Tropical Zoology 21: 109-122, 2008

Year-round spermatogenic activity in an oviparous subterranean caecilian, *Boulengerula taitanus* Loveridge 1935 (Amphibia Gymnophiona Caeciliidae)

G.J. MEASEY [1,2,3,5], M. SMITA [4], R.S. BEYO [4] and O.V. OOMMEN [4]

[1] *Molecular Ecology and Evolution Program, Kirstenbosch Research Centre, South African National Biodiversity Institute, Private Bag X7, Claremont 7735, South Africa*
[2] *Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa*
[3] *Department of Herpetology, National Museums of Kenya, P.O. Box 40658-00100, GPO Nairobi, Kenya*
[4] *Department of Zoology, University of Kerala, Kariavattom, 695 581 Thiruvananthapuram, India*

Received 27 July 2007, accepted 24 January 2008

Amphibian spermatogenic cycles are best known from mass breeding anurans and urodeles which have annual cycles featuring both active and quiescent periods. Spermatogenesis of caecilian amphibians is less well known, but recent studies have revealed similar annual cycles for tropical Indian species which have aquatic larvae. Here we report our findings for a direct-developing, oviparous caeciliid from the Taita Hills, Kenya. We investigated the testes of a year-round collection of *Boulengerula taitanus* Loveridge 1935 using morphological and histological testicular features to determine the size at maturity and presence of seasonal activity in this species. Testes volume, but not number, was directly related to total body volume, and a slight but significant asymmetry was found. Histological investigations revealed active spermatogenesis throughout the year, with subtle seasonal variations which are described. The smallest male showing signs of active testes was 227 mm long while the proportion of testes to body volume increased dramatically between 220 and 240 mm total length. This is the first caecilian to be examined which shows year round spermatogenic activity with no regression or recrudescence of the testes. Our study highlights the paradox between clearly seasonal female reproductive activity and continuously spermatogenically active males. This in turn prompts questions concerning the decoupling of mating and egg-laying in this and other caecilian species.

---

[5] Author for correspondence: Molecular Ecology and Evolution Program, Kirstenbosch Research Centre, South African National Biodiversity Institute, Private Bag X7, Claremont 7735, South Africa (Tel +27 21 799 8860; E-mail: john@measey.com).

KEY WORDS: amphibians, Gymnophiona, seasonality, testis, spermatogenesis.

INTRODUCTION

Amphibians have been most frequently studied in temperate habitats where their seasonal reproductive activities are a familiar spectacle; including migrations of large numbers of adults to breeding grounds in early spring (e.g. BEEBEE & GRIFFITHS 2000). Annual sexual cycles evidenced in both females and males follow seasonal change allowing such brief but synchronous mass mating activities. Preparation for annual spawning is evidenced in the reproductive organs and has been the subject for many studies of amphibians which elucidate both energetic and ecological cycles for these animals.

Male amphibians generally have an annual testicular cycle characterized by a quiescent period (late autumn and winter) and a spermatogenic period (spring and summer) (PANIAGUA et al. 1990). GALLIEN (1959) reviewed the evidence for seasonality of reproduction in anurans and urodeles, and concluded that reproduction is invariably correlated with spring and regimes of increased or optimum temperature in both temperate and tropical species.

In the tropics, prominent cycles are often influenced by wet/dry seasonality which produces dry periods unfavourable for some amphibians which pass an inactive quiescent phase. However, some studies have suggested that abundance of tropical frogs may be better related to changes in temperature than to rainfall (WATANABE et al. 2005). A similar conclusion was reached for the caecilian amphibian *Boulengerula boulengeri* Tornier 1896 in the rainforests of the Eastern Arc Mountains, Tanzania (MEASEY & BAROT 2006).

Caecilian amphibians (Gymnophiona) have a predominantly tropical distribution, excluding Madagascar and Australasia. Except for one South American subfamily of semi- and fully aquatic species (Typhlonectinae), all caecilians are believed to be terrestrial burrowers in soil for all or a substantial part of their adult life. Male and female reproductive activity has been shown to be seasonal in all of the species studied so far (see Smita et al. 2006 for a recent review). Caecilians exhibit some fundamental differences in their male urogenital systems which are of evolutionary significance (Wake 1981). In caecilians, sperm transport from the testes occurs via kidney tubules into the Wolffian or Mullerian duct which carries sperm toward the cloaca (Wake 1981). The Mullerian glands secrete a cocktail of nutrients similar to the semen of birds and mammals, and each gland has a duct which joins the central Wolffian duct (Wake 1981). The distal part of the male cloaca is modified to form an intromittent organ (the phallodeum) whereby males are able to insert this into the female cloaca (Exbrayat 2006). Through ejaculation of sperm and Mullerian gland secretions, all caecilians are able to practice internal fertilization.

