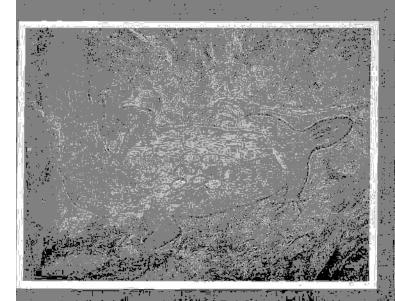
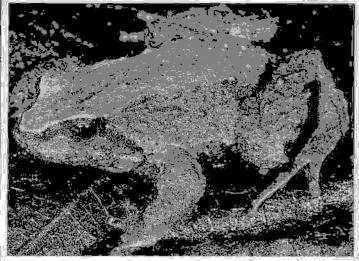
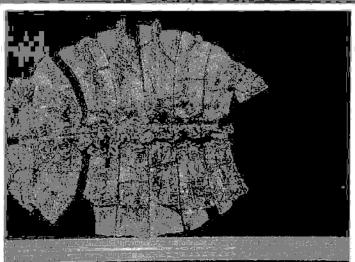
HERPETOLOGY AUSTRALIA

A DIVERSE DISCIPLINE









The state of the

Daniel Lunney and Danielle Ayers

Transactions of the Royal Zoological Society of New South Wales

- - Backyard

blues

Urbanisation is usually disastrous for wildlife, but the blue-tongued lizard seems to be a striking exception. In Sydney, for example, where lace monitors, bearded dragons, diamond pythons, red-bellied black snakes and other large reptiles have disappeared or retreated to remnant bushland, the bluetongued lizard remains abundant.

So how has this large, defenceless, slow-moving lizard managed to persist in suburbia?

Scientists at the University of Sydney -Jennifer Koenig, Professor Rick Shine and Dr Glenn Shea - have been hunting for an answer in Sydney backyards.

They suspected that the lizards were not just common, but more abundant in these disturbed areas than in more pristine adjacent habitats such as remnant forest. So they decided to implant transmitters into 17 adult lizards and track them for six months in the suburb of Hornsby.

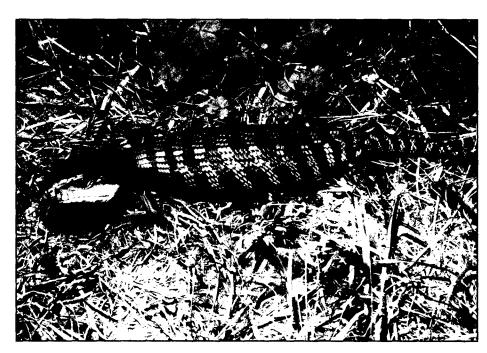
'We found that each radio-tracked lizard used many (5-17) suburban backyards, but each animal spent most of its time in a few core areas near several shelter sites in its home range, Koenig says.

'Males had larger home ranges than females (average of 12 700 versus 5100 square metres) and moved further between shelter sites. Pregnant females were more sedentary, with home ranges averaging just 1000 square metres.'

The scientists found that blue-tongued lizards seemed to show a distinct preference for garden habitats over more natural remnant habitats in the vicinity.

Within this environment, they did not move about at random. They used corridors of dense vegetation to move between familiar retreat sites and actively avoided crossing roads, perhaps showing more road sense than their domesticated enemies, cats and dogs.

Koenig and her colleagues say a combination of ecological factors enable the lizards to live in suburbia.



Male blue-tongued lizards are much more likely to roam than their pregnant partners, which prefer the relative safety of a backyard compost heap.

Firstly, the reproductively important pregnant females are highly sedentary, remaining at sites with abundant food and shelter (such as compost heaps), and are therefore less likely to encounter dangers like vehicles, cats, dogs, birds and humans.

The more expendable males move about much more, probably to locate females, but mostly in times and places that involve minimal risks from humans and pets. Peak activity periods coincide with the times when many humans are at work.

Information from a wildlife rescue database and museum specimens suggests that adult male blue-tongues are the group most likely to suffer injuries and fatalities, especially in spring, but this has less impact on populations than if it were, say, pregnant females coming to grief.

