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Source: Southeastern Naturalist, 10(1) : 95-108

Published By: Eagle Hill Institute

URL: https://doi.org/10.1656/058.010.0108

2011 SOUTHEASTERN NATURALIST 10(1):95–108

Reproduction in Male *Crotalus adamanteus* Beauvois (Eastern Diamond-backed Rattlesnake): Relationship of Plasma Testosterone to Testis and Kidney Dimensions and the Mating Season

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Abstract - The reproductive ecology of male *Crotalus adamanteus* Beauvois (Eastern Diamond-backed Rattlesnake) from southwestern Georgia (Joseph W. Jones Ecological Research Center, Baker County) was studied from 14 August 2003 to 14 August 2006. Few studies provide information on reproduction of free-living Eastern Diamond-backed Rattlesnakes, and no information is available on the seasonal relationship of plasma sex steroids of males to changes in the reproductive organs (e.g., mass of the testis and kidney) and the mating season. Here, the main goals were to determine whether: (i) males show variation in concentrations of plasma testosterone (T) during the active season (late March through November), (ii) males have elevated (peak) concentrations of plasma T during the single mating season, which has been documented to occur from late summer through early fall in nearby populations, and (iii) there are seasonal changes in length, width, and mass of the testis and kidney during the active season, particularly during the breeding period. There was a significant difference in mean concentrations of plasma T among seasons, with levels in summer significantly greater than those of spring, and levels in spring and fall were not significantly different. Testis mass and width, but not length, varied significantly across seasons. Testis mass paralleled elevated levels of plasma T, with peak mass occurring in the summer. Similarly, testis width was significantly greater in summer than in fall, but there was no significant difference between summer and spring, nor between spring and fall. We found no significant seasonal changes in any of the kidney measurements. Bisexual pairings were coincident or followed the occurrence of elevated levels of plasma T and changes in testis size; however, despite frequent observations, copulations were not observed. Nonetheless, our results support a mating season of late summer/early fall for the present population of Eastern Diamond-backed Rattlesnakes.

Introduction

For various reasons, including taxonomic chauvinism (Bonnet et al. 2002, Shine and Bonnet 2000), research on proximate determinants of reproduction in snakes, particularly studies of seasonal cycles, has lagged when compared to advances in other vertebrate groups (Aldridge and Duvall 2002, Moore and

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Lindzey 1992, Schuett 1992, Seigel and Ford 1987, Shine 2003, Whittier and Tokarz 1992). Significant gains, nonetheless, have been achieved in recent years, particularly for several taxa of natricines (Moore and Lindzey 1992, Moore et al. 2000, Whittier and Tokarz 1992) and viperids (Graham et al. 2008; Lind et al. 2010; Schuett et al. 1997, 2005, 2006; Smith et al. 2010; Taylor et al. 2004). Despite these important gains, certain deficiencies identified by Seigel and Ford (1987) over 20 years ago, such as the relationship of hormones and reproductive behavior, especially in nature, remain largely unstudied in most of the nearly 3000 species of snakes (Graham et al. 2008). Experimental studies of the relationship of sexual behavior and hormones of snakes are, moreover, limited to few taxa (Taylor and DeNardo 2010).

Among New World species, rattlesnakes (*Crotalus* and *Sistrurus*) and other pitvipers (e.g., *Agkistrodon*) have been recent subjects of long-term, field-based endocrinological studies concerning reproduction and seasonal shifts in peripheral (circulating) steroid concentrations (Almeida-Santos et al. 2004; Graham et al. 2008; Lind et al. 2010; Schuett et al. 2002, 2005; Smith et al. 2010; Taylor et al. 2004; Zaidan et al. 2003). In male subjects, the emerging pattern from these studies reveals that elevated levels of circulating androgens and other sex steroids (e.g., estrogens, progestins) are closely associated with behavioral activities that define the mating season (i.e., bisexual pairing, courtship, copulation, male-male combat), physiological activities of the testis (spermatogenesis, steroidogenesis) and, in some cases, histological and histochemical changes of specific regions of the kidney (e.g., hypertrophy of the renal sexual segment, sexual granules). The sexual segment of kidney in males of squamate reptiles is important in that it supplies materials (e.g., nutrients) to semen, likely for sustenance of spermatozoa, which is under androgen control (Burtner et al. 1956, Fox 1977, Krohmer 2004, Sever et al. 2008). Furthermore, the kidney can undergo seasonal changes in size and mass associated with the period of reproduction and/or endocrine activity (Graham et al. 2008, Prestt 1971, Schuett et al. 2002).