In the few detailed investigations of caecilians so far, the spermatogenic cycle, sperm release, fertilization and hatching are synchronous and seasonal (Smita et al. 2006). Typically males undergo an intense period of sexual activity following the onset of rains, followed by an immediate quiescent period (e.g. Smita et al. 2005). While the associated behaviour has not been observed, evidence comes from testes of males which have been collected throughout the year. Testes during active periods appear large, swollen and lobulated, while inactive testes are flattened with the locules smaller in size (see Seshachar 1936). After histological preparation, detailed examination of testes reveals the timing of intense spermatogenic activity and subsequent quiescent phases.

For example, Smita et al. (2005) identified three phases in spermatogenic activity for *Ichthyophis tricolor* Annandale 1909, a fairly abundant ichthyophid caecilian in the Western Ghats of Kerala, India (Measey et al. 2003). Spermatogenic quiescence during the first monsoon was followed by active spermatogenesis commensurate with the onset of the second monsoon, whereas early regression corresponded to the long inter-monsoonal period during which the germ cells die through apoptosis (Smita et al. 2005). However, male *Dermophis mexicanus* (Duméril & Bibron 1841) from the upland areas of Guatemala were found to undertake near continuous spermatogenesis, while females have a distinct annual cycle (Wake 1980, 1995).

Boulengerula taitanus Loveridge 1935 is a direct developing subterranean caeciliid caecilian and has been relatively well studied (e.g. Wood et al. 1975, Glaser 1984, Maina & Maloiy 1988, Nussbaum & Hinkel 1994, Gaborieau & Measey 2004, Measey 2004, Measey & Herrel 2006, Raquet et al. 2006), including the structure of male spermatozoa (Wake 1994). Malonza & Measey's (2005) study of the life history of this species suggested that reproduction is seasonal and timed to start with the onset of the short monsoonal rains in East Africa (known locally as Vuli). However, rainfall in the Taita Hills is never completely absent and *B. taitanus* can be collected year round with relative ease (Malonza & Measey 2005).

In this study we use the testes from males collected in monthly samples by Malonza & Measey (2005) to determine (i) seasonality of testicular activity, and (ii) whether maturity of male testes corresponded to the ontogeny classified through the use of a condition index.

MATERIALS AND METHODS

Study site

The Taita Hills has a monsoonal climate with two distinct wet and dry periods (see Malonza & Measey 2005). The climate is characterised by strong seasonal variations in precipitation. Rainfall is monsoonal, with a wetter south-easterly monsoon from April to October and a drier north-easterly monsoon from November to March. This results in two distinct rainy periods: long rains ("Masika") from March to May, and short rains ("Vuli") from October to December, with an annual rainfall above 1300 mm (Fig. 1). However, no month is completely dry. See Malonza & Measey (2005) for a detailed description of the study site and methods used for collection of specimens.

Study animals

We used a selection of males collected by Malonza & Measey (2005) to represent monthly samples of adult males (n = 2 for each month) to determine seasonal changes in reproductive activity. In addition, a selection of sub-adult and adult males of different sizes from June and January (n = 11 for each month) were chosen in order to determine the size of sexual maturity in male *B. taitanus*. Note that this study does not include any juvenile individuals (sensu Malonza & Measey 2005) as testes for these individuals were not discernable. Animals had originally been fixed in formalin (10% of stock solution), and were stored in 70% alcohol.

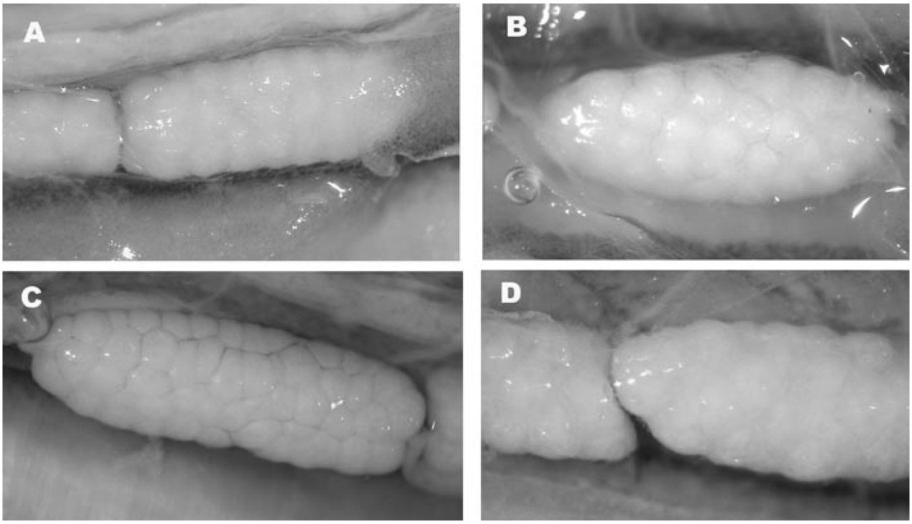

Fig. 1. — Images of testes from four *Boulengerula taitanus* males from four different seasons as seen during dissection. (A) Caught during the Masika rains (May, JM00506) has lobulate testes but lacks vascularization. (B) Caught during the end of the Kusi dry period (September, JM00602) is lobulate and shows signs of vascularisation. (C) Caught during the Vuli rains (November, JM00824) is lobulate and highly vascularised. (D) Caught during the short Kaskazi dry period (February, JM01073) is lobulate but lacks vascularization.