Newborn lizards are also vulnerable, but these losses are more or less countered by large litter sizes (up to 18 per litter) and a potentially long life span. Captive lizards sometimes live to 30 years or more. This means that populations of adult blue-tongued lizards may persist for many years, even without recruitment.

In addition to largely spurning roads, the radio-tracked lizards showed strong 'site fidelity', spending up to 70% of their time in safe locations, including drainage pipes, sheds, thick vegetation, concrete crevices, car tyres and overgrown wood piles. They have no qualms about using this artificial shelter, or about consuming unnatural food such as introduced garden snails, dog food, newspaper and kitchen scraps such as water melon!

Only one of the radio-fitted lizards was killed during the study. Data from the Wildlife Information and Rescue Service, however, indicate a massive rate of death and injury in urban bluetongued lizards. More than 2000 'rescues' were recorded in Sydney in three years.

Paradoxically, the scientists see this as an encouraging result because it suggests that population densities of the lizards are still high in many areas of the city, for the reasons outlined above.

Their use of non-territorial, overlapping home ranges in a food-rich environment means that the likeable blue-tongued lizard can potentially reach large numbers in suburban areas.

More about blue-tongued lizards

Koenig J Shine R and Shea G (2001) The ecology of an Australian reptile icon:

how do blue-tongued lizards (*Tiliqua* sdncoides) survive in suburbia? Wildlife Research. 28:215-227.

Steve DavitIson

The male reproductive cycle of the Eastern Blue-tongued Lizard Tiliqua scincoides scincoides (Squamata: Scincidae)

Glenn M. Shea

Department of Veterinary Anatomy, University of Sydney, New South Wales 2006

ABSTRACT

The male reproductive cycle of *Tiliqua scincoides scincoides* is described on the basis of variation in testis dimensions and testicular and epididymal histology of museum specimens. Sexual maturity occurs at smaller body sizes in southern Australia than in Queensland. Spermatogenesis commences in early autumn, spermiogenesis peaks in early spring, and a resting period occurs in summer. Spermiogenesis in northern populations may peak a few weeks earlier than in southern populations. The pattern of variation in testis size closely parallels the histological changes, suggesting that testis size may be used as an indicator of Spermatogenesis in *Tiliqua*.

INTRODUCTION

The reproductive cycles of skinks have received much attention in recent decades, revealing a wide variety of reproductive strategies in this speciose family (Shine 1985; Heatwole and Taylor 1987). By far the greater mass of data is on female reproduction, with an abundance of anecdotal and single records of gravid females. Even Fitch (1970), in his landmark review of the reproductive cycles in squamates, concentrated on female reproduction, assuming (p.6) "when fecund females are present in the population there are usually males available to inseminate them".

Yet it is the male reproductive cycle that shows the greatest interspecific variation in squamates. In a recent classification, Heatwole and Taylor (1987) recognize 11 reproductive patterns in Australian reptiles. In 10 of these categories, ovulation is restricted to spring or early summer, or peaks at that time. Variations in male reproductive cycle and mating season provide the remaining divisions.

Further, while studies of female reproductive cycles are generally comparable, concentrating on easily-observed changes, such as diameter and degree of yolking of follicles, and presence of oviductal eggs or embryos, a wide variety of criteria have been used to study male reproductive cycles in skinks, including histological changes and/or variation in gonad mass (Reynolds 1943; Baker 1947; Wilhoft 1963;

Wilhoft and Reiter 1965; Smyth and Smith 1968, 1974; Veron 1969; Pengilley 1972; Zug *etal.* 1982; Joss and Minard 1985; Taylor 1985;

Bourne *et al.* 1986), variation in testis length or volume (Tanner 1957; Mount 1963; Robertson *et al.* 1965; Davidge 1980; Schwaner 1980;

Simbotwe 1980, 1985; James and Shine 1985)

or a combination of these criteria (Barwick 1959, 1965; Towns 1975; Robertson 1981; Vitt and Blackburn 1983). The majority of authors using gross linear or volumetric data to define reproductive cycles have assumed that changes in testis size are strongly correlated with sperm production, using as justification correlations in distantly related taxa. However, Barwick (1965) found that in the skink Egernia cunning-hami, mean testis length varied only slightly on a seasonal basis, while testis volume and mass varied markedly in association with successive stages of sperm production. Conversely, in the skinks Bassiana duperreyi (as Leiolopisma trilineatum) and Eulamprus tympanum (as Sphenomorphus tympanum), the rapid increase in testis mass in autumn is due to spermatocytogenesis, and is not correlated with the onset of mating (Pengilley 1972).

