Energy Metrics of Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*) Vary with Sex but Not Life-History Stage

Rachel C. Wilson[1,*]
Deborah I. Lutterschmidt[1,2]
[1]Department of Biology, Portland State University, 1719 SW 10th Avenue, SRTC Room 246, Portland, Oregon 97201;
[2]Department of Ecology and Evolutionary Biology, University of California Irvine, 321 Steinhaus Hall, Irvine, California 92697

Accepted 5/16/2020; Electronically Published 7/30/2020

ABSTRACT

Because reproduction is energetically expensive, an organism's energy stores are likely involved in mediating transitions between reproductive and self-maintenance activities. We investigated whether body condition index, adipocyte follicle size, and liver glycogen differ with the life-history transition from reproduction to migration and foraging in red-sided garter snakes (*Thamnophis sirtalis parietalis*). Females primarily investing in mating behavior located at the den had a significantly higher body condition index than females migrating to summer feeding grounds. The body condition index of male snakes did not differ between snakes located at the den and those migrating to summer feeding grounds. Neither adipocyte follicle area nor liver glycogen stores differed significantly between snakes performing mating activities at the den and those migrating to summer feeding grounds. We did find a sexual dimorphism in that female red-sided garter snakes had significantly larger adipocyte follicles and higher liver glycogen compared with males. Our findings support the across-species phenomenon of females and males displaying a sexual dimorphism in stored energy substrates. Conversely, we did not find evidence to suggest that red-sided garter snakes primarily utilize fatty acids to fuel the initiation of migration, a finding that is not consistent with other long-distance migrators, such as birds. Because we did not find evidence to suggest that stored energy metrics influence the decision to migrate, a physiological mechanism that induces migration in red-sided garter snakes remains elusive.

Keywords: body condition index, adipocyte follicle size, liver glycogen, energetics, life-history transitions, migration, trade-offs, sexual dimorphism, reptile.

*Corresponding author; email: rwilson@pdx.edu.

Physiological and Biochemical Zoology 93(5):347–357. 2020. 1522-2152/2020/9305-20005$15.00. DOI: 10.1086/709994

Introduction

A common, well-established biological paradigm concerns the trade-offs associated with investment in either reproduction or self-maintenance (Bonnet et al. 2002; Fletcher et al. 2013; Lourdais et al. 2013; Dupoué and Lourdais 2014). To increase Darwinian fitness, an organism must both survive and reproduce, and deciding the appropriate time to invest in reproductive activities can affect survival (Jouventin and Dobson 2002; Bohec et al. 2007). Organisms exhibiting seasonal and/or biennial reproductive cycles must coordinate the timing of reproduction with appropriate environmental conditions. Multiple exogenous cues, such as photoperiod and temperature, affect physiology (Pinter and Negus 1965; Björnsson et al. 1989; Bartness 1996; Demas and Nelson 1998; Larsen et al. 2001; Niva and Takeda 2003; Lutterschmidt and Mason 2009; Zajac et al. 2011; Lutterschmidt 2012). However, the extent to which these exogenous cues affect physiology may be context dependent (Ozaki et al. 1978; San Martin and Touitou 2000; Barrett et al. 2007). Some factors that may contribute to the context dependency of exogenous cues include resource availability and energy status of an organism (i.e., high body condition index, large fat stores, etc.). As reproduction is an energetically expensive activity, endogenous signals that reflect energy status likely influence an organism's sensitivity to exogenous signals and perhaps facilitate transitions between life-history stages.

A multitude of evidence across taxa suggests that energy availability relates to life histories (Bronson and Marsteller 1985; Farley and Robbins 1995; De Block and Stoks 2005; Stallings et al. 2010; Alonso-Fernández and Saborido-Rey 2012; Becker et al. 2013; Costanzo et al. 2013; Muir et al. 2013; McBride et al. 2015; McCann and Padilla 2015). Reproduction has been arguably the most commonly studied life-history stage as it relates to energy availability, with higher energy stores associated with higher investment in reproduction (Bronson and Marsteller 1985; Doughty and Shine 1997, 1998; Kirk 1997; Barron and Andraso 2001; Smith and Moore 2003; Groscolas et al. 2008; Alonso-Fernández and Saborido-Rey 2012; Becker et al. 2013). Furthermore, food deprivation delayed reproductive activities (Bronson and Marsteller 1985), and insufficient energy stores resulted in premature cessation of reproduction (Groscolas et al. 2008). This suggests that energy status could potentially equip an organism with the ability to "decide" when to prioritize investment in either reproduction or self-maintenance activities. To date, little is known about the physiological mechanisms that allow organisms to prioritize life-history processes.

We investigated whether metrics of energy balance, as measured by body condition index, adipocyte follicle size, and liver glycogen, differ with the life-history transition from reproduction to foraging in a well-studied population of red-sided garter snakes (*Thamnophis sirtalis parietalis*). The annual life-history stages exhibited by northern populations of red-sided garter snakes allow for easy identification of individuals that are and that are not primarily investing in mating behaviors. In Manitoba, Canada, red-sided garter snakes overwinter in underground dens for up to 8 mo and upon emergence enter into an intense and shortened spring mating season, all while being aphagic (Gregory and Stewart 1975). Individuals spend a variable amount of time performing mating behaviors at the den, and as the mating season ends, they migrate up to 17 km to summer feeding grounds (Gregory and Stewart 1975; Gregory 1977). Male snakes emerge before females, and as single females or small groups of females emerge, males immediately and intensely begin to court attractive females. Most female snakes remain at the den for less than 1 d before dispersing, while male snakes spend upward of 2 wk courting and attempting to mate with females (Shine et al. 2001; Lutterschmidt and Mason 2009). Because this population of red-sided garter snakes exhibits a distinct behavioral transition from mating to feeding activities that is necessarily linked with migration, we easily identified whether individuals are primarily investing in reproduction or whether they transitioned to self-maintenance activities (Cease et al. 2007).

Here, we present data to determine whether body condition index, adipocyte follicle area, and liver glycogen differ between red-sided garter snakes that were primarily involved in reproductive activities and snakes that initiated migration to summer feeding grounds. Body condition index is used as a noninvasive method to obtain information about an organism's energy balance by examining the relationship between body mass and body length (Hayes and Shonkwiler 2001). We also aimed to determine whether direct measurements of stored energy (i.e., adipocyte follicle size and liver glycogen) differ between individuals at the den and those that initiated migration to summer feeding grounds. We predicted that energy metrics would be higher in snakes located at the den compared with individuals collected during migration. We expected to see a greater magnitude of change in adipose tissue compared with liver glycogen because of the preferential usage of fat stores in migrating birds (Jenni-Eiermann 2017). We also expected that males would show larger differences in energy metrics because of the sex-specific differences in the timing of migration of red-sided garter snakes, in which males spend a greater time at the den compared with females. Finally, we also explored relationships among the energy metrics quantified in this study. Because organisms utilize fat as a fuel when glucose and glycogen levels are low, we did not expect to see a significant relationship between adipocyte follicle area and liver glycogen. Conversely, we did expect to see a significant positive relationship between body condition index and both adipocyte follicle area and liver glycogen.

