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

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ABSTRACT

In male mammals that provide care for their offspring, fatherhood can lead to changes in behavioral, morphological, and physiological traits, some of which might constitute trade-offs. However, relatively little is known about these changes, especially across multiple reproductive bouts, which are expected to magnify differences between fathers and nonreproductive males. We evaluated consequences of fatherhood in the monogamous, biparental California mouse (*Peromsycus californicus*) across seven consecutive reproductive bouts. We compared breeding adult males (housed with sham-ovariectomized females) with two control groups: nonbreeding males (housed with ovariectomized females treated with estrogen and progesterone to induce estrous behavior) and virgin males (housed with untreated ovariectomized females). At 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 or comparable time points for nonbreeding and virgin males), we measured males' body composition, hematocrit, predatory aggression, resting metabolic rate, maximal oxygen consumption ($\dot{V}O_2$ max), grip strength, and sprint speed. We also weighed organs at the final time point. We predicted that fathers would have lower relative body fat and lower performance abilities compared with control groups and that these effects would become more pronounced with increasing parity. Contrary to predictions, breeding and control males differed in surprisingly few measures, and the number and magnitude of differences did not increase with parity. Thus, our expectations regarding trade-offs were not met. As reported in studies of single reproductive events, these results suggest that fatherhood has few costs in this species when housed under standard laboratory conditions, even across multiple reproductive bouts.

Keywords: body composition, costs of reproduction, energetics, exercise performance, fatherhood, paternal care.

Introduction

The complex suite of behavioral, physiological, and morphological changes associated with motherhood has been well studied in mammals (e.g., Gittleman and Thompson 1988; Hammond 1997; Speakman 2008). However, similar studies are largely lacking for mammalian fathers. Although paternal care is relatively rare in mammals (occurring in 5%–10% of mammalian species; Kleiman and Malcom 1981), it can play a crucial role in reproductive success. Care by fathers, such as huddling, grooming, protection, and transport 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). At the same time, paternal care can have a variety of direct effects on fathers, some of which can be adverse (Achenbach and Snowdon 2002). Understanding these effects on fathers and how they trade off with the benefits of paternal care might provide new insight into the evolution of paternal care in mammals. In this study, therefore, we tested the hypothesis that fatherhood in biparental species is associated with costly effects on morphology, metabolism, and exercise performance in a biparental mammal, the California mouse (*Peromyscus californicus*).

In several biparental species, fathers undergo morphological changes in association with the birth of their offspring. In the biparental common marmoset (*Callithrix jacchus*), cotton-top tamarin (*Saguinus oedipus*), and California mouse, for example, 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, presumably due to providing care for offspring, could potentially increase mortality rates in fathers, especially under such adverse conditions as

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extreme temperatures or low food availability (e.g., Fontanillas et al. 2005; Boratynski and Koteja 2009).

In addition to the morphological changes that 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; Horrell et al. 2018). Additionally, prolactin levels are higher in fathers than in nonfathers in many biparental mammals (Saltzman and Ziegler 2014; Horrell et al. 2018). Androgens, glucocorticoids, and prolactin have metabolic effects that can influence energy utilization (Moore and Hopkins 2009), body composition (Dallman et al. 2007; Blouin et al. 2008; Schibli-Rahhal and Schlechte 2009), physical activity (Ibebunjo et al. 2011), and exercise physiology (Husak and Irschick 2009; Moore and Hopkins 2009; Garland et al. 2016; Singleton and Garland 2019) in complex ways. 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).

Morphological, physiological, and behavioral changes in fathers might have short- and long-term effects on whole-organism energetics and performance (i.e., the ability of an individual to conduct a task when maximally motivated; Careau and Garland 2012) that could impact aspects of Darwinian fitness (reproductive success; Orr and Garland 2017). In one study of California mice, first-time fathers showed few differences from nonbreeding males in several measures of energy metabolism and exercise capacity under laboratory housing conditions (Andrew et al. 2016). Fathers did, however, have larger hind limb muscles and heavier subcutaneous fat pads. Although the functional significance of these effects of fatherhood are not clear, larger hind limb muscles could potentially benefit locomotor performance and provide protein reserves, whereas larger fat pads would provide energy reserves but might hinder locomotor abilities, which could be viewed as a trade-off (Garland 2014). A limitation of that study was that only first-time fathers were evaluated; thus, it did not address the possibility that effects of fatherhood might become evident only after longer periods or experience with multiple litters, as a result of cumulative energetic, physiological, or morphological effects of providing offspring care (i.e., similar to "wear and tear" theories of aging; Goldsmith 2006; Toescu 2013). 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 to after the second litter of pups was born, whereas circulating corticosterone concentrations and home-cage activity levels did not change significantly.

Our goal in the present study was to identify effects of fatherhood on energetics, morphology, and exercise performance in the monogamous, biparental California mouse and to determine whether these effects increase with increasing parity (Gubernick and Alberts 1987; 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 1974*a*, 1974*b*; Gubernick et al. 1993; Cantoni and Brown 1997; Gubernick and Teferi 2000; Wright and Brown 2002). To test for effects of high parity, we followed males from before pair formation to 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 or comparable time points for nonreproducing control males), we measured body composition (body mass, fat mass, lean muscle mass, and organ masses), hematocrit, predatory aggression, thermoneutral resting metabolic rate (RMR), maximal oxygen consumption during forced exercise ($\dot{V}O_2$ max), grip strength, and maximum sprint speed. We compared fathers with two groups of nonreproducing 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 with nonreproducing males and that these differences would become more pronounced with increasing parity.

Methods

Animals

Mice were born and reared in our 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) that were derived from a wild population in the Santa Monica Mountains in southern California. Animals were housed in polycarbonate cages (44 cm × 24 cm × 20 cm) with aspen shavings as bedding; food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN) and water were available ad lib. Lighting was on a 14L : 10D cycle (lights on at 0500 hours and off at 1900 hours), with humidity at approximately 55% and ambient temperature at approximately 21°C. Mice were checked twice daily, and cages were changed weekly. At weaning (27–31 d of age; 27.5 ± 0.2 d; mean ± SEM), animals were ear punched for identification and placed in same-sex groups of three or four 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. UCR is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Experimental Design

At 100–125 d of age (115.1 ± 3.1 d), each male underwent a series of test procedures over a 7-d period (fig. 1). Five to ten days after testing concluded, the males were randomly paired with females, 111–148 d old (127.5 ± 3.2 d), in one of three conditions. Breeding males ($N = 21$) were paired with a sham-ovariectomized

