Ecology and reproductive biology of two species of *Aplastodiscus* (Anura: Hylidae) in the Atlantic forest, Brazil

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Abstract

The present study was conducted in the Serra do Japi in the State of São Paulo, Brazil. From March 2004 to January 2006 we studied the ecology and reproductive biology of two sympatric species of *Aplastodiscus* in three different habitats: lake, stream, and swamp. The majority of *A. leucopygius* males in calling activity were recorded during the rainy season and during sporadic rains in the dry season (April to September). The same was observed for *A. arildae*. Most courtship displays of *A. leucopygius* were observed mainly during the rainy period and only one courtship behaviour was observed for *A. arildae* during a rainy night, in December 2005. *Aplastodiscus leucopygius* males were found in activity in the three habitats surveyed. In contrast, males of *A. arildae* were found in only one habitat (stream). Spatial distribution seems to be the main mechanism of reproductive segregation between the studied species.

Keywords: Anura, Aplastodiscus, ecology, reproductive biology, spatial segregation

Introduction

Studies on natural history generally focus on aspects of organism distribution, habitat use, and interactions with other organisms (Greene and Losos 1988). These studies are important for establishing taxonomic relationships between species and to understand the mechanisms that act in reproductive segregation in the community. One of the most important questions to be considered in comparative studies on natural history of sympatric species of anurans is how the species share the environment with the minimum of competition. According to Crump (1971), spatial and temporal distributions act as important mechanisms of pre-zygotic isolation among sympatric species. Toft (1985) postulated that differences in the use of habitats for reproduction are the most important mode to avoid spatial competition among sympatric species. This fact can be evidenced through the observation of different patterns of spatial distributions in species from the.
same region, which are directly related to the different reproductive adaptations of each species (Crump 1974; Duellman 1978).

*Aplastodiscus leucopygius* (Cruz and Peixoto, 1985) is a tree frog associated with mountain rivulets and temporary ponds (Haddad and Sazima 1992) at altitudes between 800 and 1200 m in the Atlantic rain forest from the State of São Paulo to the State of Rio de Janeiro (Frost 2006). Most of the information about the natural history of *A. leucopygius* was provided by Haddad and Sawaya (2000) who described a complex courtship that involves mutual tactile stimuli and egg deposition in a subterranean nest previously constructed by the male, who guides the female to the nest (mode 5 according to Haddad and Prado 2005). *Aplastodiscus arildae* (Cruz and Peixoto, 1987) is also a tree frog that calls from perches on the vegetation at the edge of rivulets (Haddad and Sazima 1992) and its geographic distribution is also restricted to the Brazilian Atlantic rain forest, from the State of São Paulo to the State of Rio de Janeiro (Frost 2006). Studies on the natural history of *A. arildae* are still rare and most of the information can be found in studies on Atlantic forest anuran communities (see Heyer et al. 1990; Haddad and Sazima 1992). Both species differ in physical parameters of their advertisement call (see Heyer et al. 1990; Haddad and Sawaya 2000).

The aim of the present study was to compare the natural history of two typical species from the Brazilian Atlantic rain forest, *A. arildae* and *A. leucopygius*, which occur in sympatry at the study site, clarifying the following aspects: reproductive and calling period, the use of habitats for reproduction, population density, and displacement of individuals.

**Material and methods**

The Serra do Japi (46°52′W, 23°11′S) is considered an ecotone of semideciduous and ombrophilous forest (Leitão-Filho 1992), with an altitude range from 700 to 1300 m and an area of 350 km². The local climate has two seasons, wet/warm from October to March and dry/mild from April to September (Pinto 1992). From March 2004 to January 2006 we visited three different kinds of habitat: lake, stream, and swamp, located at a mean distance of 2 km from each other. The water level of the three studied habitats was monitored by the use of graduated scales along the edge of each water body; we used two scales for each habitat.

Monthly observations were carried out, lasting approximately 5 days during the dry season and 10 days during the wet season. Data collection began immediately after sunset, finishing between 03:00 and 06:00 h.