Morphological measurements

In Gymnophiona, testes are paired lengthened and multilobed organs lying in the posterior third of the body alongside fat bodies and the digestive tract. Upon dissection, the external appearance of the testes was noted .We then measured the length and width of each testes lobe from both sides for all specimens. These measurements were used to calculate the volume of each lobe (using the equation for an oblate spheroid) and were totalled for each animal. The total volume for each animal was calculated using the total length (TL) and width at mid-body (using the equation for a cylinder). Total testes volumes were calculated as a fraction of total body volume.

Histological preparation

The testis lobes were dehydrated with an ascending series of alcohol and cleared with methyl benzoate and benzene. The tissue was then embedded in paraffin, serially sectioned at 4 µm using a Leica microtome (Jena, Germany), and the sections stained with Harris' haematoxylin and eosin for observation in light microscopy. Terminology and staging of spermatogenesis follows Smita et al. (2006). In addition to making observations of the status of spermatogenesis, total area of the cross section and the area occupied by lobules in five randomly chosen fields from the sections of the testis of each animal were measured using a Leica light microscope (Jena, Germany) supported with Qwin software. The software was used to calculate the total area of the testis section and areas of the lobules whose boundaries were within the given magnification.

RESULTS

Observations concerning morphology and histology of the testes were made on a total of 43 individual male *Boulengerula taitanus*. Due to damage during collection (see Malonza & Measey 2005), data either on total length or diameter (and hence volume) could not be measured on some individuals, and these were excluded from analysis where pertinent.

Morphology of testes

Males had a mean number of 12 testes lobes (± 0.44 SE; range 6-19; n = 43) arranged in two series on the left and right side of the body. Numbers on each side were found to have a slight but significant bias to the left, a difference also seen in their volume (Table 1). Generally the more posterior testes lobes were smaller than those anterior, with the most anterior lobe usually the largest. This volume of the most anterior testes lobe was found to correlate significantly with total length of the male (r = 0.58; $P < 0.001$).

Total volume of testes was found to correlate significantly with total volume of individual males (r = 0.48; $P < 0.001$), such that testes had a mean of 4.5% of total volume (SE 0.003; range 0.27-7.9%). However, the total number of testes lobes had no relationship with total length of the specimen, or its volume ($P > 0.05$).

Table 1.

The number and volume (in mm^3) of testes lobes in male *Boulengerula taitanus* show a small but significant increase to the left side. Paired t-tests were used to determine bias statistically.

	Left			Right			T stat	*P*
	mean	SE	range	mean	SE	range		
Number of testes	6.3	0.25	3-10	5.8	0.23	3-9	2.20	0.033
Volume of testes	29.7	3.12	0.5-76.2	26.7	2.85	0.62-78.21	3.80	< 0.001

On dissection, male testes lobes appeared to be either smooth (in the smallest individuals), or lobulate with little, some or high levels of vascularisation (Fig. 1). No testes were pigmented, all appearing white to pink determined by the level of vascularisation. The lobulate nature imparts the appearance of a bunch of grapes to the testis, as many others have previously reported (e.g. SMITA et al. 2004).

Histology of testes lobules

The testes of *Boulengerula taitanus* consist of lobules somewhat circular in outline and corresponding with the lobulate external appearance (Fig. 3). The germ cells are distributed throughout the lobule in cell nests or cysts at different stages of maturation, typical of Gymnophiona, but unlike other Amphibia (SMITA et al. 2006). Within a cyst the cells are found to be at the same stage of development/differentiation. Sperm ductules open into the lobule (Fig. 3). Interstitial tissue represents all areas superficial to the seminiferous epithelium. Spermiating cysts open into the collecting ducts present in the interstitium (Fig. 3A-B).

Seasonal variation in testes

External appearances of adults' testes did not have a strong relationship with the state of spermatogenesis found during histological investigations. Smooth, lobulate and vascularised testes were all found to have active spermatogenesis, as were all testes from all months sampled for *B. taitanus*.

Testes volume (corrected for total body volume) was found to be highest during the warmest months of the long Masika rains (March-May) and lowest during the short Vuli rains (Nov.-Dec.), whereas volumes during the two dry seasons were intermediary (Fig. 2A). Statistical analysis on adults from January and June show that the mean testes volume changes from 4 to 5.8% of total body volume and this difference is significant for adults (t-test t_{10} = 16.7; $P < 0.01$) but not for subadults (t-test t_3 = 6.85; $P > 0.05$; see Fig. 4). The cross-sectional area of testes was not found to correlate directly with testes volume, but was found to roughly follow the same seasonal pattern (Fig. 2B).