Material and Methods

Red-sided garter snakes were collected from the Interlake region of Manitoba, Canada, under the authority of scientific permit WB18801 issued by the Manitoba Department of Sustainable Development. All procedures were approved under Portland State University's Institutional Animal Care and Use Committee protocol 42.

Experimental Design

Male and female red-sided garter snakes were collected May 19–22, 2016, to obtain adipose and liver tissues. Brains were also collected for a separate experiment that is not part of the analyses presented here. Snakes collected from mating balls at the den were in reproductive condition, and we confirmed that females were unmated by the absence of a mating plug in the cloaca. As in Dayger and Lutterschmidt (2017) and Cease et al. (2007), we also collected migrating snakes from a road located along the migratory route approximately 1 km from the den. These snakes were migrating to summer feeding grounds and transitioning from reproductive to foraging behavior. Total sample sizes were 27 females (den: $n = 16$; road: $n = 11$) and 32 males (den: $n = 16$; road: $n = 16$).

Tissue Collection

Snakes were euthanized with 250 μL of a 1% solution of sodium Brevital administrated via injection near the heart. Mass and snout-vent length (SVL) were measured for each snake, and adipose and liver tissues were excised. Approximately 100 mg of adipose tissue was collected from the anterior portion of the abdominal cavity near the liver and gallbladder. Adipose tissue was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) overnight at 4°C. Tissues were then stored in 0.1 M phosphate buffer at 4°C until processed for paraffin embedding at Portland State University. Approximately 20 mm of the most posterior portion of the liver was excised and flash frozen on dry ice. Frozen tissues were kept on dry ice until arrival at the field station, where they were stored in liquid nitrogen. They were transferred to Portland State University on dry ice and then stored at −80°C until analyzed.

Adipose Tissue Histology

Paraffin embedding of fixed adipose tissues was conducted following the methods of Berry et al. (2014). Briefly, tissues were dehydrated in 75% ethanol for 30 min, 95% ethanol for 75 min for two incubations, and 100% ethanol for 60 min for three incubations. Tissues were then cleared in CitriSolv (22-143-975, Thermo Scientific) for 60 min for two incubations. Fresh solutions were used for all incubations. Tissues were impregnated with paraffin overnight at 58°C, during which paraffin wax was changed twice, approximately every 7 h. Specimens were sliced at 5 μm using a microtome and collected on gelatin-coated microscope slides. Because of a high density of parasites within the fat bodies of some individuals, 19% of samples were resectioned at 15 μm. Using a thicker section allowed us to increase the proportion of adipocytes visible within each tissue section, but it did not affect the measurement of follicle area ($t = 0.86$, df $= 4$, $P = 0.440$, from a paired t-test on the mean average follicle area of five snakes

measured at both 5 and 15 μm). Uhrig (2015) proposed that this encysted larval (metacercarial stage) diplostomid trematode likely belongs to the genus *Fibricola* according to morphology. Although investigations into possible sex differences in parasite load are beyond the scope of this study, it is unlikely that *Fibricola* load differs between males and females because it does not significantly relate to SVL in garter snakes (Uhrig 2015). Furthermore, pit vipers (*Bothrops jararac*) did not display any sex differences in several parasite loads among various tissues (*Caryspora* and *Sarcocystis* spp. in the gut, *Porocephalus* sp. in the lung, and *Ochetosoma heterocoelium* in the oral cavity [Grego et al. 2004]).

For each individual, we collected five sequential sections on a single slide across four series of slides (e.g., series A, B, C, D). We repeated this process four times, such that each tissue series comprised four slides; therefore, within each series, a minimum of 75 μm separated the tissues collected on each slide. Using one tissue series, we measured adipocyte follicle area in one of the five tissue sections collected on each of the four slides within the series. Because the mean diameter of adipocytes across all red-sided garter snakes in this study was 20.81 $\pm$ 0.68 μm, it is not possible that we measured an adipocyte twice.

After collection, sections were deparaffinized for 10 min in CitriSolv for two incubations. Tissues were then rehydrated in a series of ethanol incubations followed by Nanopure water, and they were counterstained with hematoxylin and eosin (fig. A1). Adipocyte follicle area was measured using the ImageJ software Adiposoft (National Institutes of Health, Bethesda, MD). Each adipocyte follicle the software recognized was assigned a unique number. We then examined each sequential adipocyte in a section to ensure that the software had accurately measured follicles. We excluded any partial adipocytes from analysis. Where possible, we measured the follicle area of 25 cells in one tissue section per slide; we repeated this process for each slide within the series and then calculated the average adipocyte follicle area of the resulting 100 cells per snake. In the case that 100 adipocytes were not present within a tissue series, we measured as many adipocytes as possible; 11 of 59 samples had fewer than 100 visible adipocytes within the tissue series. The mean number of follicles measured across nine of these individuals was 64.5 $\pm$ 9.78 SEM; the minimum number of follicles measured in any snake included in analysis was 32. Two males collected from the road had very little adipose tissue to initially collect. Because we could not obtain enough measurements (i.e., fewer than 20 visible follicles) even after reprocessing and slicing adipose tissue to accurately estimate average follicle area, we excluded these males from adipocyte analyses.

Liver Glycogen Assay

For each individual, we homogenized 100–120 mg of liver tissue with a Bio-Gen Pro200 (Oxford, CT) in 0.5 mL of 1% Halt protease inhibitor solution (1862209, Thermo Scientific, Waltham, MA) in assay buffer (0.936% Na_2PO_4, 0.4656% KPO_4, 1% NaCl) for 15 s. Liver homogenates were then centrifuged at 800 *g* for 10 min at 4°C. We transferred the supernatant to a fresh tube. To convert glycogen to glucose, we incubated 35 μL of supernatant with 175 μL of diluted glycogen hydrolysis enzyme (700483, Cayman Chemical, Ann Arbor, MI; reconstituted in 3 mL of 50 mM sodium acetate) for 30 min at 37°C. We ran a glycogen standard in each digestion assay as a positive control. We then ran these digested samples in an enzymatic colorimetric assay (10009582, Cayman Chemical, Ann Arbor, MI) following manufacturer instructions to measure glucose content. The intra-assay and interassay coefficients of variation were 3.08 $\pm$ 1.29 and 5.43, respectively. To validate this assay for snake plasma, we sequentially diluted both female and male supernatants and then subjected them first to the digestion assay and then to the colorimetric assay to verify that these samples displayed parallelism with the standard curve of the glucose colorimetric assay. Both diluted supernatants from female and male liver homogenates displayed parallelism with the standard curve of the glucose assay (data not shown), indicating that the constituents of snake plasma do not interfere with the assay.

