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ECOLOGIA REPRODUTIVA DE *Aparasphenodon arapapa* PIMENTA, NAPOLI & HADDAD, 2009 (ANURA:HYLIDAE)

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Aos meus avós queridos, Luri e Rubinho,

que deixaram saudades eternas...

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“Só o desejo inquieto, que não passa,

Faz o encanto da coisa desejada…

E terminamos desdenhando a caça

Pela doida aventura da caçada.”

Mario Quintana

RESUMO

Estudos de história natural de espécies de anuros que usam locais alternativos para a reprodução, como bromélias, ainda são escassos. Estes estudos são fundamentais já que representam um novo conjunto de adaptações ecológicas e comportamentos associados a um nicho específico. Nós estudamos a ecologia e biologia reprodutiva da espécie *Aparasphenodon arapapa*, anuro que utiliza bromélias não apenas como abrigo, mas também como sítio de vocalização e oviposição. O estudo foi realizado na Reserva Boa União localizada no sul da Bahia, no município de Ilhéus, no Brasil, durante o período de novembro 2011 a outubro 2012. A área de estudo está sob o domínio da Mata Atlântica e apresenta solo arenoso e árvores baixas (fitofisionomia do tipo Mussununga). Para avaliar que variáveis poderiam ser mais importantes nas bromélias usadas pelos machos como sítio de vocalização, nós medimos algumas características das bromélias, tais como: diâmetro do tanque central, comprimento máximo do tanque central, quantidade de detritos, inclinação das bromélias, volume de água e pH. Os machos preferiram bromélias que apresentaram maior volume de água e menos detritos. Nós discutimos estes resultados tendo em vista o cenário dinâmico das bromélias como microhabitats para adultos e girinos. Além disso, nossos resultados mostraram que a espécie tem reprodução prolongada com um comportamento de corte associado ao cuidado parental de prover ovos como comida, inclusive com uma evidencia de monogamia temporal para cuidado das larvas. Sugerimos uma nova terminologia específica para a alimentação de larvas com ovos. Por fim, descrevemos um novo modo reprodutivo para contemplar *A. arapapa* e propusemos modificações no modo #6.

ABSTRACT

Natural history studies of anuran species that use alternative sites for reproduction, like phytotelmata, are still scarce. These studies are fundamental since they represent a new set of ecological adaptations and complex behaviors associated to a specific ecological niche. We studied the ecology and reproductive biology of the anuran species *Aparasphenodon arapapa*, a bromeliad-dweller that uses the same bromeliad as a calling and oviposition site. The study was carried out from November 2011 to October 2012 at the Reserve Boa União located in the municipality of Ilhéus, southern Bahia, Brazil,. The study area is located inside the Atlantic Forest domain and corresponds to coastal sandy soil with short trees (Mussununga phytophysiognomie). To evaluate which variables could be important for the use of bromeliads by males as calling sites we measured some bromeliads characteristics, such as: diameter of the central tank, maximum central tank length, amount of debris, bromeliad inclination, water volume, and pH. Males preferred bromeliads that presented higher water volume and less debris. We discuss these results facing the dynamic scenario of bromeliads as microhabitats for adults and larvae. Furthermore, our results showed that the species is a prolonged breeder with a complex courtship behavior related to parental care, including the evidence of temporally monogamy to attend and provide food to tadpoles. We suggested a new specific terminology on eggs' consumption by larvae. Finally, we describe a new reproductive mode to contemplate *A. arapapa* reproductive characteristics and propose changes in mode #6.

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1. Introdução Geral

Os anfíbios são um grupo de vertebrados dos mais representativos em número de espécies em quase todas as comunidades terrestres (Wells 2007). O Brasil conta com mais de 870 espécies descritas e por isso é o país com a maior riqueza de anfíbios, seguido pela Colômbia e Equador (Silvano e Segalla 2005; Segalla et al. 2012). Dadas as restrições fisiológicas do grupo, tal como ovos desprotegidos contra dessecação e pele permeável, conjuntamente com aspectos reprodutivos que os limitam a determinados ambientes, os anfíbios são altamente sensíveis e susceptíveis a modificações e perda de habitat (Becker et al. 2007; Moraes, Sawaya e Barrella 2007).

