P**	**Transform**	**DF**	**F**	**Group P**	**Transform**	**DF**	**F**	**Group P**
Body Mass (Day 1)	Grams	None	2,60	0.257	0.774	None	2,55	0.306	0.738	Log_{10}	2,45	0.630	0.538
Body Mass (Day 4)	Grams	None	2,60	0.442	0.645	None	2,54	0.171	0.843	Log_{10}	2,46	0.175	0.840
Body Mass (Day 7)	Grams	None	2,60	0.135	0.874	None	2,54	0.103	0.902	Log_{10}	2,46	0.122	0.885
Fat Mass (Day 1)	Grams	None	2,60	0.134	0.875	Log_{10}	2,55	0.722	0.491	None	2,45	0.984	0.384
Percent Fat Mass (Day 1)	%	None	2,60	0.237	0.790	Log_{10}	2,55	0.917	0.407	None	2,45	1.072	0.353
Fat Mass (Day 7)	Grams	None	2,60	0.092	0.912	None	2,54	0.291	0.749	None	2,46	1.869	0.169
Percent Fat Mass (Day 7)	%	None	2,60	0.348	0.708	Log_{10}	2,54	0.511	0.603	Log_{10}	2,46	1.837	0.173
Lean Mass (Day 1)	Grams	Log_{10}	2,58	3.023	0.058	None	2,53	0.108	0.898	Log_{10}	2,45	1.131	0.334
Percent Lean Mass (Day 1)	%	None	2,58	1.250	0.295	Log_{10}	2,53	0.772	0.468	Log_{10}	2,45	0.449	0.642
Lean Mass (Day 7)	Grams	None	2,58	2.010	0.144	Log_{10}	2,53	0.182	0.835	Log_{10}	2,46	0.514	0.602
Percent Lean Mass (Day 7)	%	None	2,59	0.353	0.704	Log_{10}	2,53	0.436	0.649	Log_{10}	2,46	1.525	0.231
Hematocrit (Day 1)	%	None	2,46	2.830	0.071	Log_{10}	2,55	0.723	0.491	None	2,45	0.329	0.722
Hematocrit (Day 7)	%	Log_{10}	2,46	0.315	0.732	Log_{10}	2,53	0.089	0.915	Log_{10}	2,45	0.834	0.442
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	2,55	0.085	0.918	Rank	2,49	1.650	0.205	None	2,43	0.156	0.856
Predatory Aggression: Latency to Kill Cricket	Seconds	None	2,52	0.836	0.440	Log_{10}	2,42	0.245	0.784	Rank	2,39	0.542	0.587
Resting Metabolic Rate	ml O_2/h	Log_{10}	2,58	0.216	0.806	None	2,53	2.822	0.071	None	2,45	1.017	0.372
Maximal Oxygen Consumption	ml O_2/h	None	2,57	1.247	0.296	Log_{10}	2,54	0.818	0.448	Log_{10}	2,46	1.258	0.296
Maximum Grip Strength	Newtons	None	2,60	2.518	0.091	None	2,54	0.118	0.889	Log_{10}	2,45	1.491	0.239
Maximum Sprint Speed	m/s	Log_{10}	2,54	1.218	0.305	None	2,54	0.230	0.795	None	2,46	1.691	0.198

P values ≤ 0.05 are bolded and underlined.

Table 2.12. Comparisons among breeding males, non-breeding males, and virgin males in time point 5. Shown are transformations (where applicable), degrees of freedom, F values, and significance levels from ANCOVAs.

Trait	Unit	Transform	D.F.	F	Group P
Body Mass (Day 1)	Grams	Log_{10}	2,40	1.576	0.222
Body Mass (Day 4)	Grams	Log_{10}	2,39	1.114	0.342
Body Mass (Day 7)	Grams	Log_{10}	2,39	0.646	0.531
Fat Mass (Day 1)	Grams	Log_{10}	2,40	2.263	0.124
Percent Fat Mass (Day 1)	%	Log_{10}	2,40	2.312	0.115
Fat Mass (Day 7)	Grams	Log_{10}	2,39	1.058	0.360
Percent Fat Mass (Day 7)	%	None	2,39	1.055	0.360
Lean Mass (Day 1)	Grams	Log_{10}	2,40	2.025	0.149
Percent Lean Mass (Day 1)	%	Log_{10}	2,40	0.637	0.535
Lean Mass (Day 7)	Grams	Log_{10}	2,39	3.072	0.061
Percent Lean Mass (Day 7)	%	Log_{10}	2,39	2.887	0.071
Snout to Rump Length	Millimeters	Log_{10}	2,39	0.171	0.843
Head Length	Millimeters	None	2,39	1.883	0.170
Head Width	Millimeters	None	2,39	0.848	0.438
Right Hind Foot Length	Millimeters	None	2,39	1.117	0.340
Brain Mass	Grams	None	2,39	0.619	0.545
Subcutaneous Fat Mass	Grams	None	2,39	0.798	0.460
Heart Mass	Grams	Log_{10}	2,39	2.585	0.092
Lung Mass	Grams	None	2,39	1.130	0.336
Liver Mass	Grams	Log_{10}	2,39	3.321	**0.050**
Spleen Mass	Grams	Log_{10}	2,39	3.906	**0.031**
Pancreas Mass	Grams	None	2,39	0.591	0.560
Kidney Mass	Grams	None	2,39	1.621	0.214
Adrenal Mass	Grams	None	2,39	1.103	0.345
Stomach Mass	Grams	Log_{10}	2,39	2.550	0.095
Small + Large Intestine Mass	Grams	Log_{10}	2,39	1.879	0.170
Caecum Mass	Grams	Log_{10}	2,39	6.531	**0.004**
Testis Mass	Grams	Log_{10}	2,39	0.331	0.721
Baculum Mass	Grams	Log_{10}	2,39	0.443	0.646
Baculum Length	Millimeters	Log_{10}	2,39	0.272	0.764
Right Hind Leg Muscle Mass	Grams	None	2,39	0.400	0.674
Left Hind Thigh Muscle Mass	Grams	None	2,39	0.745	0.483
Left Hind Gastrocnemius Mass	Grams	None	2,39	1.828	0.178
Hematocrit (Day 1)	%	Log_{10}	2,40	0.900	0.417
Hematocrit (Day 7)	%	Log_{10}	2,39	0.311	0.735
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	2,38	5.194	**0.012**
Predatory Aggression: Latency to Kill Cricket	Seconds	Rank	2,34	0.553	0.582
Resting Metabolic Rate	ml O_2/h	Log_{10}	2,38	0.661	0.524
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	2,39	0.484	0.621
Maximum Grip Strength	Newtons	Log_{10}	2,39	0.675	0.517
Maximum Sprint Speed	m/s	Log_{10}	2,39	0.412	0.666

P values ≤ 0.05 are bolded and underlined.

Table 2.13. Comparisons among breeding males, non-breeding males, and virgin males for Δ values (difference from time point 1) in time points 2 – 5. Shown are transformations (where applicable), degrees of freedom, F values, and significance levels from ANCOVAs.

		Time Point 2				Time Point 3			
Trait	**Unit**	**Transform**	**DF**	**F**	**Group P**	**Transform**	**DF**	**F**	**Group P**
Δ Body Mass (Day 1)	Grams	None	2,60	0.720	0.492	None	2,55	1.359	0.267
Δ Body Mass (Day 4)	Grams	None	2,60	1.692	0.194	None	2,54	0.952	0.394
Δ Body Mass (Day 7)	Grams	None	2,60	0.005	0.995	None	2,54	0.462	0.633
Δ Fat Mass (Day 1)	Grams	Log_{10}	2,60	2.052	0.139	None	2,55	1.529	0.228
Δ Percent Fat Mass (Day 1)	%	Log_{10}	2,60	2.536	0.089	Log_{10}	2,55	2.298	0.112
Δ Fat Mass (Day 7)	Grams	Log_{10}	2,60	2.300	0.111	None	2,54	0.656	0.524
Δ Percent Fat Mass (Day 7)	%	None	2,60	3.542	**0.036**	None	2,54	1.343	0.271
Δ Lean Mass (Day 1)	Grams	None	2,58	1.407	0.255	None	2,53	3.461	**0.040**
Δ Percent Lean Mass (Day 1)	%	Log_{10}	2,58	1.178	0.316	Log_{10}	2,53	0.029	0.972
Δ Lean Mass (Day 7)	Grams	None	2,58	3.395	**0.041**	None	2,53	1.344	0.271
Δ Percent Lean Mass (Day 7)	%	Log_{10}	2,59	0.922	0.404	Log_{10}	2,53	0.364	0.697
Δ Hematocrit (Day 1)	%	Log_{10}	2,46	1.362	0.268	Log_{10}	2,55	0.126	0.882
Δ Hematocrit (Day 7)	%	Log_{10}	2,46	0.057	0.945	None	2,53	2.859	0.069
Δ Predatory Aggression: Latency to First Attack Cricket	Seconds	Log_{10}	2,55	0.091	0.914	Log_{10}	2,49	0.708	0.499
Δ Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	2,52	3.609	**0.036**	Log_{10}	2,42	1.862	0.172
Δ Resting Metabolic Rate	ml O_2/h	None	2,58	0.073	0.930	None	2,53	0.057	0.945
Δ Maximal Oxygen Consumption	ml O_2/h	None	2,57	0.670	0.517	None	2,54	0.562	0.574
Δ Maximum Grip Strength	Newtons	Log_{10}	2,60	5.165	**0.009**	None	2,54	0.228	0.797
Δ Maximum Sprint Speed	m/s	None	2,54	3.230	**0.048**	None	2,54	0.466	0.631

Table 2.13. Continued.

		Time Point 4				Time Point 5			
Trait	**Unit**	**Trans.**	**DF**	**F**	**Group P**	**Trans.**	**DF**	**F**	**Group P**
Δ Body Mass (Day 1)	Grams	None	2,45	1.090	0.347	None	2,40	0.854	0.435
Δ Body Mass (Day 4)	Grams	None	2,46	0.686	0.510	None	2,39	0.703	0.503
Δ Body Mass (Day 7)	Grams	None	2,46	0.664	0.521	None	2,39	0.223	0.801
Δ Fat Mass (Day 1)	Grams	None	2,45	1.109	0.341	None	2,40	0.037	0.963
Δ Percent Fat Mass (Day 1)	%	Log_{10}	2,45	1.350	0.272	None	2,40	0.247	0.782
Δ Fat Mass (Day 7)	Grams	None	2,46	0.915	0.409	None	2,39	0.030	0.970
Δ Percent Fat Mass (Day 7)	%	Log_{10}	2,46	0.982	0.384	None	2,39	0.041	0.960
Δ Lean Mass (Day 1)	Grams	None	2,45	4.378	**0.020**	None	2,40	4.825	**0.015**
Δ Percent Lean Mass (Day 1)	%	Log_{10}	2,45	0.177	0.838	Log_{10}	2,40	0.201	0.819
Δ Lean Mass (Day 7)	Grams	None	2,46	0.991	0.381	None	2,39	1.462	0.248
Δ Percent Lean Mass (Day 7)	%	Log_{10}	2,46	0.551	0.581	Log_{10}	2,39	0.269	0.766
Δ Hematocrit (Day 1)	%	Log_{10}	2,45	0.592	0.558	Log_{10}	2,40	0.624	0.542
Δ Hematocrit (Day 7)	%	Log_{10}	2,45	0.747	0.481	Log_{10}	2,39	1.519	0.235
Δ Predatory Aggression: Latency to First Attack Cricket	Seconds	Log_{10}	2,43	2.131	0.135	Log_{10}	2,38	0.355	0.705
Δ Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	2,39	0.386	0.683	Log_{10}	2,34	1.830	0.183
Δ Resting Metabolic Rate	ml O_2/h	None	2,45	0.435	0.651	None	2,38	0.158	0.855
Δ Maximal Oxygen Consumption	ml O_2/h	None	2,46	0.615	0.546	None	2,39	0.046	0.955
Δ Maximum Grip Strength	Newtons	None	2,45	1.489	0.239	None	2,39	0.108	0.898
Δ Maximum Sprint Speed	m/s	Log_{10}	2,46	4.012	**0.026**	Log_{10}	2,39	2.132	0.136

P values ≤ 0.05 are bolded and underlined.

Table 2.14. Correlations of residuals for energetic and performance measures with organ masses. Residuals of maximal oxygen consumption during forced exercise ($\dot{V}O_2$max), resting metabolic rate (RMR), sprint speed, grip strength, and day 1 hematocrit correlated with all organ masses and day 7 hematocrit at time point 5 only.

		VO2max	RMR	Sprint Speed	Grip Strength	Day 1 Hematocrit
RMR	Pearson Correlation	0.087				
	Sig. (2-tailed)	0.602				
	N	38				
Sprint Speed	Pearson Correlation	0.087	0.008			
	Sig. (2-tailed)	0.6	0.962			
	N	39	38			
Grip Strength	Pearson Correlation	0.048	-0.236	0.092		
	Sig. (2-tailed)	0.771	0.154	0.577		
	N	39	38	39		
Day 1 Hematocrit	Pearson Correlation	-0.153	0.187	0.292	-0.082	
	Sig. (2-tailed)	0.353	0.261	0.072	0.618	
	N	39	38	39	39	
Day 7 Hematocrit	Pearson Correlation	-0.058	0.117	0.403	-0.078	0.53
	Sig. (2-tailed)	0.727	0.485	**0.011**	0.635	**0.001**
	N	39	38	39	39	39
Right Hind Foot Length	Pearson Correlation	0.264	-0.141	-0.011	-0.139	-0.135
	Sig. (2-tailed)	0.104	0.398	0.945	0.399	0.414
	N	39	38	39	39	39
Brain Mass	Pearson Correlation	-0.08	-0.085	-0.2	0.068	-0.129
	Sig. (2-tailed)	0.63	0.613	0.223	0.682	0.436
	N	39	38	39	39	39
Subcutaneous Fat Mass	Pearson Correlation	-0.16	-0.052	-0.007	-0.302	0.288
	Sig. (2-tailed)	0.329	0.757	0.967	0.062	0.076
	N	39	38	39	39	39
Heart Mass	Pearson Correlation	0.436	0.013	0.009	0.402	-0.317
	Sig. (2-tailed)	**0.006**	0.937	0.959	**0.011**	**0.049**
	N	39	38	39	39	39
Lung Mass	Pearson Correlation	0.114	-0.136	-0.001	0.304	-0.121
	Sig. (2-tailed)	0.49	0.415	0.995	0.06	0.462
	N	39	38	39	39	39
Liver Mass	Pearson Correlation	0.174	0.031	-0.169	0.45	-0.118
	Sig. (2-tailed)	0.289	0.852	0.305	**0.004**	0.475
	N	39	38	39	39	39

Table 2.14. Continued.