As part of a broader systematic study, I am examining the reproductive patterns of the large skinks of he genus *Tiliqua*, a genus related to *Egemia* (Greer 1979; Hutchinson 1981; Shea 1990, 1992). This paper has three purposes: firstly to briefly describe the abdominal and pelvic parts of the male reproductive tract of *Tiliqua scincoides scincoides* (Hunter 1790), the type species of the genus; secondly, to describe the male reproductive cycle of *T. s. scincoides* based on variation in both histology and gross dimensions, and finally, to assess the correlation between histological changes and variation in testis dimensions, the latter feature to be used in other studies of species in the genus.

MATERIALS AND METHODS

Most male T. 5. scincoides (n=259) in Australian museum collections were examined, covering the entire geographic range of the

Herpetology in Australia 397

subspecies. All were stored whole in 70 per cent ethanol, most after fixation in 10 per cent formalin. Snout-vent length (SVL) was measured and the gonads visualized via a ventral midline incision from the liver (junction of cranial and middle thirds of axilla-groin interval) to the pelvis. The length of the right testis was measured to the nearest 0.5 mm by dial calipers, and a visual subjective estimate of testis condition made, on the following classification:

- I. testis elongate, narrow and flattened;
- II. testis elongate, moderately laterally expanded, but flattened; and
- III. testis elongate, laterally and dorsoventrally expanded (turgid).

Size at maturity was determined by plotting testis length and condition against SVL. Maturity was indicated by a sharp increase in testis length above the least-squares regression line for testis length against SVL for small individuals, and the corresponding appearance of type II and III testes. All individuals (n = 162) larger than the smallest mature male were assumed to be mature.

Mature and some subadult males in the Australian Museum (n = 62) for which dates of collection were known were further examined histologically. A 3—4 mm thick transverse section was removed from the middle of the right testis, and a similar section of epididymis at approximately mid-length. Transverse and dorsoventral diameters (width and depth respectively) of the testis section were measured to 0.1 mm with dial calipers, and both testis and epididymis were dehydrated through graded alcohols, cleared in chloroform and embedded in paraffin wax using standard techniques. Sections were cut at 5—7 /^m, mounted on glass slides and stained with Harris' haematoxylin and eosin. The following measurements and observations were made on each section, using an ocular micrometer:

mean tubule diameter; epithelial thickness and number of cell layers in the epithelium of 10 seminiferous tubules in cross-section; mean diameter and wall thickness of the largest 10 sections of epididymal duct, and the presence of spermatozoa in both tubules and ducts, on a visually subjective scale from 1 (spermatozoa absent) to 4 (maximum amounts of spermatozoa present).

In describing seasonal variation in testis size, I have incorporated components of length, width and depth in three indices:

- 1. testis length
- 2. an index incorporating variation in the two longer axes:

3. an index incorporating variation in all three axes: $VL\ X$ $W\ X\ D$.

The reductions to square and cube roots simplify data handling and comparison with testis length by reducing each index to linearity.

RESULTS

Anatomy of the male abdominal and pelvic parts of the reproductive tract

The male reproductive tract of *T. s. scincoides* is similar to that described for other skinks (Reynolds 1943; Barwick 1959, 1965; Annamalai 1966; Farag and Hashem 1980; *Zugetal.* 1983;

Taylor 1985). The paired testes are ovoid white to pale yellow organs lying on each side of the mesentery, dorsal to the voluminous colon and small intestine, suspended from the dorsal abdominal wall by a mesorchium from the medial margin of the testes. The right testis lies almost entirely cranial to the left, with the cranial pole of the right testis lateral to or just caudal to the caudate process of the liver.

The epididymis lies dorsomedial to each testis, and adjacent to the narrow, elongate, yellow adrenal. It continues caudally through the abdominal cavity, lying along the dorsal abdominal wall, and is continuous with the ductus deferens, which passes over the ventro-medial surface of the kidney, retroperitoneally in the pelvic cavity and tail base.