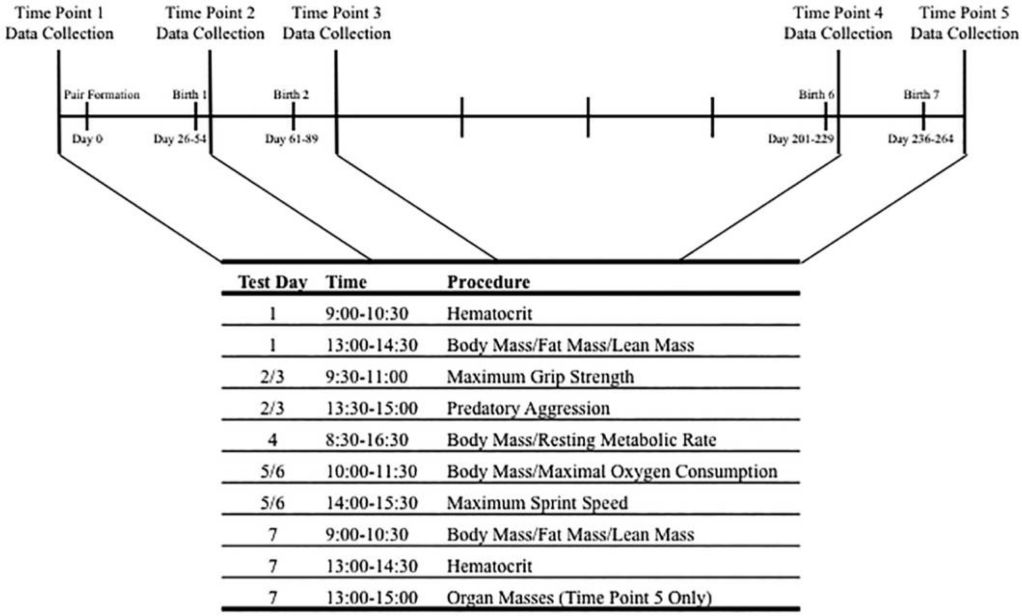

Figure 1. Study time line and design. The schedule of data collection at time points 2–5 was based on births of litters to breeding pairs, with times for nonbreeding and virgin males matched to breeding males.

female, nonbreeding males ($N = 20$) were paired with an ovariectomized female treated with estradiol benzoate and progesterone to induce estrous behavior, and virgin males ($N = 20$) were housed with an ovariectomized female that was not treated with hormones. Nonbreeding males were used to control for mating, and virgin males were used to control for cohabitation with an adult female. Mates of breeding males gave birth 30–54 d after pair formation (41.8 ± 2.0 d) and at ~35-d intervals thereafter (table 1). For breeding males, time point 2 occurred during the early postpartum period of the first litter, which corresponded to the early gestation period 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 occurred during the late postpartum period of litter 2/late gestation period of litter 3, time point 4 occurred during the early postpartum period of litter 6/early gestation period of litter 7, and time point 5 occurred during the late postpartum period of litter 7/late gestation period 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 the late (time points 3 and 5) postpartum/gestation periods. The timing of data collection in nonbreeding males and in virgin males was matched to that in breeding males. For logistical reasons, pairs were processed in three separate cohorts, and cohort was used as a cofactor in statistical analysis. All cohorts underwent an identical sequence of procedures (fig. 1).

The methods and sequence of data collection procedures were identical for each of the five 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 between 1300 and 1500 hours at 3- or 4-d intervals throughout the study, except during periods of data collection.

Ovariectomies and Estrogen/Progesterone Treatment

Females underwent bilateral ovariectomies before being paired with a virgin male or nonbreeding male, or they underwent sham ovariectomies before being paired with a breeding male. Animals were anesthetized with isoflurane, and surgeries were performed under aseptic conditions using standard procedures as previously

Table 1: Time line of the experiment

	Time point 1	Time point 2	Time point 3	Time point 4	Time point 5
Breeding males (BM)	5–11 d before pairing with female	5–10 d after birth 1	15–20 d after birth 2	5–10 d after birth 6	15–20 d after birth 7
Nonbreeding males	5–11 d before pairing with female	Matched to BM	Matched to BM	Matched to BM	Matched to BM
Virgin males	5–11 d before pairing with female	Matched to BM	Matched to BM	Matched to BM	Matched to BM

described (Zhao et al. 2018). They were then housed individually for 2 wk before being paired.

The ovarian hormones estrogen and progesterone are necessary for activating sexual behavior in many female rodents (Beach 1976), including California mice (M. Zhao, D. Chow, A. Ibarra, and W. Saltzman, unpublished data). Females from the nonbreeding male group were injected subcutaneously with estradiol benzoate (0.072 mg; suspended in sesame oil; Sigma-Aldrich, St. Louis, MO) 48 h before subcutaneous injection with progesterone (0.48 mg; suspended in sesame oil) in the afternoon (adapted from Dewsbury 1974). This study did not explicitly examine whether mating occurred after hormone treatment, but previous work in our lab (Zhao et al. 2018; M. Zhao, D. Chow, A. Ibarra, and W. Saltzman, 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, nonbreeding females were injected with estradiol benzoate and progesterone at the time of pairing and every 35 d thereafter to simulate mating cycles in the breeding male group.

At the end of the study, females from the nonbreeding and virgin pairs were euthanized by CO_2 inhalation and dissected to check for the presence of fetuses in the uterine canal. None were found in any females from either control group.

Measurements

Body Mass

During each 7-d testing period (fig. 1), males were weighed on days 1 (1300–1430 hours), 4 (0830–0930 hours), 5 (1000–1130 hours), 6 (1000–1130 hours), and 7 (0900–1030 hours).

Body Composition

On test days 1 (1300–1430 hours) and 7 (0900–1030 hours), males were weighed and scanned with a magnetic resonance whole-body analyzer (EchoMRI-100; Echo Medical Systems, Houston, TX) 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 (0900–1030 hours) and 7 (1300–1430 hours) for measurement of hematocrit. Mice were anesthetized with isoflurane, and blood was collected in heparinized microhematocrit capillary tubes (Chauke et al. 2011; Harris et al. 2013). Blood was centrifuged at 4°C and 1,300 rpm (~1,900 *g*) for 12 min (Sorvall Legend Micro 21R; Thermo Scientific, Waltham, MA), hematocrit was recorded, and plasma was removed and stored at −80°C for future use.

Predatory Aggression

On test days 2 and 3 between 1330 and 1500 hours, 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 until 7 min had elapsed. Videos were scored for latency to attack and latency to kill the cricket. If the cricket was not killed within 7 min, predatory-aggression data from that mouse was excluded from 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

RMR was measured as oxygen consumption in thermoneutral conditions on test day 4 between 0830 and 1630 hours. The procedure was identical to our previously described method for measuring basal metabolic rate (Andrew et al. 2016), except that animals were not fasted before testing. Males were separated from their cagemates and placed in a Plexiglas metabolic chamber (volume: 525 mL) with bedding inside an environmental chamber maintained at 28°–30°C. Two animals were usually tested simultaneously in separate metabolic chambers, and chamber number was used as a covariate in all statistical analyses. RMR 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 using Warthog LabHelper software (https://www.warthog.ucr.edu) and converted using the mode 1 equation in Warthog LabAnalyst ($\dot{V}O_2 = \text{STP flow rate} \cdot (FiO_2 - FeO_2)/(1 - FeO_2)$, where FiO_2 is incurrent fractional oxygen concentration [0.2095] and FeO_2 is excurrent fractional oxygen concentration). 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 1000–1130 hours on days 5 and 6 as previously described (Dlugosz et al. 2012; Andrew et al. 2016). Briefly, $\dot{V}O_2$ max was measured in a small running-wheel respirometer (circumference: 51.8 cm; effective volume: 900 mL). Mice were given a ~2-min warm-up period, then the speed was gradually increased approximately every 30 s until either oxygen concentration did not change with increasing speed or mice could not maintain their position. Flow rates (2,400 mL/min) and O_2 concentrations were measured every second using LabHelper. Measurements were taken at room temperature (22.0° ± 0.2°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 the mode 1 equation in Warthog LabAnalyst, and instantaneous corrections were used to account for the mixing and washout characteristics of the chamber (Bartholomew et al. 1981). $\dot{V}O_2$ 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 statistical analyses.