		VO2max	RMR	Sprint Speed	Grip Strength	Day 1 Hematocrit
Spleen Mass	Pearson Correlation	0.345	-0.154	-0.125	-0.06	-0.481
	Sig. (2-tailed)	**0.031**	0.355	0.45	0.717	**0.002**
	N	39	38	39	39	39
Pancreas Mass	Pearson Correlation	-0.153	0.046	-0.041	-0.31	-0.088
	Sig. (2-tailed)	0.353	0.784	0.802	0.054	0.594
	N	39	38	39	39	39
Average Kidney Mass	Pearson Correlation	0.267	0.08	-0.511	0.13	-0.463
	Sig. (2-tailed)	0.1	0.634	**0.001**	0.43	**0.003**
	N	39	38	39	39	39
Average Adrenal Mass	Pearson Correlation	0.346	0.116	-0.066	-0.01	0.079
	Sig. (2-tailed)	**0.031**	0.487	0.688	0.954	0.632
	N	39	38	39	39	39
Stomach Mass	Pearson Correlation	0.191	0.048	0.021	-0.191	-0.146
	Sig. (2-tailed)	0.245	0.773	0.901	0.245	0.374
	N	39	38	39	39	39
Intestines Mass	Pearson Correlation	0.08	-0.027	0.144	-0.071	-0.342
	Sig. (2-tailed)	0.63	0.87	0.382	0.667	**0.033**
	N	39	38	39	39	39
Caecum Mass	Pearson Correlation	0.158	0.154	-0.112	-0.181	-0.364
	Sig. (2-tailed)	0.336	0.355	0.498	0.271	**0.023**
	N	39	38	39	39	39
Baculum Mass	Pearson Correlation	0.089	0.012	0.03	0.029	0.251
	Sig. (2-tailed)	0.591	0.941	0.854	0.862	0.124
	N	39	38	39	39	39
Average Testis Mass	Pearson Correlation	-0.118	0.052	-0.086	-0.072	0.317
	Sig. (2-tailed)	0.473	0.755	0.602	0.662	**0.049**
	N	39	38	39	39	39
Thigh Mass	Pearson Correlation	0.404	-0.057	0.187	-0.011	-0.326
	Sig. (2-tailed)	**0.011**	0.734	0.255	0.947	**0.043**
	N	39	38	39	39	39
Gastrocnemius Mass	Pearson Correlation	0.112	0.122	-0.083	0.149	-0.091
	Sig. (2-tailed)	0.498	0.467	0.617	0.367	0.58
	N	39	38	39	39	39

P values ≤ 0.05 are bolded and underlined.

CHAPTER 3

Short- and Long-Term Cold Acclimation Effects on Morphology, Physiology, and Exercise Performance of California Mice (*Peromyscus californicus*): Potential Modulation by Fatherhood

Jacob R. Andrew[1], Theodore Garland, Jr.[1], Mark A. Chappell[1], Meng Zhao[1], and Wendy Saltzman[1]

[1]Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, CA 92521, USA

Abstract

California mice (*Peromyscus californicus*) are unique for the genus *Peromyscus* because they are biparental and genetically monogamous, as well as relatively large in body size. Previous work in this species has found no differences in physiological and morphological measures under typical vivarium conditions so we evaluated the effect of cold stress on metabolic rate, exercise performance, and morphology of pair-housed male California mice in both reproductive and non-reproductive conditions. In experiment 1, virgin males (VM) after approximately 25 days of acclimation to 5° or 10°C were compared with virgins housed at typical vivarium temperature of 22°C. In experiment 2, 22°C VM were compared with 3 groups of males housed at 10°C: VM, breeding males (BM; housed with an intact female and their pups), and non-breeding males (NB; housed with an ovariectomized, estrogen- and progesterone-treated female) during and after long-term acclimation (mean 243 days). In experiment 1, we additionally measured predatory aggression, resting metabolic rate (RMR), maximal oxygen consumption ($\dot{V}O_2$ max), grip strength, and sprint speed. In experiment 2, we measured basal metabolic rate (BMR), $\dot{V}O_2$max, maximal thermogenic capacity ($\dot{V}O_2$sum), and organ masses. Additionally, we measured body composition and hematocrit in both experiments. In experiment 1, VM housed at 5° and 10°C had lower body, fat, and lean masses, but higher RMR and $\dot{V}O_2$max, than VM at room temperature. In experiment 2, 10°C-acclimated groups had shorter bodies; higher body, fat, and lean masses; higher BMR and $\dot{V}O_2$sum, and generally greater morphometric measures and organ masses than VM at

22°C. For the animals at 10°C, breeding males had higher BMR, lower $\dot{V}O_2$max, longer feet, and longer, larger bacula than non-breeding and virgin males. Overall, results from this study confirm and extend previous work on cold acclimation in other species of *Peromyscus*, and indicate that the effects of fatherhood only appear under cold acclimation.

Introduction

The genus *Peromyscus* includes ~ 56 species of small-bodied rodents that are widely distributed and often locally abundant in the Western hemisphere (King, 1968; Hill, 1983; Bedford and Hoekstra, 2015). California mice (*P. californicus)* are unique among *Peromyscus* because they are genetically monogamous and biparental, with pairs forming lifelong bonds and males contributing significantly to offspring care (Gubernick and Alberts, 1987b; Ribble and Salvioni, 1990). California mouse fathers exhibit many of the same behaviors (huddling, grooming, protection, and transportation of offspring) as mothers (Dudley, 1974; Gubernick and Alberts, 1987a; Lee and Brown, 2002). Direct care by fathers can increase offspring survival and have lasting impacts on offspring development, including social, aggressive, and mating behaviors, neural and endocrine function, and cognitive ability (Braun and Champagne, 2014; Bales and Saltzman, 2016). When animals are required to "forage" for food by running in a wheel, the presence of a father significantly increases pup survival when pairs have three or more pups (Cantoni and Brown, 1997). Under high foraging demands, fathers must balance time spent interacting with their pups and time spent foraging (Bredy et al., 2007) and in conditions of chronic cold stress (8.5° - 10°C), the absence of fathers significantly decreases pup survival (Gubernick et al., 1993).

Studying paternal behavior and physiology in California mice can provide insight into how parenthood might affect fathers' biochemical function, morphology, physiology, and behavior in mammals in which fathers contribute extensive offspring care. Previous studies demonstrate that in many biparental mammals, becoming a father can

significantly alter neural circuitry (reviewed in Bales and Saltzman, 2016) and endocrine systems (reviewed in Saltzman and Ziegler, 2014), but effects of fatherhood on energetics, performance, and morphology are less clear. In the biparental common marmoset (*Callithrix jacchus*), cotton-top tamarin (*Saguinus oedipus*), and California mouse (*Peromyscus californicus*), lab studies have found that expectant fathers gain body mass during their mate's pregnancy, followed by loss of mass after parturition (Achenbach and Snowdon, 2002; Ziegler et al. 2006; Harris et al., 2011; Saltzman et al., 2015). However, other studies looking at the effects of increasing parity in California mice found no differences in body mass between breeding and non-breeding males, or any consistent effects of parity on fathers' fat or lean mass (Andrew et al., 2016; Zhao et al., 2017; Andrew et al., in review). Finally, we found that in adult males housed at 22°C, energetically challenging conditions (24-hour fasting every third day and having to climb towers to obtain food and water) increased body mass in virgin and non-breeding males but had no effect on breeding males, suggesting that reproduction limited males' ability to undergo morphological adjustments in response to an energetic challenge (Zhao et al., in press).

One possible cause of inconsistent findings on consequences of fatherhood in California mice is that typical laboratory conditions may not stress animals enough to drive extensive physiological changes in fathers. If resources are readily available (e.g., ad libitum food) and male help is not required for offspring survival, then breeding males might not be expected to differ from non-breeding males. Results from our previous experiments conducted under standard laboratory conditions (e.g. room temperature, *ad*

lib food and water, consistent lighting) found no consistent, significant differences in the energetics and exercise performance of fathers compared to non-fathers (Andrew et al., 2016; Andrew et al., in review). In the present study, therefore, we examined laboratory acclimation of adult male California mice to low ambient temperatures and how consequences of paternal status might be affected by cold stress.

Acclimatization or acclimation to reduced ambient temperatures is one component of seasonal plasticity. *Peromyscus maniculatus*, a congener of the California mouse, has been studied intensively with respect to cold acclimation (Hart, 1971). Cold-acclimating *P. maniculatus* have greatly increased $\dot{V}O_2$max (Chappell, 1984; Chappell, 1985; Hayes and Chappell, 1986; Hayes and Chappell, 1990) and increased ventilatory traits compared to warm-acclimated controls (Rezende et al., 2004), but do not show consistent differences in voluntary running speeds or maximum exercise endurance (Chappell et al., 2004). Chronic cold acclimation increases basal metabolic rate (BMR; Hart, 1971), while acute cold exposure does not affect it (Russell and Chappell, 2007). Cold acclimation also increases thermogenic capacity ($\dot{V}O_2$sum) (Chappell et al., 2007) and maximal metabolic capacity (M_{max}) in *P. maniculatus* (Heimer and Morrison, 1978). In rodent cold-acclimation studies where food is available *ad lib*, body mass typically increases in response to cold exposure while appendage lengths decrease (reviewed in Hart, 1971). In contrast, work in *P. maniculatus* (Rezende et al., 2004; Rezende et al., 2009) and *P. leucopus* (Lynch, 1973) did not find consistent decreases in body mass during cold acclimation. Finally, cold-exposed rodents, including *P. maniculatus*,

typically increase in organ size (e.g. Hart, 1971; Hammond and Kristan, 2000) and enhancing immune function (Demas and Nelson, 1996).

We chose to conduct this study in *P. californicus* in part because of their unusual mating system (described above) and in part because of their large body mass compared to *P. maniculatus*. *P. californicus* average 50 grams and have lower and upper critical temperatures of 27° and 34°C (McNab and Morrison, 1963; Hart, 1971; Merritt, 1978). *P. maniculatus*, in comparison, average 20 grams and have lower and upper critical temperatures of 28° and 37°C (Hart, 1971). No previous studies of cold acclimation have included *P. californicus*, and we are interested in whether the larger *P. californicus* shows similar changes compared to the smaller *P. maniculatus*.

We performed two experiments to evaluate the effects of both short-term and long-term cold acclimation (e.g., see Griffiths and Hunter, 2014). We first characterized relatively short-term (~ 1 month) (partial) acclimation to either 5 or 10°C to verify that *P. californicus* shows some of the expected responses to chronic cold stress, based on studies of *P. maniculatus* and other small-bodied rodents (see above; also see Hart, 1971). In this study, we measured body composition (body mass, fat mass, lean muscle mass, organ masses), hematocrit, predatory aggression, resting metabolic rate (RMR), maximal oxygen consumption ($\dot{V}O_2$max), grip strength, and sprint speed of virgin adult males. For the longer-term (~6 months) study, intended to measure full acclimatory responses, we used only 10°C because we were not confident that prolonged exposure to 5°C would be tolerated. Moreover, in addition to virgin males, we compared breeding and non-breeding males housed at 10°C. In this second study, we measured body

composition (body mass, fat mass, lean muscle mass, organ masses), hematocrit, BMR, $\dot{V}O_2$max, and maximal thermogenic capacity ($\dot{V}O_2$sum).

We hypothesized that, as in *P. maniculatus* and many other small rodents, chronic cold acclimation would have pervasive effects on the physiology, exercise performance, and morphology of male California mice. Compared with males housed at room temperature, we predicted that cold-acclimated males would have increased predatory behavior, decreased exercise performance, increased BMR or RMR, increased body, fat, and lean mass and increased relative organ masses. We expanded upon previous studies of cold acclimation in rodents by including two measures of exercise performance (maximum sprint speed and grip strength). Finally, we predicted that the effects of cold acclimation would be more pronounced in breeding males than in non-breeding and virgin males.

Methods

Animals

California mice used in this study were born and raised in our colony at the University of California, Riverside (UCR) and were descended from animals purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Animals were housed in polycarbonate cages (44 x 24 x 20 cm) with aspen shavings for bedding but no additional nesting material; food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN, USA) and water were available *ad libitum*. Lighting was on a 14:10 cycle (lights on at 05:00 h, off at 19:00 h), with humidity maintained at

approximately 55% and ambient temperature at 22.1 ± 0.9°C except where stated otherwise. Mice were checked twice daily, and cages were changed once per week. At weaning age (27– 31 days; 28.0 ± 0.3), animals were ear-punched for identification and placed in same-sex groups of 3 - 4 related and/or unrelated, age-matched individuals.

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and approved by the UCR Institutional Animal Care and Use Committee.

Experiment 1 Design – Short-Term Cold Acclimation

When males reached 60 – 114 (88.4 ± 1.1) days of age, they were moved in their virgin groups either to an environmental chamber set at 5°C (n = 29) or 10°C (n = 50) or to a new room with ambient temperature set at 22°C (n = 61) (Figure 3.1: Experiment 1). After 14 – 38 (24.8 ± 1.5) days in their respective temperature conditions, males from each group underwent a series of test procedures over a 7-day period (Table 3.1). All males remained housed with their respective cage mates throughout the period of data collection, except where indicated below.

Experiment 2 Design – Long-Term Cold Acclimation

Virgin males from the 10°C (n = 12) and 22°C (n = 12) conditions [age: 126 – 198 days (147.0 ± 5.1)] in experiment 1 were re-paired with another virgin male cagemate from their original same-sex groups and remained in their respective environmental conditions until they were 346 – 421 (381.5 ± 4.8) days of age (Figure 3.1:

Experiment 2). Additional virgin males that had been housed at 10°C in experiment 1 [age: 123 – 173 days (142.7 ± 4.4)] were randomly paired with an age-matched virgin female in one of two conditions (Figure 3.1). Breeding males (BM, n = 4) were paired with a sham-ovariectomized female and non-breeding males (NB, n = 6) were paired with an ovariectomized female treated with estradiol benzoate and progesterone (see below) to induce estrous behavior. After 207 – 295 (243.0 ± 7.4) days of acclimation, males underwent an 8-day testing period (Table 3.1), after which they were dissected (see below). BM [age: 342 – 380 days (357.3 ± 6.8)] and NB [age: 340 – 363 days (346.5 ± 5.5)] were compared with the age-matched 10°C virgin males [VM, n = 12, age: 381 – 392 days (387.8 ± 1.2)].