Statistical Analyses

We utilized *t*-tests to determine whether body condition index differed with migration status within each sex; we calculated the body condition index for female and male snakes separately because red-sided garter snakes exhibit a sexual size dimorphism, with females being larger than males. Body condition index was determined by calculating the residual from a regression of log-transformed body mass on log-transformed SVL. To determine whether adipocyte follicle area varied with sex or migration status, we utilized two-way ANOVA. We controlled for potential differences related to body size by dividing each snake's mean adipocyte follicle area by its SVL (cm); we then determined whether size-corrected adipocyte follicle area varied with either sex or migration status using a two-way ANOVA.

We accounted for the amount of liver homogenized by dividing glycogen content by liver mass homogenized and then ran a two-way ANOVA. Significant main effects from these ANOVAs were further examined using Holm-Sidak multiple comparisons tests. Last, we used linear regressions to determine whether adipocyte follicle area significantly related to liver glycogen and whether body condition index significantly related to adipocyte follicle area or liver glycogen.

Where necessary, data were square root or log transformed to meet the assumptions of parametric analysis. If data could not be transformed to meet these assumptions, nonparametric Mann-Whitney *U*-tests were utilized. All statistics were run using SigmaStat 12.0 (Systat Software). We set an α of 0.05 to determine significance; results were considered significant when $P \leq 0.05$ after rounding to two significant digits.

Results

Female snakes collected from the den had a significantly higher body condition index than migrating females collected from the road (fig. 1*A*; $t = 3.72$, $P = 0.001$). Body condition index of male snakes did not vary with collection site (fig. 1*A*; $U = 97.00$, $P = 0.250$).

During the spring, adipocyte follicle area was significantly larger in females compared with males (fig. 1*B*; $F_{1, 53} = 46.27$,

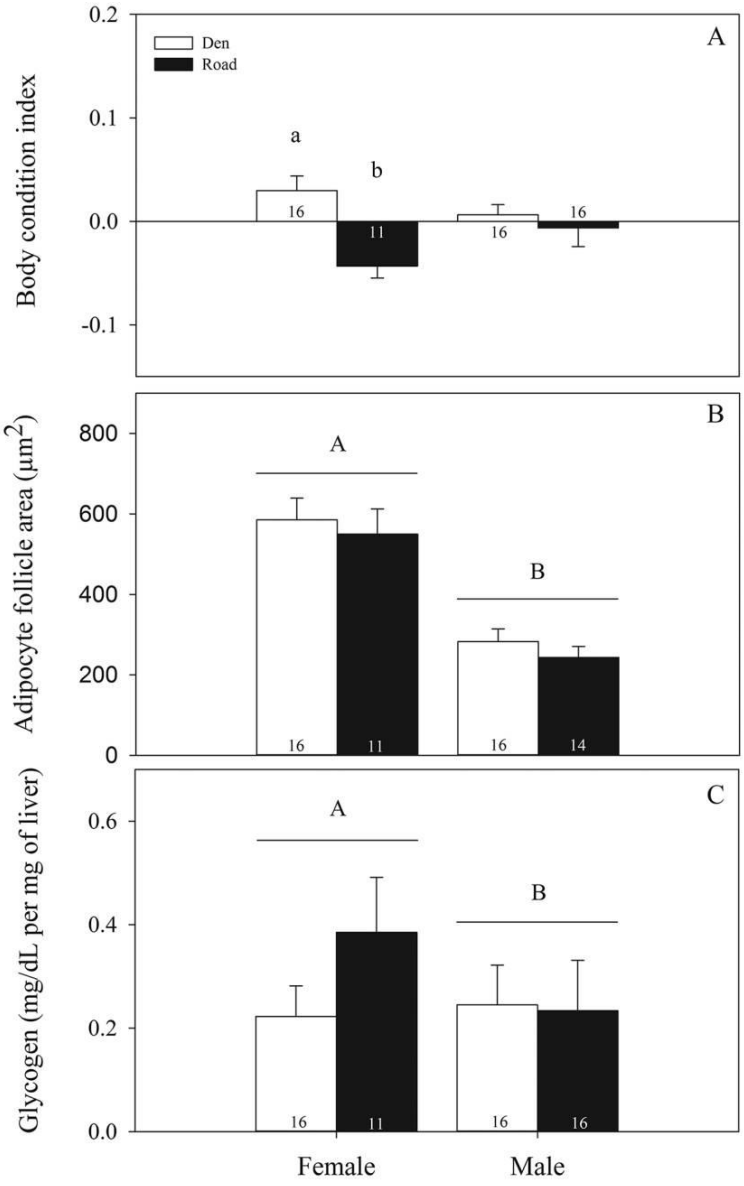

Figure 1. Influence of sex and migratory status on body condition index (*A*), adipocyte follicle area (*B*), and liver glycogen (*C*) in red-sided garter snakes (*Thamnophis sirtalis parietalis*). Nonmigratory snakes were collected from the den, and migrating snakes were collected from a road located ~1 km from the den along the migratory route. All data are mean + 1 SEM. Letters indicate significant differences between groups; numbers along the *X*-axes indicate final sample sizes.

$P < 0.001$), and this relationship persisted even after correcting for sex differences in SVL ($F_{1,53} = 15.44$, $P < 0.001$; data not shown). There were no significant differences in adipocyte follicle area between den- and road-collected individuals (fig. 1*B*; $F_{1,53} = 0.70$, $P = 0.407$), and the interaction between sex and collection site was statistically nonsignificant ($F_{1,53} = 0.03$, $P = 0.874$).

Females in the spring had significantly more glycogen per milligram of liver homogenized than male garter snakes (fig. 1*C*; $F_{1,55} = 3.89$, $P = 0.054$). Neither migratory status (fig. 1*C*; $F_{1,55} = 0.24$, $P = 0.629$) nor the interaction between migratory status and sex significantly affected liver glycogen ($F_{1,55} = 2.03$, $P = 0.160$).

