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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

ECOLOGIA E BIOLOGIA REPRODUTIVA DE DUAS ESPÉCIES SIMPÁTRICAS DO GÊNERO *Aplastodiscus* NA SERRA DO JAPI, MUNICÍPIO DE JUNDIAÍ, ESTADO DE SÃO PAULO

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Se os frutos produzidos pela terra
Ainda não são tão doces
E polpudos quanto as pêras
Da tua a ilusão
Amarra o teu arado a uma estrela
E os tempos darão
Safras e safras de sonhos
Quilos e quilos de amor
Noutros planetas risonhos
Outras espécies de dor

Se os campos cultivados neste mundo
São duros demais
E os solos assolados pela guerra
Não produzem a paz
Amarra teu arado a uma estrela
E aí tu serás
O lavrador louco dos astros
Um camponês solto nos céus
Quanto mais longe da Terra tanto mais longe de Deus

“Amarra o teu arado a uma estrela”
Gilberto Gil

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Índice

Resumo

Estudos comparativos sobre biologia reprodutiva e interações sociais em espécies simpátricas e taxonomicamente próximas de anuros ainda são escassos, embora estes aspectos tenham fundamental importância não apenas para o estabelecimento das relações filogenéticas entre espécies do mesmo grupo, mas também para a compreensão dos mecanismos que atuam na segregação das espécies no ambiente. O presente estudo foi desenvolvido na Serra do Japi, Município de Jundiaí, um dos últimos remanescentes de Mata Atlântica no Estado de São Paulo, Brasil. Durante os meses de março/04 a janeiro/06 foram coletados dados sobre a biologia reprodutiva, ecologia, repertório acústico e interações sociais de *Aplastodiscus arildae* e *A. leucopygius*. Três ambientes foram visitados (lagoa, riacho e brejo), nos quais foram coletadas as seguintes informações: características físicas dos sítios de vocalização e reprodução, temporada de vocalização e reprodução, deslocamento de indivíduos e densidade de espécimes. Quanto ao uso do ambiente, a espécie *Aplastodiscus leucopygius* foi registrada nos três ambientes estudados, enquanto *A. arildae* foi observada apenas no riacho. Ambas utilizaram a vegetação marginal ao corpo d'água como sítio de vocalização. A espécie *A. arildae* mostrou-se menos generalista quanto ao uso do ambiente para a reprodução. As duas espécies apresentaram estação reprodutiva prolongada e positivamente relacionada com o regime de chuvas na região. Este fato é muito comum entre espécies de anuros e evidencia a importância da disponibilidade de água para a reprodução. Foram identificados três tipos de canto para cada espécie: canto de anúncio, canto de corte e canto territorial. As funções sociais dos cantos foram estabelecidas de acordo com os contextos sociais em que foram emitidos. A distribuição espacial diferenciada e as diferenças quanto aos parâmetros acústicos aparentemente são os principais fatores que contribuem para o isolamento das espécies e manutenção das mesmas como unidades discretas.

Abstract

Comparative studies on reproductive biology and social interactions among closely related sympatric species of anurans are still rare. However, these aspects are fundamental, not only for the understanding of taxonomic relationships, but also to access the mechanisms responsible for maintaining segregation of species in the environment. This study was carried out at Serra do Japi, one of the last remnants of Atlantic rainforest, in the Municipality of Jundiaí, State of São Paulo, Brazil, from March/04 to January/06. During this period we collected data about ecology, reproductive biology, acoustic repertoire, and social interactions of *Aplastodiscus arildae* and *A. leucopygius*. Three habitats were visited (lake, stream, and swamp), where we collected the following information: characteristics of the calling and reproductive sites, calling and breeding periods, displacement of individuals, and species density. *Aplastodiscus leucopygius* was registered in the three studied habitats, whereas *A. arildae* was found only in the stream. Both species used the vegetation at the edge of water bodies as calling sites. *Aplastodiscus arildae* can be considered less generalist with regard to the habitat used for reproductive activity. Both species showed a prolonged breeding season positively correlated with the rainfall in the area. This fact is very common among anuran species and emphasizes the importance of water for reproduction in species of this group. We identified three different types of call for each species: advertisement call, courtship call, and territorial call. Social functions of the vocalizations were established according to the social context in which they were emitted. The different spatial distribution and the differences among call parameters of both species seem to be the main factors responsible for species segregation.

Introdução geral

Atualmente são conhecidas cerca de 5300 espécies de anuros, um dos grupos de animais proeminentes em quase todas as comunidades terrestres (FROST, 2005). Apesar da importância e da grande diversidade de espécies do táxon, especialmente nas áreas serranas da região Neotropical (veja CRUZ & PEIXOTO, 1985; HEYER, 1985; HEYER & GOCROFT, 1986; CRUZ, 1990), estudos com espécies ou comunidades de anuros ainda são escassos e a velocidade de degradação dos ambientes naturais supera os esforços dos pesquisadores na coleta de dados. No entanto, alguns trabalhos, especialmente na região Sudeste do Brasil, têm enfocado aspectos muito interessantes nas relações interespecíficas de anuros, tais como os mecanismos responsáveis pela segregação das espécies no mesmo ambiente (e.g. CARAMASCHI, 1981; CARDOSO, 1981; ANDRADE, 1987).

Um dos principais temas a ser considerado no estudo de populações de anuros é a maneira como as espécies podem ocupar um mesmo local durante a temporada reprodutiva. A competição e a predação foram durante muitos anos consideradas como mecanismos determinantes na estrutura das taxocenoses de anuros (veja DUELLMAN & TRUEB, 1986). Mais recentemente, a estrutura da comunidade e a coexistência de espécies que utilizam o mesmo ambiente para a reprodução têm sido explicadas também pela partilha de recursos reprodutivos (CARDOSO *et al.*, 1989; HADDAD, 1991; ETEROVICK & SAZIMA, 2000) que de certa forma é uma conseqüência de pressões seletivas como a competição. Neste contexto, cabe a definição de partilha de recursos como sendo o mecanismo que permite que as espécies utilizem de forma diferenciada os recursos disponíveis no ambiente (SCHOENER, 1968). CRUMP (1971) cita que algumas espécies de anfíbios e répteis da região de Belém, Brasil, possivelmente coexistem com um mínimo de competição, resultante de uma partilha de recursos espaciais e temporais. No caso de espécies simpátricas, esta partilha opera como um eficiente mecanismo de isolamento reprodutivo pré-zigótico (CRUMP, 1974). O isolamento reprodutivo pode ser resultante de divergências ecológicas, devidas, em parte, a interações comportamentais interespecíficas, envolvendo organização social, distribuição espacial e temporal nas comunidades. CARAMASCHI (1981) mostrou que espécies simpátricas de hilídeos da represa do Rio Pardo, em Botucatu, Brasil, apresentam segregação espacial, temporal e alimentar. Demonstrou ainda que as espécies estão eficientemente isoladas reprodutivamente entre si e exibem estratégias que evitam a competição interespecífica. Dados referentes a interações desse tipo em anuros da região Neotropical, embora fundamentais para a elucidação de questões em ecologia e evolução, ainda são bastante escassos.

O objetivo principal do presente trabalho foi estudar a biologia reprodutiva e ecologia de duas espécies simpátricas de hilídeos, *Aplastodiscus arildae* e *A. leucopygius*, bem como estabelecer como estas duas espécies partilham os recursos disponíveis no ambiente. Para tanto, informações como: período de atividade das espécies, caracterização dos microhábitats escolhidos, comportamentos e modos reprodutivos, repertório acústico e dados sobre esforço reprodutivo são de extrema importância.

Revisão da literatura

A família Hylidae é uma das famílias mais representativas entre os anuros e conta atualmente com cerca de 820 espécies descritas (FROST, 2005).

CRUZ & PEIXOTO (1985) descreveram a espécie *Aplastodiscus arildae* como espécie de porte mediano com a seguinte combinação de caracteres morfológicos: íris de coloração róseo-violáceo, linha amarelada percorrendo o canto rostral (do bordo da pálpebra superior até as pregas supratimpânicas), olhos de tamanho mediano, focinho arredondado em vista lateral, dorso de coloração verde pouco granulado e ventre amarelo-limão com algumas granulações, região gular de coloração verde azulada e região anal com uma estreita crista esbranquiçada superiormente e grânulos pouco numerosos inferiormente. De acordo com CRUZ & PEIXOTO (1985), *A. arildae* (referida como *Hyla arildae*) pertenceria ao complexo "*Hyla albofrenata*" e ao grupo de "*Hyla albomarginata*". Atualmente, após extensa revisão taxonômica da família Hylidae, considerando caracteres morfológicos e moleculares, FAIVOVICH *et al.* (2005) não só modificaram o gênero da espécie que passou a *Aplastodiscus* como também alocou a espécie no grupo *Aplastodiscus albofrenatus*, abolindo a classificação em complexos, proposta por CRUZ & PEIXOTO (1984; 1985). A distribuição geográfica das espécies do grupo *A. albofrenatus* é restrita a Mata Atlântica. Há registros de *A. arildae* desde o Estado de São Paulo até o Estado do Rio de Janeiro (FROST, 2005).

Aplastodiscus leucopygius foi descrita por CRUZ & PEIXOTO (1984). Segundo estes autores os caracteres morfológicos que diagnosticam a espécie incluem: porte mediano, focinho arredondado em vista lateral e pontudo em vista dorsal, coloração dorsal verde, pequeno apêndice esbranquiçado em vista dorsal na articulação tíbio-tarsal, flap anal (pequena projeção em forma de flap acima da cloaca) curto e milium anal (granulações em torno da abertura cloacal) constituído por grânulos brancos e de tamanho mediano. Assim como para *Aplastodiscus arildae*, *A. leucopygius*, anteriormente referida por CRUZ & PEIXOTO (1984) como *Hyla leucopygia* e alocada no complexo *Hyla* *albosignata* e grupo *Hyla albomarginata*, foi classificada por FAIVOVICH (2005) como espécie do gênero *Aplastodiscus*, pertencente ao grupo *Aplastodiscus albosignatus*, sendo que o complexo *Hyla albosignata* também foi abolido. O grupo *A. albosignatus* também possui distribuição geográfica restrita ao domínio da Mata Atlântica. Há registros de *A. leucopygius* desde o Estado de São Paulo até o Estado do Rio de Janeiro (FROST, 2005).

Estudos mais aprofundados com *Aplastodiscus leucopygius* e *A. arildae* são raros. O primeiro relato sobre o modo reprodutivo de *A. leucopygius* foi feito por DUELLMAN (1986, 1988), que descreveu o processo como sendo a deposição de ovos em vegetação acima da água. HEYER *et al*. (1990) consideraram a vegetação acima dos corpos de água lóticos como sendo o sítio de vocalização da espécie. Posteriormente, HADDAD & SAWAYA (2000) fizeram uma descrição detalhada do comportamento reprodutivo de *A. leucopygius*, com descrição dos cantos de anúncio, corte e “canto composto”. Estes autores descreveram o modo reprodutivo da espécie como sendo: deposição de ovos na água acumulada em ninhos subterrâneos construídos pelos machos (modo 5 *sensu* HADDAD & PRADO, 2005).

O modo reprodutivo de *A. arildae* não é conhecido e a maior parte das informações a respeito da espécie provem de estudos com comunidades, como em HEYER *et al*. (1990), que descreveram o canto de anúncio da espécie (referida como *Hyla albofrenata*) e definiram a vegetação marginal de corpos de água corrente como sendo o sítio de vocalização da espécie.

Local de estudo

A Serra do Japi (46º 52’ W; 23º 11’ S; altitude = 700 a 1300 m) está situada a oeste do Planalto Atlântico, entre os Municípios de Jundiaí, Cabreúva, Campinas e São Paulo, Estado de São Paulo (Figura 1). Possui cerca de 350 km^2 e encontra-se em sua maior parte no Município de Jundiaí, onde foi criada a Reserva Municipal da Serra do Japi em 1999 (Figura 2). A Reserva representa uma das últimas grandes áreas de floresta contínua do Estado de São Paulo e é considerada como uma região ecotonal, isto é, uma área de transição ou junção entre duas ou mais formações florestais, no caso a Mata Atlântica da Serra do Mar, as florestas do interior paulista e Matas da Serra da Mantiqueira (LEITÃO-FILHO, 1986). Por se tratar de uma área ecotonal há uma grande diversidade de espécies da fauna e da flora, mais um motivo que reforça a importância da preservação e conservação da área. A vegetação característica da área até 1000 metros de altitude é floresta mesófila semidecidual e acima dessa altitude ocorrem florestas de altitude (MORELLATO *et al.*, 1989). Nas altitudes mais elevadas as temperaturas médias anuais são de 15,7 ºC, enquanto que nas porções mais baixas as temperaturas médias são de 19,2 ºC (PINTO, 1992). No período de abril a setembro os dias são mais frios e secos e de outubro a março os dias são mais quentes e úmidos, evidenciando uma sazonalidade pronunciada (MORELLATO *et al.*, 1989).

O presente estudo foi realizado em áreas localizadas no domínio da reserva municipal da Serra do Japi no Município de Jundiaí, Estado de São Paulo (Figura 2). Foram visitados três tipos de ambientes distantes cerca de 2 km um do outro e próximos à base Ecológica da Serra do Japi (Figura 2): lagoa (23º 14’ 56,8’’ S; 46º 56’ 23,4’’ W; altitude = 1030 m) (Figura 3), brejo (23º 14’ 41,3’’ S; 46º 56’ 9,5’’ W; altitude = 900 m) (Figura 4) e riacho (23º 14’ 35’’ S; 46º 56’ 4,9’’ W; altitude = 1000 m) (Figura 5).

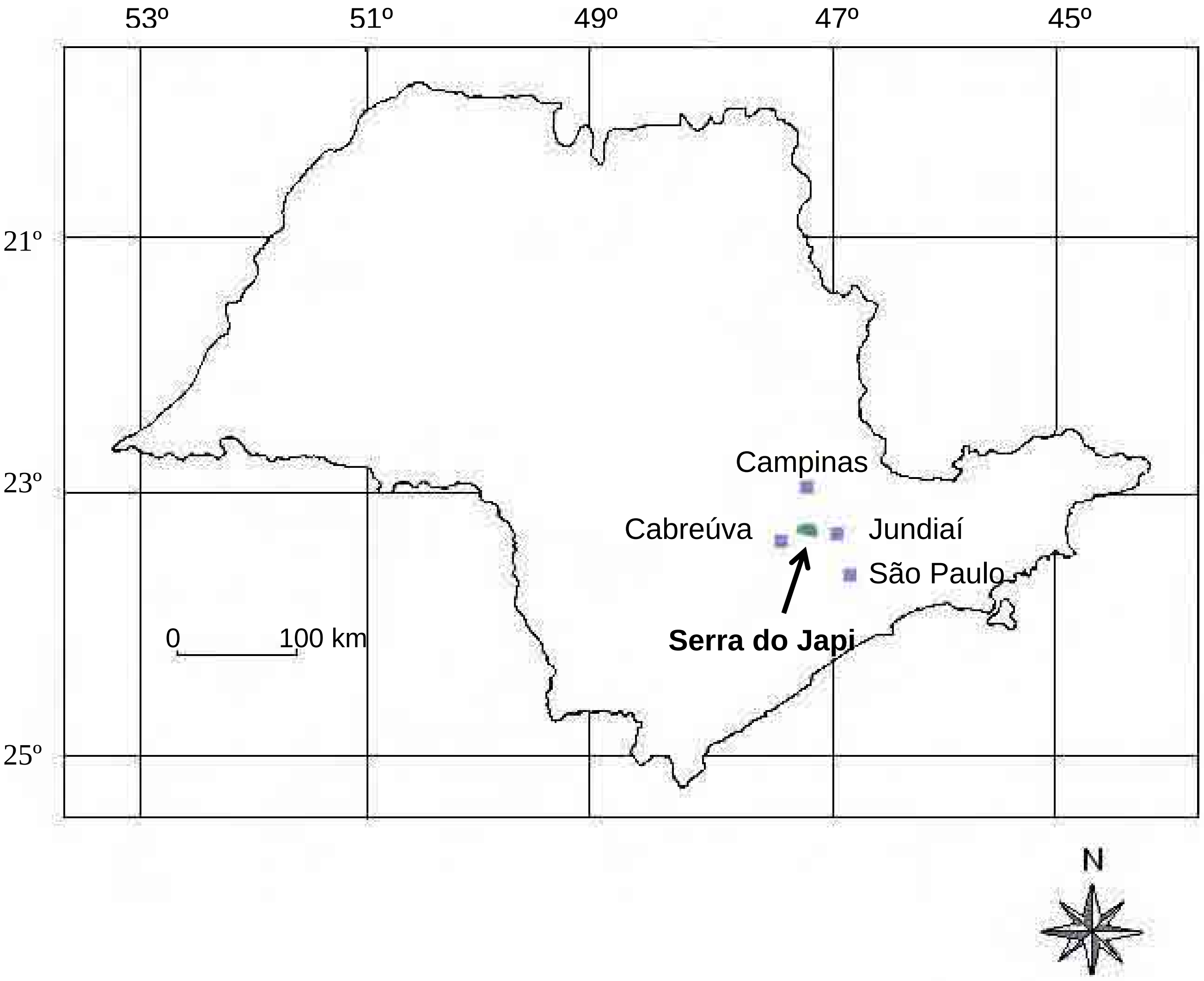

Figura 1- Mapa da localização da Serra do Japi no Estado de São Paulo em relação aos Municípios de São Paulo, Campinas, Cabreúva e Jundiaí.

