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Characteristics of Litters and Juvenile Dispersal in the Endangered Australian Skink *Tiliqua adelaidensis*

TIM MILNE², AND C. MICHAEL BULL¹, *School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia*

MARK N. HUTCHINSON, *South Australian Museum, North Terrace, Adelaide, South Australia 5001, Australia*

This paper reports on reproduction by females of the pygmy bluetongue lizard, *Tiliqua adelaidensis* (family Scincidae) from Australia. *Tiliqua adelaidensis* was the only Australian reptile considered to have become extinct in Australia following European colonization (Ehmann, 1982; Cogger, 1992). Until 1992, there were only 20 specimens recorded, the last in 1959. In 1992, a small living population of *T. adelaidensis* was discovered near Burra, in the midnorth of South Australia (Armstrong et al., 1993). Subsequently, several other isolated populations have been found nearby (Milne, 1999), but all occupy small areas on privately owned farming properties. The species is still considered rare and endangered, and management and possible translocation to conservation areas is a high priority. Knowledge of the reproductive behavior of the species is vital for conservation management.

Data on reproductive parameters are also vital in developing understanding of the evolution of life-history patterns. Australia has a rich lizard fauna, but relatively few comparative studies of demographic parameters are available (Greer, 1989). The skinks (family Scincidae) make up about 57% of all described species in Australia (Cogger, 1992), and any study of the evolution of life-history characters of the Scincidae should include data from Australian taxa.

Two common methods for investigating reproductive biology of lizards were unavailable for this study. The first is to dissect preserved specimens that have been captured at different times of the year (e.g., Vitt, 1991; Dearing and Schall, 1994). From a small sample of museum specimens of *T. adelaidensis*, Ehmann (1982) gleaned little quantitative information about female reproduction, whereas Shea (1992) reported two radio-opaque masses inside the abdominal cavity of a female *T. adelaidensis* from the British Museum, which he interpreted as enlarged ovarian follicles. Dissection of four lizards, killed by predators in spring (October and November) 1992, showed three males with enlarged or turgid testes and a female with enlarged ovarian follicles (Milne, 1999). These results suggest a spring mating period. Dissection of a larger series of *T. adelaidensis* specimens was not possible because of the endangered status of the species.

A second method sometimes employed is to collect

gravid females from the field and record their subsequent reproduction in laboratory enclosures (e.g., Schwarzkopf, 1992; Doughty, 1997). Again, conservation ethics and legislative restrictions prevented the long-term removal of this endangered species from field sites. Instead, we used observations of reproduction in a natural population. This study reports on those observations.

Individual *T. adelaidensis* could be reliably located because of their strong association with vacant vertical holes of burrowing spiders (Hutchinson et al., 1994; Milne and Bull, 2000). The lizards spend most of their day either in the burrows or basking at the burrow entrance. They use the burrows as ambush sites for passing prey, they retreat down the burrows to avoid temperature extremes, and they produce live litters of young in their burrows (Hutchinson et al., 1994; Milne, 1999).

The study area was in native grassland close to Burra, South Australia (33°42'S; 138°56'E). This was the site where the species was rediscovered in 1992 (Armstrong et al., 1993). It consists of gently undulating hills at an altitude of about 500 m above sea level and is grazed by sheep. Native plants include species of irongrass (*Lomandra*), spear grass (*Stipa*), wallaby grass (*Danthonia*), and New Holland daisies (*Vittadinia*). The area is also heavily invaded by introduced exotic plants including wild oats (*Avena barbata*), horehound (*Marrubium vulgare*), and wild sage (*Salvia verbenaca*). The area has hot, dry summers and cool, moist winters, and an average annual rainfall of 446 mm. Over six years from 1992, rainfall varied from 271 mm (1994) to 727 mm (1992).

Burrows and the burrow occupants were observed using an Olympus IF8D4X2-10L optic fibre scope and portable light source (Olympus KLS-131; Milne and Bull, 2000). A clear image of the inside of the burrow was obtained through the fibre scope with its 77 cm long, 8 mm diameter, flexible tube containing optic fibres. The tip of the tube was gently pushed down the burrow until one or more occupants, or the bottom of the burrow, was seen.

