

Contemporary Herpetology

Volume 2008, Number 3

18 June 2008

contemporaryherpetology.org

REPRODUCTION AND HABITAT OF TEN BRAZILIAN FROGS (ANURA)

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INTRODUCTION

Basic data on habitat, behavior, and reproduction are lacking for most Neotropical frog species and even higher taxonomic groups (Crump 1974; Haddad and Prado 2005), particularly for those restricted to the Atlantic Forest. Basic reproductive features are the basis of comparative studies on evolution of major natural history features (Harvey and Pagel 1998), such as the interspecific relationship between body size and egg number/size (Salthe and Duellman 1973, Crump 1974, Stearns 1992). Here, we present data on habitat, reproductive behavior and quantitative parameters such as adult sizes, egg numbers/sizes of ten sympatric frogs of an altitudinal Atlantic Forest site in Southeastern Brazil.

MATERIALS AND METHODS

The present study was carried out in the Parque Florestal do Itapetinga (PFI) and adjacent areas (approx. 23°15'S; 46°45'W, 900-1300 m alt., 1400-1700 mm rain/year), Atibaia municipality, Mantiqueira range in the State of São Paulo, Southeastern Brazil. Secondary semi-deciduous forest covers continuous 1,800 ha (Meira-Neto et al. 1989, Giaretta et al. 1999), and peripheral open areas include pastures and agricultural fields. The regional climate is seasonal, with a dry/cold season from April to September (with occasional frosts in July) and a wet/warm season from October to March. The precipitation can be zero in some months of the dry/cold season. Natural bodies of water are perennial forest rivulets which can enter open deforested areas, and seasonal oxbow ponds. Currently there are two man made lakes (< 50 x 20 m) in the area. The clean watered forest rivulets (maximum 1.5 m wide) run either over/among granitic rocks or sand.

Field data collection was performed weekly between June 1992 and June 1993; irregular trips from 1994-2005 provided additional data. In the field we recorded daily and annual time of vocal activity, annual time of occurrence of egg-bearing females and/or egg clutches, and calling sites. Data on leaf litter frogs were also based on the specimens reported in Giaretta et al. (1997) and Giaretta et al. (1999). Reproductive parameters, such

as egg number, were based on gravid females collected in the field and preserved in 5% formalin. Ovarian eggs were considered mature by comparison with eggs found in clutches or when females presented concurrently hypertrophied ovaries and oviducts (Rough 1951, Crump 1974). Size measurements were made with a caliper to the nearest 0.1 mm. For most species, samples of eggs and tadpoles were kept in aquaria until metamorphosis for specific identification. Voucher specimens are housed in the Museu de História Natural da Universidade Estadual de Campinas (ZUEC), Campinas, São Paulo, Brazil.

RESULTS

Adult females varied in size from around 20mm (*Ischnocnema parva*) to 65 mm (*Odontophrynus cf. maisuma*), and bore from around 15 (*Ischnocnema juipoca*) to 3000 (*O. cf. maisuma*) eggs/female (Table 1). Eggs were particularly large among *Ischnocnema* species (1.6 – 2.7 mm) and in *Crossodactylus* sp. (2.3 mm) (Table 1). In our sample, sexual dimorphism in size was observed in most species, being particularly pronounced among the *Ischnocnema* species (Table 2).

Considering the six species with at least three females available for analysis (Table 2), we observed a positive correlation ($p < 0.05$) between female size and fecundity just for *Ischnocnema guentheri* ($r = 0.58$; $p = 0.005$; $N = 21$) and *Crossodactylus* sp. ($r = 0.95$; $p = 0.048$; $N = 4$).

The tadpoles of all species with indirect development can be regarded as exotrophic benthic (McDiarmid and Altig 1999).

Remarks on habitat and reproductive behavior:

Ischnocnema guentheri (Steindachner 1864). Habitat- leaf litter ($N = 15$) (see also Heyer et al. 1990); annual pattern of vocalization- wet/warm season; vocalization period- crepuscular/nocturnal ($N = 20$ days) or during diurnal rain showers ($N = 10$ days); site of vocalization- perched on low (< 1.5 m) vegetation ($N = 6$ individuals).

Ischnocnema juipoca (Sazima and Cardoso 1978). Habitat- low (< 1.5 m) bushes (mostly grass) in open areas ($N = 20$ individuals) or rarely forest border ($N =$

Table 1. Egg number and egg size of ten frog species from Atibaia (São Paulo, Brazil). SD = standard deviation; N = analysed females. Ten measured eggs/female.