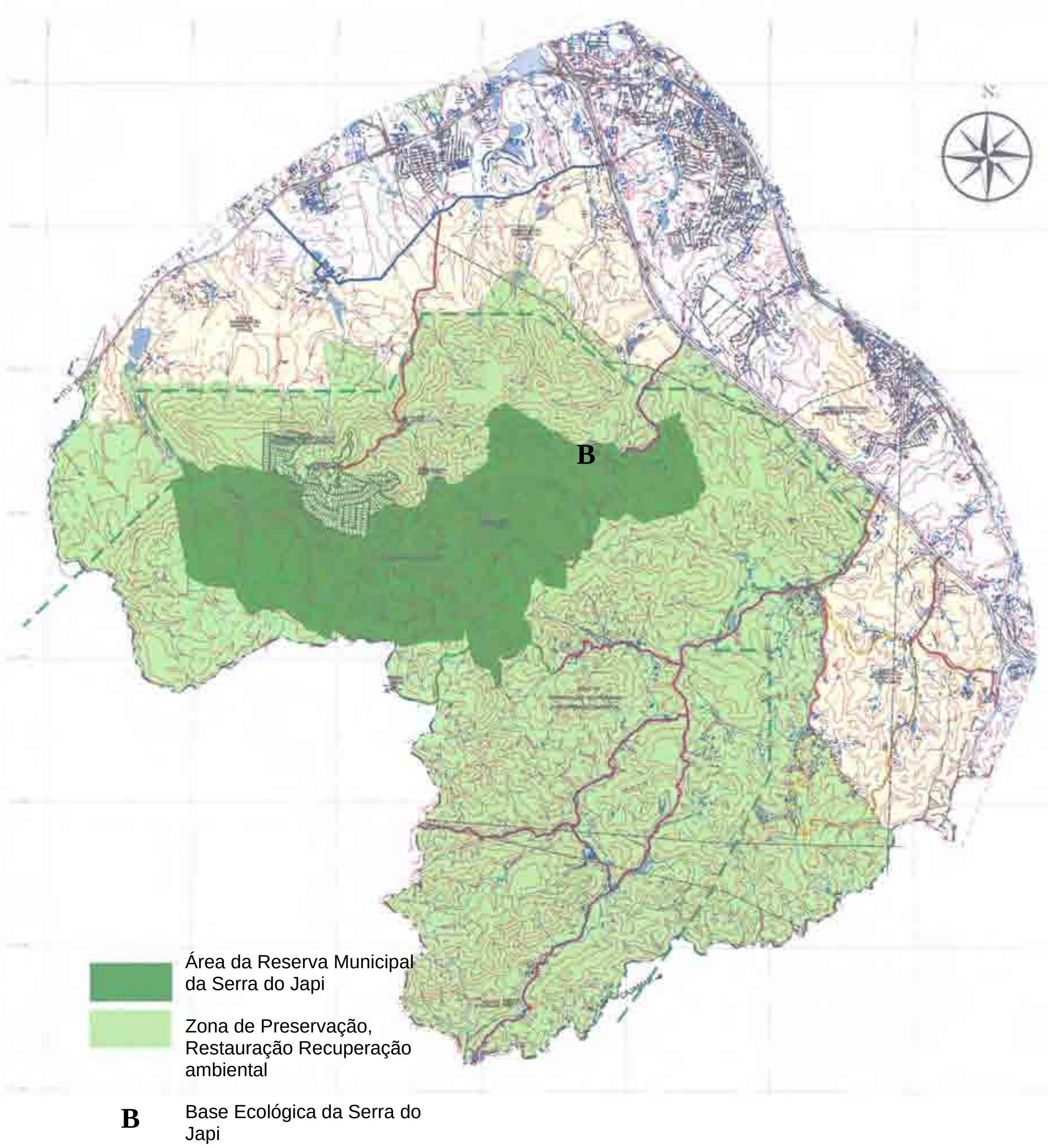

Figura 2- Mapa da Serra do Japi evidenciando as áreas de reserva municipal, zona de preservação e a localização da base ecológica da Serra do Japi, Município de Jundiaí, Estado de São Paulo. Escala: 1/25.000

Figura 3- Lagoa localizada na Serra do Japi, Município de Jundiaí, Estado de São Paulo.

Figura 4- Brejo localizado na Serra do Japi, Município de Jundiaí, Estado de São Paulo.

Figura 5- Riacho localizado na Serra do Japi, Município de Jundiaí, Estado de São Paulo.

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Chapter 1

Ecology and reproductive biology of two species of *Aplastodiscus* (Anura: Hylidae) in the Serra do Japi, Municipality of Jundiaí, State of São Paulo, Brazil

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Male of *Aplastodiscus arildae* close to its calling site

Male of *Aplastodiscus leucopygius* in its calling site

Resumo

Entre os meses de março de 2004 a janeiro de 2006 foram coletados dados ecológicos e biológicos de duas espécies simpátricas do gênero *Aplastodiscus* na Serra do Japi, um dos últimos remanescentes de Mata Atlântica no Estado de São Paulo. Três ambientes foram visitados (lagoa, riacho e brejo), nos quais foram coletadas as seguintes informações: características físicas dos sítios de vocalização e reprodução, temporada de vocalização e reprodução, deslocamento de indivíduos e densidade de espécimes. O maior número de machos em atividade de vocalização, tanto de *Aplastodiscus arildae* quanto de *A. leucopygius*, ocorreu nos meses mais chuvosos do ano e após eventos esporádicos de chuva, durante a estação seca (abril a setembro). O maior número de cortes de *A. leucopygius* foi observado também nos meses mais chuvosos e apenas uma corte foi observada para *A. arildae*, em dezembro de 2005, durante uma noite chuvosa. Quanto ao uso do ambiente, a espécie *A. leucopygius* foi registrada nos três ambientes estudados, onde foi encontrada vocalizando paralelamente ao solo em folhas de árvores ou arbustos localizados às margens dos corpos d’água. *Aplastodiscus arildae*, por outro lado, foi observada apenas no riacho, vocalizando em galhos altos da vegetação arbórea marginal ao corpo d’água, sempre em densidades populacionais baixas. A distribuição espacial diferenciada nos ambientes reprodutivos parece ser o principal mecanismo de segregação reprodutiva entre as espécies estudadas.

Palavras-chave: Ecologia, Biologia reprodutiva, *Aplastodiscus*, Dinâmica populacional.

Abstract

The present study was conducted in the Serra do Japi, one of the last remnants of Atlantic forest 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 following data were collected: physical characteristics of calling and reproductive sites, reproductive and calling period, displacement of individuals, and species density. The majority of *Aplastodiscus leucopygius* males in calling activity were registered 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, where they were observed calling from leaves at the edge of water bodies, parallel to the ground. In contrast, males of *A. arildae* were found in only one habitat (stream), where they were observed calling from leaves high in the canopy of trees located at the edge of the water body, always in low population density. Spatial distribution seems to be the main mechanism of reproductive segregation between the studied species.

Keywords: Ecology, Reproductive biology, *Aplastodiscus*, Population dynamics.

1. Introduction

Studies on natural history generally focus on aspects of organism distribution, habitat use, and interactions with other organisms (GREENE & LOSOS, 1988). These studies are important for establishing the taxonomic relationship between the species and to understand the mechanisms that act in the 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), the spatial and temporal distributions act as important mechanisms of pre-zygotic isolation among sympatric species. TOFT (1985) postulated that differences in the use of the habitats for reproduction are the most important spatial dimension being shared 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 is a tree frog associated with mountain rivulets and temporary ponds (HADDAD & 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, 2005). Most of the information about the natural history of *A. leucopygius* is provide in HADDAD & SAWAYA (2000) *Aplastodiscus arildae* is also a tree frog that calls from perches on the vegetation at the edge of rivulets (HADDAD & 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, 2005). Studies on 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 & SAZIMA, 1992).

The aim of the present study was to compare the natural history of two typical species from the Brazilian Atlantic rain forest, *Aplastodiscus 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.

2. Material and Methods

The Serra do Japi (46º 52’ W; 23º 11’ S) is considered an ecotone of Semideciduous and Ombrophilous forest (LEITÃO-FILHO, 1992), with an altitude range from 700 m to 1300 m and an area of 350 km^2. 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 habitats: 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 five days during the dry season and 10 days during the wet season. Data collecting began right after sunset, finishing between 300 h and 600 h.

For behavioural observations we used the focal animal method (LEHNER, 1996). Individuals were weighed with a field scale of 0.1 g of precision, measured with a calliper of 0.01 cm of precision, toe-clipped according to MARTOF (1953), and released in the same place where they were found. Whenever possible, all the individuals were marked in the first day of the field trip to avoid a possible negative influence of the method on the individuals’ behaviour.

To compare the SVL (snout-vent length) and body mass of the individuals we performed the student *t* test (ZAR, 1996). A *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).

3. Results

3.1 Adults

Males of *Aplastodiscus 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 *Aplastodiscus leucopygius* was 39.69 mm (SD = 2.19; range = 38.4 – 40.8 mm; N = 85) and of females was 40.19 mm (SD = 2.27; range = 38.9 – 42.1 mm; N = 20). There was no significant difference between the SVLs of both sexes (t = 0.80; P = 0.42). Females of *A. leucopygius* were observed only during the courtships 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 mm; N = 95) and of females that laid their eggs was 3.95 g (SD = 0.73; range = 3.3 – 4.1 mm; N = 15). The females that did not lay eggs had a mean body mass of 5.37 g (SD = 2.0; range = 4.9 – 6.2 mm; 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 of *Aplastodiscus leucopygius* (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).

3.2 Activity and courtship period

Both species presented a nocturnal calling activity with an activity peak between 2100 h and 2200 h.

Males of *Aplastodiscus arildae* were found in calling activity from October 2004 to February 2005 and from June 2005 to January 2006 (Figure 1). The number of calling males was positively correlated with monthly rainfall (r = 0.35; P < 0.01), but not with mean monthly air temperature (r = -0.01; P = 0.97).

Individuals began to call just after sunset, and stopped calling around 2400 h during the dry season (April to September) and around 300 h during the rainy season (October to March). The beginning of the calling activity was positively correlated with the sunset ($r = 0.54$; $P = 0.02$; N = 110).

Males of *Aplastodiscus leucopygius* exhibited a more extended calling period compared with *A. arildae*; they were found in calling activity between August 2004 and February 2005 and between June 2005 and January 2006 (Figure 2). The number of calling males of *A. leucopygius* was positively correlated with monthly rainfall ($r = 0.54$; $P = 0.02$), but not with mean monthly air temperature ($r = -0.07$; $P = 0.97$). Males of this species began their calling activities right before or after sunset, and stopped calling around 2400 h, during the dry season, and 400 h, during the rainy season. The calling activities of males that neighbored 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 500 or 600 h. The beginning of the calling activity was positively correlated with the sunset ($r = 0.68$; $P < 0.01$; N = 130).

The courtship period was only obtained for *A. leucopygius* (Figure 3) since we observed only one courtship behaviour for *A. arildae*. The courtship period of *A. leucopygius* was positively correlated with the rainfall ($r = 0.57$; $P < 0.01$), but not with the air temperature ($r = -0.05$; $P = 0.78$).

Some other abiotic factors, such as moon light intensity, strong winds, and storms, apparently negatively influenced the activities of both species. At full moon nights, the activity of both species was reduced, especially in more open habitats, such as the swamp. During strong winds and storms males of both species interrupted their calling activity.

3.4 Habitat use

Males of *Aplastodiscus arildae* were found only in the stream, where they were observed calling on the marginal vegetation. During the day, in one occasion, we observed a male of *A. arildae* resting below a large leaf close to its calling site.

Individuals of *Aplastodiscus 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. Newly metamorphosed individuals of *A. leucopygius* were found inside bromeliads in January 2005 and on 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 along this environment. The differential occupation is probably related to the characteristics of the water close to the calling sites, since *Aplastodiscus arildae* was found close to flowing water whereas *A. leucopygius* was found in sites close to calm water.

3.5 Population density and displacement of males

The toe-clipping apparently did not interfere in the activity of the individuals since they restarted their calling activity right after having been marked.

We marked 29 males of *Aplastodiscus arildae*; 14 of which were recaptured. The capture rate was higher or equal to the recapture rate during most of the studied period (Figure 4). 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 4). *Aplastodiscus arildae* occurs at low density; the maximum number of males in activity was five (Figure 1). The maximum distance between males of *A. arildae* in calling activity was 20 m and the minimum was 4 m.

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

We marked 95 individuals of *Aplastodiscus 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. 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 5). The higher capture rates were obtained at the end of the dry season and after rain events in June 2005 and July 2005. However we registered high capture rates in January 2005 in the lake, and November 2005 in the stream (Figure 5). The recaptures occurred mainly during the rainy period (stream, swamp, and lake) and during rain events that occurred from June 2005 to August 2005 (lake) (Figure 5).

The population density of *Aplastodiscus leucopygius* varied according to the habitat and period, being higher in the lake where we registered the maximum number of males in calling activity (eight males, see Figure 2).

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 it was registered during the rainy season in the lake, where we registered 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 maximum distance between males of *Aplastodiscus leucopygius* in the same environment was 10 m and the minimum was 1 m.

3.6 Operational Sex Ratio (OSR)

In general, the number of males of *Aplastodiscus leucopygius* was higher than the number of females. In 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).

4. Discussion

4.1 Adults

Sexual dimorphism in SVL is a common characteristic in anurans, being that in about 90 % of species females are larger than males (SHINE, 1979). The absence of sexual dimorphism in size may be related to the fight behaviour of males (WELLS, 1978) or could be explained by the low mass represented by the ova in females of large anuran species. We did not observe physical interaction between males of *Aplastodiscus leucopygius*, not even when the male density was higher. That said, HADDAD & 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. The lack of a significant difference between the body mass of gravid females and females that laid eggs, could be related to the low mass of the laid eggs compared with the ova mass contained in the ovary. The differences between the body mass of males and gravid females indicate that the production of gametes by the females represents a significant physiological reproductive investment.