Grip Strength

Maximum grip strength was determined on test days 2 and 3 at 0930–1100 hours. Males were suspended by their tail over a horizontal wire-mesh surface attached to a small force gauge (HF-10N, M&A Instruments, Arcadia, CA; Meyer et al. 1979; Nevins et al. 1993; Maurissen et al. 2003). The male was lowered until both the forelimbs and the hind limbs 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 the mouse 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 1400–1530 hours. Following a protocol designed to elicit maximum sprinting abilities of small rodents, including *Peromyscus* species (Djawdan and Garland 1988), males were placed on a "racetrack" (8 m long × 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 outset of each test, a mouse was placed near the start of the track and encouraged to walk or run down the track two to four 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 with a padded plastic board, triggering the photocells. Sprint speed was measured five 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; data from 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 (Djawdan and Garland 1988) for each individual was used as its maximum sprint speed.

Euthanasia and Organ Collection

On test day 7 of time point 5, between 1300 and 1500 hours, males were anesthetized with isoflurane, blood (~1 mL) was collected from the retro-orbital sinus, 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 version 24.0 to compare traits among reproductive groups (breeding, nonbreeding, and virgin males) 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 S3–S6; tables S1–S10 are available online). For each analysis, we considered the three a priori contrasts among the three groups, not the omnibus *P* value for group differences. We checked standardized residuals for normality and homogeneity of variance using Levene's test, and data were $\log_{10}$- or rank-transformed before analysis when necessary (noted in tables S3–S6). Results are presented in untransformed units (as estimated marginal mean ± SE unless otherwise noted).

Excluding the analyses of repeatability (correlations, *t*-tests), nuisance variables such as age, and results reported in the supplemental tables, this study includes 297 *P* values related to the analyses shown in tables 2–5 for the three a priori contrasts among groups (raw *P* values from overall ANCOVA and a priori contrasts are not shown in tables 2–5 but can be found in tables S3–S6). Of these 297 *P* values, 16 were nominally significant at <0.05. If all null hypotheses were in fact true, then one would expect $0.05 \cdot 297 = 15$ *P* values to be <0.05 by chance alone. Moreover, these tests include a substantial amount of nonindependence because the same individuals were measured for all traits, some traits were correlated, and many tests were interrelated. To compensate for nonindependence in multiple related tests, we used the adaptive false discovery rate (FDR) procedure as implemented in PROC MULTTEST in SAS version 9.4 (SAS, Cary, NC). Based on this procedure, none of the 16 *P* values <0.05 would be considered statistically significant (the smallest nominal *P* value was 0.0013, which had a positive FDR *Q* value of 0.2491). All *P* values reported in the text and tables are raw values not adjusted for multiple comparisons, so the reader should bear in mind that none of these would be considered statistically significant after correction for multiple comparisons.

We performed the overall *F*-test for group differences and all three a priori contrasts among the three groups; we also computed residuals from ANCOVA results, and for time point 5, we regressed energetic and performance residuals on organ masses. Because we tested mice in both the early and late postpartum

Table 2: Comparisons among breeding males (BM), nonbreeding males (NB), and virgin males (VM) at time point 2

Trait	Unit	BM			NB			VM		
		N	EMM	SE	*N*	EMM	SE	*N*	EMM	SE
Body mass (day 1)	g	20	46.81	2.14	20	45.12	1.73	20	44.69	1.76
Body mass (day 4)	g	20	47.26	2.16	20	45.25	1.75	20	44.42	1.78
Body mass (day 7)	g	20	45.82	2.14	20	44.58	1.73	20	44.29	1.76
Fat mass (day 1)	g	20	8.35	1.05	20	8.54	.85	20	7.96	.86
Percent fat mass (day 1)	%	20	16.98	1.69	20	18.27	1.37	20	17.19	1.39
Fat mass (day 7)	g	20	7.95	1.01	20	8.21	.82	20	7.74	.83
Percent fat mass (day 7)	%	20	16.50	1.64	20	17.89	1.33	20	16.90	1.35
Lean mass (day 1)	g	20	**36.48**[A]	1.12	19	**32.71**[A]	.92	19	34.04	.94
Percent lean mass (day 1)	%	20	77.06	1.56	19	74.61	1.28	19	76.95	1.30
Lean mass (day 7)	g	20	**35.57**[A]	1.12	19	**32.42**[A]	.91	20	33.44	.90
Percent lean mass (day 7)	%	20	76.62	1.59	19	74.90	1.30	20	75.94	1.28
Hematocrit (day 1)	%	16	47.87	.83	16	**47.85**[A]	.65	15	**49.66**[A]	.69
Hematocrit (day 7)	%	16	45.10	1.16	16	45.62	.91	15	46.17	.96
Predatory aggression: latency to first attack cricket	s	19	9.56	3.30	18	10.39	2.77	18	11.42	2.80
Predatory aggression: latency to kill cricket	s	17	52.53	10.17	17	47.87	8.27	18	62.00	8.22
Resting metabolic rate	mL O_2/h	18	.89	.03	20	1.27	.03	20	1.19	.06
Maximal oxygen consumption	mL O_2/h	19	6.08	.13	19	5.79	.11	19	5.91	.11
Maximum grip strength	N	20	**4.68**[A]	.19	20	**5.25**[A]	.15	20	5.14	.15
Maximum sprint speed	m/s	19	1.93	.16	19	2.19	.13	17	1.91	.14

Note. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (*N*), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs. *P* values from a priori contrasts that were ≤0.05 are shown in bold, and superscript capital letters denote where reproductive groups differed significantly from one another. Raw *P* values are shown only in corresponding supplemental tables. Cohort, age, days since pup birth, and days since pairing were used as covariates in all analyses but are not reported.

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. In the article, we discuss only the three a priori contrasts between groups within individual time points, but the *F*-test values, organ mass regressions, and Δ values are reported in tables S7–S10. For breeding males only, we also performed multiple linear regressions of each trait on relevant covariates plus litter size to determine whether 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 those results are not shown.

Results

Repeatability within Time Points

Pearson correlations indicated that all traits were repeatable (table S1) except for the two measures of predatory aggression (latency to attack and latency to kill the cricket at time points 1–3). 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 kidneys.

Time Point 2 (Litter 1 Early Postpartum/Litter 2 Early Gestation)

Breeding males had higher lean mass than nonbreeding males on days 1 ($P = 0.018$; table 2) and 7 ($P = 0.050$) of time point 2 (table 2), as well as lower grip strength than nonbreeding males ($P = 0.030$; table 2). Virgins had higher hematocrit on day 1 than nonbreeders ($P = 0.034$; table 2).

Time Point 3 (Litter 2 Late Postpartum/Litter 3 Late Gestation)

Breeding males had lower resting metabolic rate than both nonbreeding males ($P = 0.048$; table 3) and virgin males ($P = 0.031$).

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 (table 4).