Ovariectomies and Estrogen/Progesterone Treatment

Females in experiment 2 underwent bilateral ovariectomies prior to being paired with NB or sham-ovariectomies prior to being paired with BM. Briefly, females were anesthetized with isoflurane, and surgeries were performed under aseptic conditions using standard procedures as previously elsewhere (Zhao et al., in press). Females were then housed individually for two weeks to recover before being paired with males. Forty-eight hours prior to pairing, NB females were injected subcutaneously with estradiol benzoate (0.072 mg, s.c.; suspended in sesame oil, Sigma-Aldrich, St. Louis, MO, USA). On the day of pairing, females were injected with progesterone (0.48 mg, s.c.; suspended in sesame oil, Sigma-Aldrich, St. Louis, MO, USA) (Zhao et al., in press). A pilot study (unpub.) found that this treatment paradigm usually led to mating behaviors in

ovariectomized females 13h after progesterone injection, whereas untreated ovariectomized females were never observed to copulate. At the end of the current experiment, ovariectomized females were euthanized by CO_2 inhalation and dissected to check for the presence of fetuses in the uterine canal; no females had visible fetuses.

Body Mass

All males in experiments 1 and 2, as well as breeding females in experiment 2, were weighed to the nearest 0.001 g twice per week (13:00 – 15:00 h) at approximately 3- to 4-day intervals from pairing until the beginning of their test period. This procedure was used to monitor general health of the animals and pregnancies in breeding females (experiment 2), as well as to habituate animals to handling; however, these data are not presented here.

Body Composition

Body composition was measured on test days 1 (13:00 – 14:30 h) and 7 (9:00 – 10:30 h) in experiment 1 and on test day 6 (12:00 – 14:00) in experiment 2 (Table 3.1). Males were weighed before testing and then scanned with a magnetic resonance whole-body analyzer (EchoMRI-100; Echo Medical Systems, Houston, TX, USA) to assess body composition (fat mass, lean mass, free water mass, and total water mass; Zhao et al., 2017; Zhao et al., in press; Andrew et al., in review). Scans lasted ~90 s and did not require anesthesia or sedation. Here we report fat and lean masses only (unaltered and as percentages of total body mass).

Hematocrit

Blood samples (~200 µl) for measurement of hematocrit were collected on test days 1 (9:00 – 10:30 h) and 7 (13:00 – 14:30 h) in experiment 1 and on test day 8 (12:30 – 14:30 h) in experiment 2 (Table 3.1). Mice were anesthetized with isoflurane and blood was collected into heparinized microhematocrit capillary tubes from the retro-orbital sinus (Chauke et al., 2011; Harris et al., 2011; Andrew et al., 2016). Blood was centrifuged at 4°C and 1300 RPM (~1,900 g) for 12 min (Sorvall Legend Micro 21R; Thermo Scientific) and hematocrit was recorded.

Predatory Aggression

Mice were tested for predatory aggression (Thomas and Fried, 1971; Gammie et al., 2003; Zhao et al., 2017) in experiment 1 (test days 2 and 3, 13:30 – 15:00 h). Briefly, mice were placed singly in a clean cage with a thin layer of aspen shavings covering the cage floor and no food or water. After a 15-min habituation period, a live cricket of standard size (0.2 – 0.5 g) was dropped into the cage on the side opposite the mouse. Behavior was video recorded until either the cricket was killed or 7 minutes had elapsed, whichever occurred first. Videos were scored for latency to attack and latency to kill the cricket. If the mouse did not kill the cricket, the male was assigned a latency of 7 min. Predatory aggression was tested on two successive days to determine repeatability. The shorter latency of each animal's two tests was used for comparisons among temperature and housing conditions.

Resting Metabolic Rate (RMR)

RMR was measured in experiment 1 on test day 4 (8:30 – 16:30 h) as previously described (Andrew et al., 2016). Males were separated from their cage mates, weighed before testing, and placed in one of two Plexiglas metabolic chamber with bedding (volume: 525 mL; chamber 1 removed from analysis in both minimal metabolic tests due to air leaks), inside an environmental chamber maintained at 28-30°C. Oxygen consumption ($\dot{V}O_2$) was measured over 8h during the inactive period (lights-on). Subsampled excurrent air was dried (soda lime and Drierite) and sent through an oxygen analyzer. Oxygen concentration, temperature, and flow rate were measured every 5s, and 3-min reference readings were taken every 42 min (Warthog LabHelper software; www.warthog.ucr.edu). RMR was computed as the lowest 10-min average $\dot{V}O_2$ during the 8-h period.

Basal Metabolic Rate (RMR)

BMR was measured in experiment 2 on test day 1 (8:30 – 16:30 h). The procedure for measuring BMR was identical to the method for RMR used in experiment 1, except that food was removed 8h before testing began.

Maximal Oxygen Consumption

Maximal oxygen consumption during forced exercise ($\dot{V}O_2$max) was measured in experiment 1 (test days 5 and 6; 10:00 – 11:30 h) and in experiment 2 (test days 4 and 5; 11:30 – 13:30 h), using a running-wheel respirometer (circumference: 51.8 cm; effective

volume: 900 mL) as previously described (Dlugosz et al., 2012; Andrew et al., 2016). Mice were weighed beforehand and then had a ~2 min warm-up period, followed by gradually increasing the speed approximately every 30s until either oxygen concentration did not change or mice could not maintain position. Flow rates (2400 mL/min) and O_2 concentrations were measured every second using LabHelper. Measurements were taken at room temperature (20-25°C). Excurrent air was subsampled (~150 mL/min) and dried with soda lime and Drierite, and oxygen concentration was analyzed. Reference air was taken at the beginning and end of trials, and a baseline was computed by linear regression. $\dot{V}O_2$max (the highest $\dot{V}O_2$ averaged over 1 min) was determined on each of the two days to assess repeatability, and the higher of the two values for each animal was used for further statistical analyses.

Maximal Thermogenic Capacity

Thermogenic capacity or summit metabolism ($\dot{V}O_2$sum) was measured in experiment 2 on test day 7 (11:00 – 13:00 h). Males were separated from their cage mates, weighed, and placed in a Plexiglas metabolic chamber (volume: 850 mL) with bedding, inside an environmental chamber. Mice were initially exposed to moderately low temperatures (0 to -5°C) in heliox (21% O_2: 79% He by volume; Chappell et al., 2003; Rosenmann and Morrison, 1974). Flow rates (1700 mL/min), temperature, and O_2 concentrations were measured every second using LabHelper. The temperature was quickly reduced (to a minimum of -20°C) until $\dot{V}O_2$sum was elicited, as indicated by

stable or declining VO_2 despite decreasing ambient temperature. Once $\dot{V}O_2$ sum was reached, mice were removed from the chamber and a temperature probe was inserted into the anus to determine the final body temperature (to determine if cold-acclimated individuals have greater cold resistance than warm-acclimated ones). Excurrent air was subsampled (~150 mL/min) and dried with soda lime and Drierite, and oxygen concentration was computed in LabAnalyst. Reference air was taken at the beginning and end of trials, and a baseline was computed by linear regression. $\dot{V}O_2$ sum (the highest $\dot{V}O_2$ averaged over 1 min) was determined and used for further statistical analyses.

Maximum Grip Strength

Maximum grip strength was determined in experiment 1 on test days 2 and 3 (9:30 – 11:00 h). Mice were suspended by tail over a horizontal wire-mesh surface attached to a force gauge (HF – 10N, M&A Instruments Inc.). The male was lowered until both the forelimbs and hindlimbs were touching the mesh without pulling on the force gauge. Once the male had relaxed on the mesh, the end of its tail was gently pulled horizontally until it released its grip (Meyer et al., 1979; Maurissen et al., 2003). Peak force value was recorded and the test was repeated; the higher value was used for analysis. Maximum grip strength was determined on each of the two days to assess repeatability, and the higher of the two values was used for analysis.

Maximum Sprint Speed

Maximum sprint speed was measured in experiment 1 on test days 5 and 6 (14:00 – 15:30 h) using a 'racetrack' (8 m long by 10 cm wide, with 30 cm high walls) equipped with 12 sets of aligned photocells at 50-cm intervals (Andrew et al., 2016). A mouse was placed near the start of the track and encouraged to walk or run down the track 2 - 4 times for familiarization at the start of each test. The male was returned to the starting area, the photocells were activated, and the mouse was chased down the track, activating the photocells. Sprint speed was measured 5 times on each of the two days, yielding a total of 10 trials per individual; the fastest 1.0-m interval on each day was recorded. Trials were scored subjectively as 'poor', 'fair', 'okay', 'good' or 'excellent' depending on mouse cooperation, trials in which cooperation was scored as poor or fair were excluded from analysis. The highest values from each day were used for repeatability, and the single highest value for each individual was used as its maximum sprint speed.

Euthanasia and Organ Collection

On the final day of testing (day 8; 12:30 – 14:30 h) in experiment 2, males were anesthetized with isoflurane and euthanized by CO_2 inhalation. Morphometric measurements were taken [body length, head length, head width, right foot length (tip of phalanges to tibia/fibula), and baculum length]. Organs [brain, all subcutaneous fat (white adipose only), heart ventricles, lungs, spleen, pancreas, liver, stomach (emptied), small/large intestines (emptied), caecum (emptied), adrenals (left and right), kidneys (left and right), and testes (left and right)] and muscles (left thigh and left gastrocnemius) were

then removed, blot dried, and weighed.

Statistical Analysis

In both experiments, we examined repeatability for all measures that were taken on two trials or two paired organs, using Pearson's correlations and paired *t*-tests. We used single values (e.g., mean or maximum) for comparisons of group means for these measures. Analysis was conducted in the same manner as previous work (Andrew et al., 2016; Andrew et al., in review), but we do not report repeatability and correlation results for this study here. For all measures, we used analysis of covariance (ANCOVA) in SPSS 24.0 to compare traits among groups (experiment 1: 5°C, 10°C, 22°C; experiment 2: 22°C VM, 10°C VM, 10°C NB, 10°C BM). Data were $\log_{10}$- or rank-transformed prior to analysis where appropriate (noted on Tables 3.2 – 3.5); results are presented in untransformed units (as estimated marginal mean ± standard error unless otherwise noted). For experiment 1, age and cohort were significant between the 3 groups (both $P < 0.001$), while days between relocation to new housing and testing were not ($P = 0.318$), all three were used as covariates in analysis. For experiment 2, age, cohort, and days between relocation to new housing and testing were significant between the 4 groups (all $P < 0.006$), but for reasons explained below, were not used as covariates in analysis. We also used body mass, body length, and cricket mass as covariates where appropriate (denoted in Tables 3.2 – 3.5). We performed the overall *F*-test for group differences and *a priori* contrasts among all of the groups for experiments 1 and 2. We discuss only the *a*

priori contrasts between groups in experiments 1 and 2, but the overall *F*-test are reported in the tables for convenience of the reader.

Results

Adult Mortality

In experiment 1, mortality did not vary significantly among groups. Twenty-nine of 42 (69%) virgin males acclimating to 5°C survived to the last day of testing compared to 50 of 70 (71%) males acclimating to 10°C and 61 of 72 (85%) of males housed at 22°C ($\chi^2 = 4.9$, $P = 0.085$).

Mortality did vary among groups in experiment 2 ($\chi^2 = 11.2$, $P = 0.011$). Survival rates were 86% (12 of 14) for VM housed at 22°C, 86% (12 of 14) for VM housed at 10°C, 43% (6 of 14) for non-breeding males at 10°C, and 43% (6 of 14) for breeding males at 10°C. Two of the surviving breeding pairs did not give birth; when removed from analysis, the difference among groups is even more highly significant ($\chi^2 = 13.2$, $P = 0.004$).

Experiment 1 – Short-Term Cold Acclimation

Both body mass and body composition differed among temperature conditions in experiment 1. Males housed at 5°C had lower body mass than those housed at 22°C on test days 1 ($P = 0.036$) and 7 ($P = 0.024$), but not test day 4; body mass of males housed at 10°C was intermediate and did not differ significantly from either of the other two groups on any day (Tables 3.2 and 3.3). Males housed at 5°C had lower absolute fat

mass ($P = 0.041$) than those housed at 10°C on day 1 ($P = 0.034$ for analysis of fat mass as a percent of body mass). Males housed at 10°C had lower absolute lean mass ($P = 0.040$) than those at 22°C on test day 1 ($P = 0.043$ for analysis of lean mass as a percent of body mass). The 5°C males had a higher percent lean mass on day 7 than both 10°C males ($P = 0.033$) and 22°C males ($P = 0.039$).

With body mass as a covariate, RMR was significantly higher in 5°C males than in males housed at higher temperatures (10°C males: $P = 0.002$, 22°C males: $P = 3.00 \times 10^{-6}$; Table 3.2 and 3.3).

Males housed at 10°C had higher hematocrit at day 1 than males housed at 22°C ($P = 0.010$; Table 3.2 and 3.3) and at 5°C ($P = 0.071$). At day 7, however, hematocrit did not differ significantly among temperature conditions. Forced-exercise $\dot{V}O_2$max (with body mass as a covariate) was higher at both 5°C ($P = 0.001$) and 10°C ($P = 0.004$) than for males housed at 22°C. Maximum sprint speed did not differ significantly among groups, although when body mass was used as a covariate, males housed at 5°C were slower compared to 22°C males ($P = 0.041$; Table 3.2 and 3.3). We did not find any effect of temperature on maximum grip strength or on latency to attack or kill a cricket.

Experiment 2 – Long-Term Cold Acclimation to 10°C

Body length, measured at euthanasia on day 8, was shorter in 10°C NB ($P = 0.001$) and 10°C VM ($P = 5 \times 10^{-6}$) compared to 22°C VM (Figure 3.2; Table 3.4 and 3.5). Body mass did not differ statistically between 22°C VM controls and any of the three groups housed at 10°C on test days 1, 4 or 8. With body length as a covariate, 10°C NB

were heavier than 22°C VM on all three test days 1 ($P = 0.015$, $P \leq 0.001$, and $P \leq 0.001$, respectively). With body length a covariate, fat mass was greater for both 10°C NB ($P = 0.007$) and 10°C VM ($P = 0.012$) as compared with 22°C VM. Lean mass (with body length as a covariate) showed the same pattern, being significantly greater for both 10°C NB ($P = 0.002$) and 10°C VM ($P = 0.026$) as compared with 22°C VM.

BMR (with body mass as a covariate) was higher in 10°C BM ($P = 3.00\text{x}10^{-6}$) and 10°C NB ($P = 1.00\text{x}10^{-6}$) as compared with 22°C VM (Table 3.4 and 3.5). $\dot{V}O_2$max (with body mass as a covariate) was lower in 10°C BM compared to 22°C VM ($P = 0.018$; Figure 3.3). $\dot{V}O_2$sum (with body mass as a covariate) was higher in 10°C VM than 22°C VM ($P = 0.011$; Figure 3.4). Hematocrit was not affected by long-term cold acclimation.