In southern Australia, the activity season of most lizards extends over spring and summer (September–March). This study included five seasons from 1993/1994 to 1997/1998. In each season, we marked a sample of from 63–125 occupied burrows in late December. It was not always possible to identify the sex or maturity of the occupants without disturbing them. On two to four days in each week from early January until late March, we monitored all marked burrows each morning and afternoon. Some additional occupied burrows from which occupants dispersed early in the survey were not included in the analysis. We also recorded data on reproductive females from some artificial wooden burrows placed at the study site (Milne and Bull, 2000). However, the size and timing of litters from these burrows were not included in the analysis in case reproduction was affected in any way.

For each burrow, we recorded the week in which juvenile lizards were first observed in a burrow. After a period of two weeks had elapsed with no new litters recorded, we assumed that litter production had finished for that year. In the last three seasons, we attempted to collect all juveniles, and their mothers on the first occasion they were observed, usually by lur-

¹ Corresponding Author. E-mail: michael.bull@flinders.edu.au

² Present address: Nature Conservation Society of South Australia, 120 Wakefield Street, Adelaide, South Australia 5001, Australia.

TABLE 1. Number of burrows occupied by *Tiliqua adelaidensis* observed in each season, number of burrows that had litters, mean time in weeks after 1 January when litters were first detected, and mean SVL (mm) of the subset of reproducing females measured in the last three seasons. Number of females refers to those that were measured in the last three years.

Season	Occupied burrows	Burrows with litters	Mean (SE) week	Mean (SE) female SVL	No. of females
1993/1994	121	41	6.4 (0.3)		
1994/1995	90	36	6.3 (0.2)		
1995/1996	98	32	6.2 (0.2)	98.1 (2.0)	15
1996/1997	125	29	6.7 (0.2)	95.9 (1.1)	16
1997/1998	63	19	7.3 (0.3)	96.8 (1.5)	21

ing them from the burrow with a live mealworm (Milne and Bull, 2000) or, for juveniles, using a long pair of forceps. We checked burrows with the optic fibre scope to ensure no juveniles remained. We weighed and measured (snout-vent length) each female and each of her offspring, marked them by toe clips, and replaced them in the burrow. For most analyses of litter parameters, we only used litters detected in afternoon observations, from burrows where no juveniles had been detected that morning. This minimized the opportunity for juvenile dispersal from the natal burrow before recording litter size. We also removed from analysis the litters of a few females where gentle palpation revealed more additional unborn young. Observations of the burrows continued after litters were produced, and we assessed juvenile dispersal by recording how long juveniles remained associated with their natal burrows.

We recorded 157 litters over the five seasons (Table 1). Females produced litters between late January and mid-March each year. The first litters were detected from 22 January to 4 February. In four of the five years no new litters were detected after 5 March, although in 1995 one female produced a litter between 13 and 19 March. The mean week of litter production in 1997/1998 season was significantly later than in the other years (Table 1; $t_{155} = 2.63$; $P = 0.010$).

Tiliqua adelaidensis females, producing litters of live young in the late summer of each year, follow the typical pattern of other species in the genus *Tiliqua* (Greer, 1989). The timing of reproduction in *T. adelaidensis* remained consistent across years, although there was a delay of about one week in the mean time of litter production in the 1998 summer, which may have resulted from delayed mating during the unusually wet spring of 1997 (Milne, 1999).

In the last three seasons, eight individually marked females were recorded with litters in more than one year. One of these was recorded with a litter in each of three years, four with litters in consecutive years, and three with litters in alternate years. We cannot be sure whether females did not reproduce in years when they were not detected with litters, but the data show that some females can reproduce each year. There was no evidence that females produced more than one litter in any year.

In the last three seasons, we measured the mothers of 52 litters (Table 1). The smallest reproductive females had a SVL of 83 mm, whereas the largest measured 107 mm. The mean SVL of reproductive females did not differ significantly among years ($F_{2,49} = 0.48$;

$P = 0.62$), and the overall mean SVL was 96.9 mm (SE = 0.9).

We analyzed data from 22 litters that we presumed were complete. Mean litter size was 3.23 (SE = 0.16; range 2–4 young). This is larger than in *T. rugosa* (Bull et al., 1993) but smaller than in all other *Tiliqua* species (Greer, 1989).

