Herpetological Review, 2006, 37(2), 149–151.

Female Reproductive Aspects and Seasonality in the Reproduction of *Eleutherodactylus binotatus* (Spix, 1824) (Amphibia, Leptodactylidae) in an Atlantic Rainforest fragment, Southeastern Brazil

CLARISSA CANEDO*
and
ELENICE RICKLI
Departamento de Vertebrados, Museu Nacional, Quinta da Boa Vista, s/n CEP 20940-040 Rio de Janeiro, RJ, Brazil

* *Corresponding author; e-mail: canedo@mn.ufrj.br*

Most species of the genus *Eleutherodactylus* Duméril & Bibron, 1841 are direct developers with eggs laid in terrestrial sites (Wake 1978). Internal fertilization occurs in two species and may be more widespread within the genus (Townsend et al. 1981; Townsend and Stewart 1994). Live-bearing occurs in at least one species (*Eleutherodactylus jasperi*; Wake 1978). Despite these uncommon reproductive features among anurans, detailed aspects of reproductive biology are known for only a few species of the genus (e.g., Diesel et al. 1995; Donnelly 1999; Lutz 1944; Lynn and Lutz 1946a, b; Ovaska and Rand 2001).

Eleutherodactylus binotatus is a common species distributed along Atlantic Rainforest in Brazil but data on its natural history are scarce (Haddad and Sazima 1992; Pombal and Gordo 2004). The objects of this study are: A) to present data on *E. binotatus* female reproductive investment verifying the correlation between female snout–vent length and the number of ovarian follicles of the largest size class; and B) to analyze the reproductive seasonality in *E. binotatus* by evaluating the seasonal variation of total ovarian mass, the number, and mean diameter of ovarian follicles of the largest size class.

We examined 36 adult female *Eleutherodactylus binotatus* collected in a fragment of Atlantic Rainforest (22º01'S 43º31'W; ca. 600m above sea level), at Municipality of Belmiro Braga, State of Minas Gerais, Southeastern Brazil. Individuals were collected during three seasons: the first wet season from September 2000 to March 2001; the dry season from April to September 2001; and the second wet season from October 2001 to March 2002. Specimens are housed in the herpetological collection of Museu Nacional, Rio de Janeiro, Brazil (MNRJ 27371-72, 27376, 27380, 27383, 27386-87, 27394, 27397, 27404, 27409, 27411, 27414, 27417-18, 27420, 27422-24, 27446, 27448, 27451-52, 27460, 27474-78, 27490, 27492-93, 27556, 28317-18, and 28479).

Reproductive maturity of adult females was determined by oviduct condition according to Lynch and Duellman (1997). The snout-vent length (SVL) was measured with calipers to the nearest 0.1 mm. Ovaries were removed and weighed on an electronic balance to the nearest 0.001 g. Ovaries always presented several follicles grouped in different, discrete size classes. We considered only follicles of the largest size class from each ovary as we assumed these follicles constitute a single clutch. The ovarian follicles of the largest size class in ovaries were counted and measured under a stereomicroscope with an ocular micrometer.

We examined the relationship between the SVL and the number of ovarian follicles of the largest size class using a Pearson's test. We performed an Analysis of Variance (ANOVA) to test for differences among seasons regarding 1) ovarian mass, 2) number, and 3) mean diameter of follicles of the largest size class.

Females were divided into two categories according to ovarian mass (females with ovarian mass greater than 0.560 g and smaller than 0.560 g; this corresponds to the mid-point of the total range) in order to determine the frequency of females with the highest ovaries through time and to estimate the approximate time of clutching.

At the study site, female *E. binotatus* ranged in body size from 44.3 to 55.7 mm (= 49.8 ± 2.3 mm); ovarian mass ranged from 0.011 g to 1.128 g (= 0.389 ± 0.340 g); the number of follicles of the largest size class ranged from 20 to 67 (= 32.5 ± 10.1), and mean diameter of follicles of the largest size class ranged from 1.1 mm to 4.6 mm (= 2.6 ± 1.0 mm) (Table 1).