Time Point 5 (Litter 7 Late Postpartum/Litter 8 Late Gestation)

Breeding males had lower lean mass than nonbreeding males on day 7 ($P = 0.036$; table 5). Latency to attack crickets in the

Table 3: Comparisons among breeding males (BM), nonbreeding males (NB), and virgin males (VM) at time point 3

		BM			NB			VM		
Trait	Unit	*N*	EMM	SE	*N*	EMM	SE	*N*	EMM	SE
Body mass (day 1)	g	19	46.70	2.15	18	48.48	2.10	18	49.12	2.07
Body mass (day 4)	g	18	47.10	2.27	18	48.50	2.14	18	48.98	2.11
Body mass (day 7)	g	18	46.91	2.36	18	47.82	2.23	18	48.45	2.19
Fat mass (day 1)	g	19	8.30	.91	18	9.32	.89	18	9.78	.88
Percent fat mass (day 1)	%	19	17.07	1.31	18	18.68	1.28	18	19.52	1.27
Fat mass (day 7)	g	18	8.47	.96	18	9.09	.91	18	9.53	.89
Percent fat mass (day 7)	%	18	17.40	1.41	18	18.30	1.34	18	19.24	1.31
Lean mass (day 1)	g	19	35.64	1.28	18	36.00	1.26	16	36.51	1.32
Percent lean mass (day 1)	%	19	76.86	1.23	18	74.73	1.21	16	75.08	1.27
Lean mass (day 7)	g	18	35.43	1.38	17	34.90	1.34	18	35.90	1.29
Percent lean mass (day 7)	%	18	75.74	1.25	17	74.85	1.21	18	74.07	1.17
Hematocrit (day 1)	%	19	47.43	.64	18	48.66	.53	18	48.51	.95
Hematocrit (day 7)	%	17	45.45	.75	18	45.45	.68	18	45.13	.67
Predatory aggression: latency to first attack cricket	s	17	7.92	3.42	15	10.18	3.48	17	9.93	3.22
Predatory aggression: latency to kill cricket	s	14	45.27	8.84	15	52.45	8.43	13	56.61	8.89
Resting metabolic rate	mL O_2/h	18	**1.13**^{A,B}	.05	18	**1.27**^{A}	.04	17	**1.28**^{B}	.04
Maximal oxygen consumption	mL O_2/h	18	5.96	.11	18	5.79	.10	18	5.91	.10
Maximum grip strength	N	18	5.09	.18	18	5.20	.17	18	5.20	.17
Maximum sprint speed	m/s	18	1.90	.14	18	2.00	.13	18	1.89	.13

Note. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (*N*), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs. *P* values from a priori contrasts that were ≤0.05 are shown in bold, and superscript capital letters denote where reproductive groups differed significantly from one another. Raw *P* values are shown only in corresponding supplemental tables. Cohort, age, days since pup birth, and days since pairing were used as covariates in all analyses but are not reported.

predatory-aggression test was longer in breeders than in nonbreeders ($P = 0.004$) and virgins ($P = 0.008$). Nonbreeders had a greater percentage of fat than virgins on day 1 ($P = 0.040$). Virgin males also saw reductions in maximum sprint speed compared with nonbreeding males ($P = 0.048$).

Masses of several organs differed among groups. Breeding males had lower liver mass and caecum mass than both nonbreeding males (liver: $P = 0.022$; caecum: $P = 0.005$; table 5) and virgin males (liver: $P = 0.024$; caecum: $P = 0.001$). Breeders also had lower spleen mass and stomach mass (emptied of food contents) than virgins (spleen: $P = 0.009$; stomach: $P = 0.039$) and lower heart mass than nonbreeders ($P = 0.031$). No organ masses differed between nonbreeding males and virgin males.

Correlations between Organ Sizes and Energetic/Performance Measures

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 S2). $\dot{V}O_2$ 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 muscle mass ($r^2 = 0.404$, $P = 0.011$). Sprint speed correlated positively with day 7 hematocrit ($r^2 = 0.403$, $P = 0.011$) and 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 was not significantly correlated with any measured organ mass.

Discussion

Mated pairs of California mice reproduce almost continually in the lab and, except for the hot summer months, in natural habitats (Ribble 1992). Because estrus, mating, and conception occur immediately after parturition and birth of the next litter occurs shortly after the preceding litter is weaned (Gubernick 1988), parents have little or no recovery time between reproductive bouts. Given this nearly continuous investment in reproduction, and given that males are intensely involved in all aspects of parental care except lactation (Dudley 1974*a*, 1974*b*; Gubernick and Alberts 1987; Cantoni and Brown 1997), we expected that the cumulative demands of multiple sequential reproductive bouts would have substantial impacts on the physiology, morphology,

Table 4: Comparisons among breeding males (BM), nonbreeding males (NB), and virgin males (VM) at time point 4

		BM			NB			VM		
Trait	Unit	*N*	EMM	SE	*N*	EMM	SE	*N*	EMM	SE
Body mass (day 1)	g	17	52.06	3.10	13	53.17	2.85	15	55.94	2.63
Body mass (day 4)	g	17	52.16	3.36	13	53.27	3.04	16	54.43	2.73
Body mass (day 7)	g	17	52.26	3.36	13	51.98	3.04	16	53.51	2.74
Fat mass (day 1)	g	17	11.43	.83	13	10.38	.75	15	9.71	.71
Percent fat mass (day 1)	%	17	20.83	1.25	13	19.72	1.15	15	18.33	1.06
Fat mass (day 7)	g	17	11.17	.82	13	9.93	.74	16	8.91	.68
Percent fat mass (day 7)	%	17	20.45	1.28	13	19.24	1.15	16	17.32	1.04
Lean mass (day 1)	g	17	37.33	2.13	13	38.69	1.96	15	41.16	1.80
Percent lean mass (day 1)	%	17	72.20	1.20	13	72.66	1.11	15	73.64	1.02
Lean mass (day 7)	g	17	37.57	2.33	13	38.21	2.10	16	40.09	1.90
Percent lean mass (day 7)	%	17	72.47	1.21	13	73.31	1.09	16	75.09	.98
Hematocrit (day 1)	%	17	47.27	.91	12	48.42	.86	16	48.04	.75
Hematocrit (day 7)	%	16	43.11	1.06	13	44.54	.92	16	45.06	.82
Predatory aggression: latency to first attack cricket	s	16	15.13	4.46	12	11.58	4.14	15	11.67	3.72
Predatory aggression: latency to kill cricket	s	14	53.06	12.88	11	43.47	11.53	14	44.29	9.81
Resting metabolic rate	mL O_2/h	16	1.58	.20	13	1.16	.17	16	1.32	.16
Maximal oxygen consumption	mL O_2/h	17	6.11	.24	13	6.18	.22	16	6.53	.20
Maximum grip strength	N	17	5.22	.27	13	5.63	.25	15	5.91	.23
Maximum sprint speed	m/s	17	1.71	.19	13	2.18	.17	16	1.87	.16

Note. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (*N*), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs. All *P* values from a priori contrasts were >0.05; raw *P* values are shown only in corresponding supplemental tables. Cohort, age, days since pup birth, and days since pairing were used as covariates in all analyses but are not reported.

and behavior of fathers. In particular, we predicted that, compared with nonreproducing males, fathers would experience reductions in body condition (lower body mass, reduced body fat), as well as declines in "performance" (aerobic capacity [$\dot{V}O_2$ max], sprint speed, grip strength), with the differences between fathers and nonreproductive males increasing over successive litters. We also expected that shifts in organ mass and hematology would parallel the expected changes in performance and condition among the experimental groups of males.

Almost none of our expectations were supported by the results. We found a few differences in trait values for body condition, physiology, and behavior between fathers and control (nonbreeding and virgin) males at most of the five measurement time points (but not time point 4; tables 2–5). However, these differences were not consistent between successive time points. In terms of cumulative change over time (tables 5, S10), the only significant difference between fathers and the two control groups between initial measurements at the start of the experiment (before the first litter) and measurements at the last sampling time point (after the birth of the seventh successive litter produced by mated pairs) was in lean mass: fathers had a smaller increase in lean mass than nonfathers, mainly due to a difference between fathers and virgin males. Consistent with that finding, fathers also had smaller absolute lean mass than virgin males at the end of the experiment (table 5). Because fathers should have a higher metabolic "workload" than nonfathers from the energetic demands of paternal care, we expected that organs closely associated with energy metabolism would be larger in fathers. However, at the end of the experiment, we found either no difference between fathers and nonfathers (intestine, kidney, pancreas, lung) or smaller organs in fathers than in nonfathers (heart, liver, stomach, caecum)—the opposite of our predictions.