Here, we investigated the reproductive ecology of male *Crotalus adamanteus* Beauvios (Eastern Diamond-backed Rattlesnake) from southwestern Georgia. There are several ecological studies of Eastern Diamond-backed Rattlesnakes (e.g., Hoss et al. 2010, Kain 1995, Martin and Means 2000, Means 1985, Timmerman 1995, Timmerman and Martin 2003, Waldron et al. 2008), but only limited information pertaining to reproduction (e.g., Campbell and Lamar 2004, Klauber 1972, Timmerman and Martin 2003). There are, for example, no analyses of the seasonal relationship of plasma sex steroids to changes in dimensions and mass of reproductive organs (e.g., testis, kidney) in males of this species.

To address some of these deficiencies in males, we asked whether: (i) there were differences in levels of plasma testosterone (T), the predominant sex steroid in male vipers and other squamates (Norris 2006, Schuett et al. 2005), during the active season, which extends from late March/early April through November in southwestern Georgia (Hoss et al. 2010, Timmerman and Martin 2003); (ii) levels of plasma T were elevated (peaked) during the single mating season, which has been documented to occur from late summer to early fall

(August to October) in areas nearby the present study area (Aldridge and Duvall 2002, Means 1985, Timmerman 1995, Timmerman and Martin 2003); and (iii) there were seasonal changes in the mass, length, and width of testis and kidney, particularly during the mating season (Fox 1977, Graham et al. 2008, Prestt 1971, Schuett et al. 2002).

Materials and Methods

Research site

The present research site was the Joseph W. Jones Ecological Research Center (JWJERC), a 12,000-ha reserve located in Newton, GA (Baker County). The JWJERC consists primarily of *Pinus palustris* Mill (Longleaf Pine) forest, between 70 and 90 years old, in general, with some individual trees >300-years-old, with an open midstory and herbaceous understory dominated by *Aristida beyrichiana* Trin. & Rupr. (Wiregrass). Scattered throughout the property were stands of *Pinus taeda* L. (Loblolly Pine) and *Pinus elliottii* Englemann (Slash Pine), hardwood patches (mostly *Quercus* spp.), and mixed pine-hardwood forests, isolated wetlands, and riparian areas associated with the Flint River and the Ichawaynochaway Creek. Numerous food plots for *Colinus virginianus* L. (Northern Bobwhite) and *Odocoileus virginianus* Zimmermann (White-tailed Deer) were maintained throughout the forest matrix. The site is managed on a 1- or 2-year prescribed burn rotation, with approximately 4000–4900 ha burned each year, which helps maintain features of old-growth forests of Longleaf Pine (e.g., open canopy and intact understory).

Collecting procedures and radio-telemetry

From 14 August 2003 to 14 August 2006, we collected adult (snout–vent length > 700 mm) male Eastern Diamond-backed Rattlesnakes (n = 28) as they were encountered on roads or in the field. Shortly after capture, they were brought to a laboratory at Ichauway for processing (see below). From 2 September 2003 to 17 July 2004, seven of the 28 males were surgically implanted with radio-transmitters (for details see Hoss et al. 2010). All subjects were radio-tracked once per week during the active season (late-March to early-November) and twice per month during the inactive season (late-November to early-March). Radio-tracking occurred during daylight hours, and an effort was made to locate each subject at multiple times of day (i.e., early and late morning and early and late afternoon) over the course of the study. Upon location of each subject, numerous data were collected and these are presented elsewhere (Hoss et al. 2010). During radio-tracking bouts, we noted reproductive behavior, such as bisexual pairings, male-male combat, and copulations.