Correlation between the number of follicles of the largest size class and the snout–vent length of female *E. binotatus* was not significant (r = 0.0553; p = 0.78). Significant correlation between female size and clutch size is common in anurans (Kuramoto 1978; Salthe and Duellman 1973). However, Crump (1974) found this correlation in only to 26.8% of studied species in a tropical anuran community. In some species of the genus *Eleutherodactylus* there is a correlation between egg number and female size (e.g. *E. altamazonicus*, *E. lacrimosus*, and *E. lanthanites*: Crump 1974; *E. coqui*: Townsend and Stewart 1994; *E. johnstonei*: Bourne 1997; and *E. bransfordii*: Donnelly 1999) but Crump (1978) found no significant correlation in *E. conspicillatus*, *E. croceoinguinis*, *E. martiae*, *E. ockendeni*, *E. pseudoacuminatus*, and *E. variabilis*, as well *Ischnocnema quixensis*, a species that also has direct development. Species of *Eleutherodactylus* have, in general, smaller clutches with larger eggs than other genera (Salthe and Duellman 1973; Wake 1978). Probably, egg size is more important than clutch size to the reproductive success of species with direct development. Unfortunately, we did not test this hypothesis in the present study because our size data referred to follicles at different stages of development and we could not estimate the final size of eggs from our data.

There is no significant difference among wet and dry seasons, on ovarian mass (F = 0.5379; *df* = 1, 34; p = 0.47), number of follicles (F = 2.1898; *df* = 1, 26; p = 0.15), and mean diameter of follicles (F = 0.0919; *df* = 1, 26; p = 0.76). Nevertheless, females with the heaviest ovaries (ovarian mass higher than 0.560 g) were found only from September to November (Fig. 1). Furthermore, development of ovarian follicles of the largest size class (indicated by the variation in mean diameter of follicles over time; Fig. 2) showed follicles growing during the dry season (April to Sep-

TABLE 1. Mean, standard deviation (SD), and range of snout–vent length (SVL), ovarian mass, number, and mean diameter of follicles of the largest size class of female *Eleutherodactylus binotatus* (N = 36).

Variables	mean ± SD	range
SVL (mm)	49.8 ± 2.3	44.3–55.7
Ovarian mass (g)	0.389 ± 0.340	0.011–1.128
Number of follicles	32.5 ± 10.1	20–67
Follicles mean diam (mm)	2.6 ± 1.0	1.1–4.6

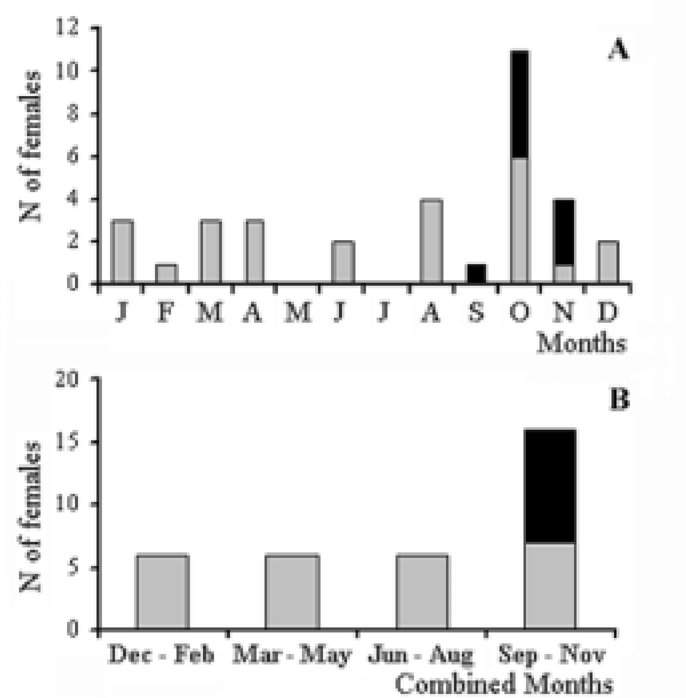

FIG. 1. Number of female *Eleutherodactylus binotatus* with ovarian mass higher than 0.560 g (black) and females with ovarian mass smaller than 0.560 g (gray), data were summed for months sampled during different years. (A) Month to month and (B) combined three-to-three months.

tember) and reaching a developmental peak at the beginning of the wet season (October to November). This peak is followed by a decrease in the mean diameter of follicles, suggestive of an end to the clutching period.

