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Reproductive cycle of the bushveld lizard *Heliobolus lugubris* (Squamata: Lacertidae) from southern Africa

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Abstract. Males of *Heliobolus lugubris* exhibit a testicular cycle with a prolonged period of sperm formation (spermatogenesis) extending from May to March (no males were examined from winter). Females were gravid during between November and February. Based on the observation of corpora lutea from a previous clutch and concomitant yolk deposition for a subsequent clutch in the same ovary of four females, *H. lugubris* may produce multiple clutches in the same reproductive season. Mean clutch size for 25 females was 3.44 ± 1.42 SD, range: 1-6. Clutch sizes of one, two and three eggs are new minimum clutch sizes for *H. lugubris.* Neonates were collected in January, February and April.

Key words. Lacertidae, *Heliobolus lugubris,* reproductive cycle, southern Africa.

Introduction

The bushveld lizard, *Heliobolus lugubris,* inhabits arid and mesic savannah from Lowveld and SE Zimbabwe, through Botswana, N. Cape and central Namibia to southern Angola; it is insectivorous (Branch 1998). There is little information about reproduction in *H. lugubris.* Branch (1998) reported females lay 4-6 eggs that hatch from December to March. Fitzsimons (1943) and Auerbach (1987) also reported clutches of 4-6 eggs. Pianka (1986) reported a mean clutch size of 3.86 ± 0.98 SD for 50 *H. lugubris* (as *Eremias lugubris).* Whiting (1996) published a note that mating occurred in the field on 20 February. The purpose of this paper is to provide additional information on the reproductive cycle of *H. lugubris* from a histological examination of museum specimens. Evidence is provided that *H. lugubris* may produce more than one clutch in a reproductive season.

Materials and methods

One hundred and sixty-eight *H. lugubris* from southern Africa were examined from the herpetology collection of the Natural History Museum of Los Angeles County, (LACM), Los Angeles, California, U.S.A. The sample consisted of 62 females (mean snout-vent length (SVL) = 57.5 mm ± 3.2 S.D., range 50-64 mm), 92 males (mean SVL = 55.0 mm ± 4.1 S.D., range 43-63 mm) and 14 neonates (mean SVL = 25.2 mm ± 2.9 S.D. range 18-29 mm). *Heliobolus lugubris* were collected 1969 and 1970 as part of an ecological study by Pianka (1971) or in 1981. Museum catalogue numbers and collection locality information are given in Appendix 1.

For histological examination, the left testis and epididymis were removed from males and the left ovary was removed from females. Enlarged follicles (> 4 mm length) or oviductal eggs were counted. Tissues were embedded in paraffin and cut into sections of 5 µm. Slides were stained with Harris hematoxylin followed by eosin counterstain. Testes slides were examined to determine the stage of the spermatogenic cycle and epididymides were examined for the presence of sperm. Ovary slides were examined for the presence of yolk deposition or corpora lutea. Statistical analyses were performed using Instat (vers. 3.0b, Graphpad Software, San Diego, CA). The relationship between body size (snout-vent length, SVL) and clutch size was examined by regression analyses; an unpaired *t*-test was used to compare *H. lugubris* male and female mean body sizes (SVL).

Heliobus lugubris is largely inactive (brumation) during winter months (HUEY et al. 1977), thus, no samples were available from this period. Seasonal changes in the testicular cycle are presented in Tab. 1. Only two stages were present in the examined samples: recrudescence and spermatogenesis. During recrudescence there was a renewal of the germinal epithelium for the next period of spermatogenesis. Primary and secondary spermatocytes were the predominant cells. During spermatogenesis, the seminiferous tubules were lined by clusters of spermatozoa and metamorphosing spermatids and the epididymides were packed with sperm. Testes undergoing spermatogenesis were observed from September to March, and May (Tab. 1). Since no males were available to examine from winter, the length of the period of spermatogenesis is not known. The smallest reproductively active male (LACM 80151) (spermatogenesis in progress) measured 43 mm SVL.