We examined several morphometric measures (body length reported above) on the final day of testing at sacrifice (Table 3.4 and 3.5). Log_{10}-transformed head length (with body length as a covariate) was longer in 10°C BM compared to 22°C VM ($P = 0.034$). Log_{10}-transformed head width was narrower in 10°C BM ($P = 0.004$) and 10°C NB ($P = 0.046$) compared to 22°C VM. When body length was used as a covariate, $\log_{10}$-transformed head width was relatively longer in 10°C BM ($P = 0.002$) and 10°C VM ($P = 0.037$) compared to 22°C VM. Foot length followed a similar pattern, regardless of whether body length was used as a covariate or not: both 10°C BM (all $P \leq 0.003$) and 10°C NB (all $P \leq 0.007$) had longer feet than 22°C VM (Figure 3.5). Finally, baculum length (without body length as a covariate) was significantly longer in 10°C BM ($P = 0.015$; with body length as a covariate $P = 0.027$) as compared to 22°C VM.

We compared organ masses (log-transformed in some analyses) among groups by ANCOVA with log-transformed body mass as a covariate (Table 3.4 and 3.5). Heart ventricle mass (body mass as a covariate) was larger in 10°C NB ($P = 0.002$) and 10°C VM ($P = 2.40\text{x}10^{-5}$) compared to 22°C VM. Kidney mass (with body mass as a covariate) was larger in 10°C VM compared to 22°C VM ($P = 1.15\text{x}10^{-4}$). Stomach mass (with body mass as a covariate) was larger in 10°C NB ($P = 0.014$) and 10°C VM ($P = 0.011$) compared to 22°C VM. Intestine mass (with body mass as a covariate) was larger in 10°C NB ($P = 0.002$) and 10°C VM ($P = 1.50\text{x}10^{-5}$) compared to 22°C VM. Caecum mass (with body mass as a covariate) was larger in 10°C BM compared to 22°C VM ($P = 0.049$). Baculum mass (with body mass as a covariate) was larger in 10°C BM ($P = 0.001$) or 10°C NB ($P = 0.038$) compared to 22°C. Thigh muscle mass (with body mass as a covariate) was smaller in 10°C VM compared 22°C VM ($P = 0.049$). Finally, gastrocnemius muscle mass (with body mass as a covariate) was smaller in 10°C NB ($P = 0.004$) and 10°C VM ($P = 3.63\text{x}10^{-4}$) compared to 22°C VM (Figure 3.6). No other organ masses were affected by long-term cold acclimation.

Experiment 2 – Effects of Reproductive Condition at 10°C

Breeding males differed from non-breeding and/or virgin males for several traits. BMR (with body mass as a covariate) was higher in 10°C BM than both 10°C NB ($P = 0.003$) and 10°C VM ($P = 1.34\text{x}10^{-7}$). $\dot{V}O_2\text{max}$ (with body mass as a covariate) was lower in 10°C BM compared to 10°C VM ($P = 0.014$; Figure 3.3). Foot length (with or without body length as a covariate) was greater in 10°C BM compared to 10°C VM (all *P*

≤ 0.005; Figure 3.4). Baculum length (without body length as a covariate) was longer in 10°C BM compared to 10°C VM ($P = 0.013$; with body length as a covariate $P = 0.014$). Ventricle mass (body mass as a covariate) was smaller in 10°C BM compared to 10°C VM ($P = 0.007$). Finally, adrenal mass (with body mass as a covariate) was smaller in 10°C BM compared to 10°C NB ($P = 0.042$).

In addition, non-breeding males differed from virgins for several traits. Body lengths of 10°C NB were longer than 10°C VM ($P = 0.001$; Figure 3.2; Table 3.4 and 3.5). BMR (with body mass as a covariate) was also higher in 10°C NB compared to 10°C VM ($P = 9.40\text{x}10^{-8}$). Foot length (with or without body length as a covariate) was greater in 10°C NB compared to 10°C VM (all $P \leq 0.015$; Figure 3.5). Baculum length (with body length as a covariate) was also longer in 10°C NB compared to 10°C VM ($P = 0.039$). Adrenal mass (with body mass as a covariate) was larger in 10°C NB compared to 10°C VM ($P = 0.014$). Intestine mass (with body mass as a covariate) was larger in 10°C NB compared to 10°C VM ($P = 0.003$). Baculum mass (with body mass as a covariate) was larger in 10°C BM compared to 10°C VM ($P = 0.015$). Thigh muscle mass (with body mass as a covariate) was smaller in 10°C NB compared to 10°C VM ($P = 0.005$). Finally, gastrocnemius muscle mass (with body mass as a covariate) was smaller in 10°C NB compared to 10°C VM ($P = 3.65\text{x}10^{-4}$; Figure 3.6).

Discussion

Overview

We hypothesized that both short-term and chronic cold acclimation would have pervasive effects on the metabolic rate, exercise performance, and morphology of male biparental California mice. This general expectation was based on results of many previous studies of small-bodied rodents, including *P. maniculatus* (Rezende et al., 2004; Rezende et al., 2009). To our knowledge, however, several of the traits we studied have not previously been considered in cold-acclimation studies of rodents (e.g., see Hart 1971; Withers et al., 2016), including sprint speed, grip strength, predatory aggression, and baculum length. We predicted that cold-acclimated males would have increased BMR or RMR, increased $\dot{V}O_2$max and $\dot{V}O_2$sum, as compared to males housed at room temperature. We also predicted that the effects of cold acclimation would be more pronounced in breeding males than in non-breeding and virgin males.

In experiment 1, short-term (24 days) housing of virgin males at 5°C and 10°C caused decreased body, fat, and lean masses, increased RMR and $\dot{V}O_2$max, and increased hematocrit compared to virgin males at 22°C. In experiment 2, chronic (243 days) 10°C housing caused non-breeding and virgin males to have higher body, fat, and lean masses (i.e., effects opposite to those measured after short-term acclimation), shorter bodies, longer feet, and larger organ masses (adjusted for body size), higher BMR, and higher $\dot{V}O_2$sum compared to virgin males housed at 22°C.

Also in experiment 2, we found several effects of reproductive condition for males housed at 10°C. For example, the 10°C breeding males had higher BMR, lower

$\dot{V}O_2$max, longer feet, and longer, heavier bacula compared to the non-breeding and virgin males housed at 10°C.

Experiment 1 – Short-Term Cold Acclimation

In experiment 1, we confirmed that *P. californicus* responds to short-term cold acclimation much in the same way as the smaller-bodied *P. maniculatus*. Previous *P. maniculatus* studies and our results confirm that California mice have increased RMR (Hart, 1971; Withers et al., 2016), increased $\dot{V}O_2$max (Hart, 1971; Chappell, 1984; Chappell, 1985; Hayes and Chappell, 1986; Hayes and Chappell, 1990; Withers et al., 2016), and decreased absolute fat mass (Rezende et al., 2004; Rezende et al., 2009) after cold acclimation. However, our results for body mass and hematocrit differed from previous studies of *P. maniculatus* and studies of rodents in general (Hart, 1971).

In the two previous studies of cold acclimation in *P. maniculatus*, body mass of males were found to be marginally lower in one study after 3 weeks (Rezende et al., 2009), whereas it was marginally higher after 3 weeks in the other study (Rezende et al., 2004). In our study, after ~ 3 weeks of cold acclimation, cold-acclimated males had lower body mass than room-temperature animals on testing days 1, 4, and 8. We are unsure why body mass decreased in our species, given that food and water were available *ad lib*, but species differences are certainly possible. In general rodent studies, body mass is found to increase even after short periods of cold acclimation when food and water are freely available (Hart, 1971).

We found that hematocrit showed little variation among groups in either experiment, except that 10°C males in experiment 1 had higher hematocrit compared to 22°C males on testing day 1 (but not on day 7). Hematocrit was marginally higher (P = 0.062) in cold-acclimated male *P. maniculatus* compared to warm-acclimated males after 7 weeks of acclimation (Rezende et al., 2009). In general, hematocrit has been found to increase with cold acclimation in rodents (Hart 1971).

Unlike previous studies of cold acclimation in *P. maniculatus*, we included measures of exercise performance and behavior in experiment 1. We found no differences between temperature conditions for grip-strength and either measure of predatory aggression. We did find that maximum sprint speed was lower in 5°C males compared to 22°C males when body mass was used as a covariate.

Experiment 2 – Long-Term Cold Acclimation to 10°C

Our results from this experiment show that California mice respond to chronic cold acclimation in ways that are similar to *P. maniculatus*, including increased BMR (Hart, 1971; Withers et al., 2016), increased $\dot{V}O_2$ sum (Chappell et al., 2007), increased visceral organ sizes (Hart, 1971; Hammond and Kristan, 2000; Rezende et al., 2009), and decreased absolute fat mass (Rezende et al., 2004; Rezende et al., 2009). Opposite to experiment 1 results though, we found that cold acclimated males had *higher* body, fat, and lean masses than room temperature virgin males. Along with the body composition results, the cold-acclimated males had shorter bodies but relatively longer feet. Altogether, this means our cold-acclimated males may have reduced in body length

(likely through shortening of vertebrae; Hart, 1971) and increased in body mass to reduce heat loss by radiation and convection (Hart, 1971), but increasing appendage length would have the opposite effect, so the result is somewhat paradoxical.

Summit metabolism, or $\dot{V}O_2$ sum, is the highest metabolic rate that can be attained in the face of cold challenge without hypothermia (Hart, 1971) and is an indicator of cold tolerance, e.g., during seasonal acclimatization (Heldmaier and Steinlechner, 1981; Heldmaier et al., 1982). Like $\dot{V}O_2$ max, $\dot{V}O_2$ sum increases during cold acclimation for *P. maniculatus* (Rezende et al., 2004; Chappell and Hammond, 2004; Chappell et al., 2007) and $\dot{V}O_2$ sum was ~42% higher than $\dot{V}O_2$ max for at least one study (Chappell et al., 2007). In contrast, we found that cold-acclimated males' $\dot{V}O_2$ sum was approximately 11% higher than their $\dot{V}O_2$ max and warm-acclimated males' $\dot{V}O_2$ sum was approximately 3% higher than their $\dot{V}O_2$ max. Our results would suggest that *P. californicus* $\dot{V}O_2$ sum does not respond to cold acclimation as strongly as for *P. maniculatus*.

Experiment 2 – Effects of Reproductive Condition at 10°C

Results from experiment 2 indicate that being a father during long-term cold acclimation has minor effects on paternal physiology and morphology compared to non-fathers. In previous work, we have examined fatherhood after a single birth (Andrew et al., 2016), throughout multiple births (Andrew et al., in review), and also under food deprivation and an exercise challenge after a single birth (Zhao et al., in press). In all

three studies, fathers were not consistently different from non-fathers. Results from the previous studies suggest that fatherhood does not have a significantly detrimental effect on *P. californicus*. In this study however, we found that cold-acclimated breeding males had higher BMR, lower $\dot{V}O_2$max, and longer, larger bacula, compared to virgin males, which differs from the previous standard laboratory studies, where we found no difference in BMR, $\dot{V}O_2$max, or baculum between reproductive conditions. Given the small breeding male sample size for BMR (N = 2), we would caution against over-interpreting those results. In any case, the $\dot{V}O_2$max and baculum results are unique compared to what we have found in the past.

Our previous studies of $\dot{V}O_2$max in this species found no differences among reproductive groups (Andrew et al., 2016, Andrew et al., in review), but in the present study breeding males had lower mass-adjusted $\dot{V}O_2$max compared to virgin males. This difference was caused by the virgins having elevated $\dot{V}O_2$max (highest of all groups, including the warm-acclimated animals), not by the breeding males having reduced values. In any case, this result provides evidence of a physiological difference between fathers and non-fathers, at least after long-term cold acclimation.

Absolute baculum length (not adjusted for body length) and baculum mass (adjusted for body mass) was greater in breeding males compared to virgin males. Aside from our previous paternal study (Andrew et al., in review), which found no differences among the same reproductive groups as studied here, but housed at room temperature, no other studies of effects of fatherhood have reported baculum length and size.

Given the higher mortality rate of the breeding (43%) and non-breeding (43%) males compared to the virgin males (86%), living with a female (most of the time) appears detrimental to a male's health in *P. californicus*. Looked at the other way, remaining a virgin reduces mortality rate, suggesting a trade-off between sex and death. Clearly something changed with our breeding males that did not happen with the other groups, but without further study, and given our small sample size, we cannot definitively say what that was.

Caveats

We caution that, when interpreting our results, some issues during testing should be kept in mind. First, repeated handling of animals and brief separations from cage mates (especially during RMR and BMR tests) might have influenced behavior and/or pup development (Vieira and Brown, 2003), but is seems unlikely, based on previous work in this species (de Jong et al., 2013; Harris et al., 2013). Second, our laboratory housing conditions – e.g., *ad lib* food and water, absence of predators and conspecific rivals, and small cages that do not require any exercise - could potentially diminish overall locomotor performance in all males and might ameliorate any energetic stress related to fatherhood, unlike what may occur for free-living males. Third, the environmental chamber we used to house the cold animals may have introduced additional stressors (e.g., fan noise, vibrations) that the warm-housed animals did not experience. Fourth, due to technical problems, the sample size for BMR was small. Finally, we initially planned to include more metabolic (daily energy expenditure),

morphological (fur density), and behavioral (food/water consumption, home cage activity, and nest-building behavior) tests, but these became infeasible due to technical problems. Nonetheless, we did obtain several "classic" measures used in cold acclimation studies of rodents, as well as some novel ones, and we did document some significant effects of fatherhood in cold-acclimated animals.

References

Achenbach, G. G. and Snowdon, C. T. (2002). Costs of caregiving: Weight loss in captive adult male cotton-top tamarins (*Saguinus oedipus*) following the birth of infants. *Int J Primatol* **23**, 179-189.

Andrew, J. R., Saltzman, W., Chappell, M. A. and Garland, T., Jr. (2016). Consequences of fatherhood in the biparental California mouse (*Peromyscus californicus*): Locomotor performance, metabolic rate, and organ masses. *Physiol Biochem Zool* **89**, 130-40.

Bales, K. L. and Saltzman, W. (2016). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Horm Behav* **77**, 249-59.

Bedford, N. L. and Hoekstra, H. E. (2015). Peromyscus mice as a model for studying natural variation. *Elife* **4**.

Braun, K. and Champagne, F. A. (2014). Paternal influences on offspring development: Behavioural and epigenetic pathways. *J Neuroendocrinol* **26**, 697-706.

Bredy, T. W., Brown, R. E. and Meaney, M. J. (2007). Effect of resource availability on biparental care, and offspring neural and behavioral development in the California mouse (*Peromyscus californicus*). *Eur J Neurosci* **25**, 567-75.

Cantoni, D. and Brown, R. E. (1997). Paternal investment and reproductive success in the California mouse, *Peromyscus californicus*. *Anim Behav* **54**, 377-86.