Adipocyte follicle area did not significantly relate to liver glycogen in females (fig. 2; $R^2 = 0.00$, $P = 0.717$) or males (fig. 2; $R^2 = 0.04$, $P = 0.139$). Neither adipocyte follicle area (fig. 3*A*; $R^2 = 0.092$, $P = 0.124$) nor liver glycogen (fig. 3*B*; $R^2 = 0.00$, $P = 0.737$) in female snakes significantly related to body condition index during the spring. Likewise, neither male adipocyte follicle area (fig. 3*A*; $R^2 = 0.02$, $P = 0.464$)

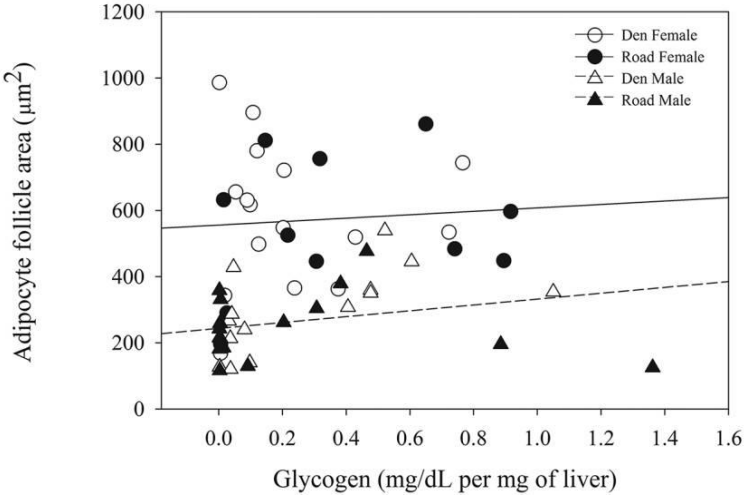

Figure 2. Relationship between adipocyte follicle area and liver glycogen in male and female red-sided garter snakes (*Thamnophis sirtalis parietalis*). Results are from separate linear regressions for each sex. Individuals collected from the den (open symbols) were primarily engaging in mating behavior, whereas individuals that initiated migration to summer feeding grounds were collected from a road ~1 km from the den (solid symbols). Because we found no significant differences in either variable between den- and road-collected individuals, we collapsed migratory status for these analyses. Adipocyte follicle area did not significantly relate to liver glycogen content in either female or male red-sided garter snakes (see text for further statistical details).

nor liver glycogen (fig. 3*B*; $R^2 = 0.00$, $P = 0.394$) significantly related to body condition index. All of the results presented in figures 2 and 3 are from simple linear regressions performed separately for each sex.

Discussion

This is the first investigation reporting liver glycogen in red-sided garter snakes. Our findings of no relationship between liver glycogen and migratory status are similar to findings from investigations in migrating fish (Tudorache et al. 2007; but see Chang and Idler 1960) and birds (McWilliams et al. 2004). In contrast to the established literature suggesting the utilization of fatty acids in many migrating animals (e.g., birds, mammals, fish, and invertebrates; Blem 1980; McWilliams et al. 2004; Weber 2009; Dingle 2014), we do not provide evidence to suggest that adipose stores influence the decision to migrate in red-sided garter snakes. However, it is possible that measuring adipocyte follicle size is not sensitive enough to assess lipid metabolism. Our results are in line with Crews et al. (1987), who found that plasma lipid content did not change over the course of 37 d after spring emergence from simulated hibernation, the time period during which individuals migrate to summer feeding grounds. Though these two lines of evidence suggest that lipid metabolism may not influence the decision to migrate in red-sided garter snakes, measuring plasma fatty acid concentration or enzymes related to lipid metabolism in den- and road-collected snakes would provide a clearer picture of whether fatty acid metabolism is involved in deciding when to initiate migration in garter snakes. A study in tree lizards (*Urosaurus ornatus*) found that one enzyme involved in fatty acid anabolism, diacyglycerol acyltransferase, differed with reproductive condition in females but not in males (Lacy et al. 2002). Because snakes located at the den were primarily performing reproductive behavior, measuring these and/or other enzymes associated with lipid metabolism could provide further evidence as to whether adipose tissue plays a role in initiating migration in red-sided garter snakes.

Other energy-related signals, such as glucose, amino acids, and other by-products of catabolism, may influence the initiation of migration. In red-sided garter snakes, total plasma protein concentrations change over 24 d after spring emergence in field-caught males, although no clear pattern of change was evident (Crews et al. 1987). Measuring total protein likely may not provide a clear picture of amino acid metabolism because of the inclusion of other plasma proteins, such as binding proteins and clotting factors. In that same study, plasma glucose levels in males did not significantly fluctuate (Crews et al. 1987), a finding similar to that from preliminary work in our lab that showed no significant differences in plasma glucose levels between den- and road-collected males (Maine et al. 2014). However, road-collected females displayed higher glucose concentrations than den-collected females (Maine et al. 2014), a finding that is reflected in our data because migrating females tended to have higher liver glycogen compared with den-collected females. This suggests that the factors regulating differences in liver glycogen between individuals primarily engaging in mating activities and those migrating to summer feeding grounds may be sexually dimorphic.

Sex Differences in Energy Stores

We found significant sex differences in adipocyte follicle size and liver glycogen stores during the spring in red-sided garter snakes, with females having significantly larger adipocyte follicles and higher glycogen per milligram of liver homogenized than males.

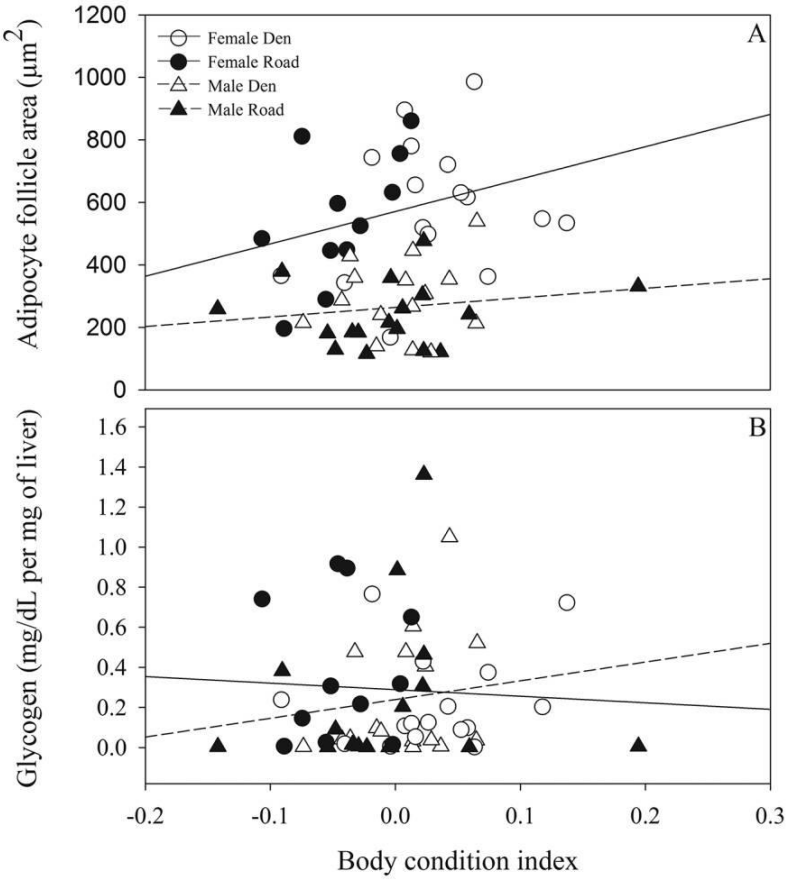

Figure 3. Relationship between adipocyte follicle area and body condition index (*A*) and between liver glycogen and body condition index (*B*) in male and female red-sided garter snakes (*Thamnophis sirtalis parietalis*). Results are from simple linear regressions run separately for each sex. Individuals collected from the den (open symbols) were primarily engaging in mating behavior, whereas individuals that initiated migration to summer feeding grounds were collected from a road ~1 km from the den (solid symbols). Body condition index was determined separately for female and male snakes by calculating the residual from a regression of log-transformed body mass on log-transformed snout-vent length. Because we found no significant differences in adipocyte follicle area or liver glycogen between den- and road-collected individuals, we collapsed migratory status for these analyses. Body condition index did not significantly relate to either adipocyte follicle area or liver glycogen in female or male red-sided garter snakes (see text for further statistical details).

