Seasonal Changes in the Physiology of Male Virginia Opossums (*Didelphis virginiana*): Signs of the Dasyurid Semelparity Syndrome?

Henri A. Woods II[1,2]
Eric C. Hellgren[2,*]
[1]Oklahoma Cooperative Fish and Wildlife Research Unit, United States Geological Survey, Biological Resources Division, Oklahoma State University, Stillwater, Oklahoma 74078; [2]Department of Zoology, Oklahoma State University, Stillwater, Oklahoma 74078

Accepted 12/11/02

ABSTRACT

Semelparity, which is multiplying once in a lifetime, is a rare reproductive strategy among mammals. Several species of the marsupial family Dasyuridae experience 100% male mortality following an intense mating period. We investigated seasonal physiological changes that may be associated with early mortality in the male Virginia opossum (*Didelphis virginiana*; Didelphidae) and compared these changes with those of semelparous, male dasyurids. Free-ranging male Virginia opossums ($n = 36$) were collected during 2001 at the Oklahoma State University Cross Timbers Experimental Range. Seasonal data were collected on hematological, morphological, and helminth parameters of these individuals. We used one-way ANOVA to determine whether there were seasonal differences among means for each parameter. It appeared that male Virginia opossums experienced some physiological changes similar to those of male dasyurids exhibiting semelparity. All males collected in summer (August) were juveniles of the year. Lack of adult males in August suggests high mortality of this cohort during the breeding season. Opossum characteristics exhibiting the dasyurid semelparity syndrome included packed cell volume, adrenal mass, and helminth numbers. Minor lymphocytopenia, neutrophilia, and testosterone concentrations also were similar to semelparous dasyurids. However, a lack of change in serum cortisol concentration and body mass and dynamics in immunoglobulin protein, serum protein, and testes mass were not consistent with previous reports of semelparous dasyurid physiology. Evolutionary divergence and differences in breeding behavior between dasyurids and didelphids may be responsible for the lack of consistency between the taxa.

* Corresponding author. Present address: 430 Life Sciences West, Oklahoma State University, Stillwater, Oklahoma 74078-3052; e-mail: ehellgr@okstate.edu.

Physiological and Biochemical Zoology 76(3):406–417. 2003. 1522-2152/2003/7603-2080$15.00

Introduction

Semelparity, defined by Cole (1954, p. 118) as "multiplying once in a lifetime," is a reproductive strategy rarely present in mammals but found in plants, insects, and fish. Semelparity is found in animals that face low or variable probabilities of survival as adults (Roff 1992; Stearns 1992) and/or if juvenile survival is consistently higher in one season (Braithwaite and Lee 1979). Several marsupial species in the Dasyuridae family of Australia exhibit semelparity, experiencing 100% adult male mortality following an intense mating period (Lee and Cockburn 1985). Currently, seven species of *Antechinus* are known to demonstrate semelparity: *A. stuartii* (Braithwaite and Lee 1979), *A. minimus* (Wilson and Bourne 1984), *A. swainsonii* (Bradley and Monamy 1990), *A. flavipes* (Inns 1976), *A. bellus* (Woolley 1981), *A. agilis* (Shimmin et al. 2000), and *A. leo* (Leung 1999). Other members of the Dasyuridae family displaying this life-history trait are *Parantechinus apicalis* (Dickman and Braithwaite 1992), *Dasyurus hallucatus* (Dickman and Braithwaite 1992), *Phascogale tapoatafa* (Wilson and Bourne 1984), and *Phascogale calura* (Bradley 1987). The syndrome does not include females, which can survive to breed a second year or more (Woolley 1966; Wood 1970; Bradley 1997). In these species, adult males are not present in the population during the time interval from complete postmating male mortality to when new male offspring are weaned and independent. The length of this interval is geographic and species specific (Wood 1970; Braithwaite 1979; McDonald et al. 1981; Bradley 1987; Dickman and Braithwaite 1992; Watt 1997).

Research into the cause of male mortality in these dasyurids revealed a sequence of events initiated with an increased concentration of plasma testosterone at the beginning of the mating season (Table 1). The testosterone increase is possibly stimulated by pheromones in male urine (Bradley et al. 1980; Millis et al. 1999; Toftegaard et al. 2002). As the concentration of testosterone rises, an unknown threshold is reached that results in a reduced concentration of corticosteroid-binding globulin

Table 1: Physiological, morphological, and immunological changes observed in dasyurids exhibiting semelparity

	Changes during Mating Season		
Parameter	Increase	Decrease	No Change
Body mass	7	1, 2, 3, 4, 10, 18, 19, 20	16
Testes mass	…	1	…
Adrenal mass	4	…	9
Spleen mass	…	14	8
Nitrogen balance	…	3	…
Cortisol concentration	5, 6, 9, 11, 14, 15, 20	11	4, 8
Androgen concentration	9, 11, 12, 14, 17, 18, 20	…	…
ACTH concentration	9, 15	…	…
CBG concentration	…	6, 9, 11, 14	…
Hematology	…	7, 17, 20	16
Parasites	9, 17, 20	…	8
Immunocompetence	9	14	…
Hemorrhaging	Yes: 7, 8, 14	No: 17	…

Sources. (1) Woolley 1966; (2) Wood 1970; (3) Woollard 1971; (4) Barnett 1973; (5) Bradley et al. 1975; (6) Bradley et al. 1976; (7) Cheal et al. 1976; (8) Barker et al. 1978; (9) Bradley et al. 1980; (10) Inns 1976; (11) McDonald et al. 1981; (12) Wilson and Bourne 1984; (13) McDonald et al. 1986; (14) Bradley 1987; (15) Bradley 1990*a*; (16) Bradley 1990*b*; (17) Oakwood et al. 2001; (18) Millis et al. 1999; (19) Bradley 1997; (20) Schmitt and Bradley 1989.