The testis is enclosed by a thin tunica albuginea, and contains large numbers of seminiferous tubules, each surrounded by a thin lamina propria, and loosely connected by connective tissue, with small numbers of interstitial cells present. In large, turgid testes (condition III), the individual seminiferous tubules are clearly visible through the tunica albuginea.

The epididymis contains a much convoluted epididymal duct, lined with a single layer of columnar epithelium, and surrounded by smooth muscle.

Size at maturity

As it had been suggested that there is geographic variation in maximum size in *T. s. scincoides* (Fleay 1931; Mitchell 1950; Wells and Wellington 1985; Covacevich 1987; Wilson and Knowles 1988), the data were analysed on a state-wide basis. The New South Wales sample, which comprised half of the available material, provided a basis for comparison (Fig. 1). The^ smallest New South Wales male with 1²¹¹"^ testes of condition II or III had SVL = 254 min^ Individuals smaller than this size showed ^

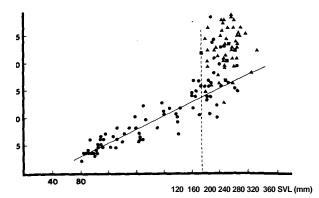


Fig. I. Relationship between testis length and condition, and snoutvent length for male *Tiliqua s. scincoides* from New South Wales. Testis condition is symbolized as: type I (•), type II (•), type III (*). Size at maturity is indicated by a dashed line. Solid line is the least squares linear regression of testis length against snout-vent length for immature material (TL = 0.061SVL - 1.849).

steady increase in testis length (up to 16.5 *mm*) with SVL, but retained the narrow, flattened form. Using the same criteria for testis size at maturity^ Victorian and South Australian adults were smaller and Queensland adults larger (Table 1). All populations were significantly different in size except for Victoria and South Australia.

Table 1. Snout-vent length (in millimetres) of mature-sized male Tiliqua s. scincoides and pair-wise statistical comparisons (Mann-Whitney U test: z values) *** = p < 0.001.

	Range	X	SD	n
Victoria South Australia New South Wales Queensland	220 02.2.0	264.7 272.3 288.2 310.4	20	27 30 84 22
	South Australia	New South Wales	Queenslan	d
Victoria South	1.32 '	4.02***	4.53***	

Relationship between testis size and body size

A number of authors have recommended the use of some form of gonosomatic index, generally testis mass as a proportion of body mass, to correct for variation in testis size due to body size. At least one study (Robertson *et al.* 1965) used testis length/SVL in the same fashion. However, the relationship between testis length and SVL in *T. s. scincoides* (Fig. 1) shows slight, but noticeable, positive allometry, and the calculated linear regression line for the immature material does not pass through the

origin. Hence, use of a gonosomatic index for testis length is invalid (de Vlaming *et al.* 1982), and unadjusted testis measurements are used.

Seasonal variation in testis size

Pooling data for mature animals throughout the distribution of T. s. scincoides, testis length and condition showed significant seasonal variation (Fig. 2). During summer (December-February), testes were at minimum length, and most were flattened (condition I-II). Testes increased in length to June. During this period, testis condition changed, flattened testes gradually disappearing, until in June, 10 of 12 testes examined were turgid. There is some evidence for a small peak in testis size in March. Data are meagre for mid-winter (late June-early August). Testis length in late August—September (up to 37.5 mm), at which time almost all testes were turgid, then decreased gradually through late .spring (October-November) concurrent with an increase in the proportion of flattened testes. However, markedly enlarged, turgid testes were recorded from a few individuals as late as 26 November.

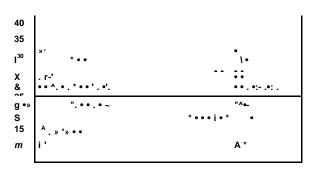


Fig. 2. Temporal variation in testis length and condition in mature T. s. scincoides. Testis condition is symbolized as: type I (A), type II (\bullet). type III (\bullet).

The differences in mean testis length between months were highly significant (oneway analysis of variance, excluding July sample (n = 1), Fio,ii6 = 4.820, P < 0.001).