For observations we used the focal animal method (Lehner 1996) when all the behavioural occurrences, including vocal and social interactions among individuals, were recorded. Individuals were weighed with a field scale of 0.1 g precision, their snout–vent length (SVL) was measured with a calliper of 0.01 cm precision, they were toe-clipped according to Martof (1953), and released in the same place where they were found. Whenever possible, all the individuals were marked on the first day of the field trip to avoid a possible negative influence of the method on the individuals’ behaviour. The toe-clipping apparently did not interfere with the activity of the individuals since they restarted their calling activity immediately after having been marked.

To compare the SVL and body mass of the individuals we performed the Student’s *t* test (Zar 1996). An *r*-correlation test was used to analyse the relationship between the climatic data (monthly rainfall and mean monthly air temperature) and the number of males in calling activity and courtship displays. Climatic data were obtained from a meteorological
station located approximately 10 km from the studied habitats. We considered significant values when \( P \leq 0.05 \) (Zar 1996).

**Results**

**Adults**

Males of *A. arildae* had a mean SVL of 36.14 mm (SD = 3.61; range = 34.1–38.2 mm; \( N = 29 \)) and a mean body mass of 2.58 g (SD = 0.64; range = 2.2–3.2 g; \( N = 29 \)). The only gravid female found had a SVL of 40.1 mm and a body mass of 3.5 g.

Mean SVL of males of *A. leucopygius* was 39.69 mm (SD = 2.19; range = 38.4–40.8 mm; \( N = 85 \)) and that of females was 40.19 mm (SD = 2.27; range = 38.9–42.1 mm; \( N = 20 \)).

There was no significant difference between the SVL values of both sexes (\( t = 0.80; \ P = 0.42 \)). Females of *A. leucopygius* were observed only during courtship and they were measured and marked as soon as they left the subterranean nests. Five females that entered the subterranean nests came out without laying their eggs. The mean body mass of *A. leucopygius* males was 4.13 g (SD = 1.21; range = 3.8–4.4 g; \( N = 95 \)) and that of females that laid their eggs was 3.95 g (SD = 0.73; range = 3.3–4.1 g; \( N = 15 \)). After they laid their eggs some of the eggs remained in the ovaries of the females. The females that did not lay eggs had a mean body mass of 5.37 g (SD = 2.0; range = 4.9–6.2 g; \( N = 5 \)). There was no significant difference between the body mass of the females that laid their eggs and the females that did not (\( t = 1.88; \ P = 0.08 \)). There was no significant difference between the body mass of the females that laid their eggs and males (\( t = 0.41; \ P = 0.68 \)). However, there was a significant difference between the body mass of the females that did not lay eggs and males (\( t = 2.56; \ P < 0.01 \)).

**Activity and courtship period**

Both species displayed a nocturnal calling activity with an activity peak between 21:00 and 22:00 h.

Males of *A. arildae* were found in calling activity from October 2004 to February 2005 and from June 2005 to January 2006 (Figure 1A). The number of calling males was positively correlated with monthly rainfall (\( r = 0.35; \ P < 0.01; \ N = 23 \)), but not with mean monthly air temperature (\( r = -0.01; \ P = 0.97; \ N = 23 \)). Individuals began to call just after sunset, and stopped calling around 24:00 h during the dry season (April to September) and around 03:00 h during the rainy season (October to March). The beginning of the calling activity was positively correlated with sunset (\( r = 0.54; \ P = 0.02; \ N = 110 \)).

Males of *A. leucopygius* exhibited a more extended calling period compared with *A. arildae*; males were found in calling activity between August 2004 and February 2005 and between June 2005 and January 2006 (Figure 1B). The number of calling males of *A. leucopygius* was positively correlated with monthly rainfall (\( r = 0.54; \ P = 0.02; \ N = 23 \)), but not with mean monthly air temperature (\( r = -0.07; \ P = 0.97; \ N = 23 \)). Males of this species began their calling activities immediately before or after sunset, and stopped calling around 24:00 h, during the dry season, and 04:00 h, during the rainy season. The calling activities of single males that neighboured couples in courtship extended longer than usual. This was because these males only stopped calling when the couple entered the subterranean nests, which usually occurred between 05:00 and 06:00 h. The beginning of the calling activity was positively correlated with sunset (\( r = 0.68; \ P < 0.01; \ N = 130 \)).
The courtship period was only obtained for *A. leucopygius* (Figure 2) since we observed only one courtship behaviour for *A. arildae*. The courtship period of *A. leucopygius* was positively correlated with monthly rainfall ($r=0.57; P<0.01; N=23$), but not with mean monthly air temperature ($r=-0.05; P=0.78; N=23$).