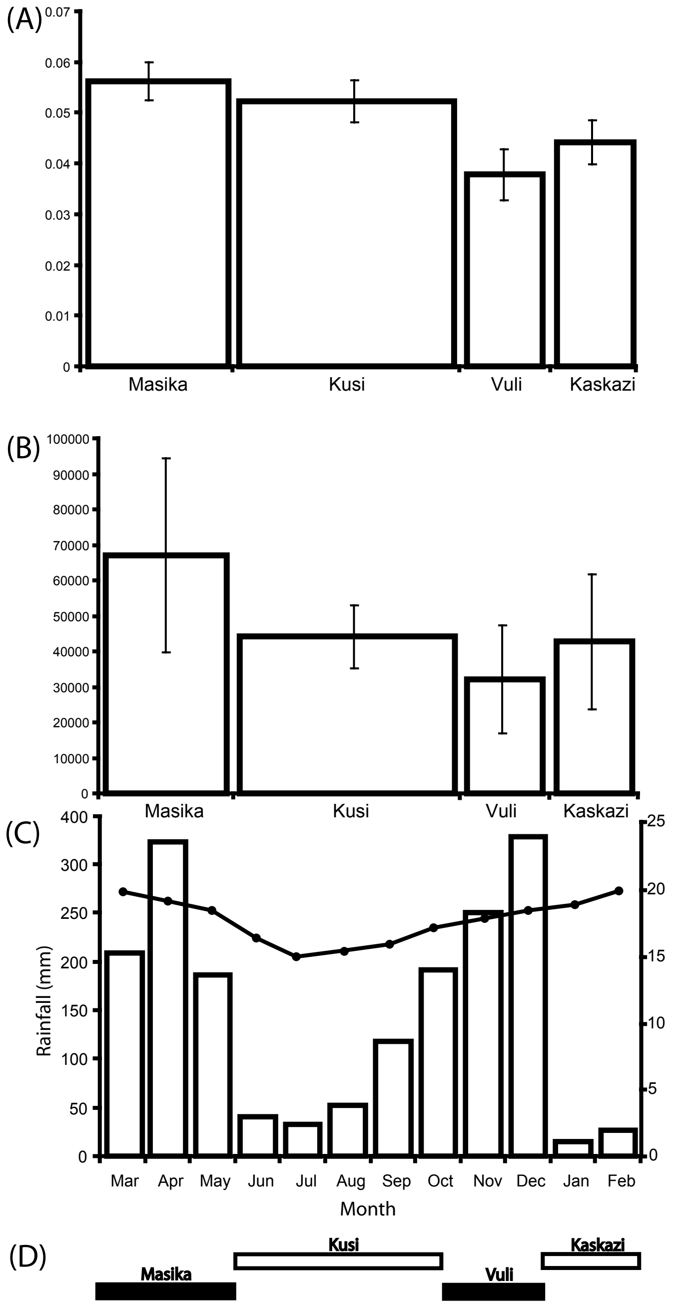

Fig. 2. — Changes in the seasonal (A) volume (B) lobular cross sectional area of *Boulengerula taitanus* males and (C) rainfall and temperature during a year in the Taita Hills, Kenya. Column heights (± SE) correspond to the y-axis, while column widths represent the length of the seasonal period (D).

In general, seasonality was not prominent from observations on histologically prepared testes. In all seasons, active testes showed different stages of spermatogonia, spermatocytes, and primary spermatids, including the presence of spermiating cysts with sperm (Fig. 3C). The conversion from round to long spermatids together with collecting ducts in the lobules (Fig. 3B-C) was seen in samples throughout the year, and was absent only in some animals collected during the longest dry season (Kusi).

The following detailed account of seasonal spermatogenic activity permits some subtle seasonal differences to be outlined:

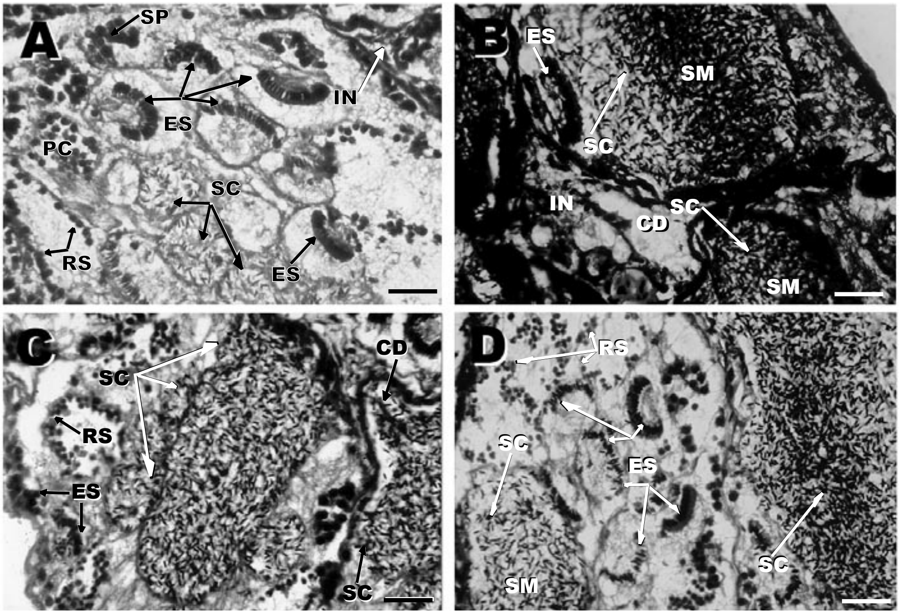

Fig. 3. — Images of testes from four *Boulengerula taitanus* individuals from each season as seen after histological preparation. (A) Caught during Kusi (long dry season; June, JM00519). Testis showing spherical lobules consisting of different cell nests or cysts. Cell cysts with primary spermatocytes (PC), secondary spermatocytes (SP), round spermatids (RS) and elongated spermatids (ES) are shown. Spermiating cysts (SC) are also present. The interstitial area is also shown (IN). Scale bar = 20 µm. (B) Caught during Kaskazi (short dry season; February, JM01071). Lobules rich in spermiating cysts (SC) full of sperms (SM). Collecting ducts (CD) are present. Elongated spermatids (ES) are also seen. The interstitial area is represented as IN. Scale bar = 12 µm. (C) Caught during the Vuli (short rainy season; December, JM00992). Stage V spermatids (round (RS) and elongated (ES) spermatids) and spermiating cysts (SC) full of sperm (SM) are observed. Some of the cysts open into collecting ducts (CD) in the interstitium. Scale bar = 10 µm. (D) Caught during Masika (long rainy season; April, JM00283). Lobules contain mainly Va and b (round (RS) and elongated (ES) spermatids). Spermiating cysts (SC) rich in sperms (SM) are also present. Scale bar = 8 µm.

Vuli (short wet season)

Stage V spermatids in different phases of differentiation are observed. Spermateleosis is prominent during this season. Spermiating cysts are also present. Some of the spermiating cysts establish contact with the collecting ducts (Fig. 3C).

Kaskazi (short dry season)

Lobules contain few cysts in the early stages of spermatogenesis and the lobule cytoplasm is richly occupied by spermiating cysts ready for spermiation. The Sertoli cell cytoplasm is also increased. The interstitial area seemed to increase marginally and collecting ducts were prominent (Fig. 3B).

Masika (long wet season)

During this period the lobules are spherical and fairly large. Cell nests in all stages of spermatogenesis are present. Lobules consist mainly of Va and b spermatids (round and elongated spermatids). Spermateleosis is very active during this season. Spermiating cysts are also abundant. Interstitial area is reduced which is inversely proportional to the lobule size (Fig. 3D).

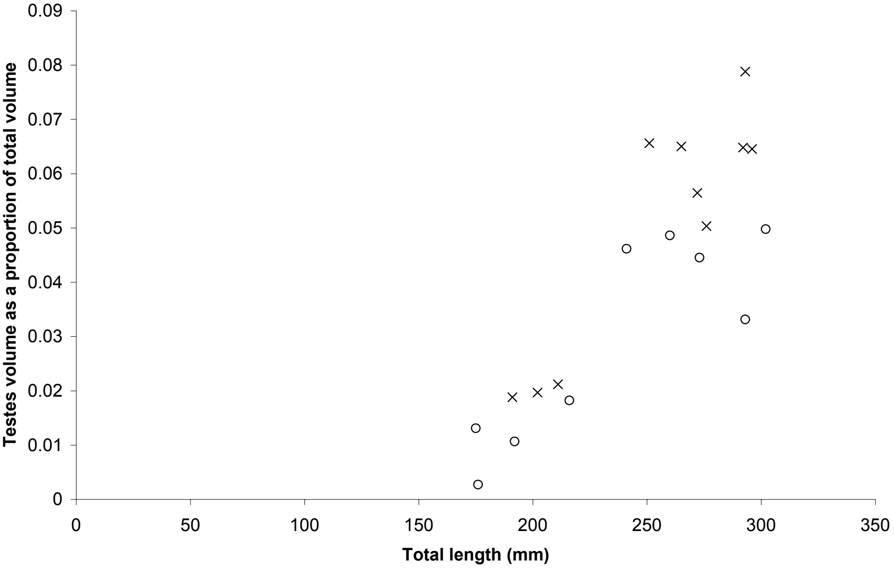

Fig. 4. — The total volume of testes can be seen to change relative to the total length of the individual between 216 and 241 TL. Animals captured in June (×) had larger testes than those captured in January (o). (Damaged individuals with missing data are omitted).

Kusi (long dry season)

Lobule size appears to be reduced compared to that in the long wet season. Spermiating cysts increased in number and establish contact with the collecting ducts in the interstitium. Cysts in stages Va and b (round and elongated spermatids) are abundant (Fig. 3A).