Obtaining blood and plasma

Blood and plasma were obtained following the methods of Schuett et al. (2005). Briefly, before a subject was processed (see below), it was restrained in a clear plastic tube and ≈2 ml of blood was collected from the caudal vessels. This was accomplished using a sterile disposable 1-cc tuberculin syringe (coated with Na heparin) and fitted with a sterile, disposable 25-gauge 5/8-inch needle. Following collection, each blood sample was centrifuged at 6000 rpm for 10 min in a labeled centrifuge tube; plasma was extracted and placed in a newly labeled 1.5-ml centrifuge tube and stored at -20°C until a steroid radioimmunoassay (RIA) for testosterone (T) was performed.

Hormone extraction and measurement using radioimmunoasay (RIA)

Stored (-20 ºC) plasma samples were thawed, transferred to 1.5 ml microcentrifuge tubes, and centrifuged for 3 min at 12,000 rpm. After centrifugation, 500 µl of plasma was removed and diluted in 16 ml ultrapure water in a 16- x125-mm borosilicate vial. The diluted plasma mixture was passed through Saint-Gobain tubing (formulation 2275, ID = 1/16, OD = 3/16, Wall = 1/16) into Sep-Pak® Plus C18 columns using a vacuum manifold to extract the steroid hormones. Columns were primed with two consecutive 2-ml washes of HPLC-grade methanol (MeOH) followed by two consecutive 2-ml washes with distilled water. After the plasma mixture was fully extracted, the columns were washed with two consecutive 2-ml washes of distilled water, and hormone was eluted from the column into labeled 12- x 75-mm borosilicate vials with two consecutive 2-ml MeOH elutions. MeOH was evaporated under a gentle stream of ultrapure nitrogen with an evaporating manifold in a water bath (37 ºC), leaving a hormone residue. The residue was stored frozen at -20 ºC for 24 h and then re-suspended in 800 µl of 5% Ethanol-95% 0.1 M phosphate buffer (37 µl of 100% ethanol added to each sample, vortexed for 2 min, followed by the addition of 713 µl 0.1 M phosphate buffer and vortexing for 40 min). Re-suspended samples were stored at 4 ºC overnight prior to performing the assay.

Samples were assayed with Diagnostic Systems Laboratories double antibody T RIA (DSL-4100); the kit protocol was strictly followed. The kit was validated with tests of parallelism and cold spike recovery using a pool of 57 adult Eastern Diamond-backed Rattlesnake plasma samples. The pool was serially diluted from 1:1 to 1:8, and the dilution curve was parallel to the standard curve of the kit (comparison of slopes [Zar 1996:355]: $t_5 = 0.536$, $P = 0.622$). Cold spike recovery entailed mixing equal volumes of kit standard with the Eastern Diamond-backed Rattlesnake plasma pool. There was a significant linear relationship between the expected and observed T concentrations (ng/ml; $F_{1, 4} = 829$, $P < 0.0001$), and the slope ($\beta = 1.25$) indicated adequate recovery.

Measurement of the testis and kidney

Recently road-killed male Eastern Diamond-backed Rattlesnakes ($n = 19$) in acceptable condition (i.e., minimally desiccated/damaged, with reproductive organs intact) were collected in southwestern Georgia (Baker and Decatur counties) between June 2002 and August 2005. Snakes were frozen until dissection and subsequently preserved and deposited in the Auburn University Museum. Also, we examined four specimens from southern Alabama (Baldwin, Covington, and Escambia counties) and four specimens from Baker County, GA, which had been previously deposited in the Auburn University Museum. Of the 27 total specimens examined, four were collected in spring, 17 in summer, and six in fall, with snout–vent lengths of 81 to 163 cm. The entire right reproductive tract of each individual was dissected out, fixed in 10% buffered formalin (2 weeks, except for museum specimens), and preserved in 95% ethanol. Gross morphological measurements of the reproductive organs were made using digital calipers (± 0.01 mm) and metric scale (± 0.001 g). Following Schuett et al. (2002), size of the right testis (i.e., mass, length, width, and height) and kidney (i.e., mass and length) were obtained from each specimen.