Note. ACTH = adrenocorticotropic hormone, CBG = corticosteroid-binding globulin.

(CBG; Bradley et al. 1976; McDonald et al. 1981). Due to loss of CBG and impaired negative feedback of the pituitary-adrenal axis (McDonald et al. 1986; Bradley 1990*a*), free corticosteroid concentrations rise. Because cortisol is the dominant corticosteroid in marsupials (Johnston et al. 1967), its concentration increases (Barnett 1973).

Cortisol is a glucocorticoid produced by the adrenal cortex that is important for dealing with stress. In mammals, cortisol causes increases in blood glucose concentrations through the process of gluconeogenesis, inhibition of glucose uptake, and lipolysis (Sherwood 2001). Chronic effects of cortisol include immunosuppression, lympholysis, reduced phagocytic and killing ability of macrophages and neutrophils, and reduction in spleen mass (Goldsby et al. 2000). If chronic supraphysiological levels persist, gastric ulceration, high blood pressure, and atherosclerosis can result (Sherwood 2001).

Physiological and morphological changes consistent with increased cortisol concentrations in semelparous, male dasyurids include enlarged adrenal glands (Barnett 1973), reduced spleen mass (Bradley 1987), reduced body mass, and negative nitrogen balance (Woolley 1966; Wood 1970; Woollard 1971; Barnett 1973; Inns 1976; Table 1). Changes in blood chemistry of semelparous, male dasyurids associated with increased cortisol included reduction in serum immunoglobulins (Bradley et al. 1980), lymphocytopenia and neutrophilia (Cheal et al. 1976; Bradley 1990*b*; Bradley and Monamy 1990), and decreased hematocrit (Cheal et al. 1976; Table 1). Postmortem examination of male *Antechinus* also revealed increased endo- and ectoparasites, gastric ulcer hemorrhaging, and anemia (Table 1; Barker et al. 1978; Bradley et al. 1980; Dickman and Braithwaite 1992; Oakwood et al. 2001). These symptoms could be replicated with injections of cortisol acetate (Bradley et al. 1980).

The Virginia opossum (*Didelphis virginiana*; Didelphidae), the only marsupial in North America, is distributed throughout most of the United States east of Colorado and in some small areas along the west coast (Gardner 1982; Seidensticker et al. 1987). Cockburn (1997) described Virginia opossums as semelparous because few live as long as 2 yr; therefore, males usually only participate in one year of two mating seasons. Many investigators have reported annual mortality of opossums to be 90%–100% (Lay 1942; Petrides 1949; Verts 1963; Llewellyn and Dale 1964; Gillette 1980; Seidensticker et al. 1987; Austad 1993; Gehrt et al. 1997). In addition, after >12,000 trap nights of effort on our study area, we have never captured an adult male opossum in two different years (Levesque 2001; Kasparian 2002). Jurgelski and Porter (1974) reported that the effective reproductive life of female opossums in captivity is 1 yr, although captive animals can survive up to 4.5 yr.

Mating seasons of opossums in the south central United States have been described in Missouri, Texas, Oklahoma, and Louisiana (Reynolds 1945; Gardner 1982; Levesque 2001; Kasparian 2002). Depending on yearly environmental stochasticity, late January is the beginning of the first mating season, with a peak in late February. The second season begins in late April and peaks in late May. Following a 13-d gestation (Gardner 1982), opossums are weaned after 100 d, with males reaching sexual maturity at approximately 8 mo (Biggers 1966) and females at approximately 6 mo (Reynolds 1952). In early winter,

male opossum testes decrease in mass and begin to produce sperm at the same time as testosterone concentrations begin to rise (Chase 1939; Biggers 1966; Winegarner 1982; Harder and Fleming 1986).

The Virginia opossum has been used for years as a research model in immunology (Taylor and Burrell 1968; review by Jurgelski 1974). However, immunological and physiological data on free-ranging Virginia opossums are scarce, and there is no previous work on seasonal variation. Studies have described levels of total serum protein (Rowlands and Dudley 1969) and hematological characteristics (Youatt et al. 1961; Mays and Loew 1968; Timmons and Marques 1969; Giacometti et al. 1972; Cutts and Krause 1980), but animals in these studies were laboratory maintained and may not reflect physiology of free-ranging populations. Other researchers have recorded data regarding immunoglobulin classes (Bell 1977), complement hemolytic activity (Ish et al. 1993), and spleen mass (Cutts and Krause 1982) but did not measure these parameters in free-ranging opossums, nor at different times of the year. Gandolfi and Culbertson (1983) described a single account of lymphocytopenia and neutrophilia by administration of cortisol acetate to a captive injured opossum in a veterinary clinic. Research regarding digestive tract helminths in Virginia opossums has been restricted to surveys of species, with no investigation of helminth effects on opossum physiology. The dominant stomach helminth of the Virginia opossum is *Physaloptera turgida* (Alden 1995).

We attempted to provide insight into seasonal physiological changes that may be associated with early mortality in the male opossum. Our working hypothesis was that Virginia opossums are semelparous, as proposed by Cockburn (1997). Therefore, we predicted that we would observe concordance for the suite of physiological measures that characterize semelparity in dasyurids with those measures in male Virginia opossums.