**Habitat use**

Males of *A. arildae* were found only in the stream, where they were observed calling on the marginal vegetation. During the day, on one occasion, we observed a male of *A. arildae* resting below a large leaf close to its calling site.
Individuals of *A. leucopygius* were found in the three studied habitats (stream, swamp, and lake). Males of this species were observed during the night in calling activity, using the marginal vegetation of water bodies as calling sites, and during the day resting below leaves or inside burrows in the ground. *Aplastodiscus leucopygius* females were observed in the studied areas only during the night, in courtship behaviour, or during the day, close to the subterranean nests where they had reproduced the night before. Before the courtship behaviour we observed that females of this species came from the forested areas adjacent to the water bodies. Newly metamorphosed individuals of *A. leucopygius* were found inside bromeliads in January 2005, and in November 2006 in the swamp and in the lake.

The stream was the only environment where the two species coexisted. However, they differed in the use of sites in this environment. The differential occupation is probably related to the characteristics of the water close to the calling sites, since *A. arildae* was found close to flowing water whereas *A. leucopygius* was found in sites close to calm water.

**Population density and displacement of males**

We marked 29 males of *A. arildae*, 14 of which were recaptured. The capture rate was higher or equal to the recapture rate during most of the studied period (Figure 3A). The higher number of recaptures occurred mainly in the middle or end of the rainy season whereas the higher capture rates occurred at the beginning of the rainy season (Figure 3A). *Aplastodiscus arildae* occurs at low density; the maximum number of males in activity was five (Figure 1A). The mean distance between males of *A. arildae* in calling activity was 10.1 m (SD=5.7; range=4–20 m; N=50).

On consecutive nights, some males of *A. arildae* were found in the same site where they had been captured. A male of this species was recorded in activity at the same site for three consecutive months. The maximum displacement distance measured on consecutive nights was 2 m.

We marked 95 individuals of *A. leucopygius* (85 males and 10 females); 55 males were recaptured and no female was recaptured. The numbers of *A. leucopygius* individuals captured and recaptured varied according to the habitat and studied period. Although we registered high capture rates in January 2005 in the lake, and in November 2005 in the stream (Figure 3B), the capture rates were higher or equal to the
Recapture rates during most of the studied period in the stream and in the swamp (Figure 3C). The higher capture rates were obtained at the end of the dry season and after rains in June 2005 and July 2005. The recaptures occurred mainly during the rainy period (stream, swamp, and lake) and during rain events that occurred from June to August 2005 (lake) (Figure 3D).

The population density of *A. leucopygius* varied according to the habitat and period, being higher in the lake where we recorded the maximum number of males in calling activity considering the area; nearly one male per metre in August 2005 and January 2006.

The marked males generally displaced only a few centimetres from the place where they were marked and some individuals were recaptured at the same calling site for three consecutive months. The maximum distance of male displacement was 10 m and was recorded during the rainy season in the lake, where we recorded the more intense water level change (approximately 10 cm higher during the rainy season). The water level did not change in the other two studied habitats and the displacement of males was not evident as in the lake. The mean distance between males of *A. leucopygius* in the same environment was 5.4 m (SD=3.7; range=1–10 m; N=279).

**Operational sex ratio (OSR)**

In general, the number of males of *A. leucopygius* was higher than the number of females. In a few situations, the number of females was equal to the number of males in calling activity. The mean operational sex ratio for this species was 0.41 (SD=0.23; range=0.12–1; N=20).

