Breeding activity of Scinax centralis (Anura, Hylidae) in Central Brazil

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ABSTRACT. From January, 1997 to April, 1998, we studied breeding activity in a population of *Scinax centralis* Pombal & Bastos, 1996, in the state of Goiás, inside the *Cerrado* Biome, Brazil. The behavior of males and females in the choruses, the relationships between environmental variables and the presence of individuals, the spatial distribution pattern, amplexus and clutches were analyzed. The breeding season occurred from November to May. The majority of males began to call when they entered the chorus; they were defending their calling sites against other males. We found a positive correlation between the number of females and males in the choruses, but no significant correlation was observed between the operational sexual ratio (OSR, number of reproducing females/number of reproducing males) and the number of calling males. OSR was highly male-biased; on average, there were 40 males for each female. The presence of individuals was positively correlated with air temperature of two previous days and negatively correlated with rainfall on the observation day. Generally, males were non-randomly distributed and satellite males were observed. Females were larger and heavier than males. Egg number, but not diameter of the egg, was correlated with the female size.

KEYWORDS. Scinax centralis, spatial distribution, breeding, aggressive behavior, Central Brazil.

RESUMO. Atividade reprodutiva de *Scinax centralis* (Anura, Hylidae) no Brasil Central. Entre janeiro de 1997 e abril de 1998, estudou-se a atividade reprodutiva em uma população de *Scinax centralis* Pombal & Bastos, 1996 no estado de Goiás, no bioma Cerrado, Brasil. Analisou-se o comportamento de machos e fêmeas nos agregados reprodutivos, as relações entre variáveis ambientais e a presença de indivíduos, o padrão de distribuição espacial, amplexos e desovas. A estação reprodutiva ocorreu de novembro a maio. A maioria dos machos começou a vocalizar quando entrou nos agregados; defendendo seus sítios de canto contra outros machos. Nós encontramos uma correlação positiva entre o número de fêmeas e de machos presentes no coro, mas não entre a razão sexual operacional (RSO, número de fêmeas reprodutivas/número de machos reprodutivos). A RSO foi altamente desviada para os machos; em média, haviam 40 machos para cada fêmea. A presença de indivíduos foi correlacionada positivamente com a temperatura do ar dos dois dias anteriores e negativamente correlacionada com a chuva do dia da observação. Geralmente, a distribuição espacial dos machos era uniforme. Machos satellites foram observados. As fêmeas eram maiores e mais pesadas que os machos. O número de ovos, mas não o diâmetro, está correlacionado com o tamanho da fêmea.

PALAVRAS-CHAVE. Scinax centralis, distribuição espacial, reprodução, comportamento de agressão, Brasil central.

Most anuran species use vocalizations to mediate social interactions during breeding (Wells, 1977; Bastos & Haddad, 1995, 2002). On successive nights, male anurans aggregate in temporary or permanent ponds and emit distinct calls (Bee *et al.*, 2000) because females may use call features as cues for evaluation of the partner fitness (Ryan & Keddy-Hector, 1992).

Generally, in the choruses, males greatly outnumber females, so they exhibit territorial behavior which may involves acoustics interactions, postures, and combat (Martins *et al.*, 1998; Bastos & Haddad, 2002). Males of prolonged or explosive breeding species (*sensu* Wells, 1977) may be territorial (Roberts, 1994; Bastos & Haddad, 2002). However, territoriality is more accentuated in prolonged breeders because females generally arrive asynchronously in choruses with males (Wells, 1977; Murphy, 1994).

A consequence of territorial behavior in anurans is the maintenance of a uniform distance between males (Arak, 1983; Dyson & Passmore, 1992a; Bastos & Haddad, 1999). The maintenance of interindividual distance can reduce the acoustic interference caused by neighbors and may facilitate the location of the calling male by the female (Whitney & Krebs, 1975; Dyson & Passmore, 1992b).

In this paper, we examined the breeding activity of *Scinax centralis* Pombal & Bastos, 1996, a small hylid of

the *S. catharinae* Boulenger, 1888 group, which is found in gallery forest, one of the physiognomies of the *Cerrado* biome (OLIVEIRA-FILHO & RATTER, 2002). Primarily, we intend to answer the following questions: (a) What are the relationships between choruses and environmental variables? (b) How are males spatially distributed in choruses? (c) Is there operational sexual ratio bias for males? (d) Are clutches correlated with physical attributes of females?