After controlling for SVL, females still had significantly larger adipocyte follicles compared with males. These data suggest that female red-sided garter snakes store energy substrates to a greater extent than males. A number of reviews have described sex-specific differences in fat distribution across body regions in mammals (Wells 2007; Valencak et al. 2017; Chang et al. 2018). In birds, female American redstarts arrived at breeding grounds with more body fat than male conspecifics (Smith and Moore 2003). Across taxa, no clear pattern of sexual dimorphisms emerged in liver glycogen. Storage of liver glycogen in female mammals may be more sensitive to fasting and feeding cycles because fed female bats stored more liver glycogen than males, but fasted female rats had lower glycogen stores than males (Deuel et al. 1937; Freitas et al. 2010; Gustavsson et al. 2010). In two species of chorus frogs (*Pseudarcris crucifer* and *Pseudarcris triseriata*), males had higher liver glycogen compared with females (Duffitt and Finkler 2011), as opposed to the common frog (*Rana temporaria*) that did not display any sexually dimorphic levels of liver glycogen (Smith 1950). Male sockeye salmon (*Onocorhynchus nerka*) had higher liver glycogen compared with females (Chang and Idler 1960). It is possible that differing methodologies in the quantification of glycogen levels confounded a clear relationship in this sexual dimorphism. What is more likely is that sexually dimorphic differences in liver glycogen are dependent on the organism's ecological niche.

Indeed, one study that investigated three snake species with differing life-history tactics found no consistent pattern in sex differences pertaining to stored forms of energy, as measured by fat and liver masses (Bonnet et al. 1998). Because the ecology of garter snakes does not perfectly align with any snake in this study but instead shares similarities with both asp vipers (*Vipera aspis*) and European whip snakes (*Coluber viridflavus*; e.g., medium vs.

large bodied, viviparous vs. oviparous, fast moving vs. slow moving), it may be difficult to draw conclusions concerning sex-specific differences in energy stores. However, both female asp vipers and female garter snakes had higher adipose and liver tissue compared with males, whereas male and female European whip snakes had equal forms of stored energy in these tissues. It is likely that certain ecological characteristics, such as body size and parity, contributed more to sex-specific differences of storing energy in snake species.

A possible mechanism underlying the sex-specific difference we found in energy stores of red-sided garter snakes is sex steroid hormones. Investigations into the presence of estrogen and androgen receptors in adipose tissue outside of mammals are sparse, with only one study that identified an androgen receptor in female orange-spotted groupers (Shi et al. 2012). In mammals, several studies have identified estrogen and androgen receptors in white adipose tissue (Mizutani et al. 1994; Dieudonné et al. 1998; O'Brien et al. 1998). Estrogen treatment decreased overall white adipose tissue mass, adipocyte size, and white adipose lipoprotein lipase, an enzyme that cleaves triglycerides into fatty acids for storage; ovariectomy increased these variables (Mead et al. 2002; Mayes and Watson 2004; Jeong et al. 2007). However, in reptiles, estradiol significantly correlated with increased fat storage (Lacy et al. 2002). This discrepancy may be due to differences in estrogen receptor subtypes expressed in adipose tissue or differential effects of estrogens on subcutaneous versus visceral adipose tissue (Mayes and Watson 2004). Because the one study in reptiles that addressed the effects of estrogens on adipose tissue conflicts with mammalian studies, it is difficult to conclude whether estrogens are responsible for the sexual dimorphism we observed in adipose tissue of red-sided garter snakes. Further complicating the potential effects of sex steroid hormones on adipose tissue in red-sided garter snakes is the fact that this population exhibits dissociated reproduction, in which snakes tend to have low levels of sex steroid hormones during the spring mating season (e.g., Krohmer et al. 1987; Whittier et al. 1987; Moore et al. 2000; Moore and Mason 2001; Lutterschmidt and Mason 2009). As such, more research should be conducted in reptiles to help determine the basis of this sexual dimorphism. Compared with estrogens, the effects of testosterone on adipose tissue were more consistent across taxa: testosterone inhibited fat deposition in mammals, lizards, and birds (Ketterson et al. 1991; Lacy et al. 2002; Mayes and Watson 2004; Rynders et al. 2018; Yao et al. 2018). It is possible that the inverse relationship between testosterone and fat deposition was due to aromatization of testosterone to estradiol, but this relationship persisted even in the presence of an aromatase inhibitor (Rynders et al. 2018).

The effects of testosterone and estradiol on promoting adipose tissue breakdown and glycogen synthesis and storage, respectively, may be more complex in red-sided garter snakes, as these snakes are dissociated breeders in which peak sex steroid hormones did not coincide with peak mating behavior. However, male snakes did exhibit declining levels of testosterone during winter dormancy and sometimes over the course of the spring mating season (Krohmer et al. 1987; Moore et al. 2000; Moore and Mason 2001; Lutterschmidt and Mason 2009). It is possible that sex steroid hormones act transseasonally during the summer, fall, and winter to influence the sex differences we observed in the energy metrics reported here. As stated previously, however, studies investigating the effects of sex steroid hormones on adipose tissue and energy-specific functions of the liver are lacking in snakes.

Body Condition Index

We found evidence to suggest that body condition index differs between snakes located at the den and females migrating to summer feeding grounds but not males, a finding similar to that of Cease et al. (2007). Female snakes located at the den had a significantly higher body condition index than road-collected females. However, neither adipocyte follicle area nor liver glycogen varied with migration status in these snakes. Because females do not ovulate until 6 wk after emergence (Whittier et al. 1987), it is unlikely that the differences in body condition index that we observed with migratory status in females were related to reproductive condition. In light of body condition index not significantly relating to either adipocyte follicle area or liver glycogen content during the spring mating season (fig. 3), other energy and non-energy factors (i.e., water content) likely contributed more to body condition index than these energy metrics. For example, in male red-sided garter snakes, body condition index significantly related to lean body mass (i.e., muscle and skeleton weight) but not to fat body mass or liver mass (Shine and Mason 2005). In contrast, the body condition index of brown tree snakes (*Boiga irregularis*) related to total fat mass (Waye and Mason 2008). These findings illustrate that body condition index did not always represent the same stored energy substrates across species. The variation in which metabolic substrates contributed to body condition index may reflect differing ecological and environmental influences and/or metabolic needs. Therefore, species-specific validation is needed to determine which form of energy substrate most relates to body condition index. The hypothesis that body condition index does not accurately reflect stored energy content is not new (Green 2001; Peig and Green 2009; Labocha et al. 2013). Peig and Green (2009) argued for the necessity of including allometric scaling in body condition index calculations to appropriately approximate energy stores. However, using residuals from regressions of body mass on SVL is appropriate to estimate body condition in snakes because these residuals were not influenced by allometry but were significantly related to measurements of fat in Burmese pythons (*Python bivittatus*; Falk et al. 2017).