**Discussion**

**Adults**

Sexual dimorphism in SVL is a common characteristic in anurans. Females, in about 90% of species, are larger than males (Shine 1979). The absence of sexual dimorphism in size may be related to the fighting behaviour of males (Wells 1978) or could be explained by the low mass represented by the ova in females of large anuran species (Haddad 1987), which is not the case for *A. leucopygius*. However, we did not observe fighting interactions between males of *A. leucopygius*, not even when the male density was higher. That said, Haddad and Sawaya (2000) did observe sexual dimorphism in SVL for the same species, also in the Serra do Japi. A possible explanation for this may be the low number of females collected compared with the number of measured males, which could influence the results of the tests. However, we cannot reject the possibility of differences related to ecology and behaviour of both examined populations. According to Halliday and Tejedo (1995), there are intraspecific differences in the degree of sexual size dimorphism between population that can be attributed to ecological and behavioural differences. The lack of a significant difference between the body mass of gravid females and females that laid eggs could also be related to the small sample size of the former group or could be related to the low mass of the eggs laid compared with the ova mass contained in the ovary. The differences between
the body mass of males and gravid females may be attributed to two factors: to excess load owing to clutch mass and/or to the small sample size of gravid females.

Activity and courtship period

Both species had a prolonged breeding season (sensu Wells 1977), evident not only by the long period of calling activities but also because they displayed typical behaviours of prolonged breeding species, such as use of vocalizations to attract females and territoriality (main reproductive tactic used by prolonged breeding species). The calling and reproductive period of both species were closely related to the rainfall in the region. Both species seem to be opportunists, benefiting from the rainy periods to initiate their calling and reproductive activities. This fact shows the importance of water as a primary resource for the reproduction of these species. Both species show a great overlap in the calling period, indicating that the temporal distribution may not act as an important aspect in the segregation of the species.

Males of *A. leucopygius* that were found near to pairs in courtship prolonged their calling activity, finishing only when the pairs entered the subterranean nests. Spending more time in calling activity could increase the chances of the neighbouring males obtaining females that might reject the males during courtship.

Habitat use

Both species used the marginal vegetation of water bodies as calling sites. However, compared with *A. leucopygius*, *A. arildae* seems to be more specialized in the use of habitats for reproduction, since it was found only in the stream. On the other hand, *A. leucopygius* was recorded in all three studied habitats and males of this species can be found in many other areas in the Serra do Japi. Even in the stream, used by both species, a spatial resource partitioning was observed between the two species. Heyer et al. (1990) observed that closely related species, sharing the same environment during the same period, show a degree of spatial segregation. According to Cardoso et al. (1989), Blamires et al. (1996) and many other former authors that worked with anuran communities, the spatial and temporal distributions represent secondary mechanisms in the reproductive isolation of the species, whereas the main mechanism responsible for a primary segregation among species would be the structure of their advertisement calls.

Females of *A. leucopygius* probably visit the habitats only for reproduction and possibly rest in the adjacent forest when they are not in reproductive activity.

The presence of newly metamorphosed individuals of *A. leucopygius* in different months may indicate the differences in the courtship period during the two studied years.

Population density and displacement of males

For both species capture rates were higher mainly at the beginning of the rainy season and after rain events in the dry season, and were also higher during the rainy season or during rainy months. This fact suggests that new individuals may appear as soon as water becomes more abundant. Apparently, there is a relative maintenance of the male population throughout the rainy season. The different patterns of capture and recapture rates and male density among the three habitats may be related to differences in the physical characteristics of the habitats, such as availability of calling sites and presence of margins to construct the subterranean nests.
The greater displacement of males of *A. leucopygius* during the rainy period in the lake may indicate limitation in the availability of margins where the males can construct their nests, and suggests that males select sites to construct them. A moist soil is easy to excavate and a medium water level is necessary for the development of the eggs. However, high water levels can interfere negatively in the integrity of the subterranean nest (J. Zina, unpublished data).

The return of the same male to the same calling site on consecutive nights is considered a territorial behaviour (Crump 1972; Rosen and Lemon 1974). For *A. leucopygius* and for *A. arildae* the return of the males to the same calling site could be a territoriality related to the localization of the subterranean nest.

**Operational sex ratio (OSR)**

The OSR calculated in the present study for *A. leucopygius* was higher when compared with other prolonged breeding species (*sensu* Wells 1977) [e.g. *Scinax rizibilis* (Bokermann, 1964): OSR=0.143; Bastos and Haddad 1999; *Scinax fuscomarginatus* (Lutz, 1925): OSR=0.083; Toledo and Haddad 2005].

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