Female *H. lugubris* were significantly larger than males (t = 3.9, d.f. = 152, P = 0.0001). The seasonal ovarian cycle is presented in Tab. 2. Ovaries without yolk deposition were found in all months except October and December. Ovaries in early yolk deposition (follicles containing basophilic yolk granules) were observed in all months (Tab. 2). Females with enlarged ovarian follicles (> 4 mm length) or oviductal eggs were found from January, November to February. Females with corpora lutea (evidence of a egg clutch being recently deposited) and yolk deposition for a subsequent clutch were found in January, February and October. This is evidence that *H. lugubris* females may produce multiple clutches in the same reproductive season. It is not known if the follicles undergoing early yolk deposition in one of the two females from March would have completed development during the current reproductive season. Linear regression analysis revealed the relationship between female SVL and clutch size was not significant (P = 0.133). Mean clutch size of 25 females was 3.44 ± 1.42 eggs, range 1-6. This is close to the value of 3.86 ± 0.98 for 50 egg clutches from *H. lugubris* reported by PIANKA (1986). His values were based on counts of oviductal eggs which he removed prior to depositing the *H. lugubris* specimens I examined at LACM. Thus, the numbers of female *H. lugubris* with oviductal eggs in Tab. 2 may be lower than occurs in the wild. The smallest reproductively active female (LACM 139004) (yolk deposition in progress) measured 50 mm SVL. Clutch sizes of one, two and three eggs are new minimum clutch sizes for *H. lugubris.* Fourteen *H. lugubris* smaller than 30 mm SVL (presumed neonates) measured 25.2 mm ± 2.9 SD in SVL and were collected during January, February and April.

Discussion

Heliobolus lugubris has a prolonged testicular cycle in which males undergoing spermatogenesis were present in all months except April (when only one male was examined). WHITING (1996) observed *H. lugubris* mating

Month	*n*	Recrudescence (%)	Spermiogenesis (%)
September	4	0	100
October	6	0	100
November	27	0	100
December	9	0	100
January	9	0	100
February	33	3	97
March	2	0	100
April	1	100	0
May	1	0	100

Tab. 1. Monthly distribution of reproductive conditions in seasonal testicular cycle of 92 *Heliobolus lugubris.* Values are the percentages of males exhibiting each of the two conditions.

Month	*n*	No yolk deposition (%)	Early yolk deposition (%)	Follicles > 4 mm length or oviductal eggs (%)	Corpora lutea (%)	Corpora lutea and early yolk deposition (%)
October	2	0	50	0	0	50
November	11	36	18	46*	0	0
December	6	0	33	67	0	0
January	11	36	9	9	9	18
February	100	27	27	40	3	3
March	2	50	50	0	0	0

Tab. 2. Monthly distribution of reproductive conditions in seasonal ovarian cycle of 62 *Heliobolus lugubris*. Values shown are the percentages of females exhibiting each of the five conditions. * Includes one female with 4 oviductal eggs.

in the field in February. Timing of events in the testicular cycle of *H. lugubris* resembles those in the testicular cycle of *Meroles anchietae* (as *Aporosaura anchietae)* in which some males undergoing spermatogenesis were found in each month (GOLDBERG & ROBINSON 1979). No *H. lugubris* males with regressed testes were found (Tab. 1), however one male each with testes in recrudescence were found in February and April. Since recrudescence follows regression, it is possible that *H. lugubris* males undergo regression in late summer. Examination of additional *H. lugubris* males from March and April are needed to answer this question. Portions of the male population of two other species of southern African lacertids (*Meroles anchietae* and *Meroles cuneirostris,* GOLDBERG & ROBINSON 1979) have a brief period of testicular regression in summer followed by recrudescence in the fall. In the African lacertids, *Pedioplanis burchelli,* (NIKOSI et al. 2004), *Pedioplanis namaquensis,* (GOLDBERG 2006a) and *Pedioplanis lineoocellata* (GOLDBERG 2006b) regression also occurs in summer, however, recrudescence appears to be completed in spring. Whether summer regression is typical for members of the Lacertidae in southern Africa must await histological examination of testes from additional species.