Mean juvenile SVL was 44.5 mm (SE = 0.2) and mean juvenile mass was 1.56 g (SE = 0.04). The mothers of those 22 litters had a mean postpartum mass of 11.2 g (SE = 0.5). The mean relative clutch mass (RCM), total litter mass as a proportion of the female postpartum mass was 0.47 (SE = 0.02). Maternal investment in young by *T. adelaidensis*, as measured by RCM, was high relative to other recorded values for the family Scincidae (average 0.22; Dunham et al., 1988). Maternal investment among species in the genus *Tiliqua* is poorly known (Greer, 1989), although Bull et al. (1993) reported a mean RCM for *T. rugosa* of 0.27. *Tiliqua adelaidensis* appears to invest more into individual litters than related species.

Litter parameters were compared by ANCOVA among years, using female SVL as the covariate. There were no differences among years in any parameters, but female SVL had a significant effect on litter size ($F_{1,18} = 43.7$; $P < 0.001$) and total litter mass ($F_{1,18} = 37.0$; $P < 0.001$). Larger females produced larger litters with greater overall mass. Female SVL did not significantly influence the average mass or the average SVL of juveniles in the litter or the RCM.

Juvenile dispersal was deduced by continued observation of 143 litters. Among those, 34.4% had no juveniles remaining in the burrow within one week of initial detection. The early time course of juvenile disappearance could not be determined because litters may have been 0–5 days old when first detected. By five weeks from first detection, 75.3% of litters had no juveniles remaining in the natal burrow, and in half of the cases where some juveniles remained, the adult female had dispersed.

Juveniles disappeared from natal burrows soon, in some cases within a week, after birth. We do not believe the disappearances resulted from predation of juveniles from the burrows of their mothers. Adults choose burrows that closely fit their own head dimensions and can probably prevent predator access (Milne and Bull, 2000). The interpretation that juveniles disperse from their natal burrow is more likely. Soon after litters are produced, juvenile lizards are found in previously unoccupied burrows, including artificial burrows placed in the study area (Milne and Bull,

2000). Juveniles prefer significantly smaller burrows than adults (Milne and Bull, 2000). By the following spring, when lizards resume activity, no burrows are cohabited by adult females and juveniles.

The rapid dispersal of young from their natal burrows implies that parental care is minimal in *T. adelaidensis*. Parent-offspring recognition has been demonstrated in *T. rugosa* (Main and Bull, 1996). Although there is no evidence for close association between adult *T. rugosa* and offspring, the young of that species tend to remain within the mother's home range (Bull and Baghurst, 1998). Dispersing juvenile *T. adelaidensis* would have no protection from the mother who remains in her burrow. Juveniles will need to locate new unoccupied burrows very soon to avoid the hazards of exposure. Dispersal occurs at the end of summer when the ground is dry and hard. *Tiliqua adelaidensis* have never been seen to dig their own holes either in the wild or in captivity and are assumed to use vacant burrows of lycosid and mygalomorph spiders (Hutchinson et al., 1994). A critical resource for the establishment of new recruits will be vacant burrows. A management strategy may be to enhance the number of new burrows of suitable size for juveniles at the time of year when they are dispersing.

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Comparisons between Toxic Effects of Tetrodotoxin Administered Orally and by Intraperitoneal Injection to the Garter Snake *Thamnophis sirtalis*

BECKY L. WILLIAMS¹, AND EDMUND D. BRODIE JR., Department of Biology, Utah State University, Logan, Utah 84322-5305, USA

EDMUND D. BRODIE III, Department of Biology, Indiana University, Bloomington, Indiana, 47405-3700, USA

Tetrodotoxin (TTX) is a potent neurotoxin that works by blocking voltage-gated sodium channels in nerve and muscle tissue, thereby inhibiting the propagation of action potentials (e.g., Narahasi et al., 1967; Lipkind and Fozzard, 1994). The rough-skinned newt, *Taricha granulosa*, contains TTX in the skin and the toxin provides an effective defense against predators (e.g., Wakely et al., 1966; Brodie, 1968; Brodie et al.,

¹ Corresponding Author. E-mail: beckyw@biology.usu.edu

TABLE 1. Mean resistance to TTX, as percent baseline crawl speed, of neonate *Thamnophis sirtalis*.