MATERIAL AND METHODS

Scinax centralis was observed in a National Forest (FLONA, Floresta Nacional; 16°39'32"S; 48°36'29"W) in the municipality of Silvânia, state of Goiás, Brazil, from January 1997 to April 1998. The study area is a patch (200m) of rivulet in a gallery forest, with insignificant water flow. Therefore, in the practice, it may be considered (a permanent lentic water body). Field observations were conducted from 17:30 h until 24:00 h or 03:00 h, depending of the activity of the individuals. The studied area was monitored for a total of 178 hours during 41 visits. Meteorological data was provided by a climatological station in FLONA.

The air temperature was recorded with a thermometer to the nearest 0.5°C. Two hundred forty-five

males and 16 females were marked by toe clipping (Martof, 1953), and measured the snout-vent length (SVL) to the nearest 0.01mm with a caliper ruler and weighed individuals to the nearest 0.05g with a spring scale. We counted the total number of males and females in the rivulet and recorded the location of these individuals, aggressive interactions, amplexus, and clutches.

In the observed aggressive interactions, we considered a male as a resident when it was calling at the beginning of the focal observations, while the winner was the male that stayed in the call site until the end of the interaction and restarted the vocalization.

All pairs found in amplexus (N=11) were collected and placed in plastic bags containing water and were maintained until the oviposition. The clutches obtained were preserved in 5% formalin and the egg diameter was measured with a micrometric ocular. Males and females were weighed and measured as described above, then released alive on the same night.

To analyze the pattern of spatial distribution, we used the methodology of CLARK & EVANS (1954), which considers the distance to the nearest individual and male density at choruses. Data on minimum air temperature and total rainfall of the day or one/two/three previous days of observation were examined to determine their influence on nightly population by stepwise multiple regression forward (ZAR, 1996). We also used the Student's t-test or Pearson correlation coefficient (ZAR, 1996) to analyze and compare the relationship between SVL/weight of female or male or SVL/weight of female and egg variables. The level of significance considered was 0.05. Voucher specimens (ZUFG 140-141, ZUFG 174-177) were deposited in the Coleção Zoológica of Universidade Federal de Goiás (ZUFG).

RESULTS

Calling males of *S. centralis* were first heard in November when the minimum air temperature and humidity were 17.6°C and 90%, respectively. Calling stopped completely in August when the minimum air temperature decreased to 9.8°C and air humidity to 65%.

Stepwise multiple regression revealed significant positive and negative correlations of the minimum air temperature and of the rainfall of the day or previous days (one/two/three), respectively, with the number of individuals (Tab. I). The strongest coefficient of determination (R²) was obtained with the minimum air temperature of the two previous days and rainfall on the observation day. Generally, the coefficient indicated that the variability of the number of individuals in choruses was explained by minimum air temperature and rainfall on the observation day or one/two/three previous day (Tab. I).

The number of males in the chorus was always greater than number of females, which generated low operational sexual ratio (OSR), varying from 0.011 to 0.048. On average, there were 40 males: 1 female. There was a positive and significant correlation between the number of males and the number of females in the chorus (r=0.356; P=0.017; N = 41), but not between OSR and number of males (r=-0.121; P=0.78; N=12).

Table I. Stepwise regression analysis of the environmental variables (air temperature and rainfall) of observation day or one/two/three previous days and number of individuals of *Scinax centralis* Pombal & Bastos, 1996. FLONA, Municipality of Silvânia, Goiás, Brazil.