Material and Methods

Study Area

The Cross Timbers ecoregion, dominated by oak (*Quercus*) forest interspersed with tallgrass prairie and invaded by eastern red cedar (*Juniperus virginiana*), covers large parts of central Oklahoma and Texas. Livestock grazing is the primary economic use of the region because the area produces few economically valuable timber products (Stritzke et al. 1991). The Cross Timbers Experimental Range (CTER), located 11 km southwest of Stillwater, Payne County, Oklahoma (36°02′40″–36°04′20″N, 97°09′30″–97°11′39″W), encompasses 712 ha. The overstory is dominated by post oak (*Quercus stellata*), blackjack oak (*Quercus marilandica*), and American elm (*Ulmus americana*) interspersed with eastern red cedar. Little bluestem (*Schizachyrium scoparium*), indiangrass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), grama grasses (*Bouteloua*), purpletop (*Tridens flavus*), ragweed (*Ambrosia*), and buckbrush (*Symphoricarpos orbiculatus*) are prevalent in the understory (Ewing et al. 1984). Beginning in 1983, combinations of prescribed fire and herbicides were applied to CTER to produce a mosaic of vegetation types. We investigated areas on CTER representative of all four major vegetation types, which were characterized as cedar forest, oak forest, grassland, and mixed brush.

Experimental Animals

Free-ranging male Virginia opossums were collected using Tomahawk wire mesh traps (25 × 31 × 81 cm) during trapping sessions in February, May, August, and November of 2001. Seven grids of eight traps each were sampled for a 10-d period during each sampling month. Traps were baited with sardines and checked daily. Traps within grids were placed in two parallel rows 300 m apart, consisting of three traps spaced at 200-m intervals. The other two traps in the grid were placed 200 m apart between the two rows.

Trapped males were anesthetized with a cocktail of ketamine hydrochloride (15 mg/kg) and xylazine (7.5 mg/kg), or Telazol (8 mg/kg). Once immobilized, body mass was recorded followed by collection of blood via cardiac puncture. Blood was collected in two Vacutainer tubes (Becton Dickinson, Franklin Lakes, N.J.) with EDTA and no additive, respectively. After blood was collected, the opossum was killed with Beuthanasia-D Special (80 mg/kg pentobarbital and 10 mg/kg phenytoin sodium; Schering-Plough Animal Health Corporation, Union, N.J.) and returned to the laboratory within 4 h. Procedures for opossum collection and handling followed Institutional Animal Care and Use Committee protocol AS-50-719 at Oklahoma State University.

Hematology

Red blood cell (RBC) and white blood cell (WBC) concentrations were determined on blood collected in the EDTA Vacutainer using a hemacytometer and Hayem's solution (RBC) or acetic acid (WBC). A heparinized, microcapillary tube and microcapillary centrifuge were used to determine packed cell volume (PCV). Mean corpuscular volume (MCV) was calculated from PCV and RBC values. Serum was isolated after placing the no-additive Vacutainer in a centrifuge at 2,500 rpm (1,250 *g*) for 8 min at 8°C. Serum was stored in microcentrifuge tubes at −70°C until appropriate assays were initiated. A smear was prepared for WBC differential counts. Diff-Quik (Baxter Healthcare, McGaw Park, Ill.) was used to stain whole blood smears. Differential WBC counts were conducted on the first 100 WBCs of a blood smear (1,000 × magnification, oil immersion lens).

Morphology and Helminths

Following collection of blood, spleen, testes, and adrenal glands were removed and weighed. Because of difficulty in locating the right adrenal (found on the dorsal side of the descending aorta) during our first sampling season, we only report data on mass of the left adrenal. The opossum was placed in a 4°C refrigerator overnight to allow helminths to detach. The next day, the gastrointestinal tract was excised, then helminths were removed and fixed in alcohol-formalin–acetic acid (AFA) for later identification (Ackerson 1992) and enumeration.

Serum Chemistry, Immunology, and Endocrinology

A microplate colorimetric method using the biuret method as described in Kingsley (1942) was used to measure total serum protein. A total of 210 μL of biuret working reagent was added to 20 μL of sample serum or 20 μL of a bovine serum albumin standard. Following a 15-min incubation at room temperature (RT), the absorbance of samples and standards were read at 550 nm. A modified protocol of Bradford's (1976) ammonium precipitation assay was used to ascertain serum immunoglobulin levels. A 0.2-mL volume of serum was added to 4.8 mL of ammonium sulfate–sodium chloride reagent and centrifuged for 30 min at 2,500 rpm (1,250 *g*) at RT. The supernatant was decanted, then the pellet was resuspended in 2.5 mL of 0.85% NaCl. A 5-μL sample of the resuspended solution was added to a microplate well, and 25 μL of reagent A and 200 μL of reagent B were added to all standard and sample wells as instructed in the DC Protein Assay kit (Biorad, Hercules, Calif.). After a 15-min incubation, plates were read at 750 nm using a bovine serum albumin standard.

We used the Davis et al. (1995) modification of De Waal et al.'s (1988) technique to assess hemolytic complement activity. Serial twofold dilutions (1/16–1/2048) of serum were placed in a 96-well plate. A 25-μL volume of 0.6% sheep red blood cells (SRBC; Colorado Serum, Denver, Colo.) in veronal buffered saline (VBS), modified for Virginia opossum complement activity (Wirtz and Westfall 1967), and 25 μL of a 1 : 80 dilution of rabbit anti-SRBC antibodies (Nordic Immunology, Tilburg, Netherlands) in VBS were added to each well. Incubation of plates occurred at 37°C for 1.5 h. Following the incubation, the plates were spun at 1,200 rpm (300 *g*), and 60 μL of supernatant was transferred to a replicate plate. Absorbances of the supernatant at 414 nm were recorded for each known standard and sample. Using a standard curve of known amounts of lysed SRBCs, we expressed the hemolytic complement activity as CH_{50} units/mL serum, where 1 CH_{50} was the amount of complement required to lyse 50% of the SRBC (Mayer 1961).

