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Author(s): Rebecca M. Calisi

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## Variation in Bidder's Organ Volume Is Attributable to Reproductive Status in *Bufo woodhousii*

REBECCA M. CALISI

Department of Biology, University of Texas at Arlington, Arlington, Texas 76019, USA; E-mail: calisiB@uta.edu

**ABSTRACT.**—A unique rudimentary ovarian structure known as the Bidder's organ (BO) occurs at the anterior end of the gonads of male bufonids. I examined BO volume in reproductively active (RA) versus nonreproductively active (NRA) males of *Bufo woodhousii* to examine changes in BO volume in relation to reproductive status. BO volume was larger in NRA males, suggesting that cues associated with sexual maturity influence BO volume.

The bidder's organ (BO) is an allotment of ovarian tissue on the anterior part of the gonads in male bufonids (Duellman and Trueb, 1994). This organ, sometimes referred to as a rudimentary ovary, forms during the larval stage of both sexes, and, in most bufonids, persists in adult males. If the testes is

removed (orchidectomized), the BO grows into functioning ovarian tissue (Pancak, 1987; Pancak-Roessler et al., 1990; Pancak-Roessler and Norris, 1991). Although BOs have long been characterized as nonfunctional vestigial structures, evidence suggests that they may have endocrinological functions (Pancak-Roessler et al.

1990; Pancak-Roessler and Norris 1991). Sullivan et al. (1996) reported a male hybrid toad (*Bufo microscaphus* × *Bufo woodhousii*) that exhibited typical male secondary sexual characteristics while simultaneously expressing functional ovarian tissue and extruding eggs.

The BO has a demonstrated gonadotropin dependence: removal of the pituitary caused BO weight to decrease (Penhos and Cardeza, 1952). BO volume also decreased when testes along with the pituitary were removed; however, upon administration of luteinizing hormone (LH), which stimulates the release of both male and female sex steroids from the gonads, BO volume increased, suggesting a role for gonadotropin regulation in the BO (Ghosh et al., 1990).

Growth responses in the BO were accelerated when solely the testes, not the pituitary gland, were orchidectomized from *B. woodhousii* (Pancak-Roessler and Norris, 1991), perhaps because LH secretion increases after the removal of the testes (Taleisnik and McCann, 1961), thus stimulating oocyte growth. Upon orchidectomy, BOs have been observed to develop into functioning ovaries with the capability of reaching vitellogenic stages (Ponse, 1926).

Zaccanti and Tognato (1976) hypothesized that androgens produced by the testes block vitellogenesis. Androgens such as testosterone may be capable of blocking ovarian or BO estradiol production and/or denying estradiol to the BO by certain processes, as implied upon atrophy of the BO upon administration of testosterone (Deb and Chatterjee, 1963). Thus, the presence of the testes may in some way inhibit the BO from collecting enough estradiol to take part in vitellogenesis.

Reproductively active (RA) males produce greater quantities of testosterone (Wada et al., 1976; Emerson, 1997), which therefore may, according to Zaccanti and Tognato (1976), inhibit growth of the BO. Because the removal of testes is correlated with growth of BO oocytes (Pancak-Roessler and Norris, 1991), it can be inferred that a difference in reproductive status, namely RA males versus nonreproductively active (NRA) males, will present a difference in BO volume.

To examine this hypothesis, I compared volume of the BO in RA to NRA males of *B. woodhousii*. In accordance with the proposed underlying endocrinological mechanisms (Zaccanti and Tognato, 1976; this study), I hypothesized that RA males have a reduced BO volume compared to NRA males because increased testosterone output appears to inhibit estradiol that is necessary for the proliferation of the ovary and the BO.

#### MATERIALS AND METHODS

Forty specimens of *B. woodhousii* were examined from the University of Texas at Arlington Collection of Amphibians (Appendix 1). Specimens were collected in Texas from Dallas, Houston, and Tarrant Counties over a 40-year time span during the breeding season months of March through July. I performed separate ANCOVAs to control for spatial (three Texas counties) and temporal (40 years and five months) components.