Statistical analysis

Generally, relatively few males were found moving frequently enough to be encountered incidentally on roads during spring; thus, we used a single sample from each of five males studied via radio-telemetry (from 7 to 21 months after implantation of radio-transmitters). To determine the appropriateness of combining samples collected from males with transmitters and those from incidentally encountered males, we compared T concentration in these two groups. Using an analysis of covariance (ANCOVA), with SVL as the covariate, we found no significant difference in mean testosterone concentrations (ng/ml) between telemetered and non-telemetered males (Group effect: $F_{1, 8} = 1.041$, $P = 0.337$; SVL covariate: $F_{1, 8} = 0.674$, $P = 0.435$) during spring. Additionally, we performed subsequent analyses both including and excluding the samples from telemetered males and found no differences. Thus, results that included both types of samples are presented. To determine whether there was seasonal variation in concentrations of plasma T, samples were classified into three seasons based on calendar dates in the Northern Hemisphere (spring: April–June, summer: July–September, fall: October–November; see Schuett 1992), and ANCOVA, using SVL as a covariate, was conducted. Tukey's post hoc tests were used to determine pairwise differences in T concentrations between seasons.

Using the same classification scheme for season, we performed a series of ANCOVAs, using SVL as the covariate, for all gross morphological measurements of the testis and kidney. For significant ANCOVAs, Tukey's post hoc tests were used to determine pairwise differences in between-season measurements.

All variables were natural log-transformed to meet the assumption of normality for parametric statistical analyses (Zar 1996). Additional assumptions for ANCOVA (normality of residuals, homogeneity of variance, homogeneity of slopes) were met. The alpha level of significance was set at $P \leq 0.05$. All statistical analyses were conducted using Systat 12 (SYSTAT Software, Inc., Richmond, CA).

Results

Plasma testosterone concentrations

There was a significant difference in mean plasma T concentrations among seasons (Season effect: $F_{2, 25} = 7.631$, $P = 0.003$; SVL covariate: $F_{1, 25} = 3.942$, $P = 0.058$; Fig. 1). Post hoc tests indicated that concentrations of plasma T in summer were significantly higher than concentrations in the spring ($P = 0.004$) and fall ($P = 0.024$). Concentrations of plasma T in the spring and fall were not significantly different ($P = 0.986$).