	IP administered TTX		Orally administered TTX		
	Dose mg TTX	30 min resistance (±SE)	Dose mg TTX	30 min resistance (±SE)	45 min resistance (±SE)
Benton Co., OR (N = 25)	0.001	45.9% (5.2)	0.04	54.7% (4.4)	71.6% (4.4)
Bear Lake, Co. ID (N = 17)	0.00005	62.6% (2.6)	0.002	71.7% (2.6)	85.8% (3.6)

1974). Only one predator of the newt is resistant to this toxin, the common garter snake, *Thamnophis sirtalis* (Brodie and Brodie, 1990, 1991, 1999a,b). The exploitative abilities of *T. sirtalis* and defenses of *T. granulosa* appear to have coevolved; snakes have become more resistant and the newts more toxic over time (Brodie and Brodie, 1990, 1999a,b; Motychak et al., 1999).

Resistance data for garter snakes have been based on intraperitoneal (IP) injections of TTX (e.g., Brodie and Brodie, 1990, 1991, 1999b). Intraperitoneal injections appear to have qualitatively similar effects to oral administration and are advantageous because they allow more accurate aliquots and quicker administration of the toxin than oral doses (Brodie, 1968; Brodie and Brodie, 1990, 1999b). Intraperitoneal injections are particularly useful because individual neonate snakes can be assayed with nonlethal doses. However, understanding the relationship between the effects of the toxin administered orally and through IP injection is critical because an oral dose is the exclusive natural mode of exposure for the snake predator.

The only information available to compare the effects of oral and IP doses of TTX comes from toxicity studies performed on mice. The oral LD₅₀ of TTX for a mouse is 334 µg/kg (Mosher et al., 1964; Kawasaki et al., 1973) and the IP LD₅₀ of TTX for a mouse is 8 µg/kg (Mosher et al., 1964). Based on this approximate translation factor of 40X for mice, we tested the relationship between IP injections and oral doses of 40 times that amount in garter snakes.

Subjects were collected from one TTX resistant (Benton County, Oregon) and one TTX nonresistant (Bear Lake County, Idaho) population of *T. sirtalis* (Brodie and Brodie, 1990). Gravid snakes were collected in the spring of 1998, housed individually, and fed fish weekly until parturition. Neonate snakes were housed in individual containers and watered daily. Resistance testing began three days after birth. Twenty-five animals from five families were used from the Benton population and 17 individuals from six families from the Bear Lake population.

A bioassay designed by Brodie and Brodie (1990) was used to determine resistance of neonate garter snakes to TTX. Individual neonate speed was measured on an 11-cm wide racetrack. The snakes were induced to crawl down the racetrack by touching them repeatedly on the tail. Infrared sensors recorded the elapsed time every 0.5 m for 2 m, and the fastest 0.5-m section speed was recorded. Each snake was tested twice, approximately 4 h apart, and the average of the two speed scores was taken as the "Baseline Speed" for that individual.

Tetrodotoxin (Sankyo lot B19481) was diluted in

amphibian ringer solution for both oral doses and IP injections. Injecting a control of 0.1 ml of physiological saline only did not alter garter snake performance in previous experiments (Brodie and Brodie, 1990). Based on earlier work (Brodie and Brodie, 1990, 1999a), individuals from the Benton population received 0.001 mg, and those from the Bear Lake population received 0.00005 mg TTX in 0.1 ml of solution intraperitoneally. Following the methods of Brodie and Brodie (1990), IP injections were administered approximately 24 h after baseline trials. Snake speed was determined exactly 30 min after injection with the fastest 0.5 m speed recorded. A repeat trial was conducted at the same dose for each individual after a 48-h recovery period. Repeated injections of TTX do not alter resistance in the subjects (Brodie and Brodie, 1990; Ridenhour et al., 1999). The mean of the two trial speeds was used as the overall "Postinjection Speed." "Resistance" is defined as the percentage of Baseline Speed crawled in the postinjection trial.