The black-pigmented throat served as an a priori indication of RA males (black throat present) versus NRA males (black throat absent). An a posteriori *t*-test demonstrated that black-throated males had significantly larger testes (mean = 49 mm<sup>2</sup>) than nonblack throated males (mean = 31 mm<sup>2</sup>;  $P < 0.001$ ), allowing

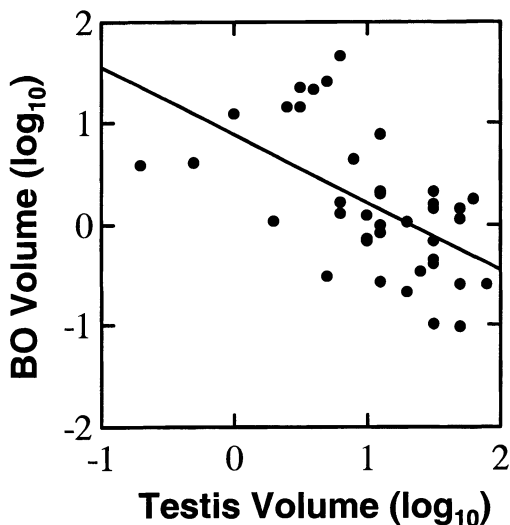


FIG. 1. A scatter plot demonstrating a positive linear relationship between  $\log_{10}$ -transformed BO volume and testis volume.

for throat pigmentation in this case to serve as a reproductive status indicator. Body size was measured by using snout-vent length (SVL). The length and width of the testes and BO were measured using dial calipers in both the right and left organs. Volume of testes and BOs were estimated using the formula for a prolate spheroid:

$$V = 4/3\pi (\text{length}/2) \cdot (\text{width}/2)^2$$

For the first 10 individuals of each group, I calculated both left and right organ volumes and tested for size differences. I found no difference between the left and right organs ( $P = 0.569$ ), and subsequently I measured only the left testes and BO and used these data in statistical analyses. A  $\log_{10}$ -transformation of BO and testes volume normalized the data and created equal variances. I used SYSTAT 8.0 (SPSS Inc., 1998) software for all analyses. To test my hypothesis, I conducted an analysis of covariance (ANCOVA) with SVL serving as the covariate to control for body size effects and determine if BO volume is related to reproductive status.

#### RESULTS

A county component showed no difference in BO volume ( $F_{3,35} = 2.358$ ,  $P = 0.088$ ) or testes volume ( $F_{3,35} = 2.307$ ,  $P = 0.094$ ) overall. Month components were also not significant in relation to BO volume ( $F_{4,34} = 1.036$ ,  $P = 0.403$ ) or testes volume ( $F_{4,34} = 0.176$ ,  $P = 0.949$ ), although a trend was noticed in an increase of BO volume during the months of March, June, and July.

BO volume was inversely related to testes volume (Fig. 1). BO volume was significantly different in RA males compared to NRA males ( $F_{1,37} = 13.765$ ,  $P = 0.023$ ), with RA males expressing a decreased BO volume (Fig. 2).

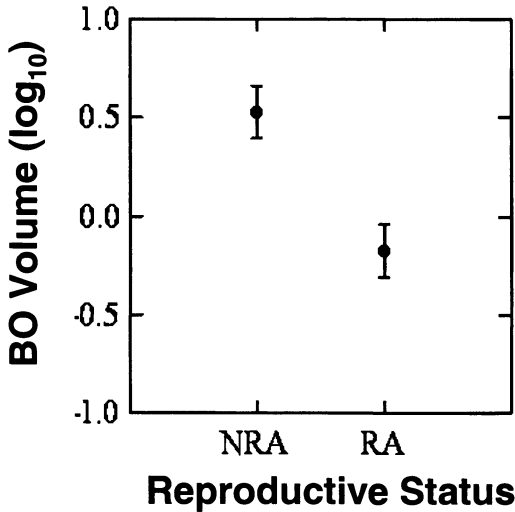


FIG. 2. A least-squares means plot of effects of RA males versus NRA males on BO volume (mean = 78.844, SD = 104.460,  $s^2 = 10911.787$ ).

#### DISCUSSION

RA male *B. woodhousii* had a reduced BO volume as compared to NRA males. Various stated hypotheses may explain the mechanisms through which testicular physiology may inhibit BO development. During the reproductive season, male amphibians have increased levels of testosterone (Wada et al., 1976; Emerson, 1997). Secondary sexual characteristics are known to correlate with increased testosterone output in amphibians (Houck and Woodley, 1995).

Androgen levels of Leopard Frogs vary on a seasonal basis, with low levels occurring before and after the breeding season (Wada et al., 1976). The testes are known to increase in size during the breeding season (Duellman and Trueb, 1994), and this coincides with an increase in testosterone levels (Emerson, 1997) that leads to reproductive behavior. Pancak-Roessler (1987) demonstrated that male *B. woodhousii* had a larger diameter of BO oocytes during the months of June, July, and August, which are months directly following the breeding season. During these months, testosterone is at its lowest level, thus possibly allowing for the proliferation of BO oocytes.