Chappell, M. A. (1984). Maximum oxygen consumption during exercise and cold exposure in deer mice, *Peromyscus maniculatus*. *Respir Physiol* **55**, 367-77.

Chappell, M. A. (1985). Effects of ambient temperature and altitude on ventilation and gas exchange in deer mice (*Peromyscus maniculatus*). *J Comp Physiol B* **155**, 751-8.

Chappell, M. A., Garland, T., Jr., Rezende, E. L. and Gomes, F. R. (2004). Voluntary running in deer mice: Speed, distance, energy costs and temperature effects. *J Exp Biol* **207**, 3839-54.

Chappell, M. A. and Hammond, K. A. (2004). Maximal aerobic performance of deer mice in combined cold and exercise challenges. *J Comp Physiol B* **174**, 41-8.

Chappell, M. A., Hammond, K. A., Cardullo, R. A., Russell, G. A., Rezende, E. L. and Miller, C. (2007). Deer mouse aerobic performance across altitudes: Effects of developmental history and temperature acclimation. *Physiol Biochem Zool* **80**, 652-62.

Chappell, M. A., Rezende, E. L. and Hammond, K. A. (2003). Age and aerobic performance in deer mice. *J Exp Biol* **206**, 1221-31.

Chauke, M., Malisch, J. L., Robinson, C., de Jong, T. R. and Saltzman, W. (2011). Effects of reproductive status on behavioral and endocrine responses to acute stress in a biparental rodent, the California mouse (*Peromyscus californicus*). *Horm Behav* **60**, 128-38.

de Jong, T. R., Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2013). Physiological and neuroendocrine responses to chronic variable stress in male California mice (*Peromyscus californicus*): Influence of social environment and paternal state. *Psychoneuroendocrinol* **38**, 2023-33.

Demas, G. E. and Nelson, R. J. (1996). Photoperiod and temperature interact to affect immune parameters in adult male deer mice: (*Peromyscus maniculatus*). *J Biol Rhythms* **11**, 94-102.

Dlugosz, E. M., Harris, B. N., Saltzman, W. and Chappell, M. A. (2012). Glucocorticoids, aerobic physiology, and locomotor behavior in California mice. *Physiol Biochem Zool* **85**, 671-83.

Dudley, D. (1974). Paternal behavior in the California mouse, *Peromyscus californicus*. *Behav Bio* **11**, 247-252.

Eisenberg, J. F. (1963). The intraspecific social behavior of some cricetine rodents of the genus *Peromyscus*. *Am Mid Nat* **69**, 240-246.

Gammie, S. C., Hasen, N. S., Rhodes, J. S., Girard, I. and Garland, T., Jr. (2003). Predatory aggression, but not maternal or intermale aggression, is associated with high voluntary wheel-running behavior in mice. *Horm Behav* **44**, 209-221.

Griffiths, B. B. and Hunter, R. G. (2014). Neuroepigenetics of stress. *Neuroscience* **275**, 420-35.

Gubernick, D. J. and Alberts, J. R. (1987a). The biparental care system of the California mouse, *Peromyscus californicus*. *J Comp Psychol* **101**, 169-177.

Gubernick, D. J. and Alberts, J. R. (1987b). "Resource" exchange in the biparental California mouse (*Peromyscus californicus*): Water transfer from pups to parents. *J Comp Psychol* **101**, 328-34.

Gubernick, D. J., Wright, S. L. and Brown, R. E. (1993). The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Anim Behav* **46**, 539-546.

Hammond, K. A. and Kristan, D. M. (2000). Responses to lactation and cold exposure by deer mice (*Peromyscus maniculatus*). *Physiol Biochem Zool* **73**, 547-56.

Harris, B. N., de Jong, T. R., Yang, V. and Saltzman, W. (2013). Chronic variable stress in fathers alters paternal and social behavior but not pup development in the biparental California mouse (*Peromyscus californicus*). *Horm Behav* **64**, 799-811.

Harris, B. N., Perea-Rodriguez, J. P. and Saltzman, W. (2011). Acute effects of corticosterone injection on paternal behavior in California mouse (*Peromyscus californicus*) fathers. *Horm Behav* **60**, 666-75.

Hart, J. S. (1971). Rodents. In *Comparative Physiology of Thermoregulation*, (ed. G. C. Whittow), pp. 1-149. New York: Academic Press.

Hayes, J. P. and Chappell, M. A. (1986). Effects of cold acclimation on maximum oxygen consumption during cold exposure and treadmill exercise in deer mice, *Peromyscus maniculatus*. *Physiol Zool* **59**, 473-481.

Hayes, J. P. and Chappell, M. A. (1990). Individual consistency of maximal oxygen consumption in deer mice. *Funct Ecol* **4**, 495-503.

Heimer, W. and Morrison, P. (1978). Effects of chronic and intermittent cold exposure on metabolic capacity of *Peromyscus* and *Microtus*. *J Biometeorol* **22**, 129-134.

Heldmaier, G. and Steinlechner, S. (1981). Seasonal control of energy requirements for thermoregulation in the Djungarian hamster (*Phodopus sungorus*), living in natural photoperiod. *J Comp Physiol B* **142**, 429-437.

Heldmaier, G., Steinlechner, S. and Rafael, J. (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *J Comp Physiol B* **149**, 1-9.

Hill, R. W. (1983). Thermal physiology and energetics of *Peromyscus*; Ontogeny, body temperature, metabolism, insulation, and microclimatology. *J Mammal* **64**, 19-37.

Lee, A. W. and Brown, R. E. (2002). The presence of the male facilitates parturition in California mice (*Peromyscus californicus*). *Can J Zool* **80**, 926-933.

Lynch, G. R. (1973). Seasonal changes in thermogenesis, organ weights, and body composition in the white-footed mouse, *Peromyscus leucopus*. *Oecologia* **13**, 363-376.

Maurissen, J. P. J., Marable, B. R., Andrus, A. K. and Stebbins, K. E. (2003). Factors affecting grip strength testing. *Neurotoxicol Teratol* **25**, 543-553.

McNab, B. K. and Morrison, P. (1963). Body temperature and metabolism in subspecies of *Peromyscus* from arid and mesic environments. *Ecological Monographs* **33**, 63-82.

Merritt, J. F. (1978). *Peromyscus californicus*. *Mamm Spec* **85**, 1-6.

Meyer, O. A., Tilson, H. A., Byrd, W. C. and Riley, M. T. (1979). A method for the routine assessment of fore-and hindlimb grip strength of rats and mice. *Neurobehav Toxicol* **1**, 233.

Rezende, E. L., Chappell, M. A. and Hammond, K. A. (2004). Cold-acclimation in *Peromyscus*: Temporal effects and individual variation in maximum metabolism and ventilatory traits. *J Exp Biol* **207**, 295-305.

Rezende, E. L., Hammond, K. A. and Chappell, M. A. (2009). Cold acclimation in *Peromyscus*: Individual variation and sex effects in maximum and daily metabolism, organ mass and body composition. *J Exp Biol* **212**, 2795-802.

Ribble, D. O. (1991). The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behav Ecol Sociobiol* **29**, 161-166.

Ribble, D. O. and Salvioni, M. (1990). Social organization and nest co-occupancy in *Peromyscus californicus*, a monogamous rodent. *Behav Ecol Sociobiol* **26**, 9-15.

Rosenmann, M. and Morrison, P. (1974). Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-O2. *Am J Physiol* **226**, 490-5.

Russell, G. A. and Chappell, M. A. (2007). Is BMR repeatable in deer mice? Organ mass correlates and the effects of cold acclimation and natal altitude. *J Comp Physiol B* **177**, 75-87.

Saltzman, W., Harris, B. N., de Jong, T. R., Nguyen, P. P., Cho, J. T., Hernandez, M. and Perea-Rodriguez, J. P. (2015). Effects of parental status on male body mass in the monogamous, biparental California mouse. *J Zool* **296**, 23-29.

Saltzman, W. and Ziegler, T. E. (2014). Functional significance of hormonal changes in mammalian fathers. *J Neuroendocrinol* **26**, 685-96.

Thomas, K. and Fried, M. (1971). The speed of predatory attacks *Mus musculus*, *Peromyscus calfifornicus* and *Onychomys torridus*. In *Amer. Soc. Mammal.* Vancouver, B.C.

Vieira, M. L. and Brown, R. E. (2003). Effects of the presence of the father on pup development in California mice (*Peromyscus californicus*). *Dev Psychobiol* **42**, 246-51.

Withers, P. C., Cooper, C. E., Maloney, S. K., Bozinovic, F. and Neto, A. P. C. (2016). Ecological and environmental physiology of mammals. OXford, UK: Oxford University Press.

Zhao, M., Garland Jr, T., Chappell, M. A., Andrew, J. R., Harris, B. N. and Saltzman, W. (in press). Effects of a physical and energetic challenge on male California mice (*Peromyscus californicus*): Modulation by reproductive condition. *J Exp Biol*.

Zhao, M., Garland, T., Jr., Chappell, M. A., Andrew, J. R. and Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiol Behav* **177**, 57-67.

Figure Legends

Figure 3.1. Timeline and use of males in experiments 1 and 2. Closed circle at the end of a line = males finished experiment, arrow at the end of a line = males re-paired and put into next experiment. Number of days indicates the lower and upper range of days between the previous "event" and the following "event." Sample sizes of males that lived through the duration of testing and were used for analysis are in parenthesis and total starting sample size is in brackets.

Figure 3.2. Body length (mm) on the x-axis versus body mass (g) on the y-axis. 22°C = closed circles (n=12), 10°C VM = open circles (n=12), 10°C NB = open squares (n=6), 10°C BM = grey triangles (n=4).

Figure 3.3. $\dot{V}O_2$ max (ml O_2/h) on the y-axis versus body mass (g) on the x-axis. 22°C = closed circles (n=12), 10°C VM = open circles (n=12), 10°C NB = open squares (n=6), 10°C BM = grey triangles (n=4).

Figure 3.4. $\dot{V}O_2$ sum (ml O_2/h) on the y-axis versus body mass (g) on the x-axis. 22°C = closed circles (n=12), 10°C VM = open circles (n=12), 10°C NB = open squares (n=6), 10°C BM = grey triangles (n=4).

Figure 3.5. Foot length (mm) on the x-axis versus body length (mm) on the y-axis. 22°C = closed circles (n=12), 10°C VM = open circles (n=12), 10°C NB = open squares (n=6), 10°C BM = grey triangles (n=4).

Figure 3.6. Gastrocnemius muscle mass (g) on the y-axis versus body mass (g) on the x-axis. 22°C = closed circles (n=12), 10°C VM = open circles (n=12), 10°C NB = open squares (n=6), 10°C BM = grey triangles (n=4).

Figure 3.1.

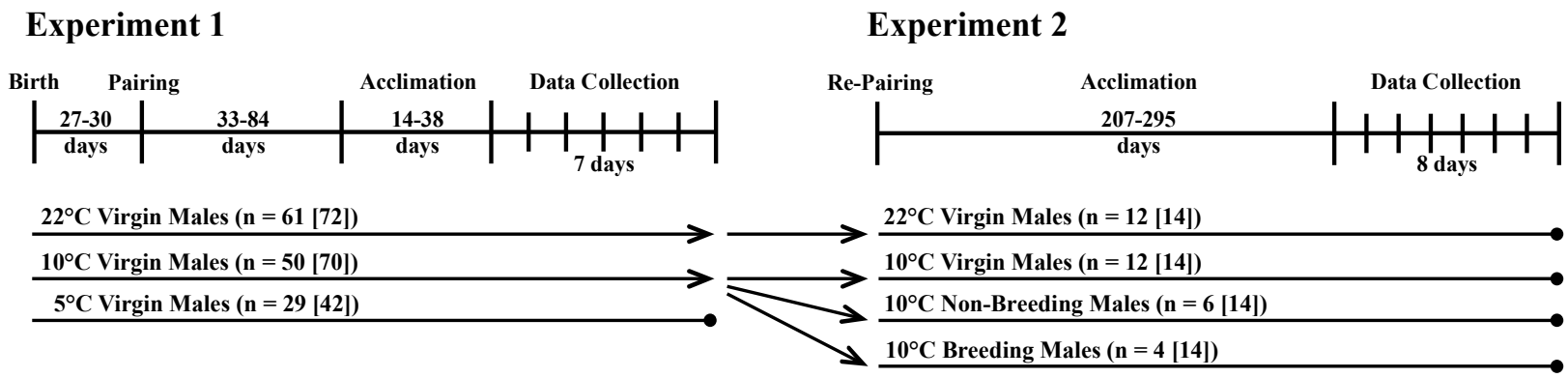

Experiment 1
Experiment 2
Birth
Pairing
Acclimation
Data Collection
27-30 days
33-84 days
14-38 days
7 days
Re-Pairing
Acclimation
Data Collection
207-295 days
8 days
22°C Virgin Males (n = 61 [72])
10°C Virgin Males (n = 50 [70])
5°C Virgin Males (n = 29 [42])
22°C Virgin Males (n = 12 [14])
10°C Virgin Males (n = 12 [14])
10°C Non-Breeding Males (n = 6 [14])
10°C Breeding Males (n = 4 [14])

Figure 3.2.

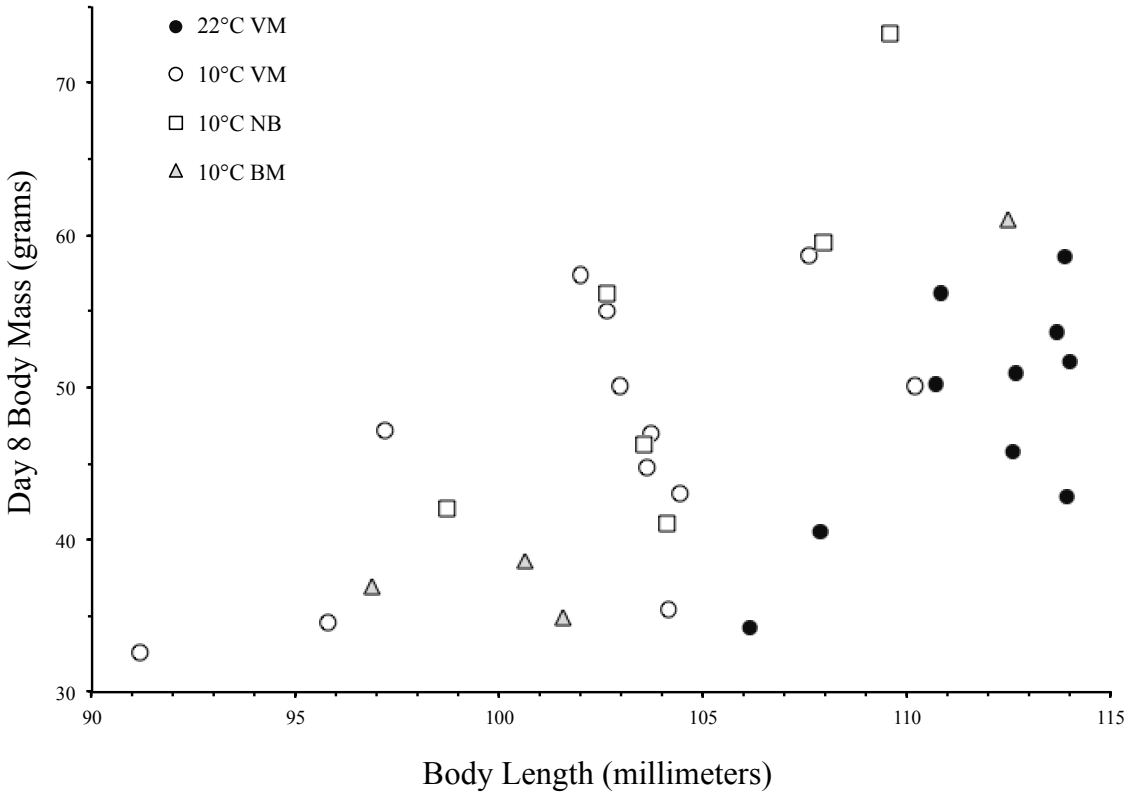

22°C VM
10°C VM
10°C NB
10°C BM
Day 8 Body Mass (grams)
70
60
50
40
30
90
95
100
105
110
115
Body Length (millimeters)

Figure 3.3.