Four to five days after the second IP injection, an oral dose of TTX was administered by introducing a 1-mm diameter tube approximately 30-mm long into the snake's upper gastrointestinal (GI) tract. The tube was attached to the end of a needle and syringe to measure the amount of solution administered accurately. Individuals from the Benton population received 0.04 mg, and those from the Bear Lake population received 0.002 mg TTX in 0.1 ml of solution. The volume of solution administered was the same for IP injections and oral doses. Each animal was tested exactly 30 min after the oral dose was administered, and the fastest 0.5 m speed was recorded. Each snake was tested again exactly 45 min after administration of the toxin to monitor snake recovery. Resistance was calculated at 30 min and 45 min.

Individual resistances to IP and to oral doses of TTX were compared using paired *t*-tests within each sample. Pearson product-moment correlations were estimated to evaluate whether individual snakes performed consistently to injected and orally administered doses relative to one another.

Injected (IP) doses of TTX reduced crawl speed to an average of 46% of baseline speed in neonate snakes from the Benton population and 63% in animals from the Bear Lake population after 30 min (Table 1). Orally administered doses of TTX reduced Baseline Speed of subjects from the Benton population to 54% and subjects from the Bear Lake population to 72% after 30 min. Forty-five min after oral administration of TTX, subjects from each population had begun to recover. Individuals from the Benton population and the Bear Lake population had recovered 17% and 14%, respectively, of their baseline crawl speed on average (Table 1).

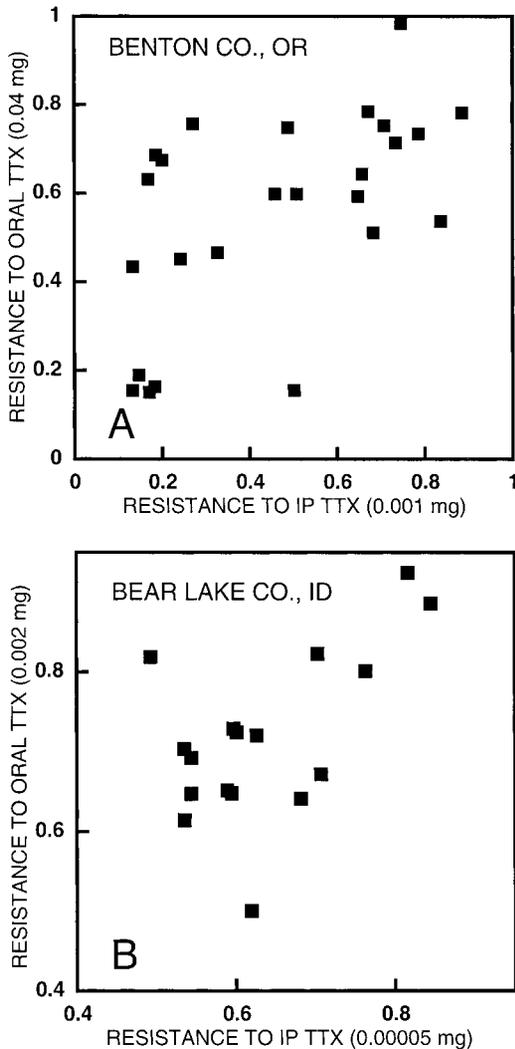


FIG. 1. Resistance of neonate snakes from resistant (A) and nonresistant (B) populations to orally administered and intraperitoneally injected doses of tetrodotoxin (TTX).

Comparisons of individual crawl speeds revealed small differences between resistance to injected and oral doses of TTX. Resistance to the oral dose was 8–9% higher than to the injected dose in each sample (Benton: $t = 1.93$, $df = 24$, $P < 0.066$; Bear Lake: $t = 3.33$, $df = 16$, $P < 0.004$).

Individual responses to orally administered TTX were positively correlated with responses to injected doses in each sample (Fig. 1). The correlation for the Benton sample was significant ($r = 0.560$, $df = 24$, $P < 0.0036$), whereas the correlation for the smaller Bear Lake sample was not as strong ($r = 0.436$, $df = 16$, $P < 0.079$). Although the Bear Lake correlation was not statistically significant at the $\alpha = 0.05$ level, because the P -value fell just outside of the predesignated α level, because crawl speed was measured in hundredths

of a second, and because the Bear Lake sample size was small, we feel that this correlation is biologically significant.