In conclusion, we present evidence to suggest that body condition index related to the decision to migrate in female, but not male, red-sided garter snakes. However, how body condition index pertained to energy substrates remains unclear in red-sided garter snakes. This research contributes to the large body of literature demonstrating sexually dimorphic storage of energy substrates across species. Because we did not find evidence to suggest that stored energy metrics influenced the decision to migrate, a physiological mechanism that induces migration in red-sided garter snakes remains elusive. Accordingly, more research will help clarify whether the prioritization of fatty acid usage to fuel migration is conserved across taxa or whether energy substrate usage is dependent on environmental or ecological conditions.

Acknowledgments

We thank the Manitoba Department of Sustainable Development for logistical support in the field. We thank Holden Anderson, Catherine Dayger, Ashley Lucas, and Robert Mason for assistance in the field and lab. We thank Jason Podrabsky for use of the TECAN plate reader. We declare no competing interests. This research was funded by Portland State University's Forbes-Lea Award to R.C.W. and by National Institutes of Health BUILD EXITO funds and National Science Foundation grants IOS-1355203 and IOS-1755427 to D.I.L.

APPENDIX

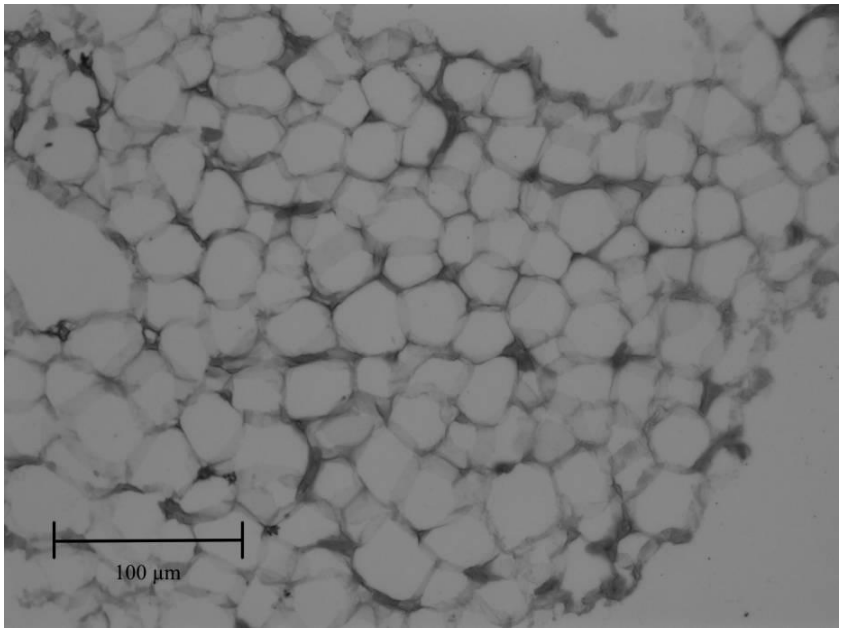

Figure A1. Adipose tissue sections from a representative male red-sided garter snake. Tissues were hematoxylin and eosin stained and photographed at ×200 magnification. A color version of this figure is available online.

Literature Cited

Alonso-Fernández A. and F. Saborido-Rey. 2012. Relationship between energy allocation and reproductive strategy in *Trisopterus luscus*. J Exp Mar Biol Ecol 416/417:8–16.

Barrett P., F.J.P Ebling, S. Schuhler, D. Wilson, A.W. Ross. A. Warner, P. Jethwa, et al. 2007. Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. Endocrinology 148:3608–3617.

Barron J.N. and G.M. Andraso. 2001. The influence of fall foraging success on follicle number in the northern water snake, *Nerodia sipedon*. J Herpetol 35:504–507.

Bartness T.J. 1996. Photoperiod, sex, gondal steroids, and housing density affect body fat in hamsters. Physiol Behav 60:517–529.

Becker J., C. Ortmann, M.A. Wetzel, C. Winkelmann, and J.H.E. Koop. 2013. Mate guarding in relation to seasonal changes in the energy reserves of two freshwater amphipods (*Gammarus fossarum* and *G. pulex*). Freshw Biol 58:372–381.

Berry R., C.D. Church, M.T. Gericke, E. Jeffery, L. Colman, and M.S. Rodeheffer. 2014. Imaging of adipose tissue. Methods Enzymol 537:47–73.

Björnsson B.T., H. Thoraresnsen, T. Hirano, T. Ogasawara, and J.B. Kristinsson. 1989. Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypoosmoregulatory ability of juvenile Atlantic salmon (*Salmo salar*) during parr-smolt transformation. Aquaculture 82:77–91.

Blem C.R. 1980. The energetics of migration. Pp. 175–224 in S.A. Gauthreaux Jr., ed. Animal migration, orientation, and navigation. Academic Press, New York.

Bohec C.L., M. Gauthier-Clerc, D. Grémillet, R. Pradel, A. Béchet, J.P. Gendner, and Y.L. Maho. 2007. Population dynamics in a long-lived seabird. I. Impact of breeding activity on survival and breeding probability in unbanded king penguins. J Anim Ecol 76:1149–1160.

Bonnet X., O. Lourdais, R. Shine, and G. Naulleau. 2002. Reproduction in a typical capital breeder: costs, currencies, and complications in the aspic viper. Ecology 83:2124–2135.

Bonnet X., R. Shine, G. Naulleau, and M. Vacher-Vallas. 1998. Sexual dimorphism in snakes: different reproductive roles favour different body plans. Proc R Soc B 265:179–183.

Bronson F.H. and F.A. Marsteller. 1985. Effect of short-term food deprivation on reproduction in female mice. Biol Reprod 33:660–667.

Cease A.J., D.I. Lutterschmidt, and R.T. Mason. 2007. Corticosterone and the transition from courtship behavior to dispersal in male red-sided garter snakes (*Thamnophis sirtalis parietalis*). Gen Comp Endocrinol 150:124–131.

Chang E., M. Varghese, and K. Singer. 2018. Gender and sex differences in adipose tissue. Curr Diabetes Rep 18:69. https://doi.org/10.1007/s11892-018-1031-3.

Chang V.M. and D.R. Idler. 1960. Biochemical studies on sockeye salmon during spawning migration. XII. Liver glycogen. Can J Biochem Physiol 38:553–558.