Maturity of testes

In appearance, testes of males under 200 mm TL were smooth, while those from 200 to 220 mm TL were slightly lobulate. The smallest male showing signs of active testes from histological examination was from the June collection with a TL of 227 mm. All larger animals were found to have active testes.

A prominent change in the proportion of testes volume per individual animal independent of seasonal change was found to occur between 220 and 240 mm TL (Fig. 4).

DISCUSSION

Boulengerula taitanus is the first species of caecilian to be examined which shows year round spermatogenic activity with no regression or recrudescence of the testes. SESHACHAR (1936) noted a period of regression in *Ichthyophis glutinosus* (Linnaeus 1758) following contribution of sperm in the mating season. Regressed testes were found to be flattened and smaller in size. Regression has also been found in testes of aquatic *Typhlonectes compressicaudus* (Duméril & Bibron 1841), and terrestrial *Gymnopis multiplicata* Peters 1874 and *Dermophis mexicanus* in each case followed by a period of recrudescence (see SMITA et al. 2006). In *I. tricolor,* testes regression is followed by a phase of spermatogenic quiescence when the spermiating cysts are completely devoid of sperm and there are only cysts in earlier stages present, before recrudescence and resumption of spermatogenesis (SMITA et al. 2005). Despite such periods without spermatogenic activity, it seems that most caecilians studied to date do have active testes for much of the year (WAKE 1995).

WAKE (1995) highlighted the paradox between the clearly synchronous and seasonal reproductive activity of females of many caecilian species, and their continuously spermatogenically active males. This paradox is well illustrated in studies by WAKE (1980, 1995) on *D. mexicanus* for which females have only a small temporal window where yolked ova are present and no apparent sperm storage site. Our study on a direct-developing oviparous species, where even a brief period of aspermatogenesis is absent, prompts the same important questions concerning the decoupling of mating and egg-laying in this and other caecilian species.

Year round testicular activity, without testes regression or quiescence, has been found in a tropical salamanders collected from the Guatemala highlands (HOUCK 1977). In a more detailed study, CHAN (2003) found that *Bolitoglossa rostrata* (Brocchi 1883) and three other plethodontid salamanders (*B. occidentalis* Taylor 1941, *Dendrotriton bromeliacius* (Schmidt 1936) and *Pseudoeurycea goebeli* (Schmidt 1936)) from the same region of Guatemala had continual year round spermatogenesis. Further, she found that there were subtle changes in histological detail of spermiation in different seasons. In one species, *B. occidentalis*, a clear increase in proportion and density of primary spermatocytes correlated with the onset of the wet season and increased surface activity and hence more mating opportunities. CHAN (2003) interpreted these variations as changes in the mating frequency of this species. Only three individuals, all from the end of the intense mating season, were found to lack sperm in the vas deferens.

Some subtle seasonal variations in the spermatogenic cycle of *B. taitanus* are suggested by the significant changes in volume of testes (see Fig. 2A) together with changes in the level of active spermatogenesis (see Fig. 3). Similarly, the proportion of individuals with prominent collecting ducts in the lobules is higher in the long dry season ("Kusi"). However, while testes relative volume appears to change for all adult males (Figs 2-3) there is clearly no synchrony in the increasing size of collecting ducts and a complete absence of regression and recrudescence seen in other caeciliid caecilians (WAKE 1995, SMITA et al. 2006).

The slight changes in testicular volume seen between the long dry season (Kusi) and the short rains (Vuli, Fig. 2) correspond to the single observation of mating in November (MALONZA & MEASEY 2005). A build up of stored sperm in the testes would increase their volume (but see SMITA et al. 2006) which would be followed by a notable decrease once they are released (see Fig. 2). Results from both testicular volume and cross-sectional area suggest a second reduction in size following the long rains (Masika). Interestingly, WAKE (1995) and EXBRAYAT (1986) both found a second peak of testicular activity in *D. mexicanus* and *T. compressicaudus*, respectively. Does this represent another discrete period of mating activity? For *B. taitanus*, we might suppose that increased activity during the long rains may lead to more male-female encounters and hence matings, as suggested by CHAN (2003) for Guatemalan salamanders. MALONZA & MEASEY (2005) found that abundance of *B. taitanus* increased in the surface soils in response to precipitation, whereas MEASEY & BAROT (2006) found that temperature and not rainfall correlated with abundance in surface soils of *B. boulengeri*. They also commented that a period where animals migrated to surface soils was likely to lead to more male-female encounters.

Despite the subtle seasonality, the finding of year-round testicular activity suggests that mating (reported by MALONZA & MEASEY 2005 in November) can occur throughout the year, giving rise to the possibility that female *B. taitanus* store sperm. Sperm storage in caecilians has long been speculated, but has yet to be reported (see SEVER 2002). It is also possible that males are able to store sperm in their Mullerian (or Wolffian) ducts, although we consider this unlikely due to their simplified nature in caecilians (GEORGE et al. 2004). An alternative possibility is that fertilisation of eggs occurs throughout the year, but that females are able to suppress development, perhaps hormonally.