temp. 0	La	ıg day	\mathbb{R}^2	F	t _{air t}	t _{rainfall}	P _{air t}	Prainfall
0 0 0.466 12.6 4.17 -3.78 0.0003 0.000 1 0 0.482 13.5 4.34 -4.21 0.0002 0.000 2 0 0.556 18.1 5.17 -4.33 0.0001 0.000 3 0 0.503 14.7 4.57 -3.80 0.0001 0.000 0 1 0.203 3.69 2.64 -0.06 0.0130 0.951* 1 1 0.167 2.91 2.33 0.19 0.0270 0.853 2 1 0.269 5.35 3.20 0.18 0.0030 0.862* 3 1 0.257 5.01 3.09 0.20 0.0040 0.845* 0 2 0.238 4.54 2.96 -1.16 0.0060 0.255* 1 2 0.200 3.62 2.64 -1.11 0.0130 0.276* 2 2 0.309 6.48 <t< td=""><td>Air</td><td>Rainfall</td><td>_</td><td></td><td>an t</td><td>rainian</td><td>an t</td><td>ramian</td></t<>	Air	Rainfall	_		an t	rainian	an t	ramian
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1 1 0.167 2.91 2.33 0.19 0.0270 0.853 2 1 0.269 5.35 3.20 0.18 0.0030 0.862 3 1 0.257 5.01 3.09 0.20 0.0040 0.845 0 2 0.238 4.54 2.96 -1.16 0.0060 0.255 1 2 0.200 3.62 2.64 -1.11 0.0130 0.276 2 2 0.309 6.48 3.55 -1.30 0.0013 0.204 3 2 0.291 5.96 3.41 -1.21 0.0019 0.237 0 3 0.222 4.15 2.65 0.85 0.0120 0.401 1 3 0.180 3.17 2.27 0.70 0.0380 0.492 2 3 0.290 5.93 3.24 0.94 0.0030 0.356								
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1 3 0.180 3.17 2.27 0.70 0.0380 0.492 2 3 0.290 5.93 3.24 0.94 0.0030 0.356	3	2	0.291	5.96	3.41	-1.21	0.0019	0.2376
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2 3 0.290 5.93 3.24 0.94 0.0030 0.356	0	3	0.222	4.15	2.65	0.85	0.0120	0.4014
	1	3	0.180	3.17	2.27	0.70	0.0380	0.4926
3 3 0.276 5.51 3.11 0.89 0.0040 0.382	2	3	0.290	5.93	3.24	0.94	0.0030	0.3565
5 0.270 5.51 5.11 0.07 0.0040 0.502	3	3	0.276	5.51	3.11	0.89	0.0040	0.3824

Of the 261 individuals marked between January and May of 1997, none were collected in the following breeding season (November/1997 - April/1998). Females $(\overline{X}_{SVL}=25.95\text{mm}\pm1.94, range: 20.85-29.45, N=19; \overline{X}_{weight}=1.02g\pm0.18, range: 0.7-1.3, N=19)$ were larger (t=12.3, P=0.000) and heavier (t=15.28, P=0.000), after the oviposition, than males $(\overline{X}_{SVL}=20.31\text{mm}\pm1.06, range: 17.05-23.45, N=243; \overline{X}_{weight}=0.52g\pm0.08, range: 0.3-0.8, N=243)$. There were no significant differences in SVL (t=0.74, P=0.45) and weight (t=1.17, P=0.17) between males found in amplexus $(\overline{X}_{SVL}=20.07\text{mm}\pm0.93, range: 18.5-21.75, N=11; \overline{X}_{weight}=0.49g\pm0.06, range: 0.4-0.6, N=11)$ and solitary males $(\overline{X}_{SVL}=20.32\text{mm}\pm1.07, range: 17.05-23.45, N=232; \overline{X}_{weight}=0.52g\pm0.08, range: 0.3-0.8, N=232)$.

Males vocalized, generally, on the branches of the marginal vegetation, from 22 cm above the ground (SD=14 cm, N=80). However, calling males were also observed on the floor or on sticks on the surface of the water. The mean distance observed between neighbor males was $1.7 \, \text{m} \pm 2.0$ (range: 0.1–19.4; N=276). A negative correlation was found between chorus size and nearest neighbor distance (r=-0.659, df=12, P=0.006, N=13; Tab. II). In most of the chorus, the distribution of males was non-random, with an observed uniform pattern in 13 nights (Tab. II).

The males of *S. centralis* were territorial during the breeding activity and defended calling sites with varied degrees of aggressiveness, including acoustic interactions or physical combat. The encounters (N=12) were always initiated by an intruder with an advertisement call. The resident male answered with territorial calls. Then, the intruder escaped (N=6) or stayed in a crouched posture (N=2). However, if the intruder emitted territorial calls near the resident calling male, both males approached each other quickly with short jumps (N=4). These aggressive interactions last 30 to 90s, and occasionally up to 3 min. Winner males ($\overline{X}_{\text{SVL}}$ =20.93mm $_{\pm}$ 1.16, 18.8-22.05, N=6; $\overline{X}_{\text{weight}}$ =0.51g $_{\pm}$ 0.05, 0.45-0.60, N=6) were not

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Table II. Results of spatial	distribution analysis on Scinax	c centralis Pombal & Basto	os, 1966 individuals i	in the chorus. FLON	A, Municipality
of Silvânia, Goiás, Brazil.	* Significant to $p \le 0.05$; **	Significant to $p \le 0.01$.			