The statistical tests only compare the means among the different seasons and the graphs demonstrate that the follicles are uniformly middle sized in the whole dry season and the largest follicles are present especially at the beginning of the wet season while the smallest follicles are present at the ending of this season. Thus, despite the not significant different means, we could assume that the reproduction in *E. binotatus* is seasonal, occurring at the beginning of the wet season. This is also the time period of the highest vocalization activity of male *E. binotatus* in the same population (C. Canedo, unpubl. data).

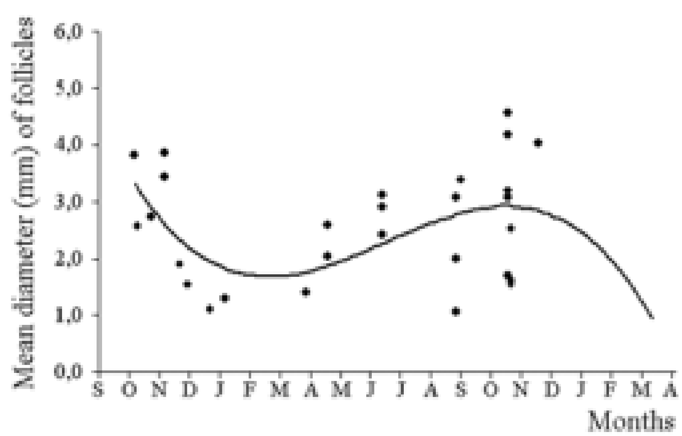

FIG. 2. Mean diameter in millimeters of ovarian follicles of the largest size class in female *Eleutherodactylus binotatus* from September 2000 (S) to March 2002 (M), presenting polynomial of third order trendline.

In the Atlantic Rainforest, in Southeastern Brazil, most anuran species reproduce only during the wet season (e.g., Pombal 1997), as we found for *E. binotatus*. However, *Eleutherodactylus* species have continuous reproduction along the year showing seasonal variation only in the frequency of reproductive activity at Amazonian Rainforest (e.g., Bourne 1997; Towsend and Stewart 1994) and Costa Rican Rainforest (Donnelly 1999).

We conclude that the studied *E. binotatus* population: 1) presents no significant correlation between number of follicles and size of females, and 2) present seasonal reproduction, occurring at the beginning of the wet season.

Acknowledgments.—We are grateful to Daniel S. Fernandes, Guilherme Muricy, José P. Pombal Jr., and Monique Van Sluys for helpful suggestions on the manuscript; to Márcia S. Couri and Carlos Frederico D. Rocha for loaning equipment; to Neil R. Canedo and M. Luzia C. C. Canedo for field assistance; to the owners of Olinda Farm for providing access permits; to Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA for providing collecting permits; to Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq and Fundação Carlos Chagas Filho de Apoio à Pesquisa do Estado do Rio de Janeiro – FAPERJ for financial support; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Capes for CC fellowship.

LITERATURE CITED

BOURNE, G. R. 1997. Reproductive behavior of terrestrial breeding frogs *Eleutherodactylus johnstonei* in Guyana. J. Herpetol. 31:221–229.

CRUMP, M. 1974. Reproductive strategies in a tropical anuran community. Misc. Publ. Nat. Hist. Mus. Univ. Kansas 61:1–68.

DIESEL, R., G. BÄURLE, AND P. VOGEL. 1995. Cave breeding and froglet transport: a novel pattern of anuran brood care in the Jamaican frog, *Eleutherodactylus cundalli*. Copeia 1995:354–360.