Costanzo J.P., M.C.F. do Amaral, A.J. Rosendale, and R.E. Lee Jr. 2013. Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog. J Exp Biol 216:3461–3473.

Crews D., M. Grassman, W.R. Garstka, A. Halpert, and B. Camazine, 1987. Sex and seasonal differences in metabolism in the red-sided garter snake, *Thamnphis sirtalis parietalis*. Can J Zool 65:2362–2368.

Dayger C.A. and D.I. Lutterschmidt. 2017. Patterns of stress responses shift during seasonal life-history transitions: an analysis comparing baseline, maximal and integrated corticosterone in female red-sided garter snakes *(Thamnophis sirtalis parietalis)*. Gen Comp Endocrinol 246:29–36.

De Block M.D. and R. Stoks. 2005. Fitness effects from egg to reproduction: bridging the life history transition. Ecology 86:185–197.

Demas G.E. and R.J. Nelson. 1998. Photoperiod, ambient temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). J Biol Rhythm 13:253–262.

Deuel H.J., Jr., J.S. Butts, L.F. Hallman, S. Murray, and H. Blunden. 1937. The sexual variation in carbohydrate metabolism. IX. The effect of age on the sex difference in the content of liver glycogen. J Biol Chem 119:617–620.

Dieudonné M.N., R. Pecquery, A. Boumediene, M.C. Leneveu, and Y. Giudicelli. 1998. Androgen receptors in human preadipocytes and adipocytes: regional specificities and regulation by sex steroids. Am J Physiol 274:C1645–C1652.

Dingle H. 2014. Physiology of migration. Pp. 96–116 in Migration: the biology of life on the move. 2nd ed. Oxford University Press, Oxford.

Doughty P. and R. Shine. 1997. Detecting life history tradeoffs: measuring energy stores in "capital" breeders reveals costs of reproduction. Oecologia 110:508–513.

———. 1998. Reproductive energy allocation and long-term energy stores in a viviparious lizard (*Eulamprus tympanum*). Ecology 79:1073–1083.

Duffitt A.D. and M.S. Finkler. 2011. Sex-related differences in somatic stored energy of *Pseudacris crucifer* and *Pseudacris triseriata* during the early breeding season. J Herpetol 45:224–229.

Dupoué A. and O. Lourdais. 2014. Relative reproductive effort drives metabolic changes and maternal emaciation during pregnancy in a viviparous snake. J Zool 293:49–56.

Falk B.G., R.W. Snow, and R.N. Reed. 2017. A validation of 11 body-condition indices in a giant snake species that exhibits positive allometry. PLoS ONE 12:e0180791. https://doi.org/10.1371/journal.pone.0180791.

Farley S.D. and C.T. Robbins. 1995. Lactation, hibernation, and mass dynamics of American black bears and grizzly bears. Can J Zool 73:2216–2222.

Fletcher Q.E., C. Selman, S. Boutin, A.G. McAdam, S.B. Woods, A.Y. Seo, C. Leeuwenburgh, J.R. Speakman, and M.M. Humphries. 2013. Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. Evol Int J Org Evol 67:1527–1536.

Freitas M.B., L.S. Goulart, M.S. Narros, D.B. Morais, T.S. Amaral, and S.L.P Matta. 2010. Energy metabolism and fasting male and female insectivorous bats *Molosssus molossus* (Chiroptera: Molossidae). Braz J Biol 70:617–621.

Green A.J. 2001. Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82:1473–1483.

Grego K.F., C.H. Gardiner, and J.L. Catão-Dias. 2004. Comparative pathology of parasitic infections in free-ranging and captive pit-vipers (*Bothrops jararac*). Vet Rec 154:559–562.

Gregory P.T. 1977. Life-history parameters of the red-sided garter snakes (*Thamnophis sirtalis parietalis*) in an extreme environment, the Interlake region of Manitoba. Nat Mus Can Publ Zool 13:1–44.

Gregory P.T. and K.W. Stewart. 1975. Long-distance dispersal and feeding strategy of the red-sided garter snake (*Thamnophis sirtalis parietalis*) in the Interlake of Manitoba. Can J Zool. 53:238–245.

Groscolas R., A. Lacroix, and J.P. Robin. 2008. Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin? Horm Behav 53:51–60.

Gustavsson C., K. Yassin, E. Wahlström, L. Cheung, J. Lindberg, K. Brismar, C. Östenson, G. Norstedt, and P. Tollet-Egnell. 2010. Sex-different hepatic glycogen content and glucose output in rats. BMC Biochem 11:38–54.

Hayes J. and J. Shonkwiler. 2001. Morphometric indicators of body condition: worthwhile or wishful thinking? Pp. 8–38 in J.R. Speakmen, ed. Body composition analysis of animals: a handbook of non-destructive methods. Cambridge University Press, Cambridge.

Jenni-Eiermann S. 2017. Energy metabolism during endurance flight and the post-flight recovery phase. J Comp Physiol A 203:431–438.

Jeong S., H.K. Choi, and M. Yoon. 2007. Morphological changes in adipose and liver tissues by 17β-estradiol in female ovariectomized C57BL/6J mice. Biomed Sci Lett 13:99–104.

Jouventin P. and F.S. Dobson. 2002. Why breed every other year? the case of albatrosses. Proc R Soc B 269:1955–1961.

Ketterson E.D., V. Nolan, L. Wolf, C. Ziegenfus, A.M. Dufty, G.F. Ball, and S.T. Johnsen. 1991. Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. Horm Behav 25:489–503.

Kirk K.L. 1997. Life-history responses to variable environments: starvation and reproduction in planktonic rotifers. Ecology 78:434–441.

Krohmer R.W., M. Grassman, and D. Crews. 1987. Annual reproductive cycle in the male red-sided garter snake, *Thamnophis sirtalis parietalis*: field and laboratory studies. Gen Comp Endocrinol 68:64–75.

Labocha M.K., H. Schutz, and J.P. Hayes. 2013. Which body condition index is best? Oikos 123:111–119.

Lacy E.L., M.A. Sheridan, and M.C. Moore. 2002. Sex differences in lipid metabolism during reproduction in free-living tree lizards (*Urosaurus ornatus*). Gen Comp Endocrinol 128:180–192.

Larsen D.A., B.R. Bechman, and W.W. Dickhoff. 2001. The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I, and thyroxine) of Coho Salmon, *Onocorhynchus kisutch*. Gen Comp Endocrinol 123:308–323.

Lourdais O., S. Lorioux, and D.F. DeNardo. 2013. Structural and performance costs of reproduction in a pure capital breeder, the children's python *Antaresia childreni*. Physiol Biochem Zool 86:176–183.

Lutterschmit D.I. 2012. Chronobiology of reproduction in garter snakes: neuroendocrine mechanisms and geographic variation. Gen Comp Endocrinol 176:448–455.