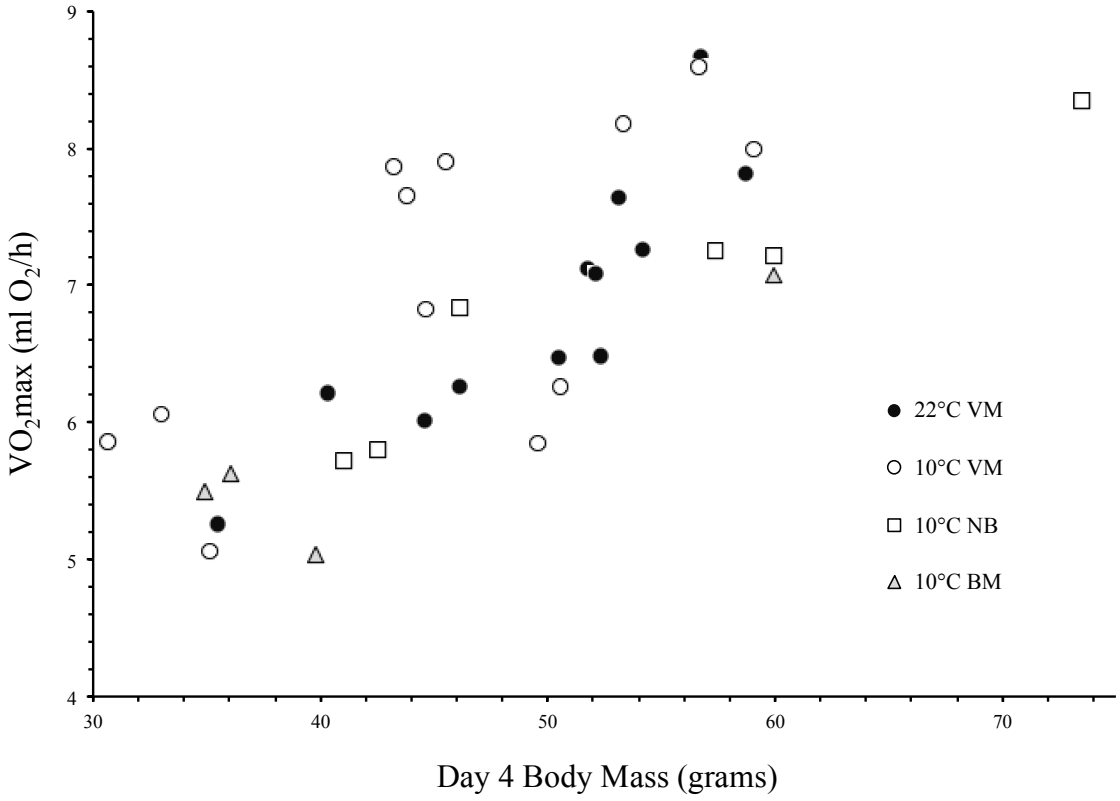

9
8
7
6
5
4
30
40
50
60
70
VO_2max (ml O_2/h)
Day 4 Body Mass (grams)
22°C VM
10°C VM
10°C NB
10°C BM

Figure 3.4.

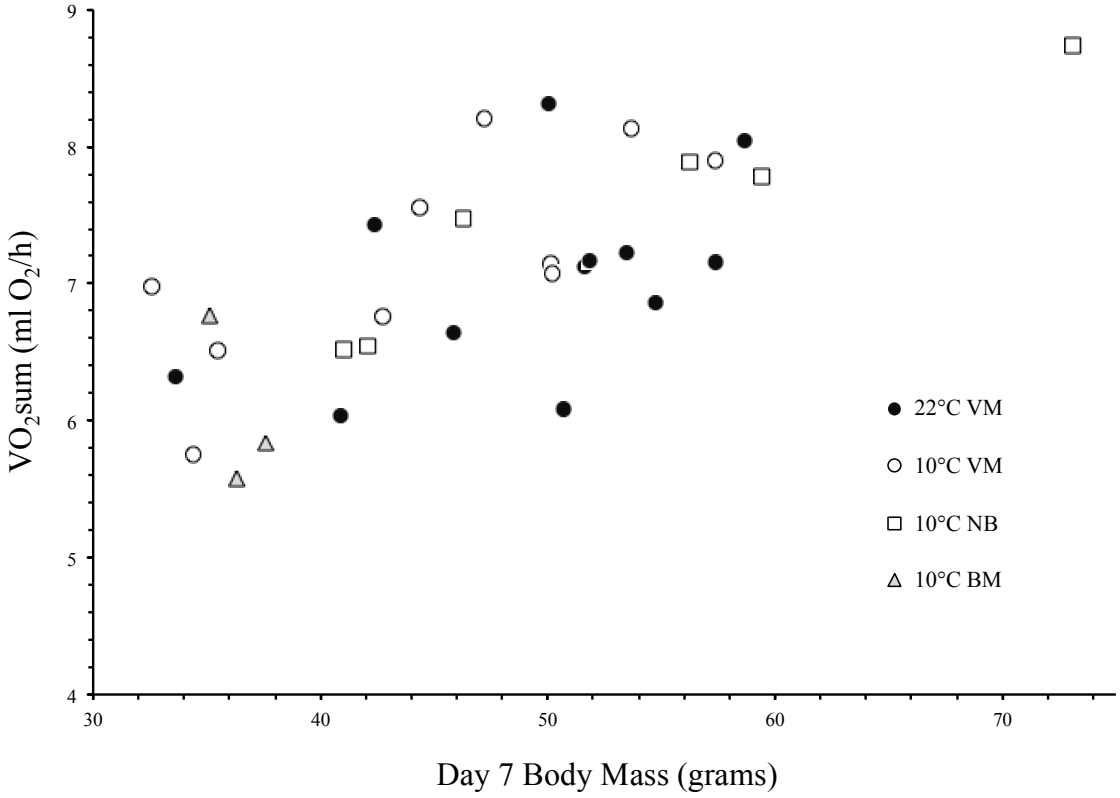

9
8
7
6
5
4
30
40
50
60
70
VO2sum (ml O2/h)
Day 7 Body Mass (grams)
22°C VM
10°C VM
10°C NB
10°C BM

Figure 3.5.

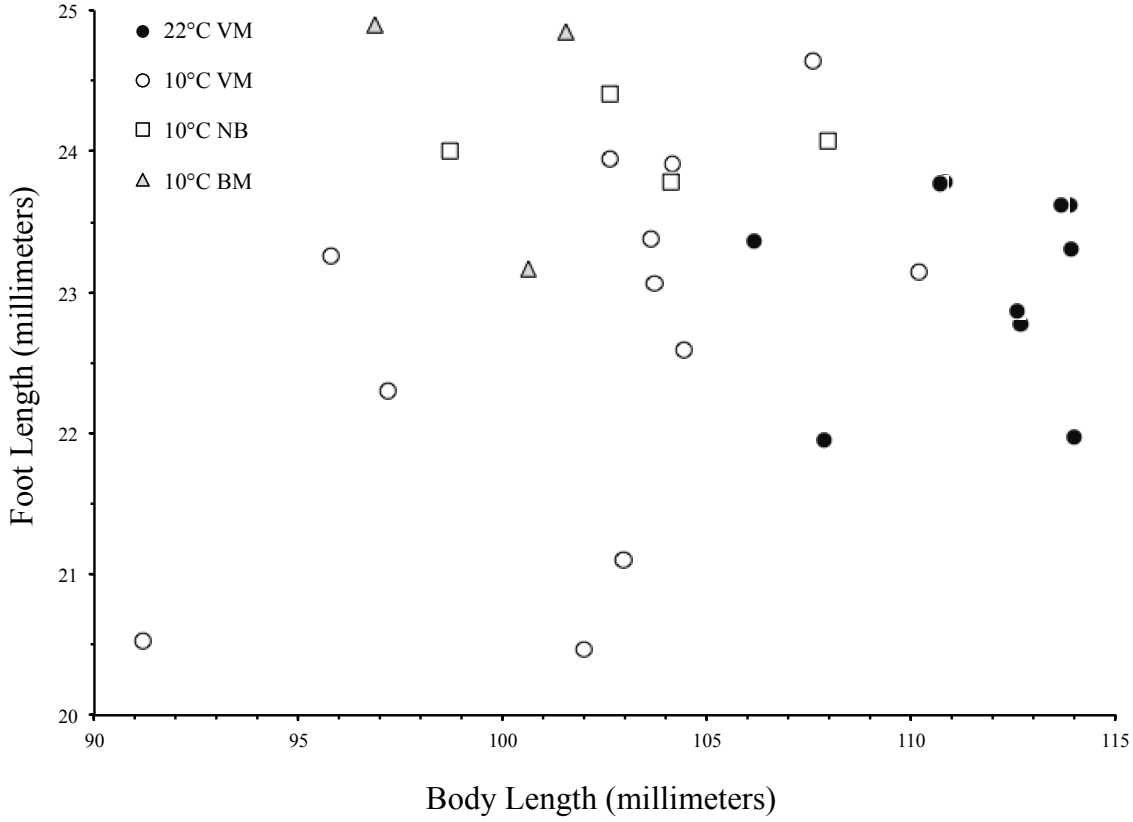

22°C VM
10°C VM
10°C NB
10°C BM
Foot Length (millimeters)
Body Length (millimeters)
25
24
23
22
21
20
90
95
100
105
110
115

Figure 3.6.

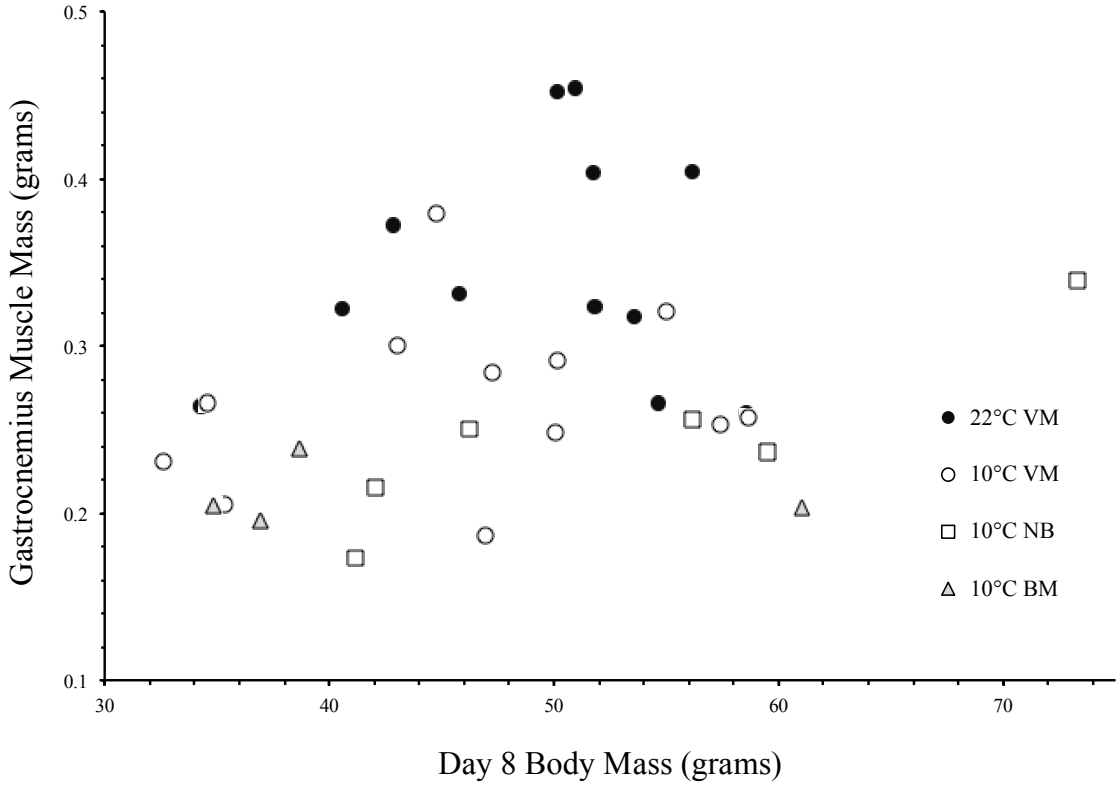

Gastrocnemius Muscle Mass (grams)
0.5
0.4
0.3
0.2
0.1
30
40
50
60
70
Day 8 Body Mass (grams)
22°C VM
10°C VM
10°C NB
10°C BM

Table 3.1. List of measures in experiments 1 and 2. Measures are listed by the day on which they were conducted and time of day conducted in each experiment.

Experiment 1			Experiment 2		
Test Day	**Time**	**Trait**	**Test Day**	**Time**	**Trait**
1	9:00-10:30	Hematocrit	1	8:30-16:30	Body Mass/Basal Metabolic Rate
1	13:00-14:30	Body Mass/Fat Mass/Lean Mass			
2/3	9:30-11:00	Maximum Grip Strength	4/5	11:30-13:30	Body Mass/Exercise VO_2max
2/3	13:30-15:00	Predatory Aggression			
4	8:30-16:30	Body Mass/Resting Metabolic Rate	6	12:00-14:00	Body Mass/Fat Mass/Lean Mass
5/6	10:00-11:30	Body Mass/VO_2max	7	11:00-13:00	Body Mass/Summit Metabolism
5/6	14:00-15:30	Maximal Sprint Speed			
7	9:00-10:30	Body Mass/Fat Mass/Lean Mass	8	12:30-14:30	Body Mass/Organ Masses
7	13:00-14:30	Hematocrit	8	12:30-14:30	Hematocrit

Table 3.2. Experiment 1 results of analysis of covariance with *a priori* contrasts comparing 5, 10, and 22°C virgin males. Table has units, transformation, covariates (B = body mass, R = RMR chamber, C = cricket mass) for each trait. Overall F-test results (DF = degrees of freedom, group *F*, and group *P*) and *a priori* contrasts *P* values (*P* values ≤ 0.05 are bolded and underlined) from ANCOVAs are reported. Cohort, age, and duration of acclimation were used as covariates in all analysis.