Family Species	N	Eggs/ Female Mean	SD	Mean ovarian egg diameter (mm)	Egg color
Brachycephalidae					
<i>Ischnocnema guentheri</i>	21	35	5	2.7	whitish
<i>Ischnocnema juipoca</i>	6	15	4	2.4	whitish
<i>Ischnocnema parva</i>	22	20	4	2.3	whitish
<i>Ischnocnema sp.</i>	2	16	0	1.6	whitish
Cycloramphidae					
<i>Odontophrynus cf. maisuma</i>	3	2980	444	1.1	black
<i>Proceratophrys boiei</i>	5	1296	284	1.8	dark gray
Hylodidae					
<i>Crossodactylus sp.</i>	4	70	8	2.3	whitish
<i>Hylodes aff. sazimai</i>	1	109	-	Immatures	whitish
Leiuperidae					
<i>Physalaemus cuvieri</i>	1	474	-	1.0	whitish
<i>Physalaemus olfersii</i>	1	648	-	1.1	whitish

1 individual); annual pattern of vocalization- wet/warm season; vocalization period- crepuscular/nocturnal (N = 20 days) or during diurnal rain showers (N = 15 days); site of vocalization- on the ground or perched on low (< 50 cm) vegetation (N = 5 individuals).

Ischnocnema parva (Girard 1853). Development mode- direct (Figure 1) (see also Lutz 1944); oviposition site- terrestrial (N = 1); habitat- leaf litter (see also Heyer et al. 1990); annual pattern of vocalization- restricted to the wet/warm season; vocalization period- crepuscular/nocturnal (N = 20 days) or during diurnal rain showers (N = 10 days); site of vocalization- leaf litter (N = 10 individuals).



Figure 1. The terrestrial egg clutch and direct development of *Ischnocnema parva*. A - Late embryos inside the eggs; B - Newly hatched juveniles. Specimens from Ubatuba, São Paulo.

Ischnocnema sp. (cf. *spanios*, [Heyer 1985]). Habitat- leaf litter (N = 4 individuals); annual pattern of vocalization- restricted to the wet/warm season (N = 10 days); vocalization period- crepuscular/nocturnal (N = 20 days) or during diurnal rain showers (N = 10 days); site of vocalization- perched on low (< 1.0 m) vegetation at the forest border (N = 3 individuals).

Odontophrynus cf. maisuma (Rosset 2008). Habitat- terrestrial, in open areas (N = 6 individuals); annual pattern of vocalization- sporadic and unpredictable in summer or winter (N = 5 events); vocalization period- nocturnal (N = 7 days); site of vocalization- beside creeks at open areas.

Proceratophrys boiei (Wied, 1825). Development mode- feeding aquatic larvae (N = 20 tadpoles); oviposition site- unknown, possibly in forest creeks, where an amplectant pair and tadpoles were found; adult habitat- leaf litter (see also Giaretta et al. 1998); annual pattern of vocalization- restricted to the wet/warm season (N = 30 days); site of vocalization- on the ground, along the margins of forest creeks; vocalization period- crepuscular/nocturnal.

Crossodactylus sp. (ex gr. *gaudichaudii* Duméril and Bibron 1885). Development mode- feeding aquatic larvae (N = 50 tadpoles); adult habitat- leaf litter along creeks (20 individuals); annual pattern of vocalization- all around the year; vocalization period- diurnal (N = 25 different days); site of vocalization- on the ground beside forest creeks (N = 16 individuals); additional remark- males keep and defend underwater galleries they dig in creeks (N = 3) to which they conduct receptive females (N = 1).

Hylodes aff. sazimai Haddad and Pombal Jr. 1995. Development mode- feeding aquatic larvae (N = 5 tadpoles); adult habitat- leaf litter along forest creeks (N = 25 individuals) and leaf litter (N = 3 individuals); annual pattern of vocalization- restricted to the wet/warm season; vocalization period- diurnal (N = 26 days); site of vocalization- on the ground beside forest creeks (N = 10 individuals).

Physalaemus cuvieri Fitzinger 1826. Development mode- feeding aquatic larvae; egg-laying site- floating foam nest in lakes or ponds (N = 3); adult habitat- collected around pond margins (N = 2), in open areas; annual pattern of vocalization- restricted to the wet/warm season (N = 25 days); vocalization period- crepuscular/nocturnal (N = 15 days); site of vocalization- in the water, in

Table 2. Size (mm) and sexual dimorphism of ten frog species from Atibaia (São Paulo, Brazil). SVL = snout-vent length; SD = standard deviation; N = sample size.