Os anuros são o maior grupo dentre os anfíbios, com cerca de 6.200 espécies descritas em todo o mundo (Frost 2013) que graças a uma maior capacidade de dispersão e colonização associaram-se aos mais diversos ambientes límnicos e desenvolveram uma grande variedade de modos de vida para isso. Os padrões de ocupação espacial e temporal das populações de anuros estão direta ou indiretamente associados a atividade reprodutiva (Wells 1977; Aichinger 1987).

Algumas espécies de anuros podem ter sua atividade reprodutiva limitada pela simples ausência do sítio reprodutivo favorável (Moser 2010). O sítio de oviposição, bem como características dos ovos e da desova, ritmo e duração do desenvolvimento, estágio e tamanho dos eclodidos e tipo de cuidado parental, caso exista (Salthe e Duellman 1973), constituem o conjunto do que caracteriza os modos reprodutivos das espécies de anuros. Para determinadas espécies de anuros o sítio de vocalização é o mesmo sítio de oviposição e assim a adequada escolha do local a serem depositados os ovos é fundamental para a sobrevivência e desenvolvimento das larvas (Silva e Giaretta 2008). A seleção de sítios reprodutivos por espécies de anuros pode ocorrer em diferentes escalas espaciais envolvendo tanto o que determina sua distribuição geográfica, quanto às características locais que regem a área de vida da espécie (Afonso e Eterovick 2007).

A alta complexidade dos ecossistemas tropicais levou a evolução de espécies de anuros com modos reprodutivos altamente dependentes de microhabitats específicos, como os que estão disponíveis em plantas (Wells 2007). Os 'fitotelmata' (grego *phytos* = planta, *telmos*= poça) são um tipo de microhabitat utilizados pelos anuros frequentemente em ambientes em que a água é um recurso escasso; podem ser ocos de bambus, frutos ocos abertos, buracos em árvores, folhas de palmeira e bromélias (Lehtinen et al. 2004). A disponibilidade, qualidade e estrutura dos fitotelmata, tal como para outros sítios reprodutivos, podem ser fatores limitantes a atividade reprodutiva das espécies de anuros que os utilizam (Heying 2004; Lin, Lehtinen e Kam 2008). No Brasil, os fitotelmata mais conspícuos são as bromélias (Juncá e Borges 2002; Cogliatti-Carvalho et al. 2008; Lacerda et al. 2009; Cogliatti-Carvalho et al. 2010).

As bromélias estão inseridas na família Bromeliaceae que está restrita às Américas (à exceção de *Pitcairnia feliciana* (A.Chev.) Harms & Mildbr., do Leste da África). Essas plantas podem funcionar como microhabitat favorável para abrigo, esconderijo, ambiente de reprodução ou forrageamento (Afonso e Eterovick 2007), pois suas folhas formam uma roseta em que há acumulo de água e ou resíduos no seu tanque central e axilas laterais (Laessle 1961). A quantidade de água que pode ser acumulada varia de acordo com sua forma, tamanho e disponibilidade de chuvas (Laessle 1961). Contudo, apenas a capacidade de armazenar água e oferecer espaço não é garantia de que será habitada por anuros (Schineider e Teixeira 2001).

Algumas variáveis como pH e tamanho da bromélia podem ser importantes na escolha do sítio reprodutivo como foi observado para *Scinax perpusillus* (Oliveira e Navas 2004). Ao mesmo tempo, pode ser que a adequação de uma planta a uma espécie de anuro esteja associada também a fatores bióticos como presença ou ausência de co-específicos (Summers 1999) ou predadores (May et al. 2009). Ademais, podem ser a única fonte de água durante estações mais secas ou até mesmo a única fonte de água em locais em que a água é um recurso escasso (Laessle 1961). Contudo, a vida em bromélias exige adaptações que podem variar de espécie para espécie, tais como: girinos endotróficos (e.g. *Syncope antenori*, Krügel e Richter 1995), girinos oófagos (e.g. *Osteopilus brunneus*, Lannoo, Townsend e Wassersug 1987), canibalismo entre larvas (e.g. *Ranitomeya ventrimaculata*, Summers, 1999), e estratégias comportamentais como tamanho de desova reduzido (e.g. *Dendrobates auratus*, Caldwell e Araújo 1998), partição de desova entre sítios (e.g. *Scinax perpusillus*, Alves-Silva e Silva 2009) e cuidado parental (Weygoldt 1980). O tipo de cuidado parental mais comum entre anuros é a manutenção de condições ideais para o desenvolvimento da prole, mas no que concerne a vida em bromélias, a atenção dispensada aos ovos e girinos geralmente é diferenciada e dispendiosa (e.g. Jungfer e Weygoldt 1999; Lin e Kam 2008).