We also tested for changes in the variability of the whole-organism traits over time. Specifically, we analyzed changes in the standard errors (as reported in tables 2–5) across the time points for all variables except organ masses using two-way (time point × group) ANCOVAs. In no case did we find a significant time point × group interaction (results not shown).

Our interpretation of these results for male California mice rearing a series of litters is similar to those of studies evaluating effects of a single breeding event on fathers (Saltzman et al. 2015; Andrew et al. 2016, 2019; Zhao et al. 2017, 2018): there are very few indications that fatherhood is stressful or costly for males, at least in terms of the traits we measured. Only the somewhat slower rate of accumulation of lean mass over increasing parity might reasonably be viewed as having a potential negative impact on Darwinian fitness. However, we emphasize the same caveat as mentioned in previous studies: it is unclear whether the conclusion of minimal fitness impact applies to California mouse fathers in their natural habitats, which presumably are considerably more demanding than the benign conditions of the laboratory environment (e.g., ad lib. food, no predation, warm and

Table 5: Comparisons among breeding males (BM), nonbreeding males (NB), and virgin males (VM) at time point 5

		BM			NB			VM		
Trait	Unit	*N*	EMM	SE	*N*	EMM	SE	*N*	EMM	SE
Body mass (day 1)	g	14	51.00	2.57	12	57.09	2.52	14	55.40	2.31
Body mass (day 4)	g	13	51.62	2.66	12	56.75	2.45	14	54.75	2.25
Body mass (day 7)	g	13	51.24	2.70	12	56.34	2.48	14	54.91	2.28
Fat mass (day 1)	g	14	10.12	.84	12	11.70	.82	14	9.99	.75
Percent fat mass (day 1)	%	14	19.55	1.26	12	**20.86**^{A}	1.23	14	**17.74**^{A}	1.13
Fat mass (day 7)	g	13	10.56	.93	12	10.71	.85	14	9.42	.77
Percent fat mass (day 7)	%	13	19.27	1.45	12	19.90	1.34	14	17.62	1.21
Lean mass (day 1)	g	14	36.96	1.87	12	42.14	1.84	14	41.34	1.68
Percent lean mass (day 1)	%	14	72.85	1.45	12	73.69	1.43	14	75.01	1.31
Lean mass (day 7)	g	13	**37.21**^{A}	2.13	12	40.66	1.96	14	**40.83**^{A}	1.78
Percent lean mass (day 7)	%	13	72.02	1.23	12	72.92	1.13	14	75.51	1.03
Body length	mm	13	113.84	1.33	12	112.98	1.23	14	113.76	1.10
Head length	mm	13	35.02	.59	12	33.58	.55	14	33.38	.49
Head width	mm	13	16.31	.73	12	17.54	.68	14	16.64	.62
Right hind foot length	mm	13	24.20	.30	12	24.77	.28	14	24.34	.25
Baculum length	mm	13	15.12	.27	12	14.85	.25	14	14.84	.22
Brain mass	g	13	.87	.02	12	.85	.02	14	.84	.02
Subcutaneous fat mass	g	13	3.29	.40	12	3.49	.37	14	2.93	.34
Heart mass	g	13	**.16**^{A}	.01	12	**.18**^{A}	.01	14	.18	.01
Lung mass	g	13	.31	.02	12	.28	.02	14	.26	.02
Liver mass	g	13	**2.56**^{A,B}	.32	12	**3.58**^{A}	.29	14	**3.51**^{B}	.26
Spleen mass	g	13	**.08**^{A}	.01	12	.09	.01	14	**.01**^{A}	.01
Pancreas mass	g	13	.18	.02	12	.16	.01	14	.18	.01
Kidney mass	g	13	.33	.04	12	.42	.03	14	.37	.03
Adrenal mass	g	13	.01	.00	12	.01	.00	14	.01	.00
Stomach mass	g	13	**.72**^{A}	.03	12	.75	.03	14	**.80**^{A}	.03
Small and large intestine mass	g	13	1.67	.12	12	1.91	.11	14	1.96	.10
Caecum mass	g	13	**.42**^{A,B}	.03	12	**.53**^{A}	.02	14	**.54**^{B}	.02
Testis mass	g	13	.29	.02	12	.28	.02	14	.27	.02
Baculum mass	g	13	.01	.00	12	.01	.00	14	.01	.00
Right hind leg muscle mass	g	13	1.94	.06	12	1.91	.05	14	1.97	.05
Left hind thigh muscle mass	g	13	1.12	.06	12	1.02	.05	14	1.07	.05
Left hind gastrocnemius mass	g	13	.29	.02	12	.35	.02	14	.35	.02
Hematocrit (day 1)	%	14	48.65	.83	12	47.07	.81	14	48.05	.74
Hematocrit (day 7)	%	13	45.77	1.59	12	44.64	1.47	14	44.23	1.33
Predatory aggression: latency to first attack cricket	s	12	**15.99**^{A,B}	5.26	12	**10.83**^{A}	4.51	14	**14.74**^{B}	3.94
Predatory aggression: latency to kill cricket	s	12	49.02	11.57	10	40.10	10.93	12	45.15	10.02
Resting metabolic rate	mL O_2/h	13	1.21	.15	12	1.39	.14	13	1.43	.13
Maximal oxygen consumption	mL O_2/h	13	6.30	.24	12	6.07	.22	14	6.27	.20
Maximum grip strength	N	13	5.38	.29	12	5.78	.27	14	5.65	.24
Maximum sprint speed	m/s	13	1.86	.21	12	2.02	.19	14	1.81	.17

Note. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (*N*), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs. *P* values from a priori contrasts that were ≤0.05 are shown in bold, and superscript capital letters denote where reproductive groups differed significantly from one another. Raw *P* values are shown only in corresponding supplemental tables. Cohort, age, days since pup birth, and days since pairing were used as covariates in all analyses but are not reported.

consistent temperatures, no requirement for extensive movement); costs of reproduction might be apparent only under physiologically challenging conditions (Roff 1993). Indeed, some studies have indicated that a more rigorous environment may differentially impact physiology and survival in breeding and nonreproductive male California mice. For example, a moderate energetic stressor (periodic 24-h fasting plus having to climb towers to obtain food and water) increased body mass and fat stores in virgin and nonbreeding males but not in first-time fathers, suggesting that fatherhood constrained males' ability to obtain, process, or accumulate energy under these conditions (Zhao et al. 2018). Also, laboratory acclimation to low temperatures typical of those in winter in natural habitats (5°–10°C) affected breeding males differently than nonbreeding males for some metabolic traits and had large negative impacts on survival and breeding success (Andrew et al. 2019).

Another caveat for our study is that the female pair mates of the males in the three study groups might have behaved differently toward their mates as a function of the females' different reproductive conditions (i.e., ovariectomized/untreated, ovariectomized/estrogen- and progesterone-treated, intact). Both estrogen and progesterone can affect aggression in female rodents and have been linked to aggression in California mice (Davis and Marler 2003; Landeros et al. 2012; Laredo et al. 2013). In this study, we did not collect data on behavioral interactions between the males and their mates; therefore, we cannot determine whether these interactions differed among the three reproductive conditions and might have influenced our results.