Heliobolus lugubris females exhibit a prolonged ovarian cycle with activity recorded in summer and spring (Tab. 2) as occurred in other lacertid species from southern Africa: *P. burchelli* (NKOSI et al. 2004), *P. namaquensis,* (GOLDBERG 2006a) and *P. lineoocellata* (GOLDBERG 2006b). *Heliobolus lugubris* females may produce more than one clutch in the same reproductive season as could females of other lacertid lizards: *Pedioplanis burchelli* (NKOSI et al. 2004); *Pedioplanis namaquensis,* GOLDBERG 2006a); *Pedioplanis lineoocellata,* (GOLDBERG 2006b). BRANCH (1998) reported *H. lugubris* deposited clutches of 4-6 eggs which hatched February-March. My data (based on collections of neonates) suggest hatching may begin somewhat earlier (January) and last until April. FITZSIMONS (1943) and AUERBACH (1987) also reported clutches of 4-6 eggs. The male and female reproductive cycles of *H. lugubris* appear to be synchronized as males are producing sperm when females are depositing yolk. The time of maximum yolk deposition and egg laying for *H. lugubris* is during the summer wet season. This ensures an abundance of insect food which will facilitate yolk deposition and will serve as food for neonates.

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Appendix 1

Natural History Museum of Los Angeles County (LACM) accession numbers and locality information for *Heliobolus lugubris.*

BOTSWANA: Kgalagadi District, 11 km S Tsabong (26°08'S, 22°28'E) LACM 80024, 80026-80031, 80033-80043, 80045, 80046, 80049-80056, 80059, 80060, 80062-80064, 80066, 80069-80086, 80091, 80092, 80094, 80096-80100, 80102, 80112, 80121, 80141, 80143, 80146, 80151. Kgalagadi District, 9 km N, 11 km E Twee Rivieren (26°23'S, 20°43'E) LACM 79980-79985, 79987-79989, 79992-79994, 79996-780005, 80008-80010, 80012, 80013, 80016-80018, 80023. Kgalagadi District, Gemsbok National Park (25°00'S, 22°00'E) LACM 138992, 138997-139000, 139002-139006, 139008, 139010-139013, 139015. Chobe District, Serondelas, ca 10 mi W. Kasane (17°81'S, 25°15'E) LACM 92522, 92523, 92525, 92526. NAMIBIA: Erongo Region, Karibib (22°00'S, 15°56'E) LACM 77454, 77455, 77458, 77459, 77460, 77462. Otjozondjupa Region, Otavi (19°40'S. 17°24'E) LACM 77508, 77510, 77511, 77517- 77519, 77520. Khomas Region, Windhoek (22°35'S, 17°04'E) LACM 77862, 77867, 77869, 77870- 77872, 77874-77877 Karas Region, 46 km N, 17 km E Aroab (26°22'S, 19°49'E) LACM 79938, 79939. Karas Region, 51 km S. 29 km E Aroab (27°14'S, 190°56'E) LACM 80152,

SOUTH AFRICA: Northern Cape Province, 129 km N, 65 km W Upington (27°17'S, 21°54'E) LACM 79905, 79907, 79908, 79911, 79933. Northern Cape Province, 121 km N, 16 km E Upington (27°22'S, 21°25'E) LACM 79948, 79951. Northern Cape Province, 12 km N, 54 km W Upington (27°22'S, 20°43'E) 79898, 79900, 79901. Northern Cape Province, 31 km N, 100 km E Upington (28°13'S, 22°16'E) LACM 79919-79921, 79928, 79934, 79935, 79936, Northern Cape Province, 6 km SE Aansluit, Kuruman River (26°45'S, 22°32'E) LACM 79968, 79970, 79972-79975. Northern Cape Province, 6 km E, 44 km N Aansluit (26°19'S, 22°31'E) LACM 79979. Northern Cape Province, 23 km S, 13 km W Witdraas (27°10'S, 20°35'E) LACM 79966.

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