4.2 Activity and courtship period

Both species had a prolonged breeding season (WELLS, 1977), evident not only by the long period of calling activities but also because they present typical behaviours of prolonged breeding species, such as: use of vocalizations to attract females (main reproductive tactic used by prolonged breeding species) and territoriality. The calling and reproductive period of both species were closely related with 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.

The interruption of vocalizations as a consequence of heavy rains or strong winds has been recorded for other frog species, such as *Dendropsophus elegans* and *Hypsiboas raniceps* (BASTOS & HADDAD, 1995; GUIMARÃES & BASTOS, 2003). These events probably exert an acoustic interference on the vocalization of frogs. According to RICHARDS & WILEY (1980), wind speed can negatively influence either the intensity of calls, especially those emitted near the ground, or the reception of the sound, since it could mask the information being transmitted. The influence of light intensity was detected by the positive correlation between dusk and the beginning of vocalization activities.

Males of *Aplastodiscus 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 enlarge the chances of the neighbouring males to obtain the females that might reject the males during courtship.

4.4 Habitat use

Both species used the marginal vegetation of water bodies as calling sites. However, compared with *Aplastodiscus 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 registered in the 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 BLAMIRES *et al*. (1996), 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 calling structure of their advertisement calls.

Females of *Aplastodiscus leucopygius* probably visit the habitats only for reproductive activity.

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

4.5 Population density and displacement of males

Both species presented higher capture rates mainly at the beginning of the rainy season and after rain events in the dry season and higher recapture rates mainly 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 among the three habitats may be related to differences in the physical characteristics of the habitats such as availably of calling sites and presence of margins to construct the subterranean nests.

The bigger displacement of males of *Aplastodiscus leucopygius* during the rainy period in the lake may indicate the 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 be excavated 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 (see “mate choice” chapter 3).

The return of the same male to the same calling site in consecutive nights is considered a territorial behaviour (CRUMP, 1972; ROSEN & LEMON, 1974). For *Aplastodiscus 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.

4.6 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* (OSR = 0.143) BASTOS & HADDAD, 1999; *Scinax fuscomarginatus* (OSR =0.083) TOLEDO & HADDAD, 2005). This fact can be related to the parceled oviposition, that enlarges the number of days spent by females in reproductive sites.

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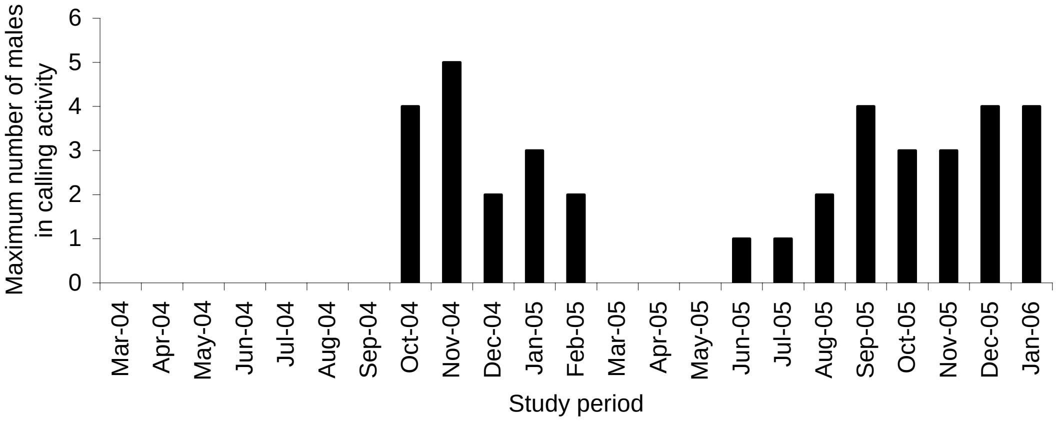

Figure 1- Maximum number of males of *Aplastodiscus arildae* in calling activity in the studied stream in the Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil.

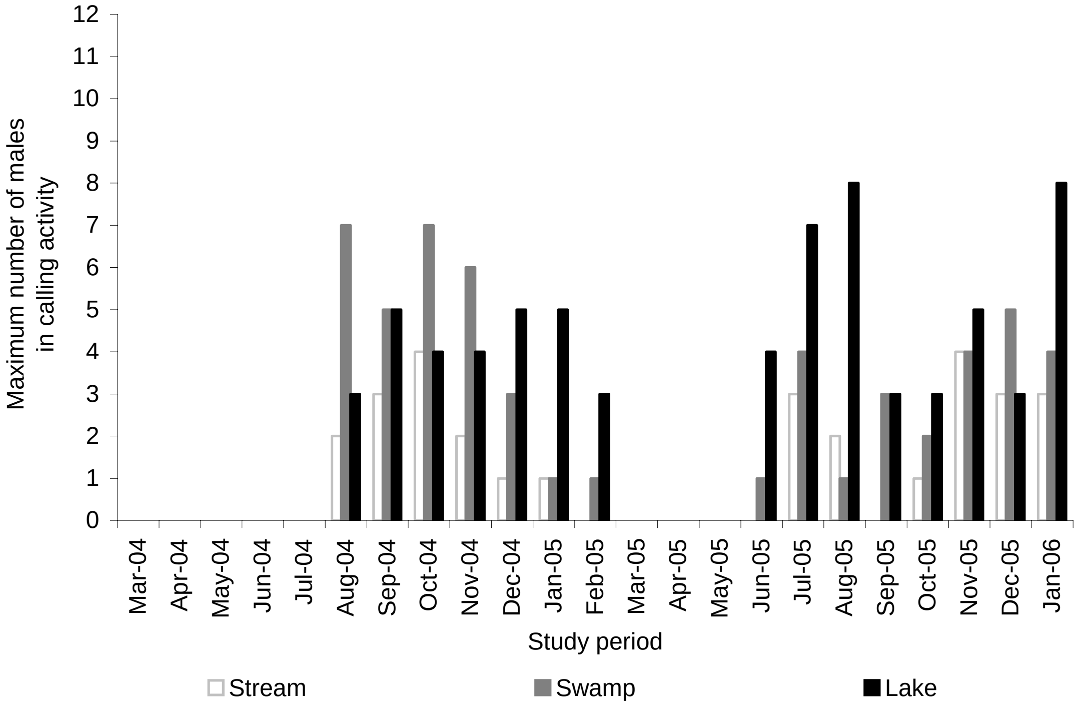

Figure 2- Maximum number of males of *Aplastodiscus leucopygius* in calling activity in three habitats (stream, swamp and lake) in the Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil.

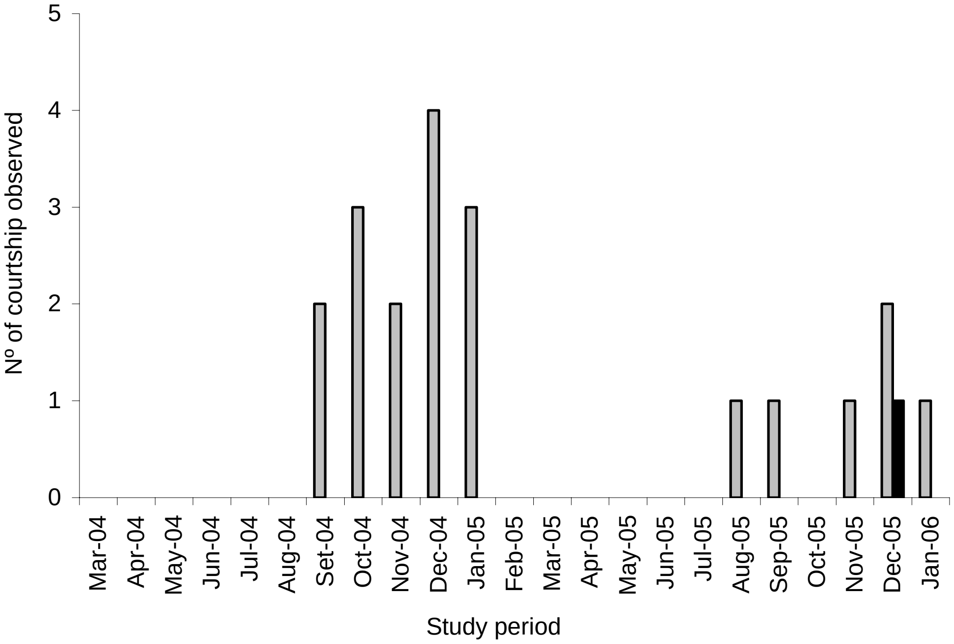

Figure 3- Number of courtship displays observed for *Aplastodiscus leucopygius* (gray columns) and *A. arildae* (black columns) in the Serra do Japi, municipalty of Jundiai, State of São Paulo, Brazil.

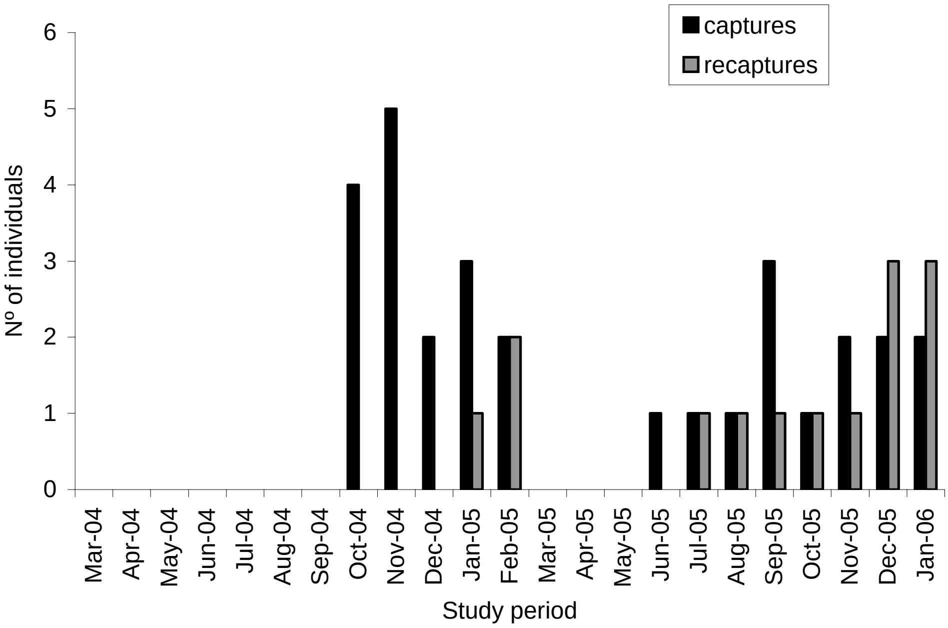

Figure 4- Number of captures and recaptures of males of *Aplastodiscus arildae* in the Serra do Japi, municipality of Jundiaí, Sate of São Paulo, Brazil.

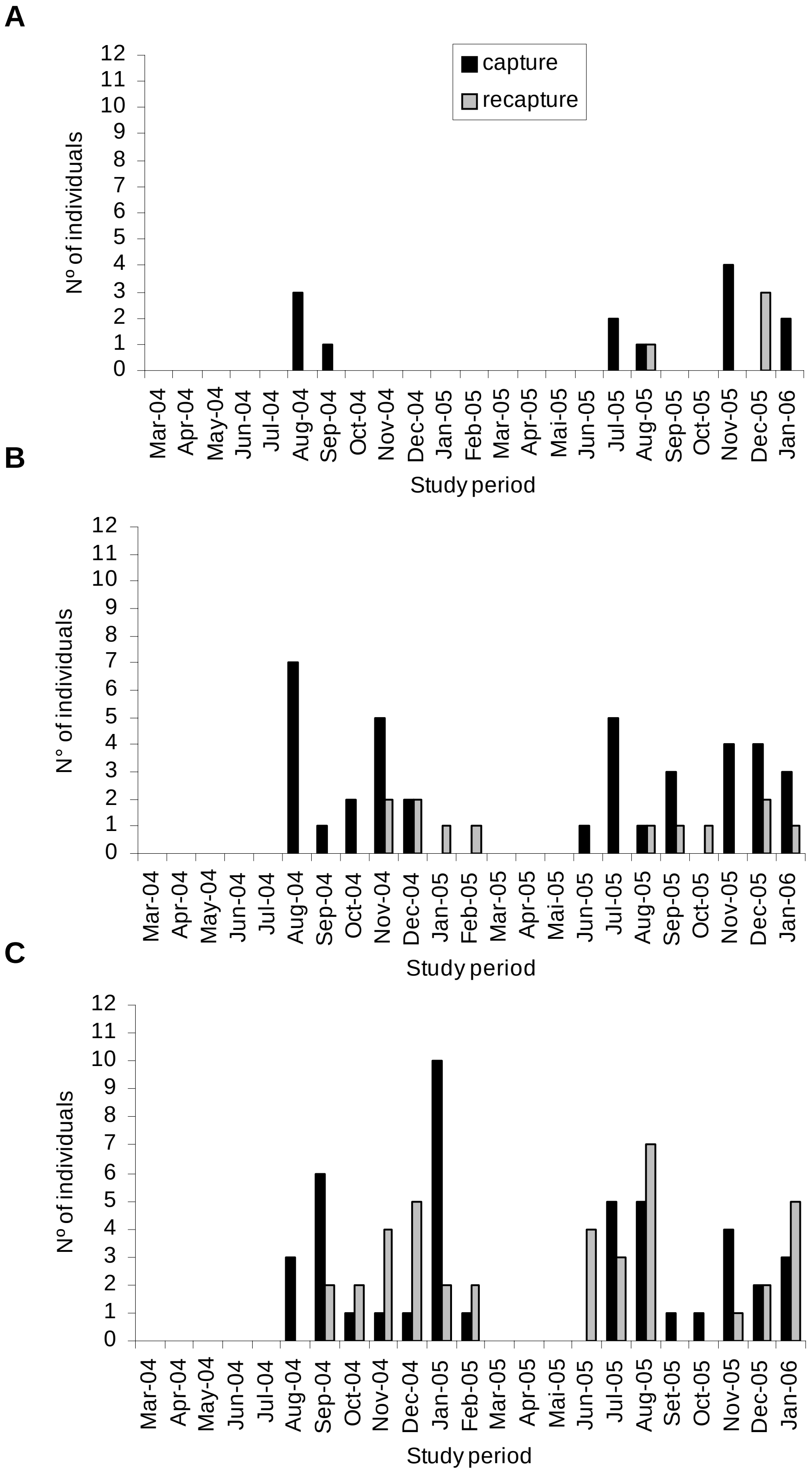

Figure 5- Number of captures and recaptures of males of *Aplastodiscus leucopygius* in the three habitats where the species was found in calling activity in the Serra do Japi, municipality of Jundiai, State of São Paulo, Brazil. A- Stream, B- Swamp, and C- Lake.

Chapter 2

Acoustic repertoire and the social context of *Aplastodiscus arildae* and *A. leucopygius* (Anura: Hylidae), in the Serra do Japi, Municipality of Jundiaí, State of São Paulo, Brazil

Juliana Zina & Célio F. B. Haddad

Calling male of *Aplastodiscus arildae*

Calling male of *Aplastodiscus leucopygius*

Resumo

As vocalizações, em muitas espécies de anuros, desempenham papel fundamental no estabelecimento das relações sociais entre os indivíduos. Durante os meses de março de 2004 a janeiro de 2006 foram gravados os cantos de machos de *Aplastodiscus arildae* e *A. leucopygius* na Serra do Japi, Município de Jundiaí, Estado de São Paulo. Foram identificados três tipos de canto para *A. arildae* e três tipos para *A. leucopygius*. As funções destes cantos foram estabelecidas conforme o contexto social em que os mesmos foram emitidos. Foram analisados os seguintes parâmetros dos cantos: freqüência mínima e máxima dos harmônicos, freqüência dominante, intensidade dos cantos, intervalo entre notas, taxa de repetição e duração das mesmas. Os dados referentes às vocalizações de anúncio de *A. arildae* e de *A. leucopygius* concordam com o que foi descrito na literatura, bem como com os dados para canto de corte de *A. leucopygius*. No entanto, no presente estudo, são apresentados dados inéditos sobre os diferentes tipos de cantos emitidos por machos das duas espécies e suas funções.