Dentro da família Hylidae há registro de 31 espécies com reprodução envolvendo fitotelmata, inclusive em bromélias (Lehtinen, Lanoo e Wassersug 2004). A família Hylidae possui cerca de 900 espécies descritas (Frost 2013) e ocupa o segundo lugar em número de modos reprodutivos (Haddad e Prado 2005); dentre estes, cinco modos estão associados a fitotelmata. Não há um clado que claramente esteja associado exclusivamente ao uso desses sítios reprodutivos e ainda são necessários estudos que elucidem a filogenia dos grupos de espécies de anuros que usam bromélias (Alves-Silva e Silva 2009) e sua distribuição (Silva e Alves-Silva 2013). Dentre os gêneros que possuem uma estreita relação com esses microhábitats está o gênero *Aparasphenodon* Miranda-Ribeiro, 1920.

As espécies do gênero *Aparasphenodon* apresentam um crânio hiperossificado (Mertens 1950; Pombal 1993; Pimenta, Napoli e Haddad 2009) que permite uma redução na taxa de perda de água por evaporação através do mecanismo de 'vedação' das bromélias com a cabeça (Andrade e Abe 1997). Em relação a espécie de anuro do presente estudo, *Aparasphenodon arapapa* Pimenta, Napoli e Haddad, 2009 existem poucas informações sobre suas relações com as bromélias que utilizam como abrigo e sítio de reprodução (Lourenço-de-Moraes et al. 2013). Além disso, dada a distribuição restrita que a espécie apresenta (são quatro localidades de ocorrência da espécie: Cairu, Ituberá, Ilhéus e Una, todas no estado da Bahia) é de interesse biológico de que sejam investigadas sua história natural, ecologia e populações afim de que se possa diagnosticar seu status de conservação.

Diante desse contexto o objetivo do estudo foi compreender como a espécie *Aparasphenodon arapapa* utiliza as bromélias para sua reprodução e deste modo: (1) avaliar se há seleção de bromélias utilizadas como sítio reprodutivo, e se houver, (2) determinar que variáveis da bromélia estão associadas a seleção, (3) descrever a relação entre a espécie de anuro e o uso de bromélias em sua reprodução.

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2. Chapter One:

A CASE OF REPRODUCTIVE SITE SELECTION OF A BROMELIAD BREEDER: *Aparasphenodon arapapa* Pimenta, Napoli and Haddad, 2009 (Anura: Hylidae)

Amanda Santiago F. Lantyer-Silva, Anthony Waldron, Juliana Zina and Mirco Solé

Abstract

Our study focused on *Aparasphenodon arapapa*, a bromeliad-dweller that uses bromeliads as a calling and oviposition site. These plants play an important role in the reproduction of *A. arapapa* and also represent one of the most dynamic habitats used for reproductive activity by anurans. To test if males exhibit preferences in the use of such habitats, we conducted a systematic study in a patch of Atlantic Forest with high bromeliad diversity and density. We measured some bromeliads characteristics, such as: diameter of the central tank, maximum central tank length, amount of debris, bromeliad inclination, water volume, and pH. Males chose bromeliads that presented higher water volume and less debris. We also found a slight preference for mildly inclined plants and smaller diameters of the central tank. Our results show that the species use spatial characteristics to choose an adequate habitat in which its aquatic larvae will develop.