				Overall *F*		*a priori* Contrasts		
Traits	**Units**	**Transform**	**Covariates**	**Group F**	**Group P**	**5° *vs.* 10°**	**5° *vs.* 22°**	**10° *vs.* 22°**
Body Mass (Day 1)	Grams	None	None	2.363	0.098	0.176	**0.036**	0.634
Body Mass (Day 4)	Grams	None	None	1.469	0.234	0.325	0.102	0.634
Body Mass (Day 7)	Grams	None	None	2.896	0.059	0.181	**0.024**	0.461
Fat Mass (Day 1)	Grams	None	None	2.269	0.107	**0.041**	0.055	0.374
Percent Fat Mass (Day 1)	%	None	None	2.393	0.095	**0.034**	0.169	0.098
Fat Mass (Day 7)	Grams	Log_{10}	None	1.371	0.257	0.175	0.101	0.919
Percent Fat Mass (Day 7)	%	None	None	1.323	0.270	0.162	0.109	0.823
Lean Mass (Day 1)	Grams	None	None	3.227	**0.043**	0.876	0.146	**0.040**
Percent Lean Mass (Day 1)	%	None	None	3.702	**0.027**	**0.008**	0.073	**0.043**
Lean Mass (Day 7)	Grams	None	None	1.863	0.159	0.972	0.253	0.125
Percent Lean Mass (Day 7)	%	None	None	2.511	0.085	**0.033**	**0.039**	0.400
Resting Metabolic Rate	ml O_2/h	None	B	12.308	**$1.30x10^{-5}$**	**0.002**	**$3.00x10^{-6}$**	0.317
Hematocrit (Day 1)	%	None	None	3.455	**0.035**	0.071	0.785	**0.010**
Hematocrit (Day 7)	%	None	None	1.664	0.194	0.265	0.079	0.671
Maximal Oxygen Consumption	ml O_2/h	None	B	10.321	**$6.90x10^{-5}$**	0.316	**0.001**	**0.004**
Maximum Sprint Speed	m/s	None	None	2.664	0.073	0.874	0.125	0.089
Maximum Sprint Speed	m/s	None	B	3.812	**0.025**	0.614	**0.041**	0.068
Maximum Grip Strength	Newtons	None	B	1.783	0.172	0.401	0.090	0.415
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	B, C	0.592	0.555	0.279	0.393	0.502
Predatory Aggression: Latency to Kill Cricket	Seconds	Rank	B, C	1.497	0.229	0.791	0.380	0.142

B = Body Mass, C = Cricket Mass

Cohort, Age and, Duration of Acclimation were used as covariates in all analysis.

P values ≤ 0.05 are bolded and underlined.

Table 3.3. Experiment 1 estimated marginal means and standard errors for 5, 10, and 22°C virgin males. Trait units, covariates (B = body mass, R = RMR chamber, C = cricket mass), sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs are reported.

			5°C			10°C			22°C		
Traits	**Units**	**Covariates**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Mass (Day 1)	Grams	None	29	37.92	1.49	50	40.71	0.97	61	41.32	0.77
Body Mass (Day 4)	Grams	None	28	38.41	1.52	49	40.47	0.99	61	41.10	0.77
Body Mass (Day 7)	Grams	None	26	37.38	1.52	49	40.15	0.96	61	41.09	0.75
Fat Mass (Day 1)	Grams	None	29	5.50	0.95	49	8.22	0.62	61	7.49	0.49
Percent Fat Mass (Day 1)	%	None	29	14.38	1.84	48	19.83	1.21	60	17.13	0.96
Fat Mass (Day 7)	Grams	None	26	5.49	0.90	50	7.25	0.57	61	7.46	0.45
Percent Fat Mass (Day 7)	%	None	26	14.38	1.70	49	17.63	1.08	61	17.31	0.85
Lean Mass (Day 1)	Grams	None	29	29.91	0.79	51	29.74	0.51	60	31.15	0.41
Percent Lean Mass (Day 1)	%	None	29	79.66	1.73	49	73.25	1.13	59	76.29	0.89
Lean Mass (Day 7)	Grams	None	26	29.87	0.85	49	29.83	0.54	60	30.91	0.42
Percent Lean Mass (Day 7)	%	None	26	79.84	1.64	49	75.02	1.04	60	76.17	0.81
Resting Metabolic Rate	ml O_2/h	B	19	1.74	0.32	34	1.48	0.25	40	1.12	0.20
Hematocrit (Day 1)	%	None	27	47.94	0.66	49	49.59	0.42	60	48.13	0.33
Hematocrit (Day 7)	%	None	26	47.28	0.70	45	46.20	0.46	61	45.94	0.35
Maximal Oxygen Consumption	ml O_2/h	B	27	6.28	0.14	50	6.09	0.09	61	5.76	0.07
Maximum Sprint Speed	m/s	None	27	1.61	0.14	50	1.64	0.09	61	1.85	0.07
Maximum Sprint Speed	m/s	B	27	1.56	0.14	50	1.65	0.09	61	1.87	0.07
Maximum Grip Strength	Newtons	B	29	4.43	0.20	50	4.67	0.13	61	4.81	0.10
Predatory Aggression: Latency to First Attack Cricket	Seconds	B, C	27	14.79	6.33	50	24.21	4.01	58	20.66	3.19
Predatory Aggression: Latency to Kill Cricket	Seconds	B, C	25	63.88	8.59	40	69.96	5.66	49	55.92	4.48

B = Body Mass, C = Cricket Mass

Cohort, Age and, Duration of Acclimation were used as covariates in all analysis.

Table 3.4. Experiment 2 results of analysis of covariance with *a priori* contrasts comparing 22°C virgin (VM), 10°C virgin, 10°C non-breeding (NB), and 10°C breeding males (BM). Table has units, transformation, covariates (B = body mass, R = RMR chamber, C = cricket mass) for each trait. Overall F-test results (DF = degrees of freedom, group *F*, and group *P*) and *a priori* contrasts *P* values (*P* values ≤ 0.05 are bolded and underlined) from ANCOVAs are reported. Cohort, age, and duration of acclimation were used as covariates in all analysis.

				Overall *F*		*a priori* Contrasts					
Traits	**Units**	**Transform**	**Covariates**	**Group F**	**Group P**	**10°C BM *vs.* 22°C VM**	**10°C NB *vs.* 22°C VM**	**10°C VM *vs.* 22°C VM**	**10°C BM *vs.* 10°C NB**	**10°C BM *vs.* 10°C VM**	**10°C NB *vs.* 10°C VM**
Body Mass (Day 1)	Grams	None	None	1.710	0.186	0.165	0.849	0.142	0.102	0.519	0.096
Body Mass (Day 1)	Grams	None	L	2.572	0.073	0.344	**0.015**	0.082	0.099	0.295	0.536
Body Mass (Day 4)	Grams	None	None	1.538	0.225	0.098	0.429	0.275	0.085	0.612	0.204
Body Mass (Day 4)	Grams	None	L	5.217	**0.005**	0.190	**0.001**	**0.010**	0.061	0.325	0.149
Body Mass (Day 8)	Grams	None	None	40.663	2.559	0.159	0.422	0.446	0.101	0.527	0.245
Body Mass (Day 8)	Grams	None	L	5.209	**0.005**	0.335	**0.001**	**0.005**	0.082	0.255	0.131
Fat Mass	Grams	None	None	0.646	0.592	0.377	0.580	0.683	0.194	0.484	0.325
Fat Mass	Grams	None	L	3.359	**0.032**	0.753	**0.007**	**0.012**	0.223	0.274	0.198
Percent Fat Mass	%	None	None	0.441	0.725	0.762	0.858	0.879	0.296	0.362	0.309
Percent Fat Mass	%	None	L	1.970	0.141	0.802	0.064	**0.037**	0.374	0.227	0.427
Lean Mass	Grams	Log_{10}	None	1.524	0.229	0.108	0.512	0.233	0.096	0.629	0.188
Lean Mass	Grams	Log_{10}	L	4.006	**0.017**	0.218	**0.002**	**0.026**	0.083	0.371	0.237
Percent Lean Mass	%	Log_{10}	None	0.657	0.585	0.377	0.589	0.671	0.191	0.478	0.315
Percent Lean Mass	%	Log_{10}	L	2.764	0.060	0.725	**0.015**	**0.025**	0.227	0.292	0.281
Basal Metabolic Rate	ml O_2/h	None	B	58.630	**$8.22x10^{-8}$**	**$3.00x10^{-6}$**	**$1.00x10^{-6}$**	0.764	**0.003**	**$1.34x10^{-7}$**	**$9.40x10^{-8}$**
Maximal Oxygen Consumption	ml O_2/h	None	B	3.556	**0.026**	**0.018**	0.324	0.062	0.781	**0.014**	0.249
Maximal Thermogenic Capacity	ml O_2/h	None	B	2.620	0.070	0.088	0.681	**0.011**	0.555	0.389	0.308
Hematocrit	%	Log_{10}	None	0.307	0.820	0.510	0.850	0.564	0.821	0.411	0.677

Table 3.4. Continued.

				Overall *F*		*a priori* Contrasts					
Traits	**Units**	**Transf.**	**Covar.**	**Group F**	**Group P**	**10°C BM *vs.* 22°C VM**	**10°C NB *vs.* 22°C VM**	**10°C VM *vs.* 22°C VM**	**10°C BM *vs.* 10°C NB**	**10°C BM *vs.* 10°C VM**	**10°C NB *vs.* 10°C VM**
Body Length	Millimeters	None	None	11.840	**2.80x10^{-5}**	0.311	**0.001**	**5.00x10^{-6}**	0.598	0.769	**0.001**
Head Length	Millimeters	Log_{10}	None	1.744	0.179	0.051	0.073	0.835	0.595	0.257	0.322
Head Length	Millimeters	Log_{10}	L	1.763	0.176	**0.034**	0.353	0.338	0.520	0.231	0.842
Head Width	Millimeters	Log_{10}	None	3.263	**0.035**	**0.004**	**0.046**	0.222	0.217	0.212	0.698
Head Width	Millimeters	Log_{10}	L	4.298	**0.013**	**0.002**	0.405	**0.037**	0.151	0.168	0.519
Foot Length	Millimeters	None	None	6.231	**0.002**	**0.002**	**0.007**	0.509	0.969	**0.005**	**0.015**
Foot Length	Millimeters	None	L	8.001	**0.000**	**0.003**	**0.001**	0.360	0.817	**0.004**	**0.002**
Baculum Length	Millimeters	None	None	3.560	**0.026**	**0.015**	0.160	0.168	0.724	**0.013**	0.110
Baculum Length	Millimeters	None	L	3.858	**0.019**	**0.027**	0.057	0.985	0.625	**0.014**	**0.039**
Brain Mass	Grams	None	B	0.995	0.409	0.337	0.795	0.133	0.417	0.930	0.253
Subcutaneous Fat Mass	Grams	Log_{10}	B	1.428	0.255	0.247	0.405	0.659	0.488	0.072	0.138
Heart Mass	Grams	None	B	10.087	**1.02x10^{-4}**	0.519	**0.002**	**2.40x10^{-5}**	0.054	**0.007**	0.532
Lung Mass	Grams	Log_{10}	B	1.721	0.185	0.176	0.166	0.997	0.663	0.091	0.096
Liver Mass	Grams	Log_{10}	B	0.724	0.546	0.198	0.185	0.989	0.322	0.999	0.992
Spleen Mass	Grams	Log_{10}	B	0.057	0.982	0.751	0.703	0.943	0.748	0.927	0.968
Pancreas Mass	Grams	None	B	0.544	0.656	0.417	0.212	0.599	0.486	0.930	0.778
Kidney Mass	Grams	None	B	6.617	0.002	0.057	0.120	**1.15x10^{-4}**	0.845	0.135	0.121
Adrenal Mass	Grams	Log_{10}	B	2.956	**0.049**	0.173	0.826	0.052	**0.042**	0.230	**0.014**
Stomach Mass	Grams	Log_{10}	B	3.476	**0.029**	0.701	**0.014**	**0.011**	0.566	0.744	0.128
Small + Large Intestine Mass	Grams	Log_{10}	B	10.327	**8.60x10^{-5}**	0.419	**0.002**	**1.50x10^{-5}**	0.756	0.719	**0.003**
Caecum Mass	Grams	Log_{10}	B	2.358	0.092	**0.049**	0.061	0.854	0.937	0.094	0.126
Testis Mass	Grams	Log_{10}	B	1.146	0.347	0.296	0.963	0.175	0.601	0.129	0.576
Baculum Mass	Grams	Log_{10}	B	5.347	**0.005**	**0.001**	**0.038**	0.073	0.588	**0.015**	0.226
Thigh Muscle Mass	Grams	Log_{10}	B	3.674	**0.023**	0.839	0.069	**0.049**	0.224	0.114	**0.005**
Gastrocnemius Muscle Mass	Grams	Log_{10}	B	8.887	**2.47x10^{-4}**	0.150	**3.63x10^{-4}**	**0.004**	0.616	0.067	**3.65x10^{-4}**

B = Log_{10}-Body Mass, L = Body Length

P values ≤ 0.05 are bolded and underlined.

Table 3.5. Experiment 2 estimated marginal means and standard errors for 22°C virgin (VM), 10°C virgin, 10°C non-breeding (NB), and 10°C breeding males (BM). Trait units, covariates (B = body mass, R = RMR chamber, C = cricket mass), sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs are reported.