We are not aware of any functional significance relating to the finding that testes number and volume are asymmetric in *B. taitanus* (see Table 1). This phenomenon may arise during development and generation of the germinal cells. The lack of correlation of total number of testes lobules with total length is of interest as this has been previously used to determine age in salamanders (e.g. SMIRINA 1994). This finding is in accordance with other studies on caecilians (e.g. EXBRAYAT & ESTABEL 2006). Our data concur with WAKE (1977) and SMITA et al. (2004), that the length of the first testes lobe appears to increase throughout the life of an individual, although it should be noted that size cannot be directly equated with age in amphibians (HALLIDAY & VERRELL 1988), including caecilians (MEASEY & WILKINSON 1998).

Maturity of male testes occurs between the sizes of 220-240 mm TL (preserved total length) as evidenced by the change in condition index (ML^{-3}) (MALONZA & MEASEY 2005). The finding that testes are active year round means that a single cohort of males is not recruited into the breeding population together, but that individuals may mature at different times commensurate with their variable growth rates and the timing of female reproduction within the short Vuli rains (see MEASEY & DI-BERNARDO 2003). It is also unlikely that a single size (i.e., length) represents a point at which males (or females) mature. Thus we prefer to give a range over which males may be expected to mature. Maturity is also evidenced by a change in the external appearance of testes from a smooth to lobulate condition in *B. taitanus* males (Fig. 1). That sub-adult males do not appear to undergo seasonal changes in their testicular volume, instead having a consistent relationship with TL (see Fig. 4), suggests that testes growth is maximised prior to spermatogenic activity.

Further studies on female sexual cycles in this species are needed in order to confirm the life history information observed by MALONZA & MEASEY (2005), and to investigate the possibility of sperm storage in *B. taitanus*. The subterranean lifestyle of caecilians means that making direct observations on sexual and reproductive behaviour in the field is impracticable. For this reason, detailed examinations of temporally collected individuals are invaluable, making a real contribution to inferring physiological, ecological and behavioural patterns. While this study elucidates the lack of seasonal cycles in the male reproductive organs of *B. taitanus*, it also serves to highlight the great dearth of information about many other caecilians for which studies are urgently needed in order to better understand the reproductive biology, ecology and phylogeny of this order of amphibians.

ACKNOWLEDGEMENTS

We gratefully acknowledge the land-owners and residents around Wundanyi in the Taita Hills for making this study possible. We thank Dave Sever, Jenny Jackson and Jean-Marie Exbrayat for making constructive comments on an earlier version. Special thanks also to Akoth Bwong, Patrick Malonza and Victor Wasonga at the National Museums of Kenya for making museum specimens available together with their help and support.

REFERENCES

Beebee T.J.C. & Griffiths R.A. 2000. Amphibians and reptiles: A natural history of the British herpetofauna. *London*: *Collins*, 270 pp.

Chan L.M. 2003. Seasonality, microhabitat, and cryptic variation in tropical salamander reproductive cycles. *Biological Journal of the Linnean Society* 78: 489-496.

Exbrayat J.-M. 1986. Le testicule de *Typhlonectes compressicaudus*; structure, ultrastructure, croissance et cycle de reproduction. *Mémoires de la Société Zoologique de France* 43: 121-132.

Exbrayat J.-M. 2006. Endocrinology of reproduction in Gymnophiona, pp. 183-229. In: Exbrayat J.-M., Edit. Reproductive biology and phylogeny of Gymnophiona. *Enfield, NH*: *Science Publishers, Inc*, 395 pp.

Exbrayat J.-M. & Estabel J. 2006. Anatomy with particular reference to the reproductive system, pp. 79-155. In: Exbrayat J.-M., Edit. Reproductive biology and phylogeny of Gymnophiona. *Enfield, NH*: *Science Publishers, Inc*, 395 pp.

Gaborieau O. & Measey G.J. 2004. Termitivore or detritivore? A quantitative investigation into the diet of the East African caecilian *Boulengerula taitanus* (Amphibia: Gymnophiona: Caeciliidae). *Animal Biology* 54: 45-56.

Gallien L. 1959. Endocrine basis for reproductive adaptations in amphibia, pp. 479-487. In: Gorbman A., Edit. Comparative endocrinology. *New York*: *Wiley*, 746 pp.

George J.M., Smita M., Oommen O.V. & Akbarsha M.A. 2004. Histology and ultrastructure of male Mullerian gland of *Uraeotyphius narayani* (Amphibia: Gymnophiona). *Journal of Morphology* 260: 33-56.

Glaser H.S.R. 1984. Observations on an abundant caecilian, *Afrocaecilia taitana*, in Kenya. *Asra Journal* 2: 47.

Halliday T.R. & Verrell P.A. 1988. Body size and age in amphibians and reptiles. *Journal of Herpetology* 22: 253-265.

Houck L.D. 1977. Reproductive biology of a neotropical salamander, *Bolitoglossa rostrata*. *Copeia*: 70-83.