We used a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles) to measure concentrations of total cortisol and testosterone in serum samples. To measure cortisol, a 25-μL sample of serum from each opossum was added to tubes coated with antibodies specific for cortisol. Next, a 1-mL volume of ^{125}I-Cortisol solution was added to compete with the cortisol in the serum sample. An incubation of 1.5 h at 37°C followed the addition of the radiolabelled cortisol. After the incubation, the solution in each tube was aspirated, and tubes were allowed to dry. Once dry, the tubes were placed in a gamma counter. The counts from the samples were then graphed on a standard curve to determine the sample cortisol concentration. We verified parallelism between the standards and a serial dilution of opossum samples. All samples were run in a single assay with each sample and standard run in triplicate. The recovery of cortisol added and intraassay coefficient of variation were 80%–113% and 6.6%, respectively. Cross reactivity of the assay antibody with other corticosteroids was as follows: corticosterone, 0.03%; cortisone, 0.98%; 11-deoxycortisol, 11.4%; aldosterone, 0.02%; other steroids, <1%. Assay sensitivity was 0.2 ng/mL.

We added a 50-μL sample of serum from each opossum to tubes coated with antibodies specific for testosterone. Next, a 1-mL volume of ^{125}I-Testosterone solution was added to compete with the testosterone in the serum sample. An incubation of 3 h at 37°C followed the addition of the radiolabelled testosterone. After the incubation, the solution in each tube was aspirated, and tubes were allowed to dry. Once dry, the tubes were placed in a gamma counter. The counts from the samples were then graphed on a standard curve to determine the sample testosterone concentration. We verified parallelism between the standards and a serial dilution of opossum samples. All samples were run in a single assay with each sample run in duplicate and each standard run in triplicate. The recovery of testosterone added and intraassay coefficient of variation were 98%–116% and 3.3%, respectively. Cross reactivity of the assay antibody with other steroids was as follows: 5-α-dihydrotestosterone, 3.3%; 19-nortestosterone, 20%; 4-estren-17-ol-3-one, 20%; all other steroids listed, <2%. Assay sensitivity was 0.04 ng/mL.

Statistical Analysis

All data were first tested for normality using PROC UNIVARIATE in SAS 8.2 (SAS Institute 2001). If the data were found to be nonparametric, then ranks were assigned to the original data using PROC RANK (SAS Institute 2001), followed by a one-way ANOVA of the ranks using PROC MIXED (SAS Institute 2001). If the data were found to be parametric, an ANOVA was conducted on the original data. Both parametric and nonparametric analyses used season as the main factor.

Means and standard errors per trapping season for PCV, RBC concentration, MCV, WBC concentration, cortisol concentration, testosterone concentration, serum complement activity, immunoglobulin concentration, total serum protein concentration, helminths per individual, testes mass, left adrenal mass, spleen mass, and WBC differentials were calculated using Proc Means (SAS Institute 2001). $\log_{10}$(spleen mass), $\log_{10}$(total tes-

Table 2: Physiological and morphological characteristics of male Virginia opossums (*Didelphis virginiana*) over three seasons from Cross Timbers Experimental Range, Payne County, Oklahoma, 2001

	February			May			November			ANOVA	
Parameter	*n*	$\overline{X}$	SE	*n*	$\overline{X}$	SE	*n*	$\overline{X}$	SE	*F*	*P*
White blood cells (10^3/mm^3)	11	14.8	2.1	5	12.3	2.3	8	12.0	1.8	.58	.57
Red blood cells (10^6/mm^3)	12	4.49	.21	5	4.22	.77	8	4.74	.17	.47	.63
Mean corpuscular volume (μm^3)[a]	12	71.2^{B}	1.1	5	66.4^{B}	5.8	8	77.1^{A}	2.1	3.58	.04
Packed cell volume (%)[a]	12	32^{B}	1	5	26^{C}	3	8	36^{A}	1	8.56	<.01
Lymphocytes (%)	11	18.5^{B}	2.4	7	24.8^{A,B}	5.4	8	35.6^{A}	4.8	4.92	.02
Neutrophils (%)	11	45.5^{A,B}	4.9	7	56.1^{A}	6.6	8	30.0^{B}	7.4	3.99	.03
Eosinophils (%)[a]	11	2.9	1.2	7	1.7	.8	8	3.3	1.3	.48	.62
Monocytes (%)	11	33.1^{A}	4.3	7	17.3^{B}	1.8	8	31.0^{A}	4.5	4.01	.03
Total protein (g/dL)[a]	12	5.57	.14	7	5.67	.24	8	5.67	.05	.84	.44
Immunoglobulin (g/dL)	12	.68^{A}	.04	7	.83^{A}	.09	8	.51^{B}	.04	7.34	<.01
Complement (CH_{50}/mL)[a]	12	36^{B}	12	7	16^{B}	0	8	60^{A}	4	14.35	<.01
Cortisol (ng/mL)[a]	12	2.60	.59	7	1.96	.6	8	2.21	.64	.31	.74
Testosterone (ng/mL)[a]	12	1.81	.77	6	1.59	.69	8	4.34	1.31	1.74	.20
Body mass (kg)	12	1.7	.1	7	1.7	.1	8	2.0	.2	2.62	.09
Left adrenal mass (g)	11	.168	.01	7	.193	.013	8	.163	.012	3.43[b]	.04[b]
Spleen mass (g)[a]	12	9.927^{A}	2.028	7	8.961^{A}	1.205	8	4.676^{B}	.613	5.61[b]	.01[b]
Testes mass (g)	6	2.939	.272	7	3.249	.166	8	2.562	.200	5.15[b]	.02[b]
Helminths[a]	12	259^{A}	51	7	61^{B}	16	8	29^{B}	7	22.69	<.01

Note. Values with the same uppercase letter are not different ($P > 0.05$).