The present study was conducted across the span of seven litters and approximately 250 d, and mice were roughly 350 d old at the final measurement. This is a substantial fraction of life span in small rodents, including the life span of *Peromyscus californicus* (Ribble 1992) in the field, and many of the differences across successive litters were likely due to aging (as indicated by changes in the nonbreeding control males). Age-related shifts in body mass and aerobic physiology similar to what we found in California mice (tables 2–5, S10) have been reported in a congener, the deer mouse (*P. maniculatus;* Chappell et al. 2003). In that species, both mass and $\dot{V}O_2$ max increased over time and then eventually declined. We did not see declines in these traits in California mice over the course of our experiments, but in *P. maniculatus*, the decrease in mass and $\dot{V}O_2$ max occurred after age exceeded 500–600 d (i.e., much older than the animals in our study).

The present findings can potentially contribute to our understanding of the evolution and maintenance of paternal care. The evolutionary factors promoting paternal behavior in California mice and other biparental mammals are not fully understood but are thought to include both reproductive benefits (e.g., enhanced offspring survival and development, increased female fecundity) and costs or trade-offs (Woodroffe and Vincent 1994; West and Capellini 2016; Rymer and Pillay 2018). The most commonly invoked cost of paternal care is forfeiture of additional mating opportunities (Seki et al. 2007; Woodroffe and Vincent 1994; West and Capellini 2016); additional potential costs, such as increased energy expenditure and reduced performance abilities, have received relatively little attention (see also Orr and Garland 2017; Rymer and Pillay 2018). Importantly, paternal care in California mice—under laboratory conditions, at least—consists almost exclusively of huddling and grooming pups, activities that presumably require very little energy expenditure; in contrast to many biparental species, fathers have low rates of transporting pups and are not known to provision them with food (Dudley 1974*a*; Gubernick and Alberts 1987). If providing paternal care in natural environments, as in our laboratory study, has few morphological, physiological, or performance costs to fathers, this would presumably favor the evolution and maintenance of biparental care, especially when paternal care can increase the number of offspring produced and/or enhance offspring survival and development (West and Capellini 2016; Requena and Alonzo 2017).

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

Running Head: Long-term effects of parenthood on California mouse fathers

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Appendices (Online Supplemental Materials)

Table S1. 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. Positive t values indicate that trial 1 > trial 2 or for paired organs, right > left.

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
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	**<u>2.06E-18</u>**	0.492	0.626
Testis Mass	Grams	39	0.985	**<u>1.34E-29</u>**	0.027	0.978

P values ≤ 0.05 are bolded and underlined.

Table S2. Correlations of residuals for energetic and performance measures with organ masses.

		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
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)	**<u>0.031</u>**	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	**<u>0.033</u>**
	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	**<u>0.023</u>**
	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	**<u>0.049</u>**
	N	39	38	39	39	39
Thigh Mass	Pearson Correlation	0.404	-0.057	0.187	-0.011	-0.326
	Sig. (2-tailed)	**<u>0.011</u>**	0.734	0.255	0.947	**<u>0.043</u>**
	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.

Table S3. Comparisons among breeding males, non-breeding males, and virgin males in time point 2. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

		Time Point 2							
Trait	**Unit**	**Transform**	**Covariates**	**DF**	**F**	**Group P**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**
Body Mass (Day 1)	Grams	None	None	2,60	0.257	0.774	0.571	0.481	0.852
Body Mass (Day 4)	Grams	None	None	2,60	0.442	0.645	0.505	0.352	0.722
Body Mass (Day 7)	Grams	None	None	2,60	0.135	0.874	0.677	0.610	0.898
Fat Mass (Day 1)	Grams	None	None	2,60	0.134	0.875	0.898	0.790	0.610
Percent Fat Mass (Day 1)	%	None	None	2,60	0.237	0.790	0.586	0.930	0.557
Fat Mass (Day 7)	Grams	None	None	2,60	0.092	0.912	0.855	0.884	0.670
Percent Fat Mass (Day 7)	%	Log_{10}	None	2,60	0.348	0.708	0.441	0.744	0.568
Lean Mass (Day 1)	Grams	Log_{10}	None	2,58	3.023	0.058	**0.018**	0.116	0.266
Percent Lean Mass (Day 1)	%	Log_{10}	None	2,58	1.250	0.295	0.241	0.933	0.159
Lean Mass (Day 7)	Grams	None	None	2,58	2.010	0.144	**0.050**	0.175	0.396
Percent Lean Mass (Day 7)	%	None	None	2,59	0.353	0.704	0.445	0.760	0.541
Hematocrit (Day 1)	%	None	None	2,46	2.830	0.071	0.982	0.096	**0.034**
Hematocrit (Day 7)	%	Log_{10}	None	2,46	0.315	0.732	0.646	0.436	0.680
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	C	2,55	0.085	0.918	0.856	0.694	0.782
Predatory Aggression: Latency to Kill Cricket	Seconds	None	C	2,52	0.836	0.440	0.738	0.511	0.205
Resting Metabolic Rate	ml O_2/h	Log_{10}	B, R	2,58	0.216	0.806	0.860	0.753	0.516
Maximal Oxygen Consumption	ml O_2/h	None	B	2,57	1.247	0.296	0.126	0.370	0.412
Maximum Grip Strength	Newtons	None	B	2,60	2.518	0.091	**0.030**	0.084	0.569
Maximum Sprint Speed	m/s	Log_{10}	B	2,54	1.218	0.305	0.145	0.505	0.327

P values $\leq$ 0.05 are bolded and underlined.

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

Table S4. Comparisons among breeding males, non-breeding males, and virgin males in time point 3. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

		Time Point 3							
Trait	**Unit**	**Transform**	**Covariates**	**DF**	**F**	**Group P**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**
Body Mass (Day 1)	Grams	None	None	2,55	0.306	0.738	0.574	0.444	0.821
Body Mass (Day 4)	Grams	None	None	2,54	0.171	0.843	0.672	0.566	0.867
Body Mass (Day 7)	Grams	None	None	2,54	0.103	0.902	0.790	0.652	0.834
Fat Mass (Day 1)	Grams	Log_{10}	None	2,55	0.722	0.491	0.432	0.237	0.656
Percent Fat Mass (Day 1)	%	Log_{10}	None	2,55	0.917	0.407	0.372	0.184	0.623
Fat Mass (Day 7)	Grams	None	None	2,54	0.291	0.749	0.658	0.450	0.724
Percent Fat Mass (Day 7)	%	Log_{10}	None	2,54	0.511	0.603	0.686	0.333	0.521
Lean Mass (Day 1)	Grams	None	None	2,53	0.108	0.898	0.849	0.651	0.766
Percent Lean Mass (Day 1)	%	Log_{10}	None	2,53	0.772	0.468	0.233	0.352	0.796
Lean Mass (Day 7)	Grams	Log_{10}	None	2,53	0.182	0.835	0.736	0.855	0.553
Percent Lean Mass (Day 7)	%	Log_{10}	None	2,53	0.436	0.649	0.593	0.356	0.668
Hematocrit (Day 1)	%	Log_{10}	None	2,55	0.723	0.491	0.525	0.668	0.237
Hematocrit (Day 7)	%	Log_{10}	None	2,53	0.089	0.915	0.997	0.742	0.709
Predatory Aggression: Latency to First Attack Cricket	Seconds	Rank	C	2,49	1.650	0.205	0.277	0.628	0.080
Predatory Aggression: Latency to Kill Cricket	Seconds	Log_{10}	C	2,42	0.245	0.784	0.898	0.636	0.504
Resting Metabolic Rate	ml O_2/h	None	B, R	2,53	2.822	0.071	<u>**0.048**</u>	<u>**0.031**</u>	0.810
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	B	2,54	0.818	0.448	0.228	0.646	0.382
Maximum Grip Strength	Newtons	None	B	2,54	0.118	0.889	0.665	0.663	0.999
Maximum Sprint Speed	m/s	None	None	2,54	0.230	0.795	0.634	0.938	0.523

P values $\leq$ 0.05 are bolded and underlined.