			10°C Breeding (BM)			10°C Non-Breeding (NB)			10°C Virgin (VM)			22°C Virgin (VM)		
Trait	**Unit**	**Covariates**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Mass (Day 1)	Grams	None	4	41.00	4.45	6	50.70	3.64	12	44.36	2.57	12	49.85	2.57
Body Mass (Day 1)	Grams	L	4	45.20	3.63	6	52.96	2.91	12	49.52	2.34	12	42.15	2.67
Body Mass (Day 4)	Grams	None	4	42.66	4.67	6	53.40	3.81	12	45.42	2.69	12	49.66	2.69
Body Mass (Day 4)	Grams	L	4	47.57	3.48	6	56.04	2.78	12	51.46	2.24	12	40.66	2.56
Body Mass (Day 8)	Grams	None	4	42.87	4.67	6	53.06	3.81	12	46.31	2.69	12	49.26	2.69
Body Mass (Day 8)	Grams	L	4	47.71	3.52	6	55.66	2.82	12	52.27	2.27	12	40.38	2.59
Fat Mass	Grams	None	4	6.83	1.98	6	10.23	1.62	12	8.45	1.14	12	9.12	1.14
Fat Mass	Grams	L	4	8.66	1.64	6	11.21	1.31	12	10.70	1.06	12	5.77	1.21
Percent Fat Mass	%	None	4	14.75	2.70	6	18.46	2.21	12	17.64	1.56	12	17.98	1.56
Percent Fat Mass	%	L	4	16.82	2.44	6	19.58	1.96	12	20.18	1.57	12	14.18	1.80
Lean Mass	Grams	None	4	33.38	2.67	6	39.40	2.18	12	34.74	1.54	12	37.27	1.54
Lean Mass	Grams	L	4	35.98	2.13	6	40.79	1.70	12	37.93	1.37	12	32.51	1.57
Percent Lean Mass	%	None	4	79.11	2.70	6	74.35	2.21	12	76.82	1.56	12	75.86	1.56
Percent Lean Mass	%	L	4	76.80	2.33	6	73.11	1.87	12	73.98	1.50	12	80.09	1.72
Basal Metabolic Rate	ml O_2/h	B	2	1.62	0.03	4	1.47	0.02	6	1.21	0.02	6	1.21	0.02
Maximal Oxygen Consumption	ml O_2/h	B	4	6.27	0.33	6	6.39	0.27	12	7.23	0.19	12	6.72	0.19
Maximal Thermogenic Capacity	ml O_2/h	B	4	7.37	0.37	6	7.08	0.30	12	7.74	0.21	12	6.93	0.21
Hematocrit	%	None	4	46.33	1.45	6	45.83	1.18	12	44.96	0.84	12	45.54	0.84

Table 3.5. Continued.

			10°C Breeding (BM)			10°C Non-Breeding (NB)			10°C Virgin (VM)			22°C Virgin (VM)		
Traits	**Units**	**Covariates**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Body Length	Millimeters	None	4	102.88	2.25	6	104.43	1.84	12	102.11	1.30	12	112.37	1.30
Head Length	Millimeters	None	4	34.41	0.99	6	35.16	0.81	12	33.15	0.57	12	33.27	0.57
Head Length	Millimeters	L	4	34.09	1.02	6	34.99	0.81	12	32.76	0.66	12	33.85	0.75
Head Width	Millimeters	None	4	15.75	0.60	6	14.82	0.49	12	16.67	0.35	12	16.02	0.35
Head Width	Millimeters	L	4	16.03	0.60	6	14.97	0.48	12	17.01	0.39	12	15.51	0.44
Foot Length	Millimeters	None	4	24.61	0.54	6	24.58	0.44	12	22.70	0.31	12	22.99	0.31
Foot Length	Millimeters	L	4	24.89	0.54	6	24.73	0.43	12	23.04	0.35	12	22.48	0.40
Baculum Length	Millimeters	None	4	15.17	0.47	6	14.95	0.38	12	13.73	0.27	12	14.28	0.27
Baculum Length	Millimeters	L	4	15.34	0.48	6	15.05	0.39	12	13.95	0.31	12	13.96	0.35
Brain Mass	Grams	B	4	0.838	0.027	6	0.867	0.022	12	0.841	0.015	12	0.874	0.015
Subcutaneous Fat Mass	Grams	B	4	2.700	0.508	6	2.524	0.417	12	3.553	0.289	12	3.129	0.288
Heart Mass	Grams	B	4	0.166	0.006	6	0.183	0.005	12	0.188	0.004	12	0.162	0.004
Lung Mass	Grams	B	4	0.323	0.026	6	0.301	0.022	12	0.265	0.015	12	0.268	0.015
Liver Mass	Grams	B	4	2.838	0.388	6	2.391	0.319	12	2.651	0.221	12	2.662	0.220
Spleen Mass	Grams	B	4	0.087	0.012	6	0.075	0.010	12	0.079	0.007	12	0.079	0.007
Pancreas Mass	Grams	B	4	0.171	0.025	6	0.194	0.020	12	0.173	0.014	12	0.163	0.014
Kidney Mass	Grams	B	4	0.362	0.032	6	0.353	0.027	12	0.418	0.018	12	0.302	0.018
Adrenal Mass	Grams	B	4	0.005	0.002	6	0.011	0.002	12	0.008	0.001	12	0.011	0.001
Stomach Mass	Grams	B	4	0.722	0.037	6	0.746	0.031	12	0.722	0.021	12	0.643	0.021
Small + Large Intestine Mass	Grams	B	4	1.676	0.119	6	1.562	0.098	12	1.682	0.068	12	1.145	0.067
Caecum Mass	Grams	B	4	0.705	0.123	6	0.754	0.101	12	0.518	0.070	12	0.582	0.070
Testis Mass	Grams	B	4	0.269	0.037	6	0.250	0.030	12	0.215	0.021	12	0.253	0.021
Baculum Mass	Grams	B	4	0.011	0.001	6	0.013	0.001	12	0.008	0.001	12	0.010	0.001
Thigh Muscle Mass	Grams	B	4	0.902	0.073	6	0.996	0.060	12	1.007	0.041	12	1.139	0.041
Gastrocnemius Muscle Mass	Grams	B	4	0.220	0.028	6	0.236	0.023	12	0.272	0.016	12	0.346	0.016

B = Log_{10}-Body Mass, L = Body Length

CONCLUSION

Summary

In this dissertation, my goal was to examine what effects fatherhood has on the physiology, exercise performance, and morphology of males in bipaternal species. I did this using the biparental, genetically monogamous, California mouse (*Peromyscus californicus*) over three studies:

Chapter 1

In chapter one, I characterized the short-term effects of fatherhood on physiology, exercise performance, and morphology of fathers housed in a relatively benign environment. I found that breeding males had significantly larger hindlimb muscles than non-breeding males (controlling for variation in body mass), and that virgin males had heavier subcutaneous fat pads than non-breeding and breeding males. I found several correlations at the level of individual variation, including positive correlations for endurance with $\dot{V}O_2$max, $\dot{V}O_2$max with testes mass, and some of the digestion-related organs with each other. These results indicate that fatherhood may not have pronounced effects on energetics, performance or morphology of fathers, at least under standard laboratory conditions and across a single breeding cycle.

Chapter 2

In chapter two, I characterized the long-term effects of fatherhood on physiology, exercise performance, and morphology of fathers across seven births. Surprisingly, I found little evidence that increasing parity exacerbated the energetic, performance, morphological, or behavioral effects of being a father. I found that fatherhood can affect morphology (lower lean mass, higher fat mass, and relatively smaller organs) and possibly exercise performance (lower grip strength), but it seems to have little effect on RMR, hematocrit or predatory aggression. Although breeding males and non-reproducing males differed for some measures, I did not find enough consistent differences between reproductive conditions to suggest that fatherhood had substantial costs, even across multiple births.

Chapter 3

In chapter three of the dissertation, I characterized the effects of cold stress on the physiology, exercise performance, and morphology of male California mice (short- and long-term cold acclimation) and whether these measures are affected by paternal status. Short-term cold-acclimating virgin males had lower body, fat, and lean masses, but higher RMR and $\dot{V}O_2$max (both adjusted for body mass), as compared with virgins at room temperature. Long-term cold-acclimated groups had shorter body lengths, higher body, fat, and lean masses, higher BMR and $\dot{V}O_2$sum (both adjusted for body mass), and generally shorter bodies, longer feet, and larger organ masses (adjusted for body size), than virgins at room temperature. For the cold-acclimated animals, breeding males had

higher BMR, lower $\dot{V}O_2$max, longer feet, and longer, larger bacula than non-breeding and virgin males.

Overview

Taking the results from all three chapters of this dissertation as a whole, fatherhood in the California mouse appears, at least under the conditions tested in this dissertation, to have rather mild and inconsistent effects on paternal physiology, exercise performance, and morphology, especially when compared to known effects of motherhood in female rodents (Speakman, 2007; Speakman, 2008). Males of biparental species have been classically thought to alter their reproductive strategies and only behave paternally if the benefits outweigh the costs (Wittenberger and Tilson, 1980; Dewsbury, 1985). It was believed that males that "choose" to behave paternally may experience additional behavioral (e.g., see Kentner et al. 2011) and hormonal changes (e.g., see Saltzman and Ziegler, 2014) that non-paternal behaving males do not experience (Dewsbury, 1985). Paternal behaving males were thought to incur greater reproductive "costs" than males that do not behave paternally, primarily because of what was known about the reproductive costs motherhood (Wittenberger and Tilson, 1980; Dewsbury, 1985; Speakman, 2008). Before this dissertation, there were virtually no direct investigations into costs of parental investment in biparental male mammals, so much of what was known came from indirect evidence. Behavior (personality, motivation, sociability, etc.) and circulating levels of key hormones (e.g., androgens, glucocorticoids, prolactin, oxytocin, and leptin) have been cited as significantly affecting

males (Saltzman and Ziegler, 2014). Circulating levels of androgens, estrogens, progesterone, glucocorticoids, prolactin, oxytocin, and leptin all change with fatherhood (Brown, 1985; Brown, 1993; Wynne-Edwards, 2001) and are all known to affect physiology, exercise performance, and morphology (discussed in the Introduction).

Due to the behavioral and hormonal changes that accompany fatherhood, becoming a father and providing paternal care seems likely to change males' physiology, exercise performance, and morphology. However, behavioral and hormonal changes in new fathers are not universal across biparental taxa. For example, some paternal species spend significant amounts of time carrying their young (e.g., *Callithrix jacchus*, *Saguinus oedipus*) while others do not (e.g., most biparental rodents).

Neural and endocrine changes, as well as behavioral changes, take place in both fathers and mothers in biparental mammals (reviewed in Saltzman et al., 2017), but only females undergo long-term, consistent changes in physiology and morphology (Krol, 2003; Sadowska et al., 2013; Speakman and McQueenie, 1996; results of this dissertation). Although fatherhood still potentially affects long-term immune function (Murakami and Ono, 1987; Dantzer et al., 1991; Gilliver et al., 2006) and psychological well-being (Zhao et al., 2017), outcomes that were not addressed in the present studies, it appears to be a less significant life-history event than motherhood (at least in terms of physiology and morphology). This is not surprising because the paternal experience is fundamentally different than the maternal experience; males cannot become pregnant, they do not lactate, and their behaviors are different.

A lack of physiological, exercise performance or morphological "costs" may have been found in paternal California mouse males for a variety of reasons. First, the costs of paternal care might be low in this species because of their small litter sizes. Second, hormonal changes may counter any physiological, exercise performance or morphological changes that fathers might undergo. Third, the particular measures considered in this dissertation may not change with paternal status. Fourth, lab studies, with any type of environmental conditions, may not be sufficient to reveal differences between fathers and control males. Fifth, reproductive costs of fatherhood may occur in California mouse males but are not physiological, exercise performance or morphological. Future paternal studies of California mice should incorporate other life-history traits such as litter size, habitat, or age. Future studies should also use wild California mice when possible. This dissertation has important implications for understanding the evolution of biparental care, the life-history of this species, and will aid further studies of paternal care.

References

Brown, R. E. (1985). Hormones and paternal behavior in vertebrates. *Am Zoo* **25**, 895-910.

Brown, R. E. (1993). Hormonal and experiential factors influencing parental behaviour in male rodents: an integrative approach *Behav Process* **30**, 1-28.

Cantoni, D. and Brown, R. E. (1997). Paternal investment and reproductive success in the California mouse, *Peromyscus californicus*. *Anim Behav* **54**, 377-86.

Dantzer, R., Bluthe, R. M. and Kelley, K. W. (1991). Androgen-dependent vasopressinergic neurotransmission attenuates interleukin-1-induced sickness behavior. *Brain Res* **557**, 115-20.

Dewsbury, D. A. (1985). Paternal behavior in rodents. *Amer Zool* **25**, 841-852.

Garland, Jr., T., Zhao, M., and Saltzman, W. (2016). Hormones and the evolution of complex traits: Insights from artificial selection on behavior. *Int Comp Biol* **56**, 207–224.

Gilliver, S. C., Ashworth, J. J., Mills, S. J., Hardman, M. J. and Ashcroft, G. S. (2006). Androgens modulate the inflammatory response during acute wound healing. *J Cell Sci* **119**, 722-32.

Gubernick, D. J., Wright, S. L. and Brown, R. E. (1993). The significance of father's presence for offspring survival in the monogamous California mouse, *Peromyscus californicus*. *Anim Behav* **46**, 539-546.

Kentner, A. C., Abizaid, A. and Bielajew, C. (2010). Modeling dad: animal models of paternal behavior. *Neurosci Biobehav Rev* **34**, 438-51.

Krol, E. (2003). Limits to sustained energy intake VIII. Resting metabolic rate and organ morphology of laboratory mice lactating at thermoneutrality. *J Exp Biol* **206**, 4283-4291.

Murakami, N. and Ono, T. (1987). Sex-related differences in fever development of rats. *Am J Physiol* **252**, R284-9.

Sadowska, J., Gebczynski, A. K. and Konarzewski, M. (2013). Basal metabolic rate is positively correlated with parental investment in laboratory mice. *Proc Biol Sci* **280**, 1-7.

Saltzman, W., Harris, B. N., De Jong, T. R., Perea-Rodriguez, J. P., Horrell, N. D., Zhao, M. and Andrew, J. R. (2017). Paternal care in biparental rodents: Intra- and inter-individual variation. *Integr Comp Biol*.

Saltzman, W. and Ziegler, T. E. (2014). Functional significance of hormonal changes in mammalian fathers. *J Neuroendocrinol* **26**, 685-96.

Speakman, J. R. (2007). The energy cost of reproduction in small rodents. *Acta Theriologica Sinica* **27**, 1-13.

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos Trans R Soc Lond B Biol Sci* **363**, 375-98.

Speakman, J. R. and McQueenie, J. (1996). Limits to sustained metabolic rate: The link between food intake, basal metabolic rate, and morphology in reproducing mice, *Mus musculus*. *Physiol Zool* **69**, 746-769.

Wynne-Edwards, K. E. (2001). Hormonal changes in mammalian fathers. *Horm Behav* **40**, 139-45.

Wittenberger, J. F. and Tilson, R. L. (1980). The evolution of monogamy: Hypothesis and evidence. *Ann Rev Ecol Syst* **11**, 197-232.

Zhao, M., Garland, T., Jr., Chappell, M. A., Andrew, J. R. and Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiol Behav* **177**, 57-67.