Histological samples were only available for August to April, and restricted to New South Wales localities with the exception of five Queensland specimens. The incorporation of measurements of testis width and depth in this more limited sample did not reveal a pattern of variation noticeably different from the pooled data on length and shape (Fig. 3).

Seasonal variation in histology

Histological changes noted were in close agreement with the variation in gross morphology. Seminiferous tubule diameter and

Herpetology in Australia 399

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*J J A S 0 N D

h"" 18 Q 16 S"^ 11 cell layers in active tubules, due to the scalloping of the luminal surface by columns of developing spermatozoa and the Sertoli cell cytoplasm, a marked increase in cell numbers concurrent with the autumn to spring increase in thickness of seminiferous epithelium was obvious (Fig. 5). Summer individuals generally had only 1-4 layers of spermatogonia and primary spermatocytes, and a relatively even luminal margin (Fig. 5a) while spring animals had large numbers of secondary spermatocytes, spermatids and maturing spermatozoa and an irregular luminal margin (Fig. 5b). A few maturing spermatozoa were seen in seminiferous tubules in February and March, but testicular spermatozoa were otherwise only seen between August and mid November.

J J A S O N D

3. Temporal variation in (a) index V testis length x width and (b) index V testis length x width x depth in the sample of mature T. s. scincoides examined histologically.

seminiferous epithelium thickness (Fig. 4) were minimal in summer (December—February:

diameter 51-111 {JLVO., x = 78 / n m, n = 8;

epithelium height 18-31 *p.m*, x = 23 jU,m, n = 8), began to increase from March, peaked in late winter/early spring (August-October:

diameter 71-250 n , x = 157; u, n = 29;

epithelium height 22-97 /Am, x = 47 /Am, n = 28), then decreased in late spring. Although it was impossible to count accurately the number of

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4. Temporal variation in (a) mean diameter of seminiferous tubules and (b) mean thickness of seminiferous epithelium in mature *T. s. scincoides*. Dots represent animals lacking spermatozoa (category 1), while the symbols *, • and T represent increasing numbers of spermatozoa (categories 2-^t respectively).

Herpetology in Australia

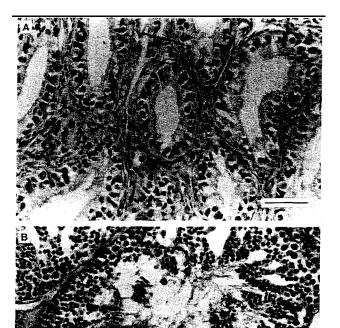


Fig. 5. Seminiferous tubules of T. s. scincoides during (a) summer resting phase (Australian Museum R103314;
6.1.1981) and (b) early spring (Australian Museum R103311;
21.ix.1980). Scale bar represents 50 /im.

There appears to be some variation in the timing of spermatogenesis, as not all spring animals had maturing spermatozoa or luminal sperm. Eight of nine specimens between 18 September and 12 October had testicular^ spermatozoa, while only three of 13 specimens^ between 21 August and 10 September, and twoja of seven specimens between 29 October and 27 November had testicular spermatozoa. Botbj

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6. Temporal variation in (a) mean diameter of epididymal ducts and (b) mean epithelium height of epididymal ducts in mature *T. s. scincoides*. Individuals with sperm in ducts are indicated by closed triangles.

August records of sperm were from Queensland animals, suggesting that at least some of this variation is geographic.

Variation in epididymal duct diameter and epithelial height, and presence of sperm in the epididymis paralleled the spermatogenic cycle (Fig. 6). Epididymal ducts containing sperm were grossly dilated (diameter 221-356/x,m, x = 304 /Jim, n = 12), with thickened epithelium (height 32-97 fim, x = 60 /^m, n = 12; Fig. 7a) and occurred between 21 August and 11 November, being more frequent between 18 September and 12 October. Epididymal ducts not bearing sperm during late winter and spring were similar in diameter to summer and autumn samples (August-November:

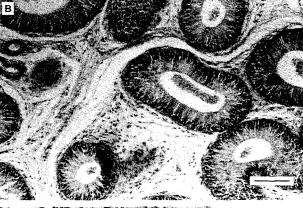
diameter 27-202 n , x = 91 n , n = 25;

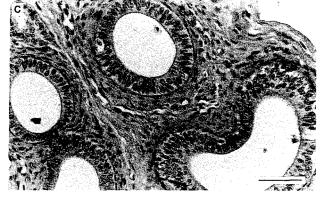
December-April: 28-173 /^m, x = 88 ^m, n = 19). However, the duct epithelium in a number of these late winter and spring individuals was noticeably higher than in summer and autumn (August—November, 8-41 fJLm, n = 25, 28% > 21 ^m; December-April, 7-51 fJLm, n = 19, 5% > 21 fim), although not as high as in sperm-bearing ducts (Fig. 7b-^).