Maina J.N. & Maloiy G.M.O. 1988. A scanning and transmission electron microscopic study of the lung of a caecilian *Boulengerula taitanus*. *Journal of Zoology, London* 215: 739-752.

Malonza P.K. & Measey G.J. 2005. Life history of an African caecilian: *Boulengerula taitanus* Loveridge 1935 (Caeciilidae Amphibia Gymnophiona). *Tropical Zoology* 18: 49-66.

Measey G.J. 2004. Are caecilians rare? An East African perspective. *Journal of East African Natural History* 93: 1-21.

Measey G.J. & Barot S. 2006. Evidence of seasonal migration in a tropical subterranean vertebrate. *Journal of Zoology, London* 269: 29-37.

Measey G.J. & Di-Bernardo M. 2003. Estimating juvenile abundance in a population of the semiaquatic caecilian, *Chthonerpeton indistinctum* (Amphibia: Gymnophiona: Typhlonectidae), in southern Brazil. *Journal of Herpetology* 37: 371-373.

Measey G.J., Gower D.J., Oommen O.V. & Wilkinson M. 2003. Quantitative surveying of endogeic limbless vertebrates — a case study of *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae) in southern India. *Applied Soil Ecology* 23: 43-53.

Measey G.J. & Herrel, A. 2006. Rotational feeding in caecilians: putting a spin on the evolution of cranial design. *Biology Letters* 2: 485-487.

Measey G.J. & Wilkinson M. 1998. Lines of arrested growth in the caecilian, *Typhlonectes natans* (Amphibia: Gymnophiona). *Amphibia-Reptilia* 19: 91-95.

Nussbaum R.A. & Hinkel H. 1994. Revision of East African caecilians of the genera *Afrocaecilia* Taylor and *Boulengerula* Tornier (Amphibia: Gymnophiona: Caeciliaidae). *Copeia*: 750-760.

Paniagua R., Fraile B. & Saez F.J. 1990. Effects of photoperiod and temperature on testicular functions in amphibians. *Histology and Histopathology* 5: 365-378.

Raquet M., Measey G.J. & Exbrayat J.M. 2006. Premières observations histologiques de l'ovaire de *Boulangerula taitanus* Loveridge, 1935, amphibien gymnophione. *Revue Française d'Histotechnologie* 19: 9-15.

Seshachar B.R. 1936. The spermatogenesis of *Ichthyophis glutinosus* L. The spermatogonia and their division. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 24: 662-706.

Sever D.M. 2002. Female sperm storage in amphibians. *Journal of Experimental Zoology* 292: 165-179.

Smirina E.M. 1994. Age determination and longevity in amphibians. *Gerontology* 40: 133-146.

Smita M., Beyo R.S., George J.M., Akbarsha M.A. & Oommen O.V. 2005. Seasonal variation in spermatogenic and androgenic activities in a caecilian testis (*Ichthyophis tricolor*). *Journal of Zoology, London* 267: 45-53.

Smita M., Jancy M.G., Akbarsha M.A., Oommen O.V. & Exbrayat J.M. 2006. Caecilian spermatogenesis, pp. 231-246. In: Exbrayat J.M., Edit. Reproductive biology and phylogeny of Gymnophiona. *Enfield, NH*: *Science Publishers, Inc*, 395 pp.

Smita M., Oommen O.V., Jancy M.G. & Akbarsha M.A. 2004. Stages in spermatogenesis of two species of caecilians, *Ichthyophis tricolor* and *Uraeotyphlus* cf. *narayani* (Amphibia: Gymnophiona): Light and electron microscopic study. *Journal of Morphology* 261: 92-104.

Wake M.H. 1977. The reproductive biology of caecilians: an evolutionary perspective, pp. 73-101. In: Taylor E.H. & S.I. Guttman, Edits. Reproductive biology of amphibians. *New York*: *Plenum*, 475 pp.

Wake M.H. 1980. Reproduction, growth and population structure of the Central American caecilian *Dermophis mexicanus*. *Herpetologica* 36: 244-256.

Wake M.H. 1981. Structure and function of the male Mullerian gland in caecilians, with comments on its evolutionary significance. *Journal of Herpetology* 15: 17-22.

Wake M.H. 1994. Comparative morphology of caecilian sperm (Amphibia: Gymnophiona). *Journal of Morphology* 221: 261-276.

Wake M.H. 1995. The spermatogenic cycle of *Dermophis mexicanus* (Amphibia: Gymnophiona). *Journal of Herpetology* 29: 119-122.

Watanabe S., Nakanishi N. & Izawa M. 2005. Seasonal abundance in the floor-dwelling frog fauna on Iriomote Island of the Ryukyu Archipelago, Japan. *Journal of Tropical Ecology* 21: 85-91.

Wood S.C., Weber R.E., Maloiy G.M. & Johansen E. 1975. Oxygen uptake and blood respiratory properties of the caecilian *Boulengerula taitanus*. *Respiration Physiology* 24: 355-363.