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

Table S5. Comparisons among breeding males, non-breeding males, and virgin males in time point 4. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

		Time Point 4							
Trait	**Unit**	**Transform**	**Covariates**	**DF**	**F**	**Group P**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**
Body Mass (Day 1)	Grams	Log_{10}	None	2,45	0.630	0.538	0.688	0.315	0.439
Body Mass (Day 4)	Grams	Log_{10}	None	2,46	0.175	0.840	0.695	0.559	0.813
Body Mass (Day 7)	Grams	Log_{10}	None	2,46	0.122	0.885	0.864	0.660	0.728
Fat Mass (Day 1)	Grams	None	E	2,45	0.984	0.384	0.413	0.175	0.485
Percent Fat Mass (Day 1)	%	None	None	2,45	1.072	0.353	0.568	0.186	0.332
Fat Mass (Day 7)	Grams	None	E	2,46	1.869	0.169	0.332	0.071	0.271
Percent Fat Mass (Day 7)	%	Log_{10}	None	2,46	1.837	0.173	0.656	0.117	0.140
Lean Mass (Day 1)	Grams	Log_{10}	None	2,45	1.131	0.334	0.601	0.183	0.296
Percent Lean Mass (Day 1)	%	Log_{10}	None	2,45	0.449	0.642	0.771	0.409	0.491
Lean Mass (Day 7)	Grams	Log_{10}	None	2,46	0.514	0.602	0.744	0.376	0.466
Percent Lean Mass (Day 7)	%	Log_{10}	None	2,46	1.525	0.231	0.605	0.137	0.201
Hematocrit (Day 1)	%	None	None	2,45	0.329	0.722	0.423	0.575	0.712
Hematocrit (Day 7)	%	Log_{10}	None	2,45	0.834	0.442	0.385	0.206	0.618
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	C	2,43	0.156	0.856	0.610	0.605	0.986
Predatory Aggression: Latency to Kill Cricket	Seconds	Rank	C	2,39	0.542	0.587	0.323	0.556	0.493
Resting Metabolic Rate	ml O_2/h	None	B, R	2,45	1.017	0.372	0.166	0.365	0.470
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	B	2,46	1.258	0.296	0.733	0.198	0.213
Maximum Grip Strength	Newtons	Log_{10}	B	2,45	1.491	0.239	0.301	0.096	0.415
Maximum Sprint Speed	m/s	None	None	2,46	1.691	0.198	0.116	0.575	0.148

P values $\leq$ 0.05 are bolded and underlined.

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

Table S6. Comparisons among breeding males, non-breeding males, and virgin males in time point 5. Shown are results of ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

		Time Point 5							
Trait	**Unit**	**Transform**	**Covariates**	**D.F.**	**F**	**Group P**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**
Body Mass (Day 1)	Grams	Log_{10}	None	2,40	1.576	0.222	0.086	0.225	0.475
Body Mass (Day 4)	Grams	Log_{10}	None	2,39	1.114	0.342	0.152	0.388	0.394
Body Mass (Day 7)	Grams	Log_{10}	L	2,39	0.646	0.531	0.288	0.592	0.446
Fat Mass (Day 1)	Grams	Log_{10}	None	2,40	2.263	0.124	0.136	0.936	0.054
Percent Fat Mass (Day 1)	%	Log_{10}	None	2,40	2.312	0.115	0.450	0.332	**0.040**
Fat Mass (Day 7)	Grams	Log_{10}	E	2,39	1.058	0.360	0.781	0.450	0.167
Percent Fat Mass (Day 7)	%	None	None	2,39	1.055	0.360	0.777	0.442	0.168
Lean Mass (Day 1)	Grams	Log_{10}	None	2,40	2.025	0.149	0.060	0.102	0.689
Percent Lean Mass (Day 1)	%	Log_{10}	None	2,40	0.637	0.535	0.754	0.317	0.412
Lean Mass (Day 7)	Grams	Log_{10}	None	2,39	3.072	0.061	0.427	**0.036**	0.088
Percent Lean Mass (Day 7)	%	Log_{10}	None	2,39	2.887	0.071	0.593	0.053	0.068
Snout to Rump Length	Millimeters	Log_{10}	B	2,39	0.171	0.843	0.645	0.926	0.606
Head Length	Millimeters	None	B	2,39	1.883	0.170	0.121	0.065	0.759
Head Width	Millimeters	None	L	2,39	0.848	0.438	0.279	0.755	0.281
Right Hind Foot Length	Millimeters	None	L	2,39	1.117	0.340	0.224	0.752	0.210
Brain Mass	Grams	None	B	2,39	0.619	0.545	0.520	0.483	0.975
Subcutaneous Fat Mass	Grams	None	B	2,39	0.798	0.460	0.525	0.284	0.592
Heart Mass	Grams	Log_{10}	B, L	2,39	2.585	0.092	0.743	0.549	0.223
Lung Mass	Grams	None	B	2,39	1.130	0.336	**0.031**	0.085	0.435
Liver Mass	Grams	Log_{10}	B	2,39	3.321	**0.050**	0.351	0.146	0.524
Spleen Mass	Grams	Log_{10}	B	2,39	3.906	**0.031**	**0.022**	**0.024**	0.832
Pancreas Mass	Grams	None	B	2,39	0.591	0.560	0.053	**0.009**	0.386
Kidney Mass	Grams	None	B	2,39	1.621	0.214	0.421	0.923	0.325
Adrenal Mass	Grams	None	B	2,39	1.103	0.345	0.096	0.389	0.235
Stomach Mass	Grams	Log_{10}	B	2,39	2.550	0.095	0.461	0.735	0.149
Small + Large Intestine Mass	Grams	Log_{10}	B	2,39	1.879	0.170	0.277	**0.039**	0.204
Caecum Mass	Grams	Log_{10}	B	2,39	6.531	**0.004**	0.211	0.063	0.452
Testis Mass	Grams	Log_{10}	B	2,39	0.331	0.721	**0.005**	**0.001**	0.691
Baculum Mass	Grams	Log_{10}	B	2,39	0.443	0.646	0.681	0.440	0.654
Baculum Length	Millimeters	Log_{10}	B	2,39	0.272	0.764	0.391	0.387	0.960

Right Hind Leg Muscle Mass	Grams	None	B	2,39	0.400	0.674	0.719	0.767	0.379
Left Hind Thigh Muscle Mass	Grams	None	B	2,39	0.745	0.483	0.248	0.534	0.429
Left Hind Gastrocnemius Mass	Grams	None	B	2,39	1.828	0.178	0.127	0.068	0.751
Hematocrit (Day 1)	%	Log_{10}	None	2,40	0.900	0.417	0.212	0.595	0.353
Hematocrit (Day 7)	%	Log_{10}	None	2,39	0.311	0.735	0.603	0.437	0.760
Predatory Aggression: Latency to First Attack Cricket	Seconds	None	C	2,38	5.194	**<u>0.012</u>**	**<u>0.004</u>**	**<u>0.008</u>**	0.537
Predatory Aggression: Latency to Kill Cricket	Seconds	Rank	C	2,34	0.553	0.582	0.303	0.438	0.676
Resting Metabolic Rate	ml O_2/h	Log_{10}	F, R	2,38	0.661	0.524	0.243	0.195	0.950
Maximal Oxygen Consumption	ml O_2/h	Log_{10}	B	2,39	0.484	0.621	0.475	0.946	0.368
Maximum Grip Strength	Newtons	Log_{10}	B	2,39	0.675	0.517	0.256	0.441	0.561
Maximum Sprint Speed	m/s	Log_{10}	None	2,39	0.412	0.666	0.877	0.613	0.388

P values $\leq$ 0.05 are bolded and underlined.