Palavras-chave: Vocalizações, *Aplastodiscus*, Comportamento reprodutivo e Escolha de parceiros.

Abstract

In many anuran species, vocalizations play an important role in the establishment of their social organization. From March 2004 to January 2006 calls of *Aplastodiscus arildae* and *A. leucopygius* were recorded in the Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. We identified three different calls for both studied species and their functions were determined according to the social context in which they were emitted. We analyzed the following call parameters: minimum and maximum frequency of the harmonics, dominant frequency, intensity of the calls, interval between calls, repetition rates, and duration of the calls. Call parameters of *A. arildae* and *A. leucopygius* agree with the data available in the literature. However, in the present study, new information on the calls and their functions were obtained.

Keywords: Vocalizations, *Aplastodiscus*, Reproductive behavior, Female choice

1. Introduction

Vocalizations in anurans evolved early in the group, playing an important role during intraspecific social interactions among the individuals (SALTHE & MECHAM, 1974). Many social functions are attributed to the calling behavior of anurans, including mate attraction (e.g. HADDAD & CARDOSO, 1992; SCHWARTZ, 1994; BRENOWITZ & ROSE, 1999), territory defense (e.g. WELLS, 1988; BASTOS & HADDAD, 2002), and defense against predators (SAZIMA, 1975). As they are species-specific, vocalizations are important pre-zygotic isolation mechanisms, maintaining the species as discrete entities in nature (OLDHAM & GERHARDT, 1975; ETGES, 1987; CARDOSO & VIELLARD, 1990; BOURNE & YORK, 2001). Variations in some parameters of the calls (dominant frequency, number of pulses, duration, and repetition rate) may result from phenotypic variations (WAGNER, 1989a; BASTOS & HADDAD, 1999; TOLEDO & HADDAD, 2005) or from environmental or social factors (WELLS, 1988; WAGNER, 1992; WILCZYNSKI & RYAN, 1999).

Aplastodiscus arildae belongs to the "*Aplastodiscus albofrenatus*" species group (FAIVOVICH *et al.*, 2005) and *A. leucopygius* to the "*A. albosignatus*" species group (FAIVOVICH *et al.*, 2005), both restricted to the Brazilian Atlantic rain forest. Descriptions of the vocalizations are available for *A. leucopygius* (referred to as *Hyla leucopygia* BOKERMANN, 1967; HEYER *et al.*, 1990; HADDAD & SAWAYA, 2000) and *A. arildae* (referred to as *Hyla albofrenata* HEYER *et al.*, 1990).

The aims of the present study were not only to describe and to identify the different calls emitted by *Aplastodiscus arildae* and *A. leucopygius*, but also to compare the characteristics of the advertisement call of both species and to recognize the social context in which the different vocalizations were emitted.

2. Material and Methods

The Serra do Japi (46º 56' W; 23º 14' S), located in the municipality of Jundiaí, is one of the last remnants of the Atlantic rain forest in the interior of the

State of São Paulo, Brazil and is considered an ecotone of Ombrofilous and Semideciduous forests (LEITÃO- FILHO, 1986).

The vocalizations of *Aplastodiscus arildae* and *A. leucopygius* were recorded from March 2004 to January 2006 with a Marantz tape recorder coupled to a Sennheiser ME 80 directional microphone. The intensity of the calls was also measured in the field with a Brüel & Kjaer sound level meter. All the recordings and call intensity measurements were made with the microphone and sound level meter placed at 70 cm from the calling male. The air temperature was measured during the recordings. The vocalizations were digitalized and analyzed in a Macintosh computer using the software Canary 1.2. We measured 10 advertisement calls from 20 males of *A. arildae* and 10 advertisement calls from 26 males of *A. leucopygius*. The courtship call of *A. leucopygius* was divided into three categories: courtship I (emitted when the female was close or touching the calling male), courtship II (emitted when the female was at least 50 cm away from the calling male), and courtship III (emitted when the male was inside or very close to the subterranean nest). We analyzed 10 calls of each type of courtship call from 19 males. Ten territorial calls from 10 males of *A. arildae* and 10 multi-note calls (*sensu* HADDAD & SAWAYA, 2000) from 10 males of *A. leucopygius* were also analyzed. Playback experiments were made with the tested male’s own advertisement calls with the speaker placed at 50 cm from the calling male. In the playbacks, the volume of the speaker was set according to the average intensity values emitted by the tested calling male. We used 20 males of *A. arildae* and 20 males of *A. leucopygius* in the experiments. The snout-vent lengths (SVLs) of the recorded males were measured with a 0.1 mm caliper and the body mass was determined with a 0.05 g field scale.

The observations began before sunset and ended when the calling activity stopped or decreased. The time when vocalization activity was more intense was registered. Information on the environment in which the species were found, calling sites used by the males, distance from the water, height above the ground, and distance to the next male, were also recorded.

To compare the physical parameters of the different call types of the same species, the advertisement calls of different individuals, and the advertisement calls of both species, we used the student *t* test. When variables were not normally distributed, we used a Mann Whitney test. For correlation between the sunset and the beginning of calling activity, we used Pearson's *r*-correlation test. The same test was used to verify possible correlations between male SVL and mass and the fundamental frequency of the advertisement call. To compare the height of the calling sites used by the same species during the dry and rainy seasons, and to compare the height of the calling sites used by both species, we used the student *t* test. We considered values to be significant when $P \leq 0.05$ (ZAR, 1996).

3. Results

3.1 Calling sites

Aplastodiscus arildae only used microhabitats located in the stream. Here, the males called parallel to the ground or in a slightly inclined position with their head directed toward the top of the trees. Calling males were usually found in high leaves on the trees from the families Araceae, Myrtaceae, Marantaceae, and Blechnaceae, at the edge of a flowing water rivulet. The height at which males were found while in calling activity did not differ between dry and wet seasons ($t = 0.03$; $P = 0.97$). The mean height throughout the studied period was 3.3 m above the ground (SD = 1.4; range = 1.2 – 7 m; N = 31). Males were observed moving, during the night, to higher leaves of the same plant. In many occasions, males of both *A. arildae* and *A. leucopygius* slowly moved their bodies in a 360º circle whilst calling. Males of *A. arildae* called from higher leaves than males of *A. leucopygius* ($t = 3.27$; $P = 0.07$).

Aplastodiscus leucopygius was found in the three studied habitats (swamp, stream, and lake). Males were found calling parallel to the ground, perched on the vegetation above the water, usually in leaves of Bromeliaceae, Blechnaceae, Poaceae, and Marantaceae, at the edge of calm water bodies. The height at which males were found in calling activity differed between dry and wet seasons (*t* = 3.43; *P* < 0.01). During the wet season, males were found calling from leaves at a mean height of 1.16 m (SD = 0.5; range = 0.5 – 2.5 m; N = 91) above the ground and during the dry season they were found calling from higher leaves, at a mean height of 2.05 m (SD = 0.7; range = 0.7 – 3.5 m; N = 55) above the ground.

3.2 Vocalizations

We identified three vocalization types for *Aplastodiscus arildae* and three for *A. leucopygius.*

3.2.1 Advertisement call

The advertisement calls of both species are similar, sharing as a common characteristic the pure sound note with a harmonic structure.

The advertisement call of *Aplastodiscus arildae* is composed of one note with two harmonics (Figure 1), emitted at a mean rate of 41.2 calls/minute (SD = 16.2; range = 12 – 73 calls/minute; N = 20). The description of the spectral and temporal parameters of the advertisement call of *A. arildae* is summarized in Table 1. The dominant frequency was in the second harmonic. There was no significant correlation between the fundamental frequency and male SVL (*r* = 0.77; *P* = 0.09; N = 20) or mass (*r* = 0.12; *P* = 0.72; N = 20).

The advertisement call of *Aplastodiscus leucopygius* is composed of one note with three harmonics (Figure 2) emitted at a mean rate of 40.9 calls/minute (SD = 23.4; range = 17 – 107 calls/minute; N = 26). The description of the spectral and temporal parameters of the advertisement call of *A. leucopygius* is summarized in Table 2. The dominant frequency of the advertisement calls can be present in the first, second, or third harmonics. This parameter varied among males and in the same male in different social contexts. In two occasions, when the same male was recorded alone, the dominant frequency was in the third harmonic, but when recorded in antiphony with another male, the dominant frequency was in the first harmonic. There was no significant correlation between fundamental frequency and male’s SVL (*r* = 0.28; *P* = 0.19; N = 26) or mass (*r* = 0.01; *P* = 0.95; N = 26) nor between position of the dominant frequency and male SVL ($r = 0.31$; $P = 0.34$; $N = 26$) or mass ($r = 0.18$; $P = 0.59$; $N = 26$).

The calling rate of the advertisement calls of both species changed according to the social and environmental context, being higher at the peak of vocalization activity during the night (2100 – 2200 h) or in the presence of a neighboring calling male. In the latter situation, males of both species were observed adjusting their calls in order to avoid overlap. At the beginning of the vocalization activity or when the analyzed male was isolated, the calling rate was lower.

The advertisement call of *Aplastodiscus arildae* has a significantly higher frequency compared with the advertisement call of *A. leucopygius* ($t = 21.5$; $P < 0.01$). The duration of the advertisement calls is also significantly different ($t = 18.5$; $P < 0.01$), being longer in *A. leucopygius*. These are the main parameters that distinguish the advertisement calls of both species.

3.2.2 Courtship call

The courtship call of *Aplastodiscus arildae* (Figure 3) was recorded in only one instance. Its structure is very similar to the advertisement call of the species and was emitted at a rate of 68 calls/minute. The spectral and temporal parameters of the courtship call of *A. arildae* are summarized in Table 1.

The courtship call of *Aplastodiscus leucopygius* was also similar to the advertisement call of the species, but was emitted at a significantly lower intensity ($U = 5541$; $P < 0.01$). There were significant differences in call duration ($U = 81567$; $P < 0.01$) and interval between calls ($U = 74049.5$; $P < 0.01$) when both calls were compared. The courtship call can be divided into three categories, according to the moment when it was emitted during courtship behavior: courtship call I (emitted when the female is close or touching the male), courtship call II (Figure 4) (emitted when the female is at least 50 cm from the male), and courtship call III (Figure 5) (emitted when the male is close or inside the subterranean nest). The differences between courtship calls I and II are related to the intensity of the calls ($t = 13.9$; $P < 0.01$) and the repetition rates ($t = 12.1$; $P < 0.01$), being significantly higher in courtship call II. Courtship call III has a particular structure; it is a trill call with three harmonics and four to five similar notes. The intensity of this call does not differ from the intensity of courtship call I (*t* = 0.53; *P* = 0.59). The spectral and temporal parameters of the courtship calls of *A. leucopygius* are summarized in Table 2.

3.2.3 Territorial call and multi-note call

The territorial call of *Aplastodiscus arildae* (Figure 6) was emitted between the advertisement calls and only during playback experiments. During the playbacks, 15 of the 20 tested males responded with a territorial call, four of them moved in the direction of the tape recorder, three males increased the repetition rate of the advertisement call, and two stopped calling.

The territorial call of *Aplastodiscus arildae* consists of three or four calls with three harmonics. The spectral and temporal parameters of the territorial call of *A. arildae* are summarized in Table 1.

The multi-note call of *Aplastodiscus leucopygius* (sensu HADDAD & SAWAYA, 2000) (Figure 7) was emitted in many circumstances, such as: before the beginning of the vocalization activities, in the presence of a male in courtship, in response to a multi-note call emitted by a neighboring male, and even during the period when the species was not found in calling activity (March/04 to July/04 and March/05 to May/05). However, none of the 20 tested males responded with this call to the playback experiments. Ten of the tested males increased the repetition rate of the advertisement call and 10 stopped calling. In one occasion, a male was observed emitting a multi-note call in the presence of a neighboring male that started to emit the same type of call; after 10 minutes the neighbor moved 10 cm away from its original calling site and started to emit advertisement calls. The other male also started to emit advertisement calls.

Three or four calls with three harmonics characterize the multi-note call. The spectral and temporal parameters of this call are summarized in Table 2.

3.3 Satellite behavior

Satellite behavior was observed only for *Aplastodiscus leucopygius* in two occasions. The satellite males had larger or similar SVL compared to calling males and after the removal of the calling male, the satellite males started calling. We also observed that the satellite males started to emit courtship calls as soon as the females approached the resident calling males, attracted by their advertisement calls. We did not observe female interception by satellite males.

4. Discussion

4.1 Calling sites

Calling sites used by *Aplastodiscus arildae* and *A. leucopygius* were similar to sites described for both species in other studies (see HEYER *et al.*, 1990; HADDAD & SAZIMA, 1992; HADDAD & SAWAYA, 2000). In the present study we observed that both species used similar microhabitats as calling sites, probably due to similarities in the vegetation of the studied habitats. However, there was a significant difference between the height from the ground at which each species was found in calling activity. The higher vocalization sites of *A. arildae* could represent a way to escape from the noisy environment produced by the fast waters that can disturb the emission of the calls. The movement of the males to higher leaves of the same plant could be related to the lower density of leaves on the tree canopies. In the absence of leaves, the reverberation index decreases, therefore, the signal can be emitted and received with less interference (RICHARDS & WILEY, 1980).

The movement in a 360° circle during calling activities, observed for *Aplastodiscus arildae* and *A. leucopygius*, and consequently, the change in the orientation of the call propagation was also observed by GUIMARÃES & BASTOS (2003) for *Hypsiboas raniceps*. This behavior can increase the probability of the calling male to be heard by nearby males and females, being more efficient at warning other males of their proximity and also increasing the likelihood of their mating success (JUNCÁ, 1998).

4.2 Vocalizations

4.2.1 Advertisement call

Comparing the advertisement calls of *Aplastodiscus arildae* and *A. leucopygius*, the advertisement call of the former is emitted with a higher frequency, which could be related to the environment used by the species as a calling site since *A. arildae* is found only in noisy environments. The evolution of an advertisement call adapted to the low frequency environmental noise could be an explanation for the higher frequency of the calls in *A. arildae*. However, frequencies up to 2.5 kHz can be attenuated by the leaves (MORTON, 1975), which could explain the great height at which the males were found in calling activity, since in higher parts of the trees the density of leaves is lower.

The advertisement call of *Aplastodiscus arildae* is similar in structure to that of other species that belong to the *A. albofrenatus* group (see CONTE *et al*., 2005). The acoustical parameters measured in the present study agree with the description of the advertisement call of *A. arildae* made by HEYER *et al.* (1990) in Boracéia, State of São Paulo, Brazil. However, there was a slight difference in the repetition rate of the calls, probably due to the different social context in which the recordings were made, since this parameter is strongly dependent on the number of calling males in the vicinity.