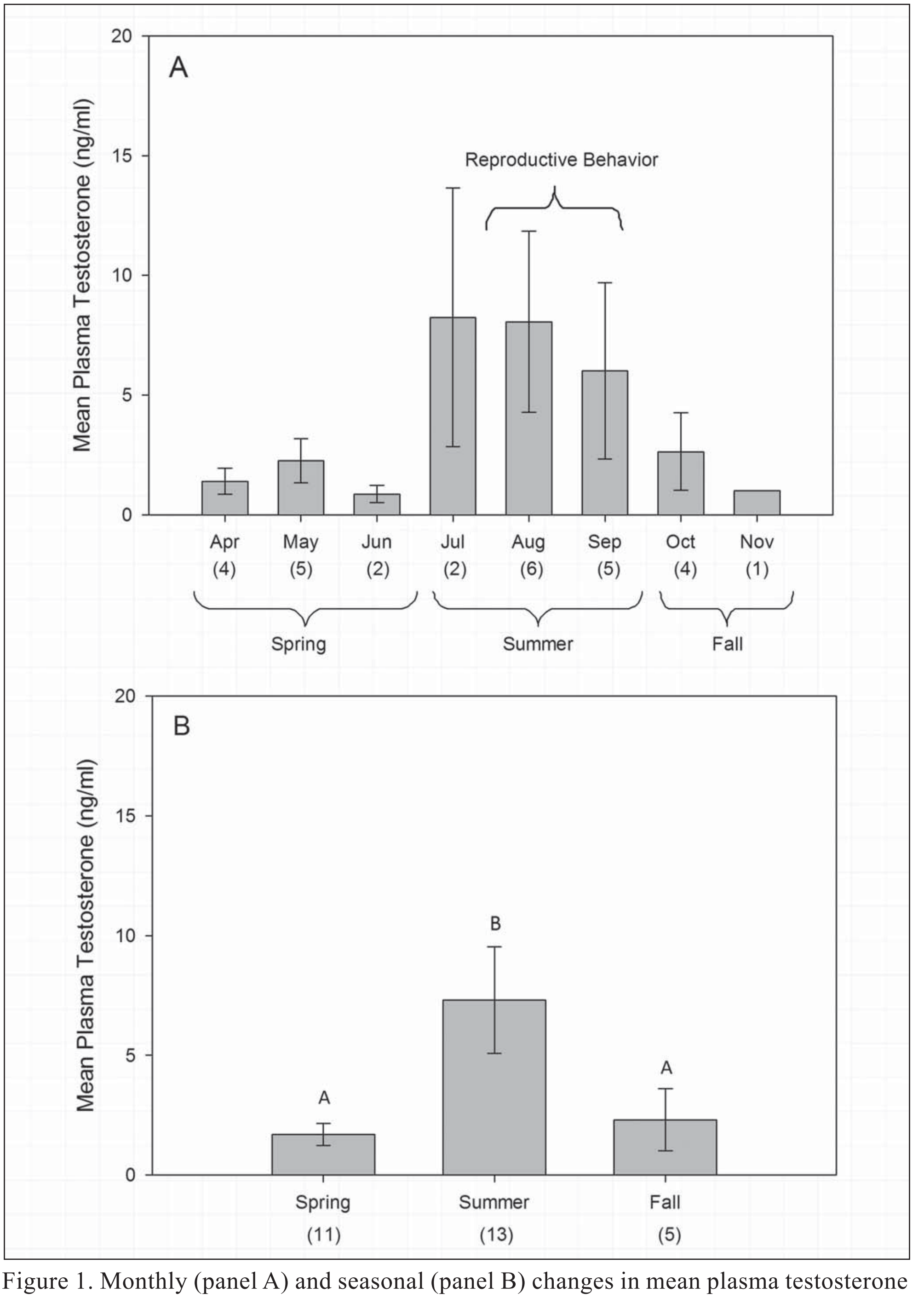

Figure 1. Monthly (panel A) and seasonal (panel B) changes in mean plasma testosterone concentrations for male *Crotalus adamanteus* (Eastern Diamond-backed Rattlesnake) from Baker County, GA. Sample sizes for each month and season are in parentheses. The months in which reproductive behavior (e.g., bisexual pairings) was observed at the study site, at a nearby area (e.g., male-male combat), or is predicted to occur in the region (e.g., the mating season is reviewed by Timmerman and Martin 2003) are indicated by the bracket.

Measurement of the testis and kidney

Gross morphological results of the testis and kidney are presented in Table 1 and Figure 2. Two measurements varied significantly across seasons: testis mass (Season effect: $F_{2, 23} = 6.045$, $P = 0.008$; SVL covariate: $F_{1, 23} = 5.318$, $P < 0.001$) and testis width (season effect: $F_{2, 23} = 3.882$, $P = 0.035$; SVL covariate: $F_{1, 23} = 3.431$, $P = 0.077$). Testis mass showed the same seasonal trend