Family Species	Female SVL			Male SVL			Dimorphism	
	N	Mean	SD	N	Mean	SD	t-test	p value
Brachycephalidae								
<i>Ischnocnema guentheri</i>	25	35.9	1.94	4	28.1	3.21	6.87	< 0.001
<i>Ischnocnema juipoca</i>	8	26.1	1.41	4	20.1	0.69	8.00	< 0.001
<i>Ischnocnema parva</i>	25	20.1	0.88	12	15.5	1.38	12.37	< 0.001
<i>Ischnocnema sp.</i>	4	21.4	1.30	5	16.2	0.38	8.75	< 0.001
Cycloramphidae								
<i>Odontophrynus cf. maisuma</i>	3	52.2	2.40	4	46.0	6.01	1.67	= 0.16
<i>Proceratophrys boiei</i>	12	65.4	3.82	6	47.9	4.52	8.60	< 0.001
Hylodidae								
<i>Crossodactylus sp.</i>	8	24.1	1.10	9	21.6	0.85	5.31	< 0.001
<i>Hylodes aff. sazimai</i>	2	30.3	3.68	4	28.6	0.91	0.98	= 0.38
Leiuperidae								
<i>Physalaemus cuvieri</i>	4	28.2	1.60	5	26.5	1.15	1.86	= 0.10
<i>Physalaemus olfersii</i>	4	29.6	1.47	8	27.2	1.52	2.56	= 0.28

open area ponds.

Physalaemus olfersii Lichtenstein and Mertens 1856. Development mode- feeding aquatic larvae (N = 30 tadpoles); oviposition site- foam nests built under leaves in shallow (< 5 mm deep) water of oxbow ponds at forest border (N = 15); adult habitat- leaf litter (see also Heyer et al. 1990); annual pattern of vocalization- restricted to the wet/warm season; vocalization period- crepuscular/nocturnal (N = 30 days); site of vocalization- on the ground, close (up to 1 m) to inside forest oxbow ponds.

DISCUSSION

Ischnocnema guentheri and *I. parva* are known to have direct development (Lynn and Lutz 1946, Lutz 1944, present work), which is also expected to occur in *I. juipoca* and *I. cf. spanios*. Pombal and Haddad (2005) presented data on egg number and size of 19 frog species from a coastal locality in southeastern Brazil. Their data on *I. guentheri*, *P. cuvieri*, *P. olfersii* and *P. boiei* are in agreement with those we found; as they considered fertilized eggs, the sizes they report tend to be larger than those reported here. *Odontophrynus* are expected to have feeding aquatic larvae (Gallardo 1963). The oviposition sites of most species of Hylodidae are not known. As we found for *Crossodactylus sp.*, Weygoldt and Carvalho e Silva (1992) and Haddad and Giaretta (1999) report males conducting females to underwater galleries. The reproductive habits of *Physalaemus cuvieri* have been detailed in Bokermann (1962), Cardoso (1980), and Menin and Giaretta (2003).

Among frogs, intraspecific variation in body size often accounts for little of the variation in egg number (Crump 1974, present work); besides size, we suggest that the number of reproductive events a female experiences within a season certainly is an important parameter to be considered in this relationship. Phylogeny (Lynch 1971, Heyer 1975) accounts for most of the observed behavioral/ecological similarities among the studied species. *Ischnocnema* frogs are terrestrial breeding species that have low egg number, large unpigmented eggs, pronounced sexual size dimorphism, direct development, and in some species perching habits. *Ischnocnema juipoca* show a rare condition within the genus (Lynch and Duellman 1997) by being an open area dweller. *Physalaemus* species are foam-nesting frogs that have median fecundity, medium size, and unpigmented or slightly pigmented eggs. *Crossodactylus* and *Hylodes* are hylodid

frogs, which breed in forest creeks, have diurnal habits, relatively low fecundity, and large and unpigmented eggs (Giaretta et al. 1993, Haddad and Giaretta 1999). The *Odontophrynus* and *Proceratophrys* (Cycloramphidae, Alsodinae) species were quite different from one another in habitat, but both were similar in having unspecialized tadpoles inhabiting slow flowing water, high fecundity, and small and dark-pigmented eggs.

The number and size of the eggs of a frog species appear to be related to its development mode, site of egg-laying, and existence of parental care (Salthe and Duellman 1973, Crump 1974, Basso 1990; Pombal Jr. and Haddad, 2005), but these hypotheses have not been tested within a phylogenetic context (Harvey and Pagel 1998). As phylogenies are still poorly resolved and controversial and basic information on the component species are insufficient such comparative studies depend on accumulation of data such as those presented here.

ACKNOWLEDGMENTS

The Fundação O Boticário de Proteção à Natureza, Fundo de Apoio ao Ensino e à Pesquisa (UNICAMP), CNPq, and FAPEMIG financially supported the project. R.J. Sawaya, J.C. Oliveira Filho, A. P. Rodrigues, W. R. da Silva, and D. Resende helped in the field works. Grants by CNPq (AAG) and CAPES (KGF). A.J. Cardoso, C.F.B. Haddad, A.S. Abe, W.R. Heyer and J. Jim made helpful suggestions on early drafts.

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