DISCUSSION

All data indicate that testicular recrudescence in *T. s. scincoides* is initiated in March and peaks in September, followed by a period of testicular regression, and a resting phase between







Fig

7. (a) Epididymal duct of *T. s. scincoides* bearing sperm at peak of spermiogenesis in early spring (AM R91931; 9.X.1974). Scale bar = 100 /^.m. (b) Non-sperm-bearing epididymal duct of *T. s. scincoides* in spring (AM R103694; 30.viii.1980). Scale bar = 100 ^m. (c) Regressed epididymal duct of *T. s. scincoides* in summer (AM R47283; 18.1.1975). Scale bar = 50/xm.

December and February. Maximum testis size occurs a few weeks before the peak in sperm production (late August—September vs late September—early October). The temporal congruence of the presence of spermatozoa in testis and epididymis indicates that there is no long-term sperm storage in the epididymis, as has been described for other skink species (Towns 1975).

The meagre data on mating in *T. s. scincoides* indicate that mating is coincident with this peak in spermiogenesis. Longley (1939)

Herpetology in Australia 401

recorded three matings in different years in captive animals kept in Sydney, all in October;

P. Harlow and A. Manning (pers. comm.) observed three instances of mating in Sydney captives on 9—10 September and 23—24 September, and Mudrack (1969) and Honegger and Schmidt (1964) record mating in September and on 31 October by northern hemisphere captives, acquired less than a year previously, and earlier that month respectively. Ovulation and embryonic development are also spring phenomena (Shea 1981, 1992).

Several classifications of squamate reproductive cycles have been proposed, mostly based on northern hemisphere taxa. The male reproductive cycle of T. 5. scincoides is closest to type III of Licht and German (1970), although the peak of spermiogenesis occurs earlier, in spring rather than summer. In the classification proposed by Heatwole and Taylor (1987), based on Australian species, T. s. scincoides conforms to Type III, although the differentiation between their Type I and III categories is not well-defined.

The only other *Tiliqua* species for which there are published data on male reproduction is T. *rugosa* (Bourne *et al.* 1986). Seasonal variation in testis mass, seminiferous tubule diameter and spermatogenesis was similar to that seen in *T. s. scincoides*, although peak diameter of seminiferous tubules was slightly lower in T. *rugosa*. In both species, there was some indication of a lesser autumn peak in testicular development, prior to the main spring peak, in March in *T. s. scincoides* and May in *T. rugosa*. A similar lesser peak in testis mass was recorded in March-April in the related species *Egemia cunninghami* (Barwick 1965), accompanied in one individual by early stages of spermiogenesis, as was seen in two individuals in this study. The significance of this early peak, if real, is unclear.

The use of museum specimens imposed two major constraints on this study. Most importantly, the pooling of material collected over many years from throughout the distribution obscures any potential annual or geographic variations in timing of reproduction. However, the purpose of this study was not to explore such variation, but to define and categorise the male reproductive cycle of the species for inter-taxon comparisons. The congruence of the data on testis size in the pooled and geographically restricted samples argues for uniformity in the reproductive cycle of T. s. scincoides, although spermiogenesis may peak a little earlier in northern populations.

402 Herpetology in Australia

The second difficulty in utilizing museum material is the necessity to minimally damage the specimen material. Use of an abdominal midventral incision allows access to both testes while damaging only skin and muscle. However, due to the deep body, thick abdominal wall and enormous colon of Tiliqua species, this approach allows little more than visualization and measurement of lenth of the testes while they remain in situ. The congruence of the data on testis length and shape with histological changes in both testis and epididymis suggests that the former measurements allow reasonable estimates of spermatogenic activity.

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