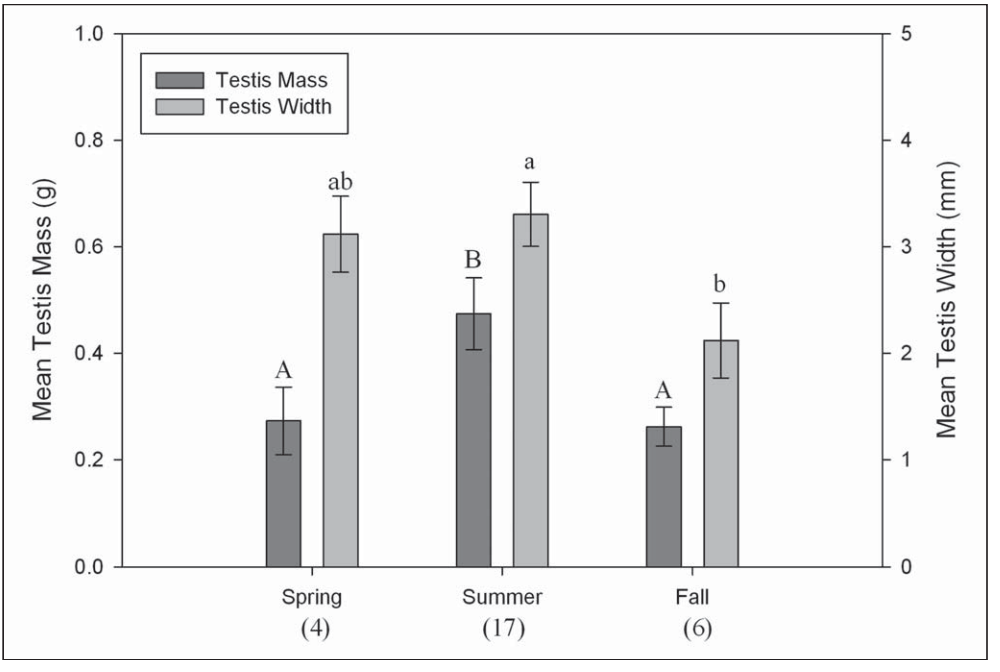

Figure 2. Seasonal changes in mean testis mass and width for road-killed male *Crotalus adamanteus* (Eastern Diamond-backed Rattlesnake) from Georgia and Alabama. Sample sizes for each season are in parentheses. Data are from predicted values generated from analysis of covariance, using snout–vent length (SVL) as a covariate.

Table 1. Summary of statistical analyses performed on gross morphological parameters of reproductive organs of adult male *Crotalus adamanteus* (Eastern Diamond-backed Rattlesnake) from southwestern Georgia and southern Alabama. Means and standard errors are for predicted values generated from the ANCOVA. *F*-values (*F*), degrees of freedom (df), and *P*-values (*P*) are from ANCOVA, in which the reproductive parameter was the dependent variable and season was the class variable. Snout–vent length (SVL) was a covariate in all analyses. Significant results are in bold. Mass parameter units are grams, others are millimeters.

Reproductive	ANCOVA (season)			Mean ± standard error		
parameter	*F*	df	*P*	Spring (*n* = 4)	Summer (*n* = 17*)	Fall (*n* = 6)
Testis mass	6.084	2,23	**0.008**	0.273 ± 0.063	0.474 ± 0.067	0.262 ± 0.037
Testis height	1.266	2,23	0.301	4.510 ± 0.429	5.687 ± 0.412	7.102 ± 2.059
Testis width	3.909	2,23	**0.035**	3.118 ± 0.357	3.304 ± 0.301	2.120 ± 0.352
Testis length	2.298	2,23	0.123	31.435 ± 5.378	34.764 ± 2.119	28.898 ± 4.358
Kidney mass	1.230	2,23	0.311	4.320 ± 0.865	5.158 ± 0.778	5.748 ± 1.252
Kidney length	1.766	2,22	0.194	165.468 ± 4.414	149.029 ± 8.454	162.060 ± 12.755

**n* = 16 for kidney length.

as testosterone concentrations, i.e., values were significantly greater in summer than in spring ($P = 0.025$) or fall ($P = 0.038$). Similarly, testis width was significantly greater in summer than in fall ($P = 0.28$), but no difference was found between summer and spring values ($P = 0.947$) or spring and fall values ($P = 0.203$). There was no significant relationship between residual testis mass (i.e., testis mass regressed against SVL) and kidney mass (Pearson's $r_{25} = 0.222$, $P = 0.267$). No significant seasonal changes in kidney mass ($P = 0.311$) or length ($P = 0.194$) were detected.

Discussion

Our results show that male Eastern Diamond-backed Rattlesnakes from southwestern Georgia exhibited a reproductive pattern that has been documented in several other species of rattlesnakes and pitvipers inhabiting temperate North America (Graham et al. 2008, Schuett et al. 2005, Smith et al. 2010, Taylor and DeNardo 2010). Specifically, the concentration of plasma testosterone (T) in males varied significantly during the active season (April through November), with basal levels in spring and elevated levels in late summer. Furthermore, elevated levels of T coincided with changes of the mass and width of the testis; however, it is important to note that, because we did not measure T in the same animals in which we measured testis mass and width, we cannot conclude direct correlations between these variables. As documented in several other snake species (Graham et al. 2008), we found no significant seasonal changes in kidney size (but see Schuett et al. 2002).