[a] One-way ANOVA of ranked data.

[b] One-way ANOVA of residuals.

tes mass), and $\log_{10}$(left adrenal mass) were regressed on $\log_{10}$(body mass). Residuals of the regression equation were calculated for each datum point using PROC REG (SAS Institute 2001). Residual data were analyzed the same as the parameters listed above.

We conducted two separate analyses, one with four seasons and another with three seasons only (February, May, and November). Both analyses provided similar results for all parameters except left adrenal mass and testosterone, which will be discussed in "Results." We present results for only the three-season analysis because all collected animals were considered to be adults in those seasons. Only immature young-of-the-year were captured in August. Some samples could not be used in data collection. For example, some blood samples in February and May were not included because of broken sample tubes, poor bloodsmear, or cell lysis after collection. Therefore, sample sizes for the appropriate parameter were adjusted accordingly. Data analysis included pairwise comparisons of seasonal means or ranks using least significant difference. Two testosterone concentration values (19.68 ng/mL in February and 10.89 ng/mL in August) were removed from analysis because they were extreme outliers according to box plot analysis (Mann 1998). Both values were greater than three times the interquartile range. Therefore, for testosterone analyses, February had a sample size of six for the three- and four-season analyses, and August had a sample size of eight for the four-season analysis.

Results

Trapping

During 2,222 trap nights, we collected 36 males (12 in February, seven in May, nine in August, and eight in November). We also captured 47 females (seven in February, eight in May, 13 in August, and 19 in November). Males captured in August were smaller in body mass ($F = 19.96$; df $= 3, 32$; $P < 0.001$; 0.9 ± 0.1 kg; $n = 9$) than males captured in other seasons (1.8 ± 0.1 kg; $n = 27$). No males captured in August weighed more than 1.2 kg. All males trapped in August were assumed to be juveniles from the spring 2001 cohort.

Hematology

Lymphocytes, neutrophils, monocytes, MCV, and PCV varied seasonally (Table 2). Mean PCV and MCV were minimal during May. During May and February, percentage lymphocytes reached a minimum, whereas percentage neutrophils peaked in February and May (Table 2). The season with the lowest mean percentage monocytes was May. RBC concentration did not vary seasonally but was smallest in May (Table 2). He-

matological results from analysis conducted including data from the August trapping season provided similar results.

Morphology and Helminths

Residuals (log[*g*]) for left adrenal mass varied seasonally in the three-season analysis ($F = 3.43$; df $= 2, 23$; $P = 0.04$), with residuals largest in May (May $= 0.053 \pm 0.033$; February $= -0.001 \pm 0.022$; November $= -0.045 \pm 0.018$; Fig. 1*a*). However, in the four-season analysis, left adrenal residuals did not vary seasonally ($F = 1.85$; df $= 3, 31$; $P = 0.15$). Residuals (log[*g*]) for spleen mass varied seasonally ($F = 5.61$; df $= 2, 24$; $P = 0.01$) in the three-season analysis and were smallest in November (November $= -0.221 \pm 0.043$; February $= 0.085 \pm 0.082$; May $= 0.108 \pm 0.069$; Fig. 1*b*). Spleen residuals also varied seasonally in the four-season analysis, with a nadir in November ($F = 5.84$; df $= 3, 32$; $P < 0.01$; -0.231 ± 0.041; Table 2). Testes mass residuals (log[*g*]) varied seasonally in both three- (Table 2) and four-season analyses ($F = 5.97$; df $= 3, 26$; $P < 0.01$). Residuals (*g*) for testes mass were largest in May (0.061 ± 0.023; Fig. 1*c*) and smallest in November (-0.062 ± 0.028; Fig. 1*c*).

Males collected in February had more helminths than males trapped in the other two seasons (Table 2). Identical results were recorded for the four-season analysis ($F = 27.75$; df $= 3, 32$; $P < 0.001$). Thirty-five of 36 males possessed gastric helminths, and all gastric helminths were *Physaloptera turgida*. Other helminths, collected from five males (four in May and one in November), belonged to the genus *Cruzia*. These helminths were located in the caecum and/or large intestine.

Serum Chemistry, Immunology, and Endocrinology

Immunoglobulin protein and complement showed seasonal variation in the three-season analysis, whereas testosterone and cortisol did not (Table 2). Immunoglobulin protein concentration was greatest in May (Table 2), whereas complement activity was lowest in May (Table 2). Serum testosterone varied seasonally ($F = 2.79$; df $= 3, 30$; $P = 0.057$) in the four-season analysis, with testosterone concentrations largest in November. Cortisol concentrations did not vary seasonally in the four-season analysis but were greatest in August (4.43 ± 0.89 ng/mL; $n = 9$). Four-season analyses for other parameters provided similar results as three-season analyses.