According to FORREST (1994), the propagation distance of long-range signals is an important selective factor that depends on background noise, signal distortion during propagation, and the ability of the individuals to receive and extract information from the emitted signal. Males of both species in the presence of calling neighboring males increased their call rates, as reported for other species (e.g. RYAN, 1985; SCHWARTZ & WELLS, 1985; SCHWARTZ, 1986; BENEDIX & NARINS, 1999). Females of *Aplastodiscus leucopygius* apparently choose their mates based on call rates (see “mate choice” Chapter 3). Therefore, males which are already socially involved are expected to change their calling parameters in order to maximize the probability of females to perceive them as more attractive than their local rivals. The background noise (biotic or abiotic) can interfere in the detection of the signal and in the transmission of the information. The heterospecific and cospecific background noises represent important sources of biological noise (FORREST, 1994). Many strategies may be used by males to avoid this interference, such as variation of some parameters of the advertisement call (intensity, duration of the call, frequency, increase in the call repetition rate, and antiphony). *Aplastodiscus arildae* and *A. leucopygius* were observed increasing their call repetition rate and adjusting their calls in order to avoid overlap. Many studies on anuran vocal interactions demonstrated that overlapping calls are less attractive to females than calls that do not overlap (WELLS & SCHWARTZ, 1984). The calling alternation and the adjustment made by males to avoid overlap can be considered a territorial behavior (TELFORD, 1985) and female choice may act as a selective pressure in the establishment of a minimum distance between males.

The parameters of the advertisement call of *Aplastodiscus leucopygius* measured in the present study are similar to those obtained by HADDAD & SAWAYA (2000). According to these authors, the dominant frequency of the advertisement call of *A. leucopygius* is concentrated in the third harmonic. However, in the present study, we observed that the dominant frequency might be concentrated in any one of the three harmonics. In anurans, males often access the fight ability of their opponents based on parameters of the rival calls (DAVIES & HALLIDAY, 1978; ARAK, 1983; RAMER *et al*., 1983; ROBERTSON, 1986; WAGNER, 1989a; BEE *et al.*, 1999). The analysis of the fight ability of an opponent could avoid physical aggression that is energetically costly (DAVIES & HALLIDAY, 1978; RIECHERT, 1978; CLUTTON-BROCK & ALBON, 1979; ROBERTSON, 1986). The dominant frequency of the call in most anurans species is inversely proportional to the size of the individuals (RYAN, 1985; SULLIVAN & WAGNER, 1988; WAGNER, 1989a). If the acoustic signals used by males to detect the fight ability of their rivals were “honest”, they would show the real size of the opponents. However, in some species, small males can deliberately change the dominant frequency of their advertisement calls (see BEE & PERRIL, 1996). WAGNER (1989a) showed that, during playback experiments, males of *Acris crepitans* lower the dominant frequency of their calls. He also demonstrated that this parameter changes according to the night in question, probably as a consequence of social interactions. BEE *et al*. (2000) considered three hypotheses that could explain the changes in the dominant frequency; 1- male shows its real size, 2- signal does not depend on the size of the calling male, but is related to the fight ability of the male based on its previous experiences or its motivation, and 3- the male can change the frequency of its call so that its opponents receive an information that does not correspond to its real fight ability; the bluffing behavior would be socially dependent. Males of *Acris crepitans* may fight after vocal interactions (WAGNER, 1989b), as may males of *Rana clamitans* (BEE & PERRIL, 1996). *Aplastodiscus leucopygius*, however, is not known as a fighting species and we did not see any physical interaction between males. Therefore, the change in the dominant frequency in *A. leucopygius* is more likely to be related to the male's attractiveness for females in the presence of a neighboring calling male than for bluffing to other males. ABRUNHOSA *et al*. (2005) suggested that the position of the dominant frequency is the most important character for constructing a phylogenetic tree based on characteristics of the advertisement call of species of the *A. albosignatus* group. As we observed here, this parameter in *A. leucopygius* is variable and probably socially dependent. Thus, this parameter is not adequate for characterizing the calls of *A. leucopygius* and perhaps of other species within the *A. albosignatus* group.

4.2.2 Courtship calls

In anurans, the courtship call is generally very similar to the advertisement call in duration and structure; however, in some cases, the courtship call is emitted at lower intensity and with higher repetition rate (WELLS, 1988).

We observed different types of courtship calls for *Aplastodiscus leucopygius* based on the different intensities and repetition rates of the calls. Different types of courtship calls were also observed for *A. perviridis* by HADDAD *et al.* (2005). For this species, these authors observed three types of courtship calls that were emitted at two different moments of the courtship behavior: “long courtship call” and “short courtship call”, being the last one divided into two different types; one of them emitted when the male was out of the subterranean nest and the other one when the male was inside the nest (HADDAD *et al*., 2005). For this species, differences between calls were mainly the repetition rate of the calls, duration of the calls, and interval between calls. In the present study, we only verified differences between the intensity of the calls and repetition rate. According to RICHARDS & WILEY (1980), the two most important variables that affect the identification and localization of an acoustic signal in a forest are intensity fluctuations and reverberation. Observational studies show that the intensity of the advertisement calls affects inter-male spacing, being this parameter the most important mediator of the space between males (see review in GERHARDT & HUBER, 2002). Apparently the intensity of the calls is the main parameter used by males to evaluate the proximity of their neighbors (BRENOWITZ *et al*., 1984; GERHARDT *et al.*, 1989). In the present study, we may extend this hypothesis to females during courtship behavior. Females may use the intensity variation of the courtship calls to evaluate the proximity of the males when following them during the journey to the subterranean nests.

4.2.3 Territorial call and multi-note call

The main function of the territorial call is to warn close neighboring males about the territorial occupation (WELLS, 1977). It has been demonstrated in a number of species (PERRIL & SHEPHERD, 1989; WAGNER, 1989 a, b; BRENOWITZ *et al*., 1984; GERHARDT *et al*., 1989; STEWART & BISHOP, 1994).

The territorial call of *Aplastodiscus arildae* had a conspicuous function in warning other males about the territorial occupation. Apparently, the multi-note calls also act to establish the territorial limits of *A. leucopygius* males, as observed during calling interactions between males. According to the results of the playback experiments, *A. arildae* seems more territorial than *A. leucopygius.*

4.3 Satellite behavior

Satellite behavior is known in many prolonged breeding species (e.g. *Physalaemus pustulosus*, GREEN, 1990; *Dendropsophus minutus*, HADDAD, 1991; *Scinax fuscomarginatus*, TOLEDO & HADDAD, 2005). Several hypotheses have been suggested to explain satellite behavior, such as an alternative reproductive tactic in high density choruses (GREEFIELD & SHELLY, 1985; ROWELL & CADE, 1993), a way to save metabolic energy (FORESTER & LYKENS, 1986, ARAK, 1988), a mechanism against predation (FOREST & LYKENS, 1986; GREEN, 1990), and as an alternative to unattractive males that face reduced fitness as callers (ARAK, 1988). The density of calling males of *Aplastodiscus leucopygius* was not higher when satellite males were observed, compared with other occasions in which we did not find satellite males. The fact that satellite males started to emit courtship calls in the presence of females close to the resident males, and the observation that after the removal of the resident male satellite males started to emit advertisement calls, reinforces the hypothesis that this behavior is performed in order to save metabolic energy or to avoid predators. The complex courtship behavior and the fact that each male probably has its own subterranean nest are two other questions to be considered in the analysis of the satellite behavior in *A. leucopygius*. As a consequence of the reproductive mode exhibited by the species, males do not amplect females as soon as they approach them; the pair exhibits a very stereotyped behavior until it reaches the subterranean nest (HADDAD & SAWAYA, 2000). In this way, females would only be intercepted by the satellite males if they stopped this alternative tactic and started to call when the female approached the territory of the dominant male.

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Table 1- Spectral and temporal parameters of the different call types of *Aplastodiscus arildae*. Maxf = Maximum frequency; Minf = Minimum frequency; CD = Call duration; IN = Interval between calls; CI = Call intensity. Values as mean ± standard deviation.

Type of call	Harmonic	Minf (Hz)	Maxf (Hz)	CD (s)	IN (s)	CI (db)
Advertisement call	1º	878.72 ± 154	1682.80 ± 115.73	0.02 ± 0.007	1.67 ± 0.09	65.65 ± 9.13
	2º	2076.98 ± 175.84	2942.70 ± 158.16			
Courtship call	1º	850 ± 97.18	1771 ± 66.4	0.01 ± 0.001	0.71 ± 0.07	45.20 ± 10.2
	2º	2034 ± 29.51	2983 ± 45.16			
Territorial call	1º	928.57 ± 125.89	1857.14 ± 194.65	0.02 ± 0.002	0.17 ± 0.001	Not measured
	2º	2107.14 ± 100.95	3012.86 ± 100.95			
	3º	2930 ± 812.44	3888.57 ± 953.49			

Table 2- Spectral and temporal parameters of the different call types of *Aplastodiscus leucopygius*. Maxf = Maximum frequency; Minf = Minimum frequency; CD = Call duration; IN = Interval between calls; CI = Call intensity. Values as mean ± standard deviation.

Call type	Harmonic	Minf (Hz)	Maxf (Hz)	CD (ms)	IN (s)	CI (db)
Advertisement call	1º	438.72 ± 105.70	978.65 ± 85,54	1.38 ± 0.26	3.17 ± 2.48	64.85 ± 4.2
	2º	1249.35 ± 145.30	1737.99 ± 169.56			
	3º	2013.89 ± 253.41	2557.07 ± 285.04			
Courtship call I	1º	515.60 ± 73.71	954.80 ± 55.70	1.29 ± 0.31	0.8 ± 0.3	60.6 ± 3.01
	2º	1273.70 ± 72.49	1714.49 ± 77.47			
	3º	2038.92 ± 95.91	2519.50 ± 124.70			
Courtship call II	1º	540.60 ± 167.45	1028.19 ± 251.22	1.19 ± 0.33	2.0 ± 1.84	45.91 ± 3.63
	2º	1261.17 ± 110.88	1721.38 ± 103.30			
	3º	2030.50 ± 103.92	2484.74 ± 138.46			
Courtship call III	1º	530.23 ± 64.5	999.45 ± 34.52	1.27 ± 0.21	1.5 ± 0.56	45.2 ± 3.56
	2º	1275.34 ± 94.5	1720.23 ± 64.30			
	3º	2045.43 ± 99.84	2490.33 ± 100.34			
Multinote call	1º	730.5 ± 199.4	1291.9 ± 386.6	0.53 ± 0.01	2.12 ± 0.32	Not measured
	2º	1538.3 ± 351.4	2135 ± 384.1			
	3º	2258.3 ± 208	2908.3 ± 298.5			

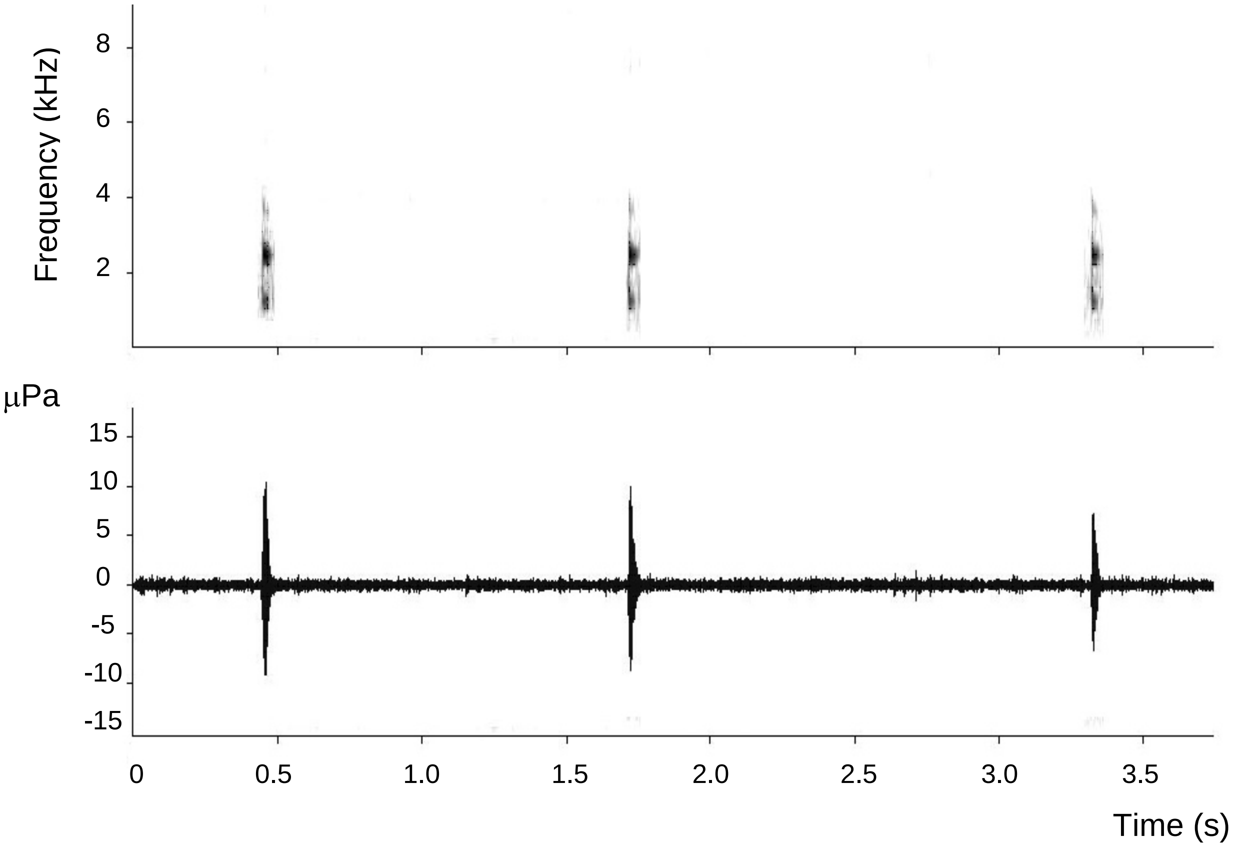

Figure 1- Advertisement call of *Aplastodiscus arildae*. Spectrogram (upper) and waveform (lower). Recorded at 2200 h at Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. Air temperature: 17 ºC.

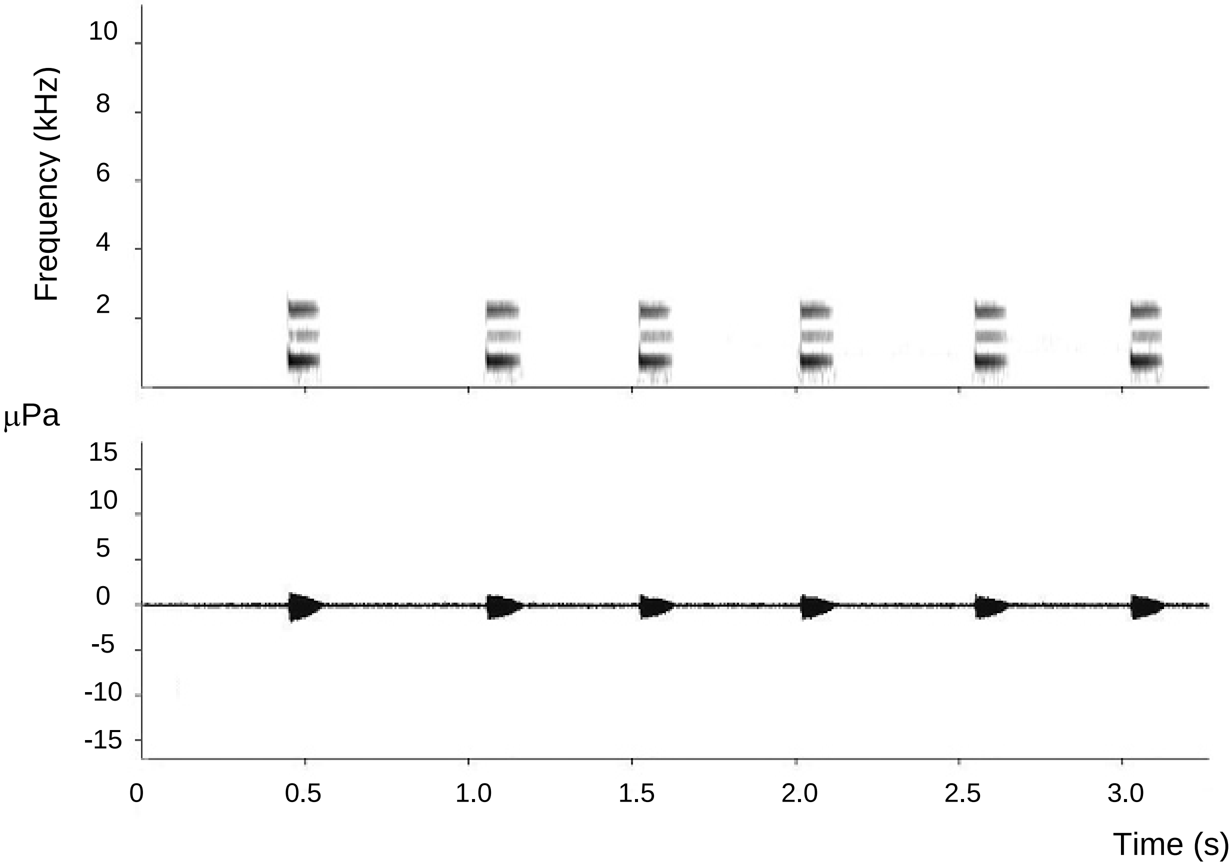

Figure 2- Advertisement call of *Aplastodiscus leucopygius*. Spectrogram (upper) and waveform (lower). Recorded at 2035 h at Serra do Japi, municipally of Jundiaí, State of São Paulo, Brazil. Air temperature: 18 ºC.