DONNELLY, A. M. 1999. Reproductive phenology of *Eleutherodactylus bransfordii* in Northeastern Costa Rica. J. Herpetol. 33:624–631.

HADDAD, C. F. D., AND I. SAZIMA. 1992. Anfíbios anuros da Serra do Japi. *In* L. P. C. Morellato (org.), História Natural da Serra do Japi: Ecologia e Preservação de uma Área Florestal no Sudeste do Brasil, pp. 188–211. Ed. Unicamp/Fapesp, Campinas, Brazil.

KURAMOTO, M. 1978. Correlations of quantitative parameters of fecundity in amphibians. Evolution 32:287–296.

LUTZ, B. 1944. The hatching of *Eleutherodactylus parvus* Girard. Copeia 1944:64.

LYNCH, J. D., AND W. E. DUELLMAN. 1997. Frogs of the genus *Eleutherodactylus* in western Ecuador: systematics, ecology, and biogeography. Spec. Publ. Nat. Hist. Mus. Univ. Kansas 23:1–236.

LYNN, W. G., AND B. LUTZ. 1946a. The development of *Eleutherodactylus nasutus*. Bol. Mus. Nac. (N.S.) Zool. 79:1–30.

———, and ———. 1946b. The development of *Eleutherodactylus guentheri* Stdnr, 1864. Bol. Mus. Nac. (N.S.) Zool. 71:1–46.

OVASKA, K., AND A. S. RAND. 2001. Courtship and reproductive behavior of the frog *Eleutherodactylus diastema* (Anura: Leptodactylidae) in Gamboa, Panama. J. Herpetol. 35:44–50.

POMBAL, J. P., JR. 1997. Distribuição especial e temporal de anuros (Amphibia) em uma poça permanente na Serra de Paranapiacaba, Sudeste do Brasil. Rev. Brasil. Biol. 57:583–597.

———, AND M. GORDO. 2004. Anfíbios anuros da Juréia. *In* O. A. V. Marques and W. Duleba (eds.), Estação Ecológica Juréia-Itatins: Ambiente Físico, Flora e Fauna, pp. 243–256. Holos Editora, Ribeirão Preto, Brazil.

SALTHE, S. N., AND W. E. DUELLMAN. 1973. Quantitative constraints asso-

ciated with reproductive mode in anurans. *In* J. L.Vial (ed.), Evolutionary Biology of the Anurans: Contemporary Research on Major Problems, pp. 229–249. Univ. of Missouri Press, Columbia.
TOWNSEND, D. S., AND M. M. STEWART. 1994. Reproductive ecology of the Puerto Rican frog *Eleutherodactylus coqui*. J. Herpetol. 28:34–40.
———, ———, F. H. POUGH, AND P. F. BRUSSARD. 1981. Internal fertilization in an oviparous frog. Science 212:469–471.
WAKE, M. H. 1978. The reproductive biology of *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), with comments on the evolution of live-bearing systems. J. Herpetol. 12:121–133.

Herpetological Review, 2006, 37(2), xxx–xxx.

Longevity and Breeding Site Fidelity in the California Newt (*Taricha torosa*): A Long-Term Study Showing the Efficacy of PIT Tagging

TYLER S. WATTERS
and
LEE B. KATS
Natural Science Division, Pepperdine University
24255 Pacific Coast Hwy, Malibu, California 90263, USA
e-mail: tyler.watters@pepperdine.edu, lee.kats@pepperdine.edu

California Newts (*Taricha torosa*) inhabit the streams and surrounding terrestrial habitats along the California coast. In the winter and spring, adults migrate from terrestrial habitats to nearby streams or ponds for breeding. Few studies have examined the behavioral patterns of adult *T. torosa* in any great detail, but extensive population and migration studies on *T. rivularis* have demonstrated site fidelity and homing behavior in individuals displaced from capture locations during the breeding season (Twitty 1966). Salamanders in other families (e.g., Ambystomatidae, Plethodontidae) have been shown to possess strong breeding pond fidelity from season to season (Mathis 1989; Trenham et al. 2000), and we suspected that this characteristic exists in *T. torosa* as well.