Key-words: Bromeliaceae, Habitat Selection, Habitat use, Phytotelmata, Phytotelm-breeder

Introduction

Ecology studies the spatial and temporal patterns of the distribution and abundance of organisms (Scheiner and Willig 2007), and in this context, the habitat selection is one sub-area of great interest, as it interprets conditions and available resources that can be selected by animals (Morris 2003). Differences exist in habitat quality in whichever environment to whatever animal activity (foraging, reproduction, dispersion, sheltering) thus, studies on habitat selection are crucial to understand the patterns of distribution and abundance of organisms.

In anurans, reproduction is the most conspicuous feature (Wells 2007) pointed out by many studies as responsible for the spatial and temporal distribution of species, especially in seasonal environments (Aichinger 1987; Bertoluci and Rodrigues 2002; Prado, Uetanabaro and Haddad 2005). Reproductive site selection can be viewed as an important behavioral adaptation, considering physiological constraints (permeable skin and eggs unprotected from desiccation) and reproductive mode restrictions (Heying 2004; Resetarits Jr. and Wilbur 1989; Rudolf and Rödel 2005). In fact, habitat selection for reproduction is mostly studied for anurans that use larger water bodies (e.g. ponds, streams, lakes), while species that use smaller water bodies, like bromeliads, are still understudied. Although bromeliads present a wide geographical distribution and occur in many morfo-climatic domains of America (Laessle 1961), there is still a lack of knowledge on mechanisms and environmental features that lead some species, not only anurans, to choose the microhabitats (Juncá and Borges 2002; Osses, Martins and Machado 2007).

Bromeliads are known to be biodiversity amplifiers, due to their structure and shape, which allows for aggregation of resources such as debris and/or water. As so they are considered phytotelm; a special type of temporary water bodies, that are parts of plants or water holding plants (Lehtinen, Lanoo and Wassersug 2004). Although anurans that use phytotelmata to reproduce have to deal with different limitations such as low levels of dissolved oxygen, water availability and limited food resources (Laessle 1961; Kitching 2001) these plants may represent an alternative site with less competitors and predators (von May et al. 2009). Not surprisingly, many anuran species use bromeliads as shelter and at least 63 species use bromeliads as a site for reproduction of some form (Moser 2010).

Some anuran species use the same site for both vocalization and oviposition (Duellman and Trueb 1986). However, the mechanisms that lead bromeliad anuran breeders to choose a specific bromeliad are still not understood (Giaretta 1996; Eterovick 1999; Silva, Carvalho and Bittencourt-Silva 2011). Therefore, in the present study we used a systematic approach to determine the characteristics involved in the calling site selection of a tree frog (Hylidae).

Species of the genus *Aparasphenodon* Miranda-Ribeiro, 1920 are known for their close relationship with bromeliad microhabitats, at least as shelter, once their co-ossified cranium and phragmotic behavior (use of the head to seal the entrance of bromeliad) are striking adaptations associated to this habitat (Andrade and Abe 1997). The species *Aparasphenodon arapapa* Pimenta, Napoli and Haddad, 2009 was the last described species of it genus and presents a restricted geographic distribution (Lourenço-de-Moraes et al. 2013) as the others species of the genus, except for *A. brunoi* (Frost 2013). It uses bromeliads both as calling and oviposition site and tadpoles develop and metamorphose inside of them (Chapter Two).

As a bromeligen species, we expected that some plant characteristics would lead males of *A. arapapa* to choose a specific bromeliad as a calling site, since the oviposition site is the same as the calling site, such as: (1) bromeliad dimensions (minimum diameter and maximum central tank length), once it could limit the physical occupation of the plant and interfere with the phragmotic behavior (Mesquita, Costa and Zatz 2004); (2) amount of debris and (3) bromeliad inclination, both which could interfere with individuals' access to the central tank (Osses, Martins and Machado 2007), (4) water volume and (5) pH, that could be of interest to tadpole survival and growth (McDiarmid and Altig 1999).

Material and Methods

Study area

The study site (Reserve Boa União - 15º04' S; 39º03' W, 95 m a.s.l., 112ha) is located in the municipality of Ilhéus, state of Bahia, Brazil (Figure 1). The local vegetation is classified as Mussununga Forest, an Atlantic Forest phytophysiognomie of southern Bahia characterized by shorter trees on sandy soil (*sensu* Thomas 2003), resembling an arboreal 'restinga'. According to Köppen (1936), the local climate corresponds to Af; warm and humid with no defined dry season. Mean annual rainfall from the nearest municipality (Una, 22 km) is about 1,919mm (Santos and França 2009).