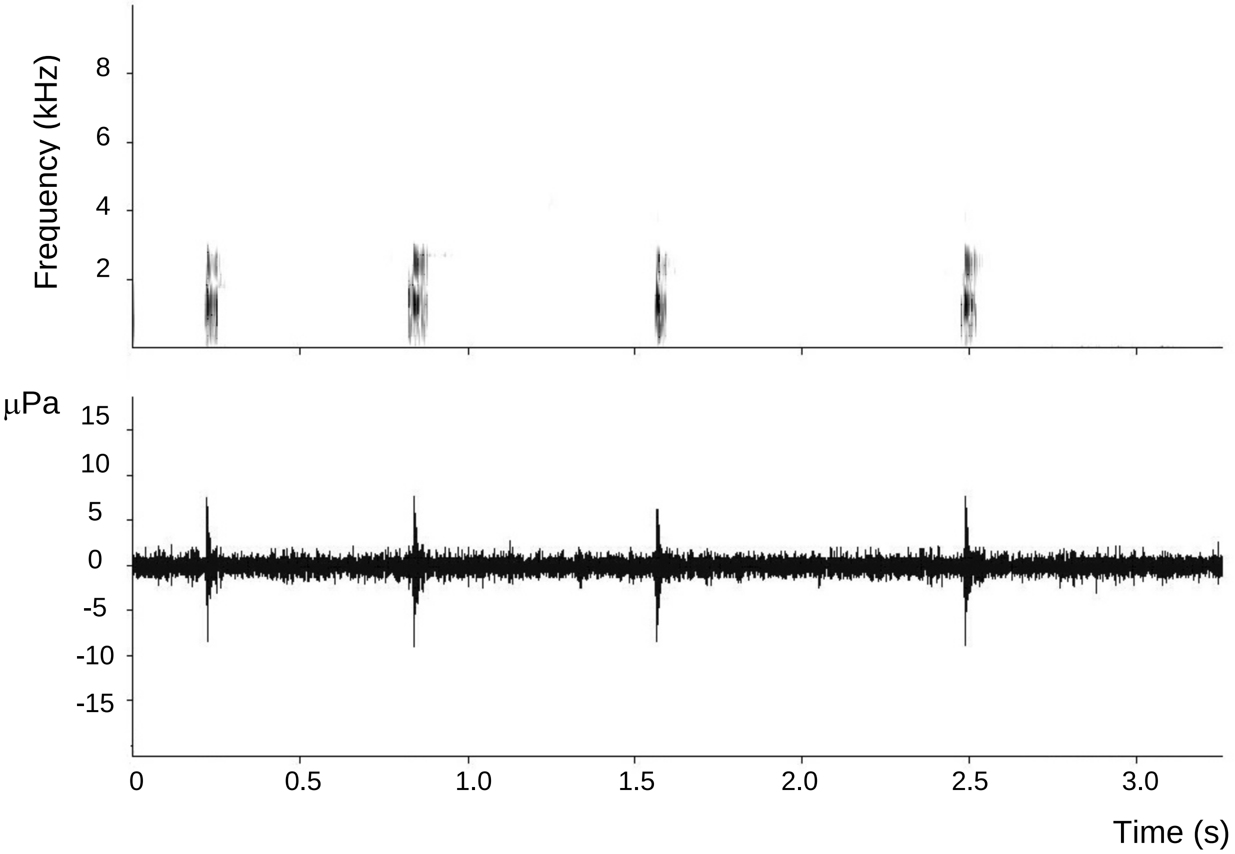

Figure 3- Courtship call of *Aplastodiscus arildae*. Spectrogram (upper) and waveform (lower). Recorded at 2300 h at Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. Air temperature: 17 ºC.

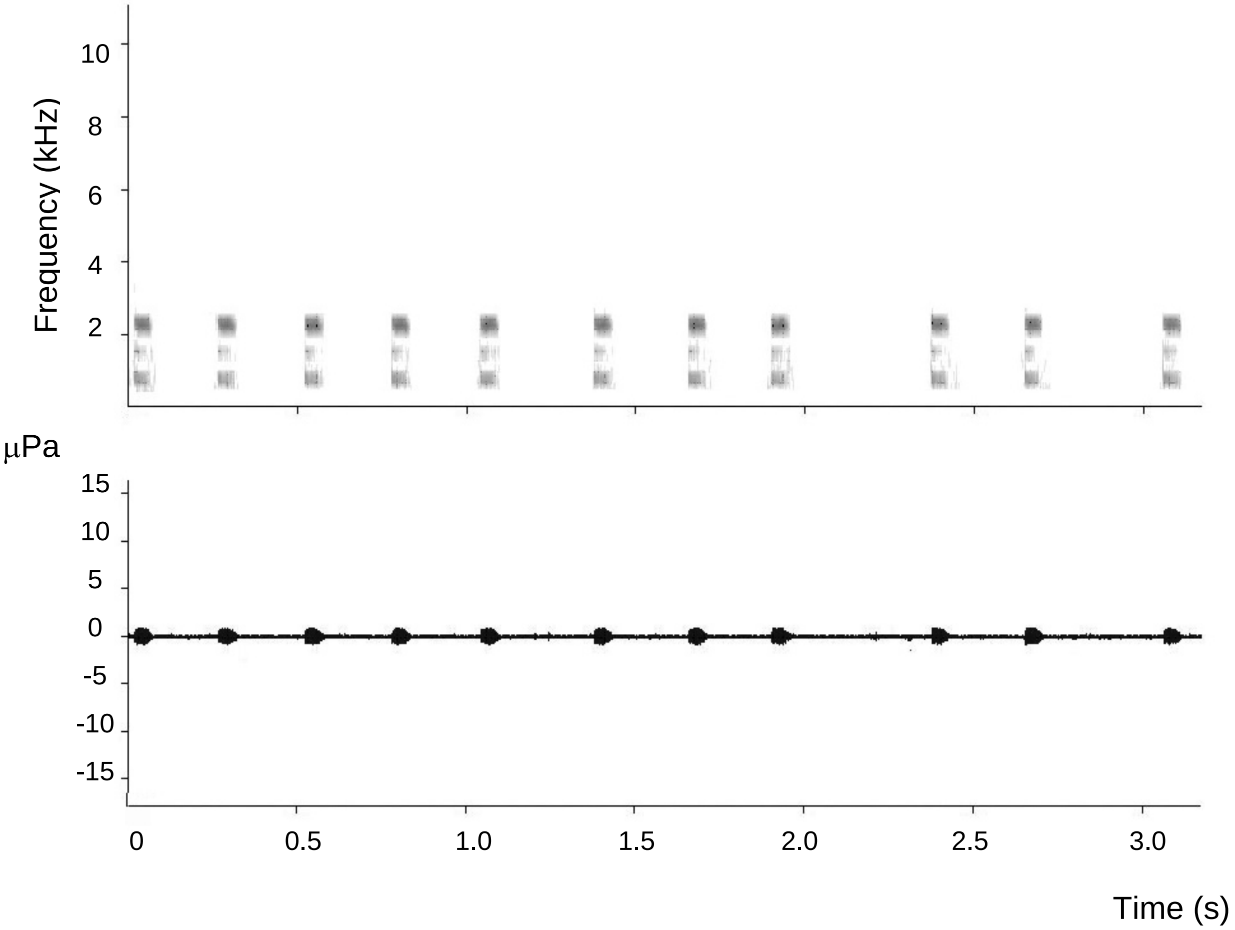

Figure 4- Courtship call II of *Aplastodiscus leucopygius*. Spectrogram (upper) and waveform (lower). Recorded at 2300 h at Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. Air temperature: 17 ºC.

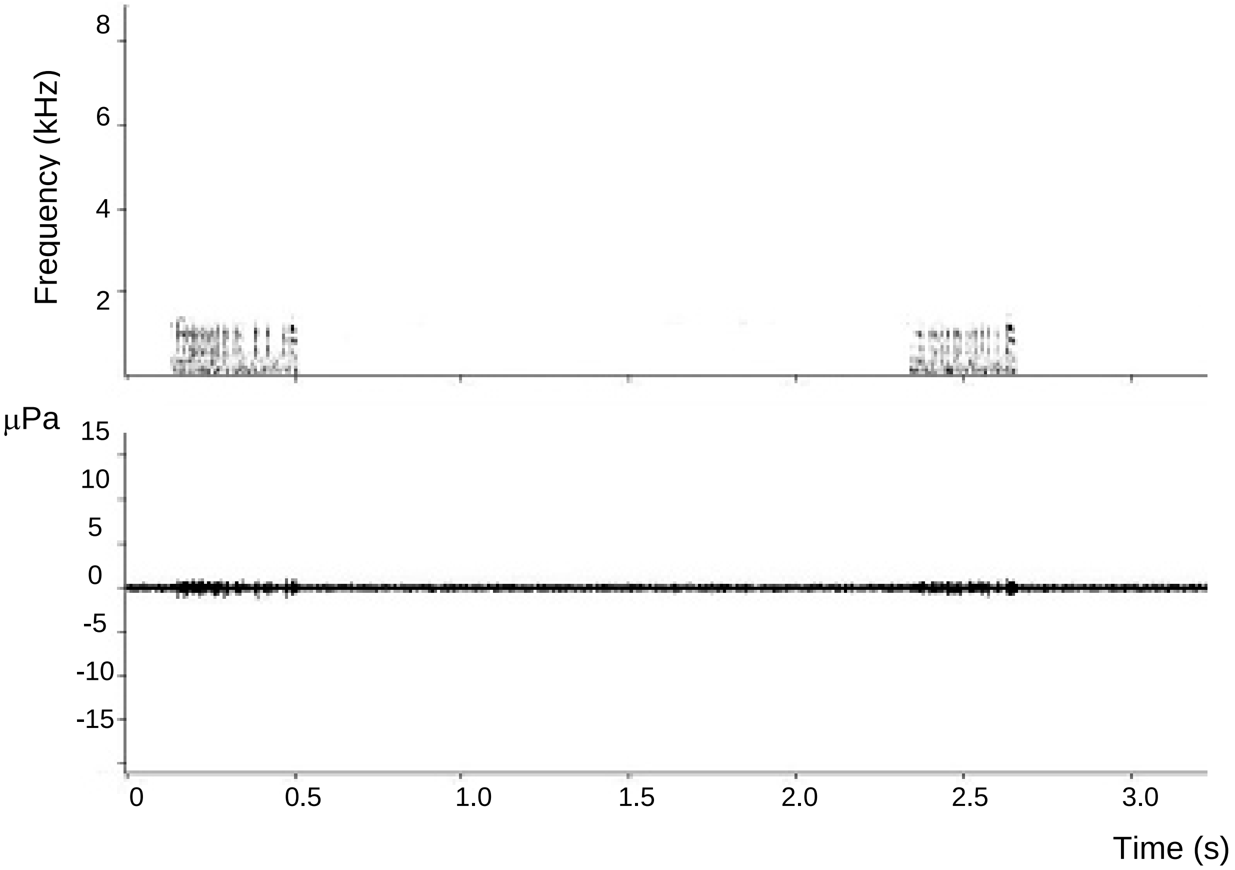

Figure 5- Courtship call III of *Aplastodiscus leucopygius*. Spectrogram (upper) and waveform (lower). Recorded at 2300 h at Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. Air temperature: 17 ºC.

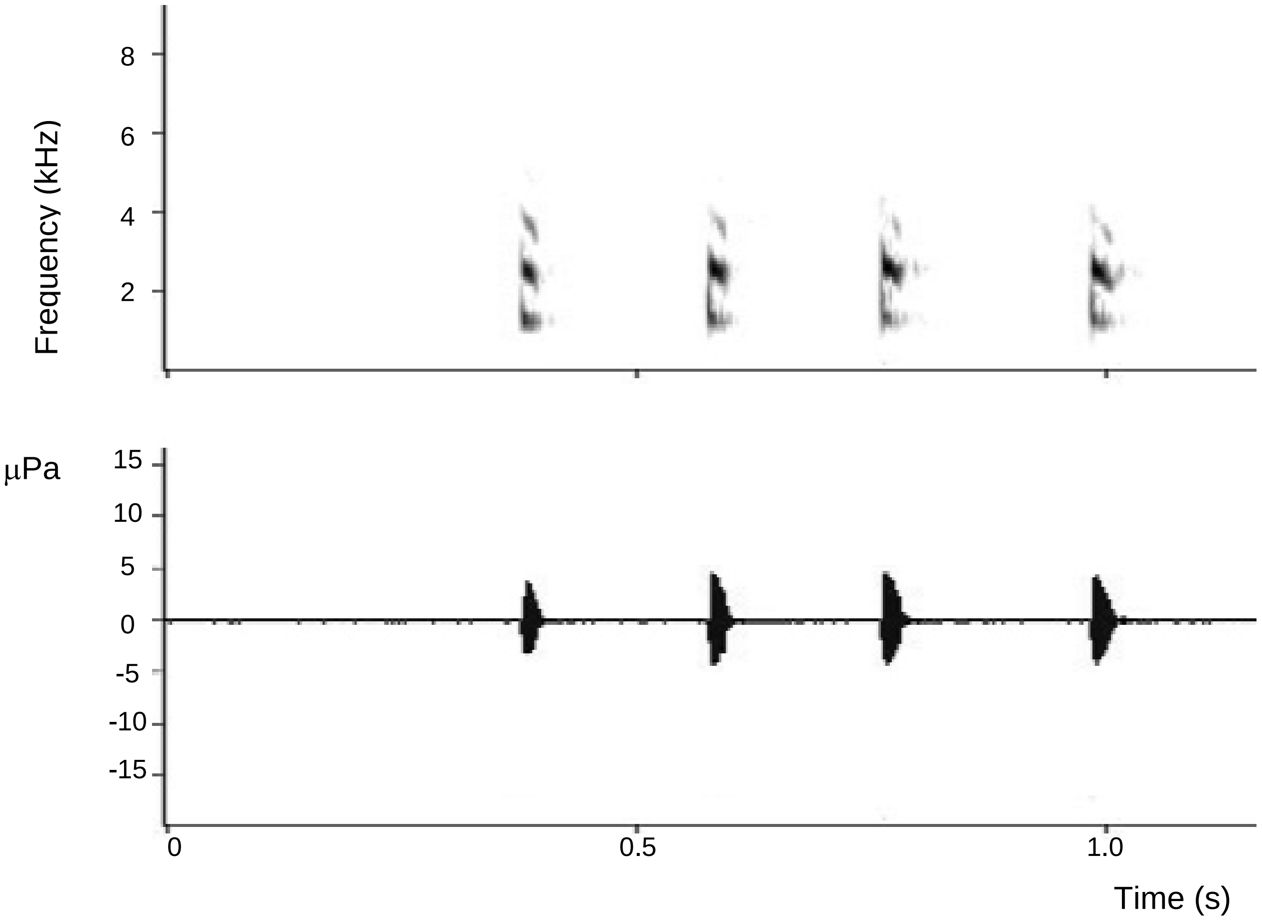

Figure 6- Territorial call of *Aplastodiscus arildae*. Spectrogram (upper) and waveform (lower). Recorded at 2300 h at Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. Air temperature: 19 ºC.