However, demonstrating breeding site fidelity in *Taricha* is challenging experimentally, due to both their longevity and the difficulty that lies in identifying individual newts and their migrations from year to year. We examined PIT tagging as a marking method for identification in *T. torosa*. This marking technique was successful over our long-term study and enabled us to confirm breeding site fidelity in this species.

Site fidelity in newts was first verified by the use of coded toe clipping, but that technique was reliable for only a couple years following marking because newts regenerated amputated appendages and limbs (Twitty 1966). Radio transmitters, either surgically implanted or inserted into the salamanders' abdomen (Jehle 2000; Madison 1997), are not practical for long-term studies due to their limited battery life. While heat branding can be used to identify individuals years after their initial capture (Peterson et al. 1983), the use of passive integrated transponders (PIT tags) has been introduced recently as a more reliable technique (Trenham et al. 2000).

We conducted a mark-recapture study of *T. torosa* in Cold Creek, California (Los Angeles Co.), a stream located in the Santa Monica Mountains Using permanent geographic land markers, we mapped 700 m of the stream. We surveyed this stream section annually from 1991 to 2003 and recorded the location, sex, SVL, and mass of adult newts. From 1991 to 1994, 36 adults were marked with PIT tags manufactured by AVID. Newts were anesthetized using MS222 and tags were inserted into the abdomen using a syringe. The incision was closed using New Skin® (Medtech). Each subsequent year until 2003, on several days during the breeding season, the stream was mapped and surveyed as before. Newts encountered during surveys were captured and scanned for a PIT tag. Recaptures were measured and weighed, and the distance from their first location was determined. Many individuals in this study were captured several times in a single breeding season, but only the data for their first capture of the season was included in analyses.

Through 2003, 14 of 36 (39%) tagged *T. torosa* adults were recaptured among years, up to 11 years post-tagging (mean 763 d). A few adults were captured more than once for a total of 22 recaptures. Each recapture increased in length (mean (SD): 0.12 (0.16) cm) and body mass (mean (SD): 1.11 (2.47) g) from when they had most recently been collected. Upon recapture, the mean distance between an individual's recapture site and site of initial capture was 15.5 m (SD: 30.1 m). Fourteen of the 22 total recaptures occurred at or within 10 m of an individual's original capture.

Our data demonstrate the utility of PIT tags for long term studies in newts and documentation of life history and population demography information. Our study is the first to document the potential lifespan of the California newt in the wild. One female, recovered in 2000, was originally tagged in 1991 and was last recaptured in May of 1993. Two males, originally marked in 1994, were recaptured and recorded in April of 2003, along with another male who had not been observed in the field since his original capture in 1992, 11 years earlier. Given that *Taricha* do not reach sexual maturity until at least three to four years of age in males, and slightly longer in females (Twitty 1959), the four individuals captured 9–11 years after their initial marking are likely, at minimum, 12–14 years of age.

In Southern California, the 6–12 week breeding period for *T. torosa* can last into May, but most adults leave the streams within several weeks following this period and densities drop to near zero over the course of the summer (Kats et. al 1994; Petranka 1998). Adults emerge from below ground and do not reenter streams, ponds, and reservoirs until after late Fall rains (Stebbins 2003). Our findings suggest that many individuals return to almost the exact locations within the stream among years. Exactly how *T. torosa* orient to the same location along a stream year in and year out is unknown. In *T. rivularis* and *T. granulosa*, Twitty (1959) ruled out visual navigation using landmarks and memorization of the topographical pattern of an individual's home range, and suggested navigation by a chemical mechanism. Extra-ocular magnetic orientation is now known in the red spotted newt, *Notophthalmus viridescens* (Deutschlander et. al 1999).

Acknowledgments.—We thank Tom Vandergon for his valuable field assistance, the Mountains Restoration Trust for access to Cold Creek and continuing support of stream ecology research in the Santa Monica Mountains, and the Natural Science Division at Pepperdine University for providing the equipment used in this study.