Data	Number of Individuals	Distance to the closest individual $\overline{X} \pm DP (Min - Máx)$	R	С	Interpretation
19/02/97	64	$1.76 \pm 1.33 \ (0.25\text{-}10.6)$	1.83	7.61**	Uniform
19/03/97	40	$2.44 \pm 1.88 \; (0.10 8.0)$	2.178	12.57**	Uniform
21/03/97	46	$1.12 \pm 0.53 \ (0.40 - 2.3)$	1.49	4.20**	Uniform
25/04/97	31	$1.94 \pm 1.16 \; (0.40\text{-}14.4)$	1.87	7.75**	Uniform
23/05/97	17	$2.64 \pm 0.77 \; (1.90 \text{-} 19.4)$	2.28	9.15**	Uniform
28/12/97	40	$1.36 \pm 0.52 \ (1.0 - 6.0)$	1.72	2.75**	Uniform
23/01/98	85	$0.74 \pm 0.77 \; (0.20 \text{-} 3.4)$	1.09	1.00	Random
22/02/98	84	$1.27 \pm 1.06 \ (0.25 \text{-} 3.5)$	1.648	5.68**	Uniform
20/03/98	85	$1.06 \pm 0.81 \ (0.10 3.5)$	1.644	6.76**	Uniform
03/04/98	55	$1.26 \pm 1.02 \ (0.30 3.5)$	2.31	11.58**	Uniform
04/04/98	54	$1.14 \pm 0.99 \; (0.20 \text{-} 3.5)$	1.62	5.91**	Uniform
09/04/98	75	$1.21 \pm 0.95 \ (0.45 - 3.5)$	1.537	4.83*	Uniform
11/04/98	60	$2.08 \pm 1.52 \ (0.15\text{-}4.1)$	2.24	9.51**	Uniform
25/04/98	45	$3.07 \pm 0.60 \ (2.2-3.5)$	2.43	8.31**	Uniform

significantly larger (t=-1.36, P=0.25) or heavier (t=-0.97, P= 0.37) than losers (\overline{X}_{SVL} =19.94mm \pm 1.0, range: 18.0-20.9, N=6; \overline{X}_{weight} =0.47g \pm 0.06, 0.40-0.55, N=6). We did not observe any aggressive interaction involving females and concluded that they are not territorial.

We also found satellites males (N=6) that adopted a crouched postured near a calling male. The mean distance between calling and satellite individuals was 27 cm \pm 20.66 (N=3). Calling males (\$\overline{X}_{SVL}\$=20.35mm \pm 0.6, 19.75-21.15, N=5; \$\overline{X}_{weight}\$=0.52g \pm 0.04, 0.50-0.60, N=5) were not significantly larger (t=0.99, P=0.35) or heavier (t=1.25, P=0.21) than satellites (\$\overline{X}_{SVL}\$=19.74mm \pm 1.39, range: 19.45-21.15, N=5; \$\overline{X}_{weight}\$=0.48g \pm 0.07, 0.35-0.55, N=5). When the calling male was removed, the satellite emitted advertisement calls (N=5).

We observed the initiation of amplexus twice. A female approximated to the calling male until a distance of 10cm, staying in front of him and with her body flattened on the substrate. When the male noticed her presence, he stopped the calls and jumped above her without previous physical contact by the female before clasping. The amplexus is axillar and lasted up to 4.5 hours. The clutch was deposited under leaf litter of the rivulet. We did not find a significant correlation between SVL or weight of the males and females in amplexus (r_{SVL} =0.549, P>0.05, N=11; r_{weight} =0.07, P>0.05, N=11).

The eggs have a black pigmented animal pole and a cream vegetative pole including a transparent gelatinous layer, being submerged or adhered to the marginal vegetation. Females deposited an average of 64 eggs \pm 15 (N=17 clutches). The egg diameter range from 1.33 to 1.64mm (\$\overline{X}\$=1.49mm \pm 0.091). We found a significant positive correlation between SVL (r=0.62, P=0.005, N=17) and weight (r=0.61, P=0.0043, N=17) of females and the number of eggs by clutch. However, the egg diameter was not correlated with SVL of females (r=0.29, P=0.34, N=12) and weight (r=0.43, P=0.12, N=12) or with the number of eggs (r=0.009, P=0.85, N=12).

DISCUSSION

Our results indicate that *Scinax centralis* is a typical prolonged breeding species (*sensu* Wells, 1977), with individuals congregating at permanent water bodies, where males defend territories and vocalize to attract females, as other anurans (Martins, 1988; Bastos & Haddad, 1996, 1999; Menin *et al.*, 2004). In the breeding aggregations, the number of males was greater than that of females, arising low nightly estimates of OSRs (40 males:1 female), as registered in many prolonged breeders (Bastos & Haddad, 1996; 1999; Dyson *et al.*, 1992; Wogel *et al.*, 2002). Low OSR can explain the small number of males (N=11) encountered in amplexus, because malemale competition (intrasexual competition) is very intense. Otherwise, it is possible that females mate with preferred males.