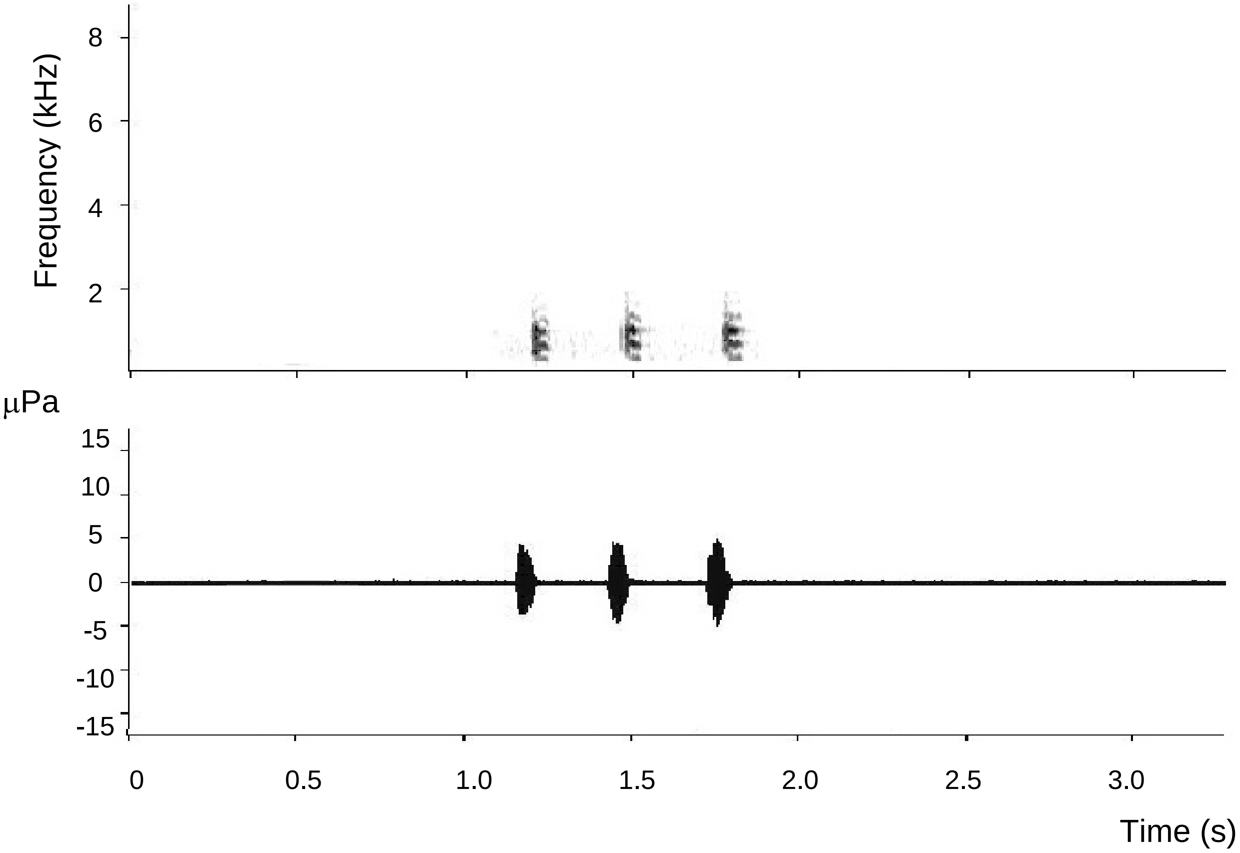

Figure 7- Multi-note call of *Aplastodiscus leucopygius*. Spectrogram (upper) and waveform (lower). Recorded at 2100 h at Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil. Air temperature 19 ºC.

Chapter 3

Courtship behaviour of *Aplastodiscus arildae* and *A. leucopygius* (Anura: Hylidae)

Juliana Zina & Célio F. B. Haddad

Courtship of *Aplastodiscus arildae*

Courtship of *Aplastodiscus leucopygius*

Resumo

Durante março de 2004 a janeiro de 2006 estudamos o comportamento de corte e modo reprodutivo de duas espécies simpátricas do gênero *Aplastodiscus*, *A. arildae* e *A. leucopygius*, pertencentes respectivamente aos grupos de *A. albofrenatus* e *A. albosignatus*. O modo reprodutivo de *Aplastodiscus leucopygius* concorda com o descrito na literatura; no entanto, novas informações foram obtidas sobre o comportamento de corte da espécie. O comportamento de corte de *Aplastodiscus arildae* foi observado, descrito e comparado com o comportamento exibido por *A. leucopygius*. Ambos envolvem seqüências comportamentais especializadas, caracterizadas pela emissão de diferentes tipos de vocalizações e uma seqüência estereotipada de estímulos táteis mútuos entre macho e fêmea.

Palavras-chave: Modo reprodutivo, *Aplastodiscus*, Seleção sexual e Comportamento de corte.

Abstract

From March 2004 to January 2006 we studied the courtship behaviour and reproductive mode of two sympatric species of *Aplastodiscus*, *A. arildae* and *A. leucopygius*, belonging to the *A. albofrenatus* and *A. albosignatus* groups, respectively. The reproductive mode observed for *Aplastodiscus leucopygius* agrees with the description available in the literature. However, new information about the courtship behaviour of this species is described here. The courtship behaviour of *Aplastodiscus arildae* is described and compared with that of *A. leucopygius*. Both involve a specialized sequence of behaviours, characterized by the emission of different vocalizations and a stereotyped sequence of mutual tactile stimuli between male and female.

Keywords: Reproductive mode, *Aplastodiscus*, Sexual selection, Courtship behaviour

1. Introduction

Reproductive mode can be defined as a combination of traits that includes clutch and ovum characteristics, oviposition sites, rate and duration of development, stage and size of hatchling, and type of parental care, if any (SALTHE & DUELLMAN, 1973). According to the review by HADDAD & PRADO (2005), there are 39 different reproductive modes registered for anurans, the greatest reproductive diversity among the tetrapods (DUELLMAN & TRUEB, 1986). The family Hylidae exhibits 12 modes, 11 of which occur in the Atlantic forest (HADDAD & SAWAYA, 2000). The high diversity of reproductive modes observed for the Atlantic forest frogs can be attributed mainly to the diversified microhabitats and high humidity present in this biome (HADDAD & PRADO, 2005).

Most examples of complex courtship behaviours come from studies on dendrobatids (e.g. WELLS, 1977; LIMERICK, 1980; ROITHMAIR, 1992; JUNCÁ, 1998). Nevertheless, recent studies on reproductive behaviour of hylids revealed complex courtship behaviours, mostly in the genus *Aplastodiscus* (e.g. *Aplastodiscus leucopygius*, HADDAD & SAWAYA, 2000; *Aplastodiscus eugenioi*, HARTMANN *et al.*, 2004). The courtship of these hylids involves mutual tactile stimuli and egg deposition in a subterranean nest previously constructed by the male, who guides the female to the nest.

Sexual selection is a fundamental concept in the evolution of social behaviour since it affects many biological phenomena, including parental care, mate system, degree of sexual dimorphism, age of sexual maturity, and variation in mortality and sexual rates (HOWARD, 1980). In many anuran species, females choose their mates based on certain characteristics of their calls (see RYAN & KEDDY-HECTOR, 1992). Mate choice frequently involves selection by the female, which makes it a non random process (OLSON *et al.*, 1986). Female mate choice has been demonstrated as an important determinant of male reproductive success in several species of anurans (RYAN, 1985).

The aims of this study were to compare the courtship behaviour between two sympatric species of *Aplastodiscus* and to verify the characteristics used by the females to evaluate males during the mate choice process.

2. Material and methods

This study was carried out in the Serra do Japi (46º 56’ W; 23º 14’ S) municipality of Jundiaí, State of São Paulo, south-eastern Brazil, between March 2004 and January 2006. The observations began right after sunset and finished when the reproductive activity ended. Focal animal and all occurrence samplings were used for behavioural records (LEHNER, 1979). We counted the number of calls/minute for each male of *Aplastodiscus leucopygius* in calling activity in the habitats during two minutes per male in the same night, between 2100 and 2200 h, when calling activities of the species reach the peak (see “activity and courtship period” chapter 1). During the observation of the courtship behaviour, we used head lights with weak batteries to reduce the disturbance on the behaviour of the pairs. Neighbouring males that were observed close to the pairs were measured to the nearest 0.1 mm with a caliper ruler, weighed to the nearest 0.1 g with a field scale, and toe-clipped for permanent identification, allowing repeated observations of the same individuals. The same procedure was carried out with the pairs in courtship after they finished their activities.

For *Aplastodiscus leucopygius*, five gravid females and five females that deposited their eggs were dissected to obtain the mean number of ovarian eggs, ovarian egg diameter, and mass of ovary. The same information was obtained for collected *A. leucopygius* females that did not lay their eggs and the only gravid female of *A. arildae*. Egg diameter was measured to the nearest 0.1 mm with an ocular micrometer under a Zeiss SV 11 stereomicroscope. Two males of *A. leucopygius* and two males of *A. arildae* were dissected to obtain the relative mass of their testicles. We collected three clutches of *A. leucopygius*, counted the total number of eggs and measured 10 eggs from each. Females, males were deposited in the CFBH collection (Appendix 1) at Universidade Estadual Paulista, UNESP, Rio Claro, SP, Brazil.

We used the Person *r*-correlation to verify the relationship between the snout-vent length (SVL) and body mass of males and females that lay their eggs during courtship behaviour. A Pearson *r*-correlation test was also used to verify the relationship between monthly rainfall and number of courtship displays of *Aplastodiscus leucopygius*. Monthly rainfall was obtained from a meteorological station located approximately 10 km from the studied habitats. To compare the SVL and mass of males in courtship with that of solitary males, we used the student *t* test; we considered values to be significant when $P \leq 0.05$ (ZAR, 1996).

3. Results

3.1 Courtship behaviour

During the study period we observed 20 courtship sequences of *Aplastodiscus leucopygius* and only one courtship behaviour of *A. arildae*.

Aplastodiscus arildae

The courtship behaviour of *Aplastodiscus arildae* consisted of a complex sequence of tactile stimuli. The behavioural sequence of the observed pair (Figure 1) can be summarized as follows: 1- the male was calling parallel to the ground on a leaf at 1.5 m from the ground; 2- the female jumped onto a leaf next to the leaf where the male was calling from when the male noticed the presence of the female, it stopped emitting the advertisement call; 3- the female touched the male’s snout with its hand; 4- the female repeatedly beat the male with its snout; 5- the female touched the male’s snout with its hand; 6- the male moved itself slightly and started to move one of its feet up and down repetitively, beating it against the leaf; 7- the male moved so that it positioned itself face to face with the female; 8- the female moved one of its feet up and down repetitively, beating it against the leaf; 9- the male repeated this behaviour; 10- the female positioned its head above the male’s head; 11- the male moved slightly preparing itself to get down; 12- after two hours, the male started descending from its perch, emitting the courtship call; 13- the male stopped emitting courtship calls constantly and the female followed the male, touching it with its hand; 14- after three hours from the beginning of courtship, the pair reached the ground, the female continued following the male, touching its dorsum with its hand. This courtship description is based on one observation that occurred during a rainy night and took more than nine hours. The pair separated after a branch fell down next to them.

Aplastodiscus leucopygius

We only observed 10 courtship sequences from the beginning. The other 10 were observed from the moment when the females were already close to the males.

The process has a mean duration of 4 h and 30 min from the arrival of the female to the entrance of the pair into the subterranean nest (SD = 80 min; range = 2 – 7 h; N = 10). The courtship sequence starts with the female approaching the male, attracted by its advertisement call, and ends when the male enters the subterranean nest followed by the female. After oviposition, males abandon the subterranean nests; females do the same right after the males. From the entrance of the pair into the subterranean nest until the exit of the male, the process has a mean duration of 4 hours (SD = 90 min; range = 3 – 5 h; N = 20).

We observed that both females and males passed their hands over their eyes or blinked when facing each other in the middle of the journey to the subterranean nest. During this interaction, we also observed that males executed toe trembling.

On two occasions, an individual of *Hypsiboas bischoffi* (Hylidae) jumped close to the leaf where males of *Aplastodiscus leucopygius* were calling. After that, males of *A. leucopygius* started to emit courtship calls.

Subterranean nests were located at a mean distance of 3.7 m from the male calling site (SD = 1.3; range = 2 – 8 m; N = 10).

3.2 Mate choice

There was no significant correlation between SVLs of females and males of pairs involved in courtship behaviour (r = 0.29; P = 0.32; N = 15) (Figure 2A), neither between their masses (r = 0.44; P = 0.13; N = 15) (Figure 2B). The mean SVL of solitary males was 39.93 mm (SD = 1.8; range = 37 – 44 mm; N = 37) and their mean mass was 4.15 g (SD = 0.87; range = 3.5 – 5.5 g, N = 37). Solitary males did not present significant differences in SVL (t = 0.59; P = 0.11) when compared with males involved in courtship and also did not present significant differences in mass (t = 0.13; P = 0.89). Seventy five percent of females were observed choosing males with the highest repetition rate of advertisement calls.

From the 20 courtship sequences observed for *Aplastodiscus leucopygius*, 15 finished with oviposition. The number of courtship behaviours of *A. leucopygius* was positively correlated with the monthly rainfall (r = 0.57; P < 0.01). In three courtship sequences of *A. leucopygius* observed in December 2005, females came out of the subterranean nest right after they had entered, and reproduction did not occur. Nests in these occasions were almost entirely full of water. Another two courtship sequences were interrupted, at the moment when the pairs were inside the subterranean nest, due to the incidental interference of the observer. This was necessary as we could not observe the oviposition behaviour inside the subterranean nests. However, in these two situations, when we interrupted the behaviour inside the nests, we observed the males at the entrance to the nests, while the females were inside the nests and their snouts were covered in mud.

3.3 Reproductive investment and nests

We measured 20 subterranean nests of *Aplastodiscus leucopygius*. The mean diameter was 6.5 cm (SD = 1.3; range = 4.5 – 7.1 cm; N = 20) and the mean height was 6.9 cm (SD = 1.7; range = 5.4 – 7.5 cm; N = 20). Some subterranean nests had a short corridor with a mean length of 3.1 cm (SD = 0.7; range = 2.1 – 3.7 cm; N = 7). Mean water depth inside the subterranean nest was 3.5 cm (SD = 1.5; range = 2 – 4.2 cm; N = 17) and three nests measured in December 2005 were almost entirely filled with water.