Relative differences in individual resistance were conserved between modes of exposure to TTX in both resistant and nonresistant samples of *T. sirtalis*. The average level of resistance to the oral dose was only slightly higher (less than 10% of Baseline Speed) than to the IP dose in each sample. The greater level of resistance of snakes to the oral dose differs slightly from the published quantitative relationship between oral and injected doses in mice (Mosher et al., 1964) and may result from differences in assay and approach. For example, the approximate translation factor of 40 \times for mice is based on population level average effects (LD_{50} s), whereas our assay is based on fine scale individual differences in locomotor performance. Honma and Suda (1998) postulated that the route of exposure had less influence on toxicity at high doses, such as those used to determine LD_{50} s. Alternatively, species level differences in tolerance to TTX (Brodie 1968; Motychak et al., 1999) may account for the difference in the reaction to oral doses for mice and snakes. Regardless of the cause of such differences, the results of our study indicate that for *T. sirtalis* oral and injected doses are qualitatively similar in effect and that the quantitative difference in effect is slightly more than 40 times greater for IP doses.

Generally, IP doses of toxins produce a more rapid response than oral doses (Eaton and Klaassen, 1996). However, the time for peak effect of the toxin was similar for both oral administration and IP injection. Snakes were well on their way to recovery at 45 min after oral dosing. This result is consistent with the observed recovery times of snakes subjected to IP trials (Brodie et al., 2002). The similarity of timing in peak effect suggests that the mode of intoxication and processing of TTX were similar for both oral doses and IP injections. Injected IP doses generally result in a higher bioavailability of xenobiotics delivered by the systemic circulation than ingested oral doses because of such factors as limited absorption of oral doses, hepatic first-pass effect (Medinsky and Klaassen, 1996) and excretion of unmodified toxin in waste products (Honma and Suda, 1998). However, if an adequate portion of the toxin is directly absorbed by the upper GI tract (including the esophagus and oral lining) rather than first passed through the stomach and liver, the timing of peak effect would be similar for oral doses and IP injections of TTX.

It is unknown what routes of absorption occur before TTX binds to sodium channels at the final sites of action, and the portion of TTX that is not absorbed in the upper GI tract may have little to do with the effect of the toxin. Tetrodotoxin exhibits reduced stability at low pH (Kao, 1966); thus, the toxin may be degraded by the acidity of the stomach. Also, high concentrations of TTX are found in rat kidney after exposure (Ogura, 1958), suggesting that much of the toxin that remains undegraded may be excreted unchanged (Kao, 1966). Finally, additional detoxification may occur in the liver where elevated concentrations of the toxin also occur in mice (Kao, 1966). These physiological factors may explain why larger amounts of toxin are required when administered orally to pro-

duce the same effect as TTX administered through IP injection in *T. sirtalis*.

When considering ecological and evolutionary aspects of garter snake response to TTX, one would expect the variation in resistance among populations of snakes to be roughly correlated with the toxicity of sympatric newt populations. This seems to be the case, because snake populations sympatric with extremely toxic newts are highly resistant to TTX (Benton County, OR) and nonresistant snake populations are either allopatric to the newts (Bear Lake County, ID) or inhabit areas (Vancouver Island, BC) where the populations of *T. granulosa* do not possess TTX (Brodie and Brodie, 1991; Hanifin et al., 1999). However, the results of this study enable us to make quantitative comparisons between the amount of TTX a snake of average resistance can safely ingest and the amount of TTX contained in an average newt of a sympatric population.

Assays of newt toxicity indicate that adult newts from Benton County, Oregon, contain an average of 1.0 mg TTX/g skin (Hanifin et al., 1999) and have approximately 1 g of skin. Based on TTX resistance estimated by IP injection (Brodie and Brodie, 1990; unpubl.), an adult *T. sirtalis* (70 g) from this population that consumed such a newt would suffer a reduction in crawl speed to approximately 60% of baseline speed. This quantitative estimate is consistent with observations of snakes found in the field that are physically impaired but not completely immobilized after eating adult newts (Brodie and Brodie, 1990).

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