Data Collection

From November 2011 to May 2012, we visited the study site four to six days each month. We conducted a systematic sample in an area of 80m *per* 350m, characterized by high bromeliad density. We sampled 24 circular plots of 2m radius each (totaling 12.5m^2 of sampled area) and in each we measured features of five to seven bromeliads (n=165) with no *Aparasphenodon arapapa* (absence bromeliads, AB) in calling activity. We measured the same parameters of 124 bromeliads which we registered, throughout auditory searching, the *A. arapapa* males in calling activity (presence bromeliads, PB). Only the bromeliads marked and that later presented inflorescences were possible to be identified. Thus, the species of bromeliads used by *Aparasphenodon arapapa* were *Aechmea* gr. *lingulata*, *Aechmea blanchetiana* and *Araeococcus* sp . We also verified that no bromeliad classified as AB presented males of *A. arapapa* in calling activity during the whole survey day.

We used the following procedure to select plot position (Figure 2): first of all, we sorted the position of the first plot in an interval of 1 to 10 first meters along longitudinal axis (parallel) of the principal trail. Next, we sorted the direction (right or left) from the principal trail. After that, we sorted the latitudinal distance from the principal trail (1 to 10m) plus 5m always summed to the sorted value. The next 23 plots, with a longitudinal distance of 12 m from each other, followed the same procedures to determine the direction and latitudinal distance from the principal trail. We had to mirror one plot (#11) to the other side (left to right) because there were no bromeliads on that site.

All bromeliads sampled (AB and PB) were marked with a red tag, numbered, and had their location registered and the following data collected: (1) minimum diameter of central tank (cm) (MD), (2) maximum length of the central tank (cm) (ML), (3) amount of debris, (4) inclination, (5) water volume from central tank (ml), and (6) pH from water inside central tank. The amount of debris (leaves and branches) was considered as an ordinal variable (from 0 to 3) adapted from a similar procedure used by Osses, Martins e Machado (2007): 0- no debris, 1- small amount of debris, 2- large amount of debris but not enough to prevent an individual of *A. arapapa* from passing through the bromeliad central tank; 3- very large amount of debris with no passage free through the bromeliad central tank. Inclination of the bromeliad was considered a categorical variable: 0- upright plants, 1- slightly inclined, 2- heavily inclined (less than 5cm from the ground). Water volume was measured in a graduated cylinder to the nearest 1.0 ml. Water from bromeliads was sucked from the central tank with a tube connected to a recipient. The pH values were measured with a water quality sensor with an precision of 0.1. Individuals of *A. arapapa* were toe-clipped (*sensu* Waichman 1992) and each one had its location registered. Two males were collected as voucher specimens and deposited at Zoological Collection of Universidade de Estadual Santa Cruz – Herpetological section (MZUESC 11085-6) under the collecting permit provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) to MS (13708-1).

Data analysis

We pooled all observations into a single group, representing the fact that a bromeliad was either used or not used at the moment of observation, rather than using a multi-season analysis (Manly et al. 2002).

We analyzed only one bromeliad *per* male (n=72) due to the possible bias effect that individual choice idiosyncrasies could cause in the general results. Bromeliads registered with *A. arapapa* in calling activity that were not included in the analysis represented recaptured males (n = 46), epiphytic bromeliads (n = 2) or when the frog used lateral axils rather than the central tank (n = 2).

To test which variables explain calling sites used by *Aparasphenodon arapapa*, we initially used logistic regression models to explore data (Manly et al. 2002). To account for the non-linear effects in a presence/absence framework, we used Generalized Additive Models (GAMs) with binomial errors (Zuur et al. 2009) using R Core Team for Windows version 2.15.2 (2012). Plants with no water volume were not tested for pH. We therefore ran an initial set of candidate models on only plants with non-zero water volume (n=98 AB; n=58 PB). However, none of these models showed any support for pH as an important explanatory variable, so we extended the dataset to include zero water volumes (n=165 AB; n=72 PB) and removed pH from all candidate regression models.