In addition, the number of males was positively correlated with the number of females, which is a common result in anurans (Bastos & Haddad, 1996; 1999; Rico *et al.*, 2004). However, as there was no significant correlation between the number of males and OSR, males in large choruses do not have a higher probability of mating than males in smaller choruses, as observed for other species (Bastos & Haddad, 1996; 1999).

Minimum air temperature and daily rainfall seem to dictate the breeding activity in *S. centralis*, as other species of amphibians (Heatwole, 1962; Salvador & Carrascal, 1990; Lizana *et al.*, 1994). As it is expected, the correlation between number of individuals and minimum air temperature was positive because direct insolation generates an increase in soil temperature in the refuge, and the individuals migrate to the breeding area as observed in other species (Salvador & Carrascal, 1990). Otherwise, the effect of daily rainfall is negative because they stop the calling activity as verified in other species (e.g. *Hyla elegans* Wied, 1924, Bastos & Haddad 2002).

In this study, we did not recaptured none marked male (N=261) in the following breeding season. This result may be due to the method of marking by toe clipping, because occasional infections caused the death of

animals (see Golay & Durrer, 1994; Lemckert, 1996). However, effects of toe-clipping vary among species, with return rates not decreasing (Funk *et al.*, 2005). As the seasonality (dry and rainy seasons) in the Cerrado biome is very intense, it is possible that many individuals died or individuals may have relocated to other patches of rivulet, which were not studied (our study area was only a patch of 200m) and not recaptured. Alternative marking techniques can not be used on the smaller species (as *Scinax centralis*) and toe clipping is the only efficient method (Funk *et al.*, 2005). So, we can not conclude that toe clipping had some influence on the results.

Males of *S. centralis* showed site fidelity and defended their territories with postures, vocalizations and fights, indicating that the agonistic interactions presented an increasing escalated aggressiveness, this being a similar system found in other species (Dyson and Passmore, 1992a; Pombal *et al.*, 1994, Martins *et al.*, 1998; Wogel *et al.*, 2002).

In other anuran species, when aggressive interactions escalate to physical combats, size (Howard, 1978, Robertson, 1986), residence (Fellers, 1979; Wogel et al., 2002), or both (Wells, 1978; Bastos & Haddad, 2002) are the main determinant of the result. For *S. centralis*, it is possible that residence was a decisive factor, because the winners of agonistic interactions were always the residents.

An evident consequence of territoriality observed for S. centralis, was the maintenance of a uniform spatial pattern of distribution among males in choruses, as observed for other anuran species (Whitney & Krebs, 1975; Wells, 1977; Arak, 1983; Bastos & Haddad, 1999). The negative correlation between chorus size and nearest neighbor distance found for S. centralis is also characteristic of anuran species (GERHARDT et al., 1989; Bastos & Haddad, 1999). However, for S. centralis, the accommodation of additional males into a chorus did not result in alteration of spatial distribution, indicating that males could tolerate a neighbor when the number of individuals in the chorus increased, as observed in other anurans (Wells, 1988). According to Wells (1977), the maintenance of space can reduce the acoustic interference of neighbors therefore, facilitating the location of the calling individual by female, being a compromise between territoriality and mating.

The occurrence of satellite male in *S. centralis* was registered a few times, making difficult to relate this strategy to population density. However, as we did not find a significant difference between the SVLs of calling and satellite males, and because the satellite male began to emit an advertisement call only when the resident male left, it is possible that the determinant factor that decides between the satellite or calling tactic could be related to the physiological state, as suggested for *Dendropsophus minutus* (Peters, 1872) (HADDAD, 1991).

Clutch size in *S. centralis* was positively correlated with weight and SVL of females, as found in other species (Bastos & Haddad, 1996; 1999; Rico *et al.* 2004). However, in *S. centralis* the eggs diameter was not correlated with weight or SVL of the females or with the number of eggs by clutch as observed in others anuran species (Crump, 1974; Ricco *et al.*, 2004). These last results are not

according to Salthe & Duellman (1973), because expected is a positive correlation between egg diameter and female SVL, or a negative correlation between egg diameter and clutch size.

We conclude that *Scinax centralis* is a prolonged breeder, with OSR male-biased, with a non-random pattern of spatial distribution, where rainfall and air minimum temperature influences reproductive activity.

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