We prepared and sectioned the testis and kidney for histological analysis, but upon examination found that the tissues had undergone significant cellular damage, which prevented us from making reliable measurements. Thus, whether or not seasonal changes occur in the renal sexual segment in male Eastern Diamond-backed Rattlesnakes remains for future studies (see Graham et al. 2008, Schuett et al. 2002, Sever et al. 2008). Likewise, the seasonal pattern of spermatogenesis was not possible to ascertain, though an aestival cycle (Saint Girons 1982, Schuett 1992) is suspected based on a pilot analysis of a small sample of museum specimens (S.K. Hoss, Auburn University, Auburn, AL, unpubl. data). We attribute the failure of our histological analysis to cellular damage caused by the specimens having been stored frozen for extended periods of time (i.e., >1 yr) and suggest that specimens intended for histological examination be preserved as soon as possible or stored in a laboratory-grade freezer (i.e., minimum -20 ºC) prior to processing. Ideally, monthly samples of snakes should be sacrificed after blood extraction, to ensure freshness of the tissues and enable the measurement of hormone concentrations and dimensional and histological parameters of the gonads from the same individuals (e.g., Graham et al. 2008).

Recent studies of temperate North American pitvipers, including rattlesnakes (*Crotalus* and *Sistrurus*), reveal that the mating season occurs either once (unimodal pattern) or twice (bimodal pattern) within an annual cycle (Aldridge and Duvall 2002, Cardwell 2007, Dugan et al. 2008, Graham et al. 2008, Lind et al.

2010, Prival et al. 2002, Schuett 1992, Schuett et al. 2006, Smith et al. 2009). For example, in cases where a unimodal pattern is exhibited, its occurrence is in: (i) early to mid-summer, (ii) late summer through early fall, or (iii) spring (Graham et al. 2008). In patterns (i) and (ii), long-term sperm storage by females is obligatory (Schuett 1992). Furthermore, whereas unimodal patterns (i) and (ii) are frequently reported (Aldridge and Duvall 2002, Graham et al. 2008, Hill and Beaupre 2008, Schuett 1992, Schuett et al. 2005, Smith et al. 2009), a mating season restricted to spring (iii) is documented only in one taxon, *Crotalus ruber* Cope (Red Diamond Rattlesnake; Aldridge and Duvall 2002), though this conclusion will require further investigation.

In species exhibiting the bimodal pattern mating season, the first period of breeding typically occurs from late summer through early fall (e.g., mid-August to mid-October), followed by a period of quiescence during winter; the second breeding period occurs from late winter to mid-spring (e.g., March to mid-May). Several taxa of North American pitvipers are documented to show the bimodal pattern (Aldridge and Duvall 2002; Graham et al. 2008; Lind et al. 2010; Schuett 1992; Schuett et al. 2002, 2005; Taylor and DeNardo 2010). In both unimodal and bimodal patterns of mating seasons for North American pitvipers, ovulation and fertilization occur from mid- to late spring, followed by parturition from early July to mid-September (Aldridge and Duvall 2002, Schuett 1992, Taylor and DeNardo 2010).

Evidence thus far indicates that the Eastern Diamond-backed Rattlesnake exhibits a unimodal pattern (type ii), with breeding occurring from late summer through early fall (Aldridge and Duvall 2002, Kain 1995, Klauber 1972, Means 1985, Schuett 1992, Timmerman and Martin 2003). However, the mating season of populations from extreme southern locations (e.g., Everglades, Florida Keys) possibly extends into late fall or even early winter (Timmerman and Martin 2003). Although copulations were not documented in any of the present subjects, radio-tracked individuals were observed to be in bisexual pairings on several occasions during the season when mating was expected, i.e., late summer and early fall (August and late September). Moreover, male-male combat was observed in a nearby county (Seminole County, GA) on 11 August 2008 (K. McKean, JWJERC, Newton, GA). In viperid snakes, male agonism is commonly associated with dominance and priority of access to females (Madsen et al. 1993, Schuett 1992). Accordingly, we show that elevated levels of circulating plasma T and increased testis size were coincident with anecdotal observations of bisexual pairings and male agonism, thus reflecting additional evidence of a single mating season (i.e., late summer to early fall) in the present population of Eastern Diamond-backed Rattlesnakes (see Graham et al. 2008).