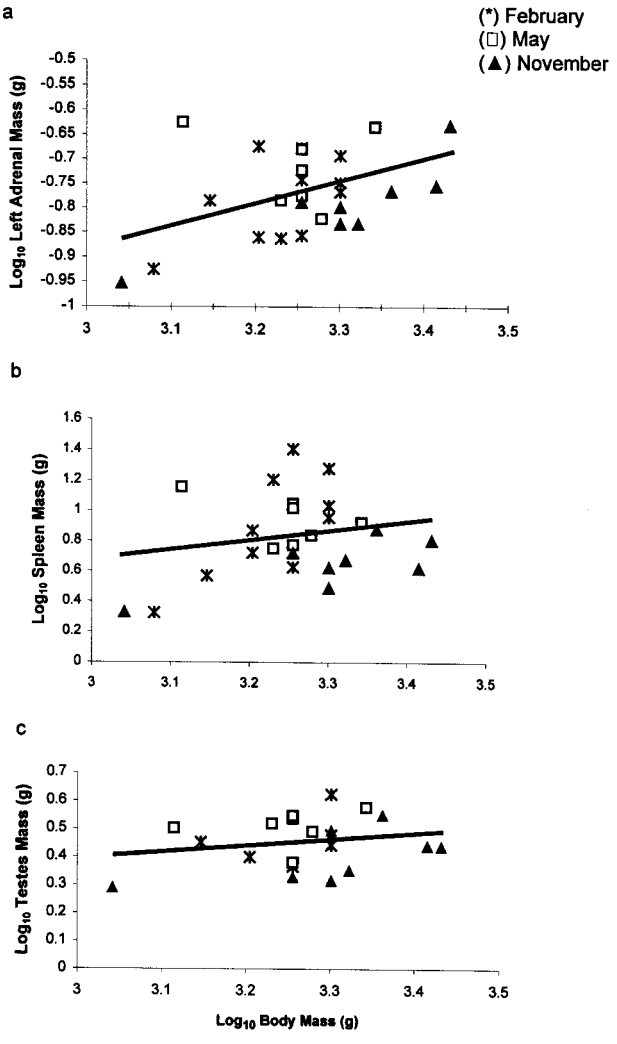

Figure 1. Linear regression of $\log_{10}$ tissue mass on $\log_{10}$ body mass from seasonal data for male Virginia opossums collected at Cross Timbers Experimental Range, Payne County, Oklahoma, 2001. Tissue masses were (*a*) left adrenal ($r^2 = 0.18$), (*b*) spleen ($r^2 = 0.06$), and (*c*) testes ($r^2 = 0.07$).

Discussion

Male Virginia opossums experienced physiological changes similar to those of male dasyurids exhibiting semelparity. Opossum characteristics consistent with the dasyurid semelparity syndrome included adrenal mass, PCV, helminth numbers, and complement activity. Minor lymphocytopenia and neutrophilia during February and May and testosterone concentration during November also were consistent with characteristics of semelparous dasyurids. However, the lack of change in serum cortisol concentration and body mass, along with dynamics in immunoglobulin protein, serum protein, and testes mass, was not consistent with previous reports of dasyurid physiology.

Demographically, the total lack of adult male opossums captured during August and results from two previous studies on the same study area in summer (Levesque 2001; Kasparian 2002) supported the hypothesis of male opossum semelparity. On our study area, sex ratios are female biased during the July–August period after the mating season (Levesque 2001). In 3 yr of trapping preceding this study, the ratio of adult males to females decreased from 1.2 : 1.0 ($n = 62$) in

spring (April–May) to 0.4 : 1.0 ($n = 42$) in summer (July–August). However, we note that it is highly likely in the present study that the absence of adult males in the August sample was influenced by the removal trapping in February and May. Nevertheless, multiple studies have reported low annual survival of adult opossums (Gillette 1980; Seidensticker et al. 1987), with work on our study area showing lower male survival ($s = 0.08$) than female survival ($s = 0.41$) during the breeding season (March–July; Kasparian 2002). We had no evidence that male behavior changed during the year, causing lower capture probabilities in certain seasons. In addition, if males survived and dispersed, emigration should have been balanced by immigration from the surrounding landscape, which was composed of similar habitat.

Adrenal mass residuals were largest in May. Barnett (1973) also reported increased adrenal mass in *Antechinus stuartii* during mating season, although Bradley et al. (1980) did not record a change in adrenal mass in mating season. However, we did not observe a rise in cortisol concentrations during the breeding season in male Virginia opossums, in contrast to results recorded in semelparous male dasyurids (Bradley et al. 1975; Bradley 1987, 1990*a*; Schmitt and Bradley 1989). The lack of increased cortisol in the male opossum is not unprecedented in medium-sized semelparous marsupials. *Dasyurus hallucatus*, a 1-kg marsupial, did not show significant changes in cortisol concentrations during the mating season (Oakwood et al. 2001).

Given the lack of concomitant increases between seasonal cortisol concentration and adrenal mass, it is unclear which index, serum cortisol or adrenal gland weight, is most useful to monitor adrenocortical activity in male opossums. Many influencing factors, such as age, sex, and body size, are important in size variation of the adrenal cortex. These factors are also the source of debate of whether the adrenal mass is a good index of adrenocortical activity in small mammals (reviewed in Lee and McDonald 1985). Acute increases in cortisol concentrations, normally attributed to stress, have been reported in handled and/or trapped mammals (Hellgren et al. 1985; Harlow et al. 1990; Waas et al. 1999; Osadchuk et al. 2001). These rapid changes make it difficult to identify chronic increases in serum concentrations of cortisol. The increase in cortisol for males in August was likely due to evasive activity of opossums in the trap (Dallman and Jones 1973). August was the only month during which males performed such active behavior.