Males returned to their calling sites three days or more after having been observed in courtship.

Females laid a mean number of 102 eggs (SD = 27; range = 127 – 157 eggs; N = 3). Eggs had a mean diameter of 5.04 mm (SD = 0.56 mm; range = 4.5 – 5.5 mm; N = 30) and each egg had an individual gelatinous capsule. Females that did not lay their eggs (N = 5) had a mean number of 279 ova (SD = 73; range = 181 – 327 ova; N = 5) in their ovaries with different diameters that varied from 0.5 mm to 1.4 mm. Ovarian mass of females that did not lay their eggs corresponded on average to 23.72 % of the female's mass (SD = 1.34; range = 21.8 – 25.3 %; N = 5) whereas the testes mass of males corresponded to 0.034 % (SD = 0.002; range = 0.02 – 0.05 %; N = 2) of the body mass of males.

The only *Aplastodiscus arildae* female collected had a relative ovarian mass of 25.5% of the body mass and contained 307 eggs with diameters that varied from 0.4 mm to 1.3 mm. Testes mass corresponded to 0.026% of the males' body mass (SD = 0.016; range = 0.012 – 0.020 %; N = 2).

4. Discussion

4.1 Courtship behaviour

Tactile stimuli between male and female and the behaviour of guiding the female to the oviposition site are typical of anuran species in which males construct the nest before the arrival of the female (e.g., *Leptodactylus fuscus*, MARTINS, 1988; *Hylodes phyllodes*, FARIA *et al.*, 1993; *Hylodes asper*, HADDAD & GIARETTA, 1999; *Aplastodiscus leucopygius*, HADDAD & SAWAYA, 2000; *Aplastodiscus eugenioi*, HARTMANN *et al*., 2004; *Aplastodiscus perviridis*, HADDAD *et al*., 2005).

The observed courtship behaviour was similar to the one described in the literature by HADDAD & SAWAYA (2000). Until now, specialized courtship behaviour similar to that observed for *Aplastodiscus leucopygius* and *A. arildae* was described for *A. perviridis* (HADDAD *et al.*, 2005) and *A. eugenioi* (HARTMANN *et al*., 2004). According to HARTMANN *et al*. (2004), *A. eugenioi* performed visual signals during courtship, which is considered an unusual communication mode for nocturnal anurans. We observed that, during courtship, the male and the female blinked at each other, a behaviour that can be considered a type of visual communication. Descriptions of visual signals are uncommon in frogs and are predicted to be predominantly employed by diurnal species at sites with an unobstructed view (ENDLER, 1992). Limb shaking is also considered a type of visual communication (see HÖDL & AMÉZQUITA, 2001) and was described for *A. eugenioi* (HARTMANN *et al*., 2004). In this study we observed limb shaking behaviour for *A. arildae* during the courtship sequence. Apparently, this is a visual signal as described by HÖDL & AMÉZQUITA (2001), but we cannot discard the possibility of seismic communication, when the female or male perceive the presence of other individuals by the propagation of low frequency signals in the substrate. The perception of an individual by its movement on the vegetation was observed for males of *A. leucopygius*, which that started to emit courtship calls as soon as another individual jumped next to them. However, experiments using appropriate equipment to measure seismic signals are necessary to confirm that seismic communication is used by this species. For *A. leucopygius* we observed at least three types of male-female communication during courtship behaviour: acoustic, visual, and tactile.

4.2 Mate choice

Many investigators of sexual selection in anurans have postulated that females choose the largest males in the chorus (see FAIRCHILD, 1981; HOWARD & KLUGE, 1985; OLSON *et al.*, 1986). On the other hand, some researches proposed that, in some species, females select males with an ideal SVL for a better cloacal superposition during amplexus, minimizing the loss of gametes ("assortative mating" *sensu* PARTRIDGE, 1983) (see BASTOS & HADDAD, 1999). For *Aplastodiscus leucopygius* we observed that females did not select males based on their SVL or mass, but apparently the selection was based on the repetition rate of the advertisement calls, as observed for other anuran species (e.g. *Hyperolius marmoratus*, PASSMORE *et al*., 1992; *Dendropsophus microcephalus*, SCHWARTZ, 1994; *Eleuterodactylus coqui*, LOPEZ & NARINS, 1991). According to RYAN (1985), this is the most usual parameter by which females choose males and this trait does not depend on male size. LOPEZ (1996) suggested that, for *Eleutherodactylus coqui*, the repetition rate of the call could be related to the metabolic energy loss during the behaviour, this being the result of a complex relationship between energy supplies, physiological efficiency, ability to capture prey, and other characteristics restricted to the individuals' genome. For *A. leucopygius* the long period of courtship behaviour probably requires a great amount of energy from both sexes during the courtship process.

The courtship period of *Aplastodiscus leucopygius* was positively correlated with the rainfall in the study area. However, during intense rainy periods, the courtship sequences did not finish in oviposition. Nests, in these occasions, were almost entirely filled with water. Male rejection by females suggests that the females inspect the nest and may reject males if the nests are not adequate. Another fact which suggests that the nests are inspected by females was their dirty snouts, observed when they were already inside the nest. This could mean that females may not only inspect the nest, but also arrange it before oviposition, a behaviour observed for other species like *Hypsiboas faber* (MARTINS & HADDAD, 1988).

4.3 Reproductive investment and nests

The eggs of *Aplastodiscus leucopygius* are unpigmented, a characteristic also observed in many species that lay their eggs in subterranean nests (e.g. *Leptodactylus fuscus*, MARTINS, 1988; *Aplastodiscus eugenoi*, HARTMANN *et al*., 2004; *Aplastodiscus perviridis*, HADDAD *et al.*, 2005). Lack of pigmentation may be due to the absence of harmful effects of UVB, since the subterranean nest protects the eggs from sun rays. The nest can also be considered a good protection against predators for the adults, during the oviposition process, and for the eggs (HADDAD & SAWAYA, 2000).

The relative mass of the gonads reflects a physiological reproductive investment that was considerably higher in females than in males. Nevertheless, we have to consider the investment made by the male in the construction of subterranean nests and the energy spent during calling behaviour and the courtship process. Males returned to reproductive activity only a few days after courtship, suggesting that they need time to recover from the energy expenditure during reproduction. Therefore, both sexes apparently had a great investment in reproduction. The low investment in testes mass in *Aplastodiscus leucopygius* may be explained by the lack of sperm competition, since the oviposition behaviour occurred inside subterranean nests to which other males do not have access, as observed in species of the *Leptodactylus fuscus* group (see PRADO & HADDAD, 2003).

The presence of eggs with different diameters in the ovaries of the females suggests that the females of both species may produce more than one clutch per reproductive season, a characteristic that has been observed for other species of anurans (see PRADO & HADDAD, 2005).

We did not observe the end of the courtship behaviour in *Aplastodiscus arildae*, but we presume that it finishes in a subterranean nest where oviposition occurs, as observed for other species of the genus *Aplastodiscus* (*Aplastodiscus leucopygius*, HADDAD & SAWYAYA, 2000; *Aplastodiscus eugenoi*, HARTMANN *et al*., 2004; *Aplastodiscus perviridis*, HADDAD *et al.*, 2005), since the courtship behaviour observed for *A. arildae* is similar to that performed by the other species.

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Appendix 1- Collection number of vouchers used in the present study.

Adults of *Aplastodiscus leucopygius* (CFBH 8310, 8311, 8312, 8313, 9441, 9566, 10001, 10002, 10003, 10004, 10151, 10152), adults of *Aplastodiscus arildae* (CFBH 10190, 10191, 10842).

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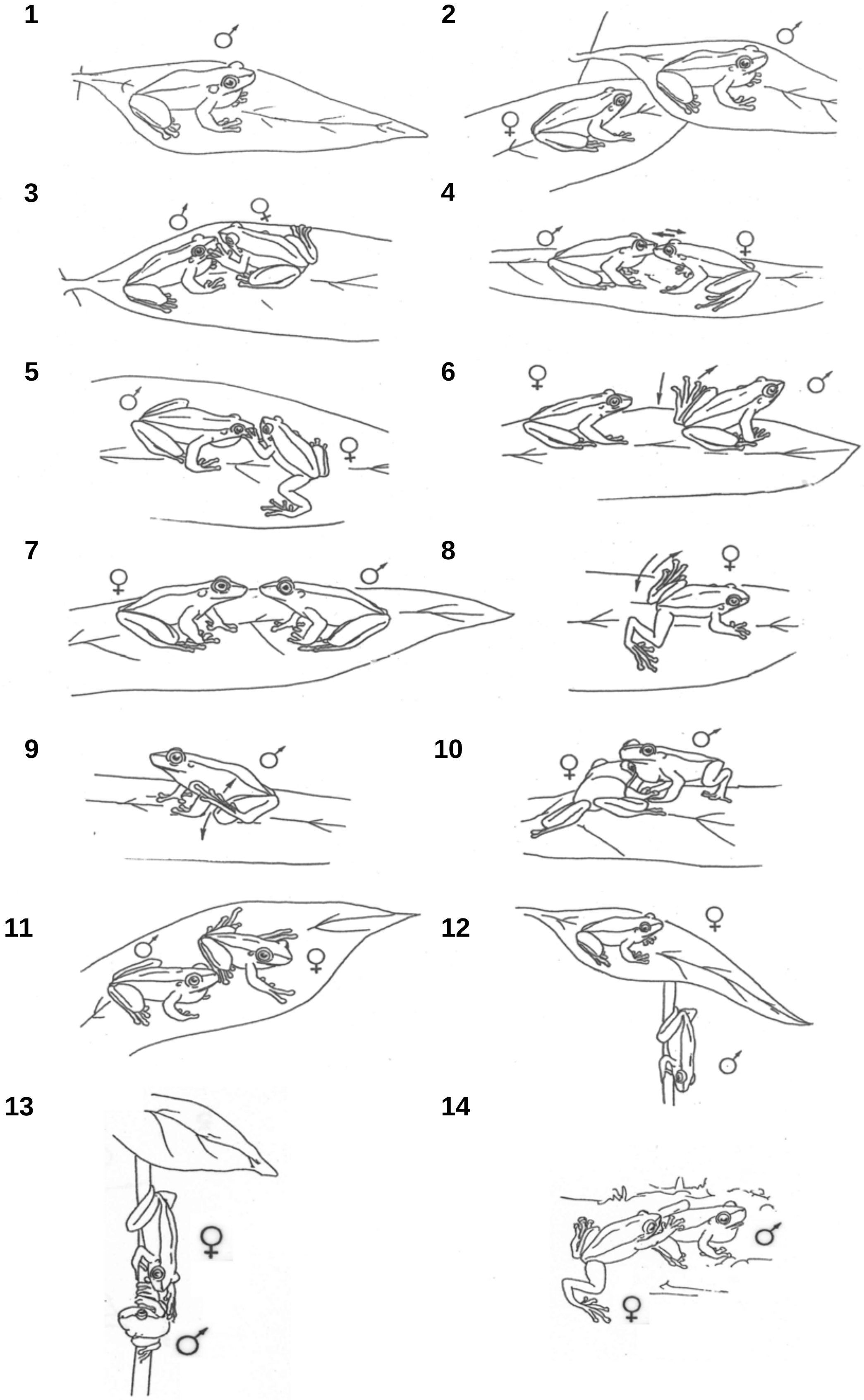

Figure 1-Behavioural sequence of the courtship behaviour of *Aplastodiscus arildae* based on one courtship observed in the Serra do Japi, municipality of Jundiaí, São Paulo, Brazil. Numbers 1– 14 refers to the behavioural sequence (see text).

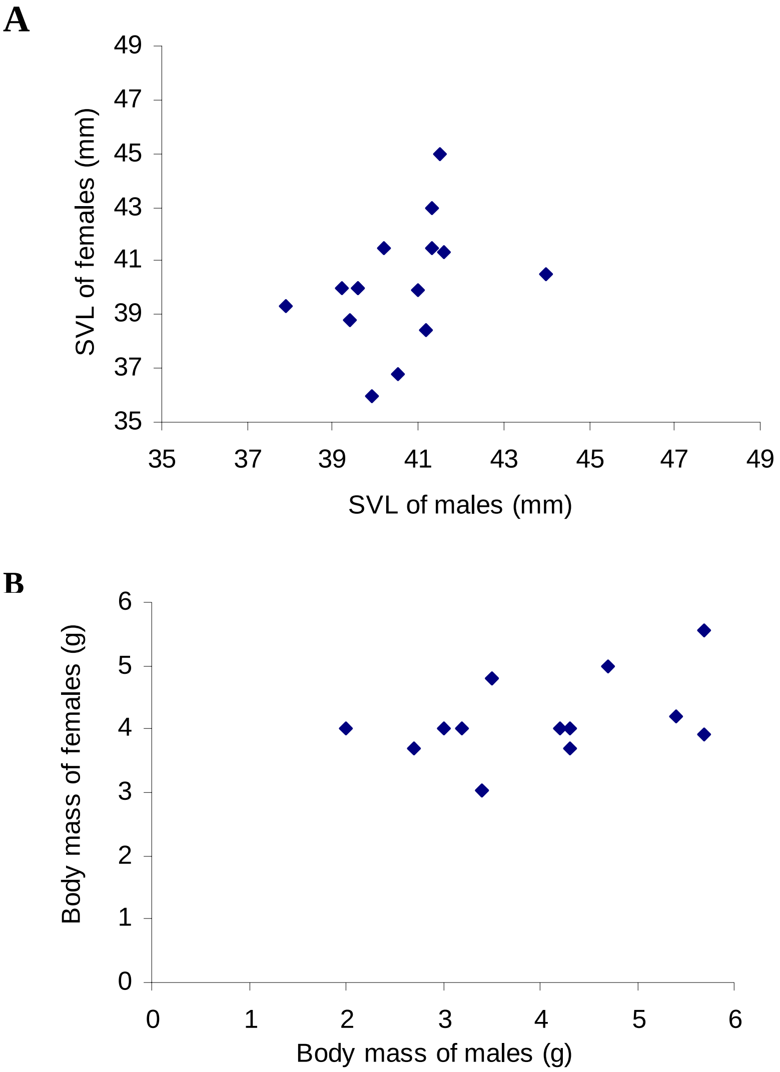

Figure 2- SVL (A) and body mass (B) of females and males of *Aplastodiscus leucopygius* found in courtship in the Serra do Japi, municipality of Jundiaí, State of São Paulo, Brazil.

Considerações finais

- *Aplastodiscus arildae* e *A. leucopygius* podem ser consideradas como espécies de estação reprodutiva prolongada com atividade de vocalizações e atividade reprodutiva relacionada positivamente com o regime de chuvas na região.

- As espécies se diferenciaram quanto ao uso dos ambientes para as atividades de vocalização sendo esta uma das principais dimensões responsáveis pela segregação entre as espécies estudadas.

- *Aplastodiscus leucopygius* mostrou-se mais plástica quanto ao uso do ambiente para atividades de vocalização e reprodução.

- As vocalizações de anúncio de ambas as espécies apresentaram diferenças conspícuas relacionadas principalmente aos ambientes utilizados pelas espécies para atividade reprodutiva.

- Diferenças com relação aos parâmetros dos cantos de anúncio de *Aplastodiscus arildae* e *A. leucopygius* atuam também como mecanismo responsável pela segregação das espécies no ambiente

- Variações nos parâmetros dos cantos de anúncio de *Aplastodiscus arildae* e *A. leucopygius* foram dependentes dos contextos sociais.

- O comportamento de corte de ambas as espécies pode ser caracterizado por uma seqüência complexa de toques mútuos entre macho e fêmea.

- Provavelmente a espécie *A. arildae* também desova em tocas construída pelos machos da espécie.