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

Table S7. Comparisons among breeding males (BM), non-breeding males (NB), and virgin males (VM) for time point 2 Δ values (time point 2 minus time point 1). Shown are results of overall ANCOVAs with *a priori* contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

			a priori Contrasts			Overall			BM			NB			VM		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**DF**	**F**	**Group P**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.239	0.461	0.563	2,60	0.720	0.492	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	2,60	1.692	0.194	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	2,60	0.005	0.995	20	3.64	1.19	20	3.75	0.94	20	3.64	0.96
Δ Fat Mass (Day 1)	Grams	Log_{10}	**<u>0.049</u>**	0.190	0.373	2,60	2.052	0.139	20	2.30	1.00	20	-0.47	0.79	20	0.48	0.80
Δ Percent Fat Mass (Day 1)	%	Log_{10}	**<u>0.034</u>**	0.255	0.185	2,60	2.536	0.089	20	3.05	1.99	20	-2.91	1.58	20	-0.10	1.60
Δ Fat Mass (Day 7)	Grams	Log_{10}	**<u>0.037</u>**	0.122	0.463	2,60	2.300	0.111	20	1.95	0.82	20	-0.48	0.65	20	0.16	0.66
Δ Percent Fat Mass (Day 7)	%	None	**<u>0.011</u>**	0.102	0.208	2,60	3.542	**<u>0.036</u>**	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	2,58	1.407	0.255	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	2,58	1.178	0.316	20	-2.83	2.31	19	0.68	1.84	19	2.00	1.87
Δ Lean Mass (Day 7)	Grams	None	**<u>0.013</u>**	**<u>0.037</u>**	0.596	2,58	3.395	**<u>0.041</u>**	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	2,59	0.922	0.404	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	2,46	1.362	0.268	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	2,46	0.057	0.945	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	2,55	0.091	0.914	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}	**<u>0.015</u>**	0.260	0.079	2,52	3.609	**<u>0.036</u>**	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	2,58	0.073	0.930	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	2,57	0.670	0.517	19	0.15	0.14	19	0.18	0.11	19	0.02	0.11
Δ Maximum Grip Strength	Newtons	Log_{10}	**<u>0.005</u>**	**<u>0.004</u>**	0.945	2,60	5.165	**<u>0.009</u>**	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	**<u>0.015</u>**	2,54	3.230	**<u>0.048</u>**	19	0.10	0.17	19	0.40	0.13	17	-0.06	0.14

P values ≤ 0.05 are bolded and underlined.

Table S8. Comparisons among breeding males (BM), non-breeding males (NB), and virgin males (VM) for time point 3 Δ values (time point 3 minus time point 1). Shown are results of overall ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

			a priori Contrasts			Overall			BM			NB			VM		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**DF**	**F**	**Group P**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.478	0.113	0.320	2,55	1.359	0.267	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	2,54	0.952	0.394	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	2,54	0.462	0.633	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	2,55	1.529	0.228	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	**<u>0.038</u>**	2,55	2.298	0.112	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	2,54	0.656	0.524	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	2,54	1.343	0.271	18	0.72	1.81	18	-1.01	1.69	18	2.67	1.66
Δ Lean Mass (Day 1)	Grams	None	0.122	**<u>0.012</u>**	0.228	2,53	3.461	**<u>0.040</u>**	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	2,53	0.029	0.972	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	2,53	1.344	0.271	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	2,53	0.364	0.697	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	2,55	0.126	0.882	19	0.10	0.92	18	-0.48	0.87	18	-0.50	0.86
Δ Hematocrit (Day 7)	%	None	0.882	0.077	**<u>0.033</u>**	2,53	2.859	0.069	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	2,49	0.708	0.499	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	2,42	1.862	0.172	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	2,53	0.057	0.945	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	2,54	0.562	0.574	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	2,54	0.228	0.797	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	2,54	0.466	0.631	18	0.14	0.11	18	0.22	0.10	18	0.09	0.10

P values $\leq$ 0.05 are bolded and underlined.

Table S9. Comparisons among breeding males (BM), non-breeding males (NB), and virgin males (VM) for time point 4 Δ values (time point 4 minus time point 1). Shown are results of overall ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

			a priori Contrasts			Overall			BM			NB			VM		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**D.F.**	**F**	**Group P**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.631	0.541	0.150	2,45	1.090	0.347	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	2,46	0.686	0.510	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	2,46	0.664	0.521	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	2,45	1.109	0.341	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	2,45	1.350	0.272	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	2,46	0.915	0.409	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	2,46	0.982	0.384	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	**<u>0.009</u>**	2,45	4.378	**<u>0.020</u>**	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	2,45	0.177	0.838	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	2,46	0.991	0.381	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	2,46	0.551	0.581	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	2,45	0.592	0.558	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	2,45	0.747	0.481	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	2,43	2.131	0.135	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	2,39	0.386	0.683	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	2,45	0.435	0.651	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	2,46	0.615	0.546	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	2,45	1.489	0.239	17	0.32	0.35	13	0.67	0.33	15	1.15	0.30
Δ Maximum Sprint Speed	m/s	Log_{10}	**<u>0.021</u>**	0.462	**<u>0.024</u>**	2,46	4.012	**<u>0.026</u>**	17	-0.17	0.17	13	0.45	0.15	16	0.02	0.14

P values ≤ 0.05 are bolded and underlined.

Table S10. Comparisons among breeding males (BM), non-breeding males (NB), and virgin males (VM) for time point 5 Δ values (time point 5 minus time point 1). Shown are results of overall ANCOVAs with a priori contrasts, as well as significance levels, sample sizes (N), untransformed estimated marginal means (EMM), and associated standard errors (SE) from ANCOVAs.

			a priori **Contrasts**			**Overall**			**BM**			**NB**			**VM**		
Trait	**Unit**	**Transform**	**BM *vs.* NB**	**BM *vs.* VM**	**NB *vs.* VM**	**D.F.**	**F**	**Group P**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**	**N**	**EMM**	**SE**
Δ Body Mass (Day 1)	Grams	None	0.505	0.208	0.494	2,40	0.854	0.435	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	2,39	0.703	0.503	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	2,39	0.223	0.801	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	2,40	0.037	0.963	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	2,40	0.247	0.782	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	2,39	0.030	0.970	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	2,39	0.041	0.960	13	1.67	2.83	12	0.86	2.58	14	1.72	2.33
Δ Lean Mass (Day 1)	Grams	None	0.097	**<u>0.004</u>**	0.135	2,40	4.825	**<u>0.015</u>**	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	2,40	0.201	0.819	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	2,39	1.462	0.248	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	2,39	0.269	0.766	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	2,40	0.624	0.542	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	2,39	1.519	0.235	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	2,38	0.355	0.705	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	2,34	1.830	0.183	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	2,38	0.158	0.855	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	2,39	0.046	0.955	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	2,39	0.108	0.898	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	**<u>0.048</u>**	2,39	2.132	0.136	13	-0.01	0.18	12	0.19	0.16	14	-0.22	0.15

P values $\leq$ 0.05 are bolded and underlined.