Changes in adrenal mass of mammals during breeding season are species specific. For example, increases in adrenal mass during the breeding season have been reported in male meadow voles (*Microtus pennsylvanicus*; Seabloom et al. 1978); decreases have been reported in male bobcats (*Lynx rufus*; McKinney and Dunbar 1976), male pikas (*Ochotona princeps*; Millar 1970), and male muskrats (*Ondatra zibethica*; Beer and Meyer 1951); and no mass change has been reported in male prairie deer mice (*Peromyscus maniculatus bairdii*; Christian 1967), male white-footed mice (*Peromyscus leucopus noveboracensis*; Christian 1967), male cotton rats (*Sigmodon hispidus*; Goertz 1965), and male golden-mantled ground squirrels (*Spermophilus lateralis lateralis*; Skryja and Clark 1970).

An increase in cortisol concentrations of semelparous, male dasyurids may ensure sufficient glucose concentrations in the blood during the mating season. Male dasyurids spend enormous amounts of resources performing agonistic behavior, locating females or, in the case of *A. stuartii*, possibly lekking (Barnett 1973; Inns 1976; Braithwaite 1979; Lazenby-Cohen and Cockburn 1988). These resource-demanding behaviors can place male dasyurids in negative nitrogen balance (Woollard 1971; Bradley 1997) due to protein catabolism for gluconeogenesis but maximize their time spent in competing for access to mates (Lee and Cockburn 1985). Male Virginia opossums also will seek and defend a female in estrus. If multiple males approach a female in estrus, the largest male usually will win any male-male combat and the opportunity to copulate with the female (Ryser 1992). The large size (2–3 kg) of male Virginia opossums may allow for larger proportions of adipose deposition than smaller dasyurids. Therefore, protein may be less required for gluconeogenesis. In contrast to the loss of body mass by male dasyurids during the mating season (Woolley 1966; Wood 1970; Barnett 1973; Inns 1976; Bradley 1997), male Virginia opossums did not show a decrease in body mass between February and May. Sustained body mass may be a result of males foraging and acquiring energy resources during and between the two long mating seasons. Such a strategy also would be consistent with a model that males having reproductive opportunities spread over an extended breeding season should derive energy from food rather than from protein catabolism for gluconeogenesis (Boonstra and Boag 1992).

An increase in testosterone concentrations before the mating season leads to increased concentrations of cortisol in semelparous, male dasyurids (McDonald et al. 1981; Wilson and Bourne 1984; Millis et al. 1999; Oakwood et al. 2001). Testosterone concentrations in the male Virginia opossum did not increase during the mating season; however, testosterone increased in November before the mating season. November is when sperm production of male Virginia opossums would be expected to begin in Oklahoma (Winegarner 1982). Harder and Fleming (1986) also reported an increased testosterone concentration in November for male opossums. Mean testosterone concentrations for February and May of this study were similar to the mean testosterone concentration from January to June (2.0 ng/mL) reported by Harder and Fleming (1986).

Reduction in testes mass during November is in agreement with Biggers' (1966) report of opossum testes mass but is in disagreement with increased testes mass reported by Woolley (1966). Winegarner (1982) described no seasonal variation in testes mass of male opossums in early winter, the time of sperm production in Florida. However, Winegarner's (1982) conclu-

sion of no seasonal difference in testes mass was founded on testes mass only, not on a ratio or regression of testes mass on body mass.

The decline in PCV observed in May in opossums was associated with a nonsignificant decrease in RBC concentration. *Dasyurus hallucatus* (Oakwood et al. 2001) and *A. stuartii* (Cheal et al. 1976), both dasyurids, also exhibited a significant decrease in PCV during the later portion of their breeding seasons. Authors of the dasyurid literature do not explain the decreased PCV, except for one instance where *Babesia* spp. (a blood parasite) was thought to be the culprit (Cheal et al. 1976). Boonstra et al. (2001) cited laboratory work indicating that glucocorticoids could directly inhibit production of red blood cells. A possible explanation for seasonal PCV variation in male opossums could be the combination of decreased RBC concentration and reduction of MCV.

Minor lymphocytopenia and neutrophilia during both mating seasons (February and May) in opossums were consistent with results observed during the mating season of semelparous dasyurids (Cheal et al. 1976; Bradley 1990*b*). Previously, neutrophilia and lymphocytopenia had been recorded only in an opossum repeatedly injected with cortisol acetate (Gandolfi and Culbertson 1983). In this article, we report a 2 : 1 ratio of neutrophils to lymphocytes during the mating months of opossums. Boonstra et al. (2001) also reported a 2 : 1 ratio of neutrophils to lymphocytes during the breeding season for male arctic ground squirrels. Low lymphocyte concentrations in male opossums may be a result of age and immunosenescence. Reports in mice and humans have shown decreases in populations of T-cells with age, possibly caused by involution of the thymus (Hirokawa 1992; Miller 1996; McFarlane et al. 2001). However, because captive opossums may live for several years, it seems more likely that chronic high concentrations of cortisol (as indexed by increased adrenal mass) could be responsible for the decreased percentage lymphocytes. The mean percentage of lymphocytes in August (55.8) was similar to that reported by Cutts and Krause (1980; 58.5) in juvenile opossums.