In several snake species, the occurrence of mating coincides with or follows the period of elevated levels of circulating T in males. Here, we document that pattern in male Eastern Diamond-backed Rattlesnakes. Specifically, we show that T peaked in July/ August and declined thereafter until the early fall. Accordingly, elevated T both preceded and coincided with the onset of the mating season, a pattern that has been observed in both Old- and New-World viperids (Graham et al. 2008, Naulleau et al. 1987, Saint Girons et al. 1993, Schuett et al. 1997, Smith et al. 2010). Our results corroborate the growing view that circulating T (and other sex steroids) influences male sexual behavior in snakes (Graham et al. 2008; Lind et al. 2010; Schuett et al. 2002, 2005; Smith et al. 2010; Taylor and DeNardo 2010).

In male rattlesnakes and other vipers, particularly those from temperate regions, seasonal shifts have been documented in dimensions of the testis and kidney (Fox 1977, Lofts 1969, Moore and Lindzey 1992, Prestt 1971, Schuett et al. 2002). Studies on spermatogenic and renal sexual segment (RSS) cycles in both Old- and New World vipers (and other snake taxa) have placed emphasis on histological, histochemical, and, more recently, ultrastructural changes (Krohmer 2004, Graham et al. 2008, Gribbins et al. 2008, Sever et al. 2008). However, with respect to gross morphology, the testis and the kidney have shown increases in mass and linear dimensions (e.g., length, width) associated with: (i) spermatogenesis, (ii) elevated levels of circulating sex steroids (e.g., androgens, estrogens, progestins) and/or (iii) the mating season (see Schuett et al. 2002). In this study, lack of significant seasonal changes in the kidney might have resulted from two factors. First, our within-season sample sizes were relatively small, especially for spring, which might have hindered our ability to find smaller differences in kidney size. Second, and perhaps more important, because the RSS region only accounts for the anterior one-quarter of the kidney, inclusion of the remaining region in our measurements likely obscured changes since it is not expected to undergo similar seasonal changes in morphology (Fox 1977). Thus, we suggest measurement of the kidney in males include both the mass of the entire kidney and isolation of the RSS region.

Additional studies on the reproductive ecology of male Eastern Diamond-backed Rattlesnakes should document seasonal changes (histological and ultrastructural) of the testis (e.g., spermatogenesis) and RSS of the kidney (Burtner et al. 1956, Sever et al. 2008). Additionally, it would be desirable to gain information on geographic variation in the reproductive patterns/traits we inspected (see Graham et al. 2008). For examples, a recent study of male *Agkistrodon piscivorus* Lacépède (Cottonmouth) from southeastern Louisiana provides evidence for the first time in a snake species that two cycles of spermatogenesis can occur within a single active season (Gribbins et al. 2008). Although the functional and evolutionary significance of this pattern has yet to be fully elucidated, it is an important finding and should be explored with regard to male mating capacity and mating seasons. Accordingly, it remains for future research whether this bimodal-type of spermatogenesis occurs in other snakes from certain regions of the southern United States. We suggest that the Eastern Diamond-backed Rattlesnake is a good candidate species to inspect for this pattern of spermatogenesis, especially in populations occurring in the Everglades and Florida Keys. These kinds of studies, and the novel data they might produce, will be invaluable additions in the construction of a robust synthesis of the reproductive ecology and mating systems of New World pitvipers

(Aldridge and Duvall 2002; Duvall et al. 1993; Graham et al. 2008; Schuett et al. 2002, 2005; Taylor and DeNardo 2010).

Acknowledgments

We appreciate the assistance of G. Miller with radio-telemetry and dissection of specimens. The Herpetology Laboratory at JWJERC helped us capture and bleed snakes and collect road-killed specimens. Craig Guyer provided access to museum specimens at Auburn University. Surgical supplies were donated by Zoo Atlanta, courtesy of Dwight Lawson. All Eastern Diamond-backed Rattlesnakes were collected under the Georgia Department of Natural Resources scientific collecting permit numbers 29-WMB-04-188 and 29-WTN-05-166. All procedures were approved by the IACUC of Auburn University (protocol number 2004-06311).

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