Lutterschmidt D.I. and R.T. Mason. 2009. Endocrine mechanisms mediating temperature-induced reproductive behavior in red-sided garter snakes (*Thamnophis sirtalis parietalis*). J Exp Biol 212:3108–3118.

Maine A.R., C. Dayger, D.Y. Richards, L.M. Ramierz, and D.I. Lutterschmidt. 2014. Migration to summer feeding grounds is associated with changes in plasma glucocorticoids and glucose in red-sided garter snakes (*Thamnophis sirtalis parietalis*). Soc Integr Comp Biol P3.30.

Mayes J.S. and G.H. Watson. 2004. Direct effects of sex steroid hormones in adipose tissues and obesity. Obes Rev 5:197–216.

McBride R.S., S. Somarakis, G.R. Fitzhugh, A. Albert, N.A. Yaragina, M.J. Wuenschel, A. Alonso-Fernández, and G. Basilone. 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish Fish 16:23–57.

McCann M.J. and D.K. Padilla. 2015. Effects of a patchy food environment across life history stages. J Exp Mar Biol Ecol 472:135–141.

McWilliams S.R., C. Guglielmo, B. Pierce, and M. Klaasen. 2004. Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J Avian Biol 35:377–393.

Mead J.R., S.A. Irvine, and D.P. Ramji. 2002. Lipoprotein lipase: structure, function, regulation, and role in disease. J Mol Med 80:753–769.

Mizutani T., Y. Nishikawa, H. Adachi, T. Enomoto, H. Ikegami, H. Kurachi, T. Nomure, and A. Miyake. 1994. Identification of estrogen receptor in human adipose tissue and adipocytes. J Clin Endocrinol Metab 78:950–954.

Moore I.T., M.P. LeMaster, and R.T. Mason. 2000. Behavioral and hormonal responses to capture stress in male red-sided garter snake, *Thamnophis sirtalis parietalis*. Anim Behav 59:529–534.

Moore I.T. and R.T. Mason. 2001. Behavioral and hormonal responses to corticosterone in male red-sided garter snakes, *Thamnophis sirtalis parietalis*. Physiol Behav 72:669–674.

Muir T.J., B.D. Dishong, R.E. Lee, and J.P. Costanzo. 2013. Energy use and management of energy reserves in hatchling turtles (*Chrysemys picta*) exposed to variable winter conditions. J Therm Biol 38:324–330.

Niva C.C. and M. Takeda. 2003. Effects of photoperiod, temperature and melatonin on nymphal development, polyphenism and reproduction in *Halyomorpha halys* (Heteroptera: Pentatomidae). Zool Sci 20:963–970.

O'Brien S.N., B.H. Welter, K.A. Mantzke, and T.M. Price. 1998. Identification of progesterone receptor in human subcutaneous adipose tissue. J Clin Endocrinol Metab 83:509–513.

Ozaki Y., R.J. Wurtman, R. Alonso, and H.J. Lynch. 1978. Melatonin secretion decreases during the proestrous stage of the rat estrous cycle. Proc Natl Acad Sci USA 75:531–534.

Peig J. and A. Green. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–1891.

Pinter A.J. and N.C. Negus. 1965. Effects of nutrition and photoperiod on reproductive physiology of *Microtus montanus*. Am J Physiol 208:633–638.

Rynders C.A., S.L. Schmidt, A. Bergouignan, T.J. Horton, and D.H. Bessesen. 2018. Effects of short-term sex steroid suppression on dietary fat storage patterns in healthy males. Physiol Rep 6:e13533.

San Martin M. and Y. Touitou. 2000. DHEA-sulfate causes a phase-dependent increase in melatonin secretion: a study of perfused rat pineal glands. Steroids 65:491–496.

Shi Y., X. Liu, H. Zhang, Y. Zhang, D. Lu, and H. Lin. 2012. Molecular identification of an androgen receptor and its changes in mRNA levels during 17α-methyltestosterone-induced sex reversal in the orange-spotted grouper *Epinephelus coioides*. Comp Biochem Physiol B 163:43–50.

Shine R., M.J. Elphick, P.S. Harlow, I.T. Moore, M.P. LeMaster, R.T. Mason and A.H. Price. 2001. Movements, mating, and dispersal of red-sided garter snakes (*Thamnophis sirtalis parietalis*) from a communal den in Manitoba. Copeia 2001:82–91.

Shine R. and R.T. Mason. 2005. Do a male garter snake's energy stores limit his reproductive effort? Can J Zool 83:1265–1270.

Smith C.L. 1950. Seasonal changes in blood sugar, fat body, liver glycogen, and gonads in the common frog, *Rana temporaria*. J Exp Biol 26:412–429.

Smith R.J. and F.R. Moore. 2003. Arrival fat and reproductive performance in a long-distance passerine migrant. Oecologia 134:325–331.

Stallings C.D., F.C. Coleman, C.C. Koenig, and D.A. Markiewicz. 2010. Energy allocation in juveniles of a warm-temperate reef fish. Environ Biol Fishes 88:389–398.

Tudorache C., R. Blust, and G. De Boeck. 2007. Swimming capacity and energetics of migrating and non-migrating morphs of three-spined stickleback *Gasterosteus aculeatus* L. and their ecological implications. J Fish Biol 71:1448–1456.

Uhrig E. 2015. Reproductive implications of parasitic infections and immune challenges in garter snakes. PhD diss. Oregon State University, Corvallis.

Valencak T., A. Osterrieder, and T.J. Schulz. 2017. Sex matters: the effects of biological sex on adipose tissue biology and energy metabolism. Redox Biol 12:806–813.

Waye H.L. and R.T. Mason. 2008. A combination of body condition measurements is more informative than conventional condition indices: temporal variation in body condition and corticosterone in brown tree snakes (*Boiga irregularis*). Gen Comp Endocrinol 155:607–612.

Weber J.M. 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. J Exp Biol 212:593–597.

Wells J.C.K. 2007. Sexual dimorphism of body composition. Best Pract Res Clin Endocrinol Metab 21:415–430.

Whittier J.M., R.T. Mason, and D. Crews. 1987. Plasma steroid hormone levels of female red-sided garter snakes (*Thamnophis sirtalis parietalis*); relationship to mating and gestation. Gen Comp Endocrinol 67:33–43.

Yao Y., H. Ma, K. Wu, Y. Shao, W. Han, Z. Cai, N. Xu, M. Qi, C. Zhao, and C. Wu. 2018. Body composition, serum lipid levels, and transcriptomic characterization in the adipose tissue of male pigs in response to sex hormone deficiency. Gene 646:74–82.

Zajac D.M., D.J. Cerasale, S. Landman, and C.G. Guglielmo. 2011. Behavioral and physiology effects of photoperoid-induced migratory state and leptin on *Zonotrichia albicollis*. II. Effects of fatty acid metabolism. Gen Comp Endocrinol 174:269–275.