The relationship between mating season and increased helminth populations observed in male *A. stuartii* (Bradley et al. 1980) was replicated in male opossums in February but not May. Blumenthal and Kirkland (1976) reported *Physaloptera turgida* in the stomach of Virginia opossums from Pennsylvania but did not find seasonal variation in burdens of *P. turgida*. *Physaloptera turgida* uses arthropods as an intermediate host and can use frogs, snakes, and small mammals as paratenic hosts (Anderson 1988). Arthropods and small mammals are important components of the opossum's diet during winter months (Kasparian 2002). A large proportion of worms during February was small adults or juveniles. During the other three trapping seasons, most worms were large adults. The decrease in helminth numbers from February to May could be a reflection of diet change. Between February and May, worms excreted in the feces will not be replaced if opossums are not consuming the intermediate or paratenic host of *P. turgida*.

Our observations of increased immunoglobulin protein and serum protein seem to conflict with the decreased complement activity of male opossums during May. Immunoglobulins are used in initiation of the complement cascade (Goldsby et al. 2000); therefore, if immunoglobulin protein concentrations are largest in May, then possibly the concentration of complement proteins was decreased in May. Evidence to support the decrease in complement protein concentrations is the reduced number of monocytes in May. Monocytes are a significant source of complement proteins (Goldsby et al. 2000). Lack of seasonal variation in serum protein concentrations in male opossums is contrary to increased plasma protein concentrations of male, semelparous dasyurids during mating (Bradley 1990*b*).

A large proportion of lymphocyte activity occurs in the spleen (Goldsby et al. 2000), so spleen mass should adjust to changing lymphocyte concentrations. This adjustment did not seem to be the situation for adult male Virginia opossums, since the highest percentage lymphocytes and smallest spleen masses were observed in November. Spleen mass residuals for opossums were largest during May, which was also a time of lymphocytopenia. Large spleens during May could perhaps be better explained by increased removal of damaged RBCs with age (Ferrant et al. 1987; Zocchi et al. 1987; Grossman and Jolow 1988). Research has shown that increased metabolic activity can increase the fragility of RBCs (Hanzawa and Watanabe 2000; Senturk et al. 2001). May is several months into the breeding season, and male opossums have increased their searching, agonistic, and mating activity. This increased metabolic activity could produce damaged RBCs that have to be removed from the circulating population.

Differences in physiological changes in Virginia opossums and semelparous dasyurids may be explained by several factors: difference in the length of their mating seasons, number of estrous cycles in the females, and evolutionary origin. Mating seasons of dasyurids last about 1 mo (McDonald et al. 1981; Wilson and Bourne 1984; Scott 1986; Bradley 1987; Dickman and Braithwaite 1992), whereas the Virginia opossum's mating season lasts about 5 mo (Gardner 1982). Therefore, the process that leads to early mortality of opossums may occur at a more gradual pace in opossums than dasyurids and allow males to have sufficient opportunity to inseminate estrous females in both mating seasons. Estrous cycles also differ between female dasyurids and female opossums. Female dasyurids are a synchronized monoestrous group (Woolley 1966; Wood 1970), as opposed to female Virginia opossums, which are polyestrous. Therefore, males have several opportunities of mating with an individual female opossum (Gardner 1982). Evolutionary lineage may contribute to the different physiological differences. Didelphids originated in South America approximately 7.5×10^7 yr ago, whereas the Dasyuridae family did not orig-

inate in Australia until approximately 4.5×10^7 yr ago (Austad 1988).

Are opossums semelparous? Technically no, if they survive to breed in the two mating periods between January and May. However, their life history seems to fit into existing models of physiological stress-influenced life histories. Boonstra and Boag (1992) proposed a model that accounted for differences in hormonal and physiological responses between species with semelparous males and those with iteroparous males. They termed the strategy associated with semelparity in small mammals the "adaptive stress response," assuming it maximized energy for a brief period of reproductive effort at a cost of virtually no survival to the next reproductive period. Death results from immunosuppression and anti-inflammatory responses due to excess glucocorticoid activity. Physiological and demographic results from a study of Arctic ground squirrels (*Spermophilus parryii*), a species with a single brief, intense breeding opportunity each year and low between-year survival of males, supported their hypothesis of an adaptive stress response that trades off survival for reproduction in males (Boonstra et al. 2001).

The alternative strategy, seen in other seasonally breeding small mammals, was termed the "homeostasis stress response" (Boonstra and Boag 1992). This strategy includes spreading out reproductive effort over a longer breeding season and retaining normal function of the hypothalamic-pituitary-adrenal axis. Bradley (1997, p. 753) added to this discussion by proposing the adaptive-stress senescence hypothesis, which proposes that accelerated senescence may occur as "a consequence of experiencing a different hormonal *milieu* during a significant or hormonally sensitive part of its life history." Our data on adrenal mass, PCV, helminths, complement activity, lymphocytopenia, and neutrophilia suggest that the male opossum is experiencing the consequences of elevated adrenal activity, surviving long enough to reproduce during the extended mating season of the species, and finally succumbing to the adaptive-stress syndrome. Sampling individual opossums repeatedly through the mating season would help to support or reject these suppositions. At a more general scale, future work in this area of interplay between physiology and life history should include investigation of physiological and immunological parameters from mammals varying in size and demography.

Acknowledgments

Funding was provided by the Department of Zoology and Department of Plant and Soil Sciences at Oklahoma State University (OSU). Logistical support was provided by D. M. Leslie, Jr., and the Oklahoma Cooperative Fish and Wildlife Research Unit (OSU, Oklahoma Department of Wildlife Conservation, United States Geological Survey Biological Resources Division, and Wildlife Management Institute cooperating). We thank the many graduate and undergraduate students who helped with fieldwork.

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