For consistency in model comparisons, MD and ML were always square root transformed and water volume was always cube root transformed. Variables were then centered and standardized by a (x-mean(x))/sd(x)) transformation). It was not clear a *priori* whether the distances between the classifications for inclination and debris (0,1,2,3) were biologically significant, so we tested two sets of models:

models in which these terms were treated as continuous variables, and models in which they were treated as categorical factors. A further advantage of the categorical factor approach is that it is more flexible in dealing with non-linear responses to changes in the levels (e.g. a preference for mid-level inclination over both zero inclination and strong inclination). Model selection approach was based on the Akaike Information Criterion (AIC) (Johnson and Omland 2004) and Un-Biased Risk Estimator (UBRE) (Wood 2004).

We used logistic regression models initially to define the subclass of resource selection (Manly et al. 2002). Early exploration of the data suggested a strong, positively non-linear response to water volume and a negatively non-linear response to the diameter of the bromeliad central tank.

There was a strong correlation between plant ML and plant water volume (Pearson's r = 0.54). Early exploration suggested that volume was a stronger predictor of plant use by calling males than ML, but that changes in use probability with ML closely mirror changes in use with volume. We therefore chose volume as the main variable for further statistical modeling of the preference function.

Additionally, there were complex associations among other covariates (Figure 3). Debris tended to be greater in wider and more upright plants. At the same time, less debris tended to be registered for plants with a higher water volume. Also, wider plants tended to be less inclined while high-volume plants tended to be more inclined. Water volume showed a humped relationship with bromeliad diameter, rather than the monotonic relationship expected (Damgaard 2006). These relationships were generally non-linear and thus did not always generate high correlations among covariates. See descriptive statistics in Tables 1 and 2.

Results

Candidate models were compiled using debris and inclination as continuous variables initially, and were compared by UBRE scores (Table 3). The best-fitting model (lowest score) showed a strongly positive, asymptotic response to increasing water volume (Figure 4) and a highly significant negative response to debris. The effective degrees of freedom for the diameter smoother was 1, so the smoother was replaced *post hoc* with a parametric linear term. Although inclination and diameter had weak effects on bromeliad choice, we noticed that *Aparasphenodon arapapa* males tended to use inclined bromeliads over upright ones and plants with smaller diameter openings over larger ones (Table 4). Overall, this model explained approximately 64% of the variation in bromeliad choice (adjusted R^2).

Treating debris and inclination as categorical variables revealed a similar pattern, suggesting that bromeliads with low and intermediate levels of debris (levels 1 and 2) had lower probabilities of use than debris-free bromeliads ($p=0.02$, $p<0.001$, intercept difference = -1.39, -2.75 on a logit scale). Furthermore, the models suggested that slightly inclined bromeliads (level 1) had a higher probability of use than upright bromeliads (level 0, $p=0.046$, intercept difference = 1.18 on a logit scale), but that inclinations steeper than this (level 2) did not lead to additional changes in preference ($p=0.59$).

We also tested the possibility of interactions between water volume and debris, and water volume and inclination. However, the strong non-linear associations between variables caused model problems, including convergence and models performed better in terms of AIC but produced highly non-significant p-values for the smoothers. Two interaction models are included in Table 3 (2 & 7) but in general, the problems of non-linear covariation suggest that these are not accurate.

The highly complex and non-linear covariance among variables suggest that statistical modeling may miss subtle interactive effects (even when modeling interactions, Table 3). To further investigate such patterns, we graphically represented the environmental envelope of the species (the subset of the universe of possible values for habitat variables that was associated with a record of use, compared to the approach of Hirzel et al. 2002) (Figure 5). These graphical representations suggest a more complex set of preferences than the statistical model, although they do not provide significance tests.

It was not feasible to check every bromeliad in the area on every field visit and a bromeliad observed as AB on one day may have been PB on other days. Therefore, we performed an estimate of sensitivity to undetected presences, when we arbitrarily modeled 100 situations in which a random 10% of the AB bromeliads were converted to PB