The trend in increase of BO volume during the months of June and July coincides with androgen seasonal level variations (as discussed in Wada et al., 1976) and with observations of Pancak-Roessler (1987) in which *B. woodhousii* maintained BO oocytes with increased diameter at this time. It can be inferred that the commencement of androgen level increase in March, the beginning of the breeding season, would not yet demonstrate a significant effect on BO volume; instead, repeated androgen level exposure would allow for a postponed size effect causing increased oocyte diameter in the following month(s). Androgen levels in April and May, a time when breeding is in full force, demonstrated a decreased trend in BO volume,

supporting the idea that increased testosterone levels at this time, can possibly inhibit BO oocyte growth.

BO volume did increase in NRA individuals, suggesting low testosterone levels influence the size of the BO. Zaccanti et al. (1994) treated *Bufo bufo* with androsten-3 one 17 $\beta$ -carbostilic acid at early stages of development to reduce testosterone levels. Lower levels of testosterone accelerated the differentiation of BO oogonia, which resulted in a BO volume increase. In another example, Pancak-Roessler and Norris (1991) demonstrated that gonadotropins produced no stimulating effect on the BO in *B. woodhousii* with intact testes; but upon orchidectomy, the BO increased in size. These results support the dependence of the BO on gonadotropins and also the inhibitory effect of intact testes.

Estradiol may be responsible for BO oogonia proliferation, but high levels of estradiols have been found in male toads during the breeding season (Pancak-Roessler, 1987), which does not coincide with results of the BO volume decrease trend during this time. The Sertoli cells of the seminiferous tubules have been shown to possess an aromatase enzyme that converts testosterone into estradiols (Dorrington and Armstrong, 1975), and gonadotropin follicle-stimulating hormone (FSH) has been linked to the fostering of these Sertoli cells. The presence of this hormone may be indirectly responsible for BO growth by association with decreased usage or absence of the testes. However, the steroidogenic enzymes  $\Delta^5-3\beta$ -hydroxysteroid dehydrogenase and 17 $\beta$ -hydroxysteroid dehydrogenase present in the corpus luteum and follicle have been noted to also occur in the BO at this time (Ghosh et al., 1984), possibly denying the BO of processing estradiol.

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#### APPENDIX 1

Collection abbreviation: UTA-A: The University of Texas at Arlington Collection of Amphibians. UTA-A- Houston County, TX (UTA-A- 789, 915, 919, 926, 928, 930, 933, 934, 45971); Dallas County, TX (UTA-A- 1492, 1498, 1500, 2995, 2996, 2999, 3000, 3001); Tarrant County, TX (UTA-A- 34281-34284, 34286, 34287, 34293, 34295, 34299, 34302, 34303, 34305-34307, 34313, 34316, 34320, 34324, 34325, 34331, 34337, 34358).

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## Spatial Mobilization of Calcium and Magnesium from the Eggshell of the Snapping Turtle, *Chelydra serpentina*

CYNTHIA J. LAWNICZAK AND MARK A. TEECE<sup>1</sup>

*Department of Chemistry, State University of New York College of Environmental Science and Forestry, Syracuse, New York 13210, USA*

**ABSTRACT.**—Reptilian eggshells are a potential source of nutrients for developing embryos. Embryonic Snapping Turtles (*Chelydra serpentina*) used calcium and magnesium from the eggshell during development. This supplemental source of calcium provided more than twice the amount of calcium present in freshly laid egg yolk to developing embryos. Calcium was preferentially mobilized from the sides and bottom of the eggshell. Scanning electron micrographs of eggshells indicated that this preferential use affected the structural integrity of the eggshell, which presumably facilitates pipping. The mass and concentration of calcium in the chorioallantoic membrane increased significantly during later development, suggesting that this membrane plays a critical role in mobilization of calcium during development. This is the first study to demonstrate mobilization of eggshell magnesium by embryonic turtles.

The eggshell is an important source of nutrients, including calcium and possibly magnesium, for developing reptilian and avian embryos. During embryonic development of *Chelydra serpentina* (Snapping Turtle), over 50% of required calcium was mobilized from the eggshell (Packard et al., 1984). Calcium is necessary for proper skeletal and carapacial development (Ewert, 1985) as well as essential for the proper

function of numerous enzymes (Kaim and Schwederski, 1994). Although turtles use calcium from the eggshell during incubation, the details of this mechanism remain unclear. By the time of hatching, the calcareous layer of eggshells appears flaky and more granular than freshly laid eggshells (Booth, 2002; Ewert, 1985; Packard, 1980; present study). Reasons for the morphological changes are unknown, but calcium mobilization is suspected (Packard, 1980).

We propose that the morphological changes in eggshell structure are indeed caused by the mobiliza-

<sup>1</sup> Corresponding Author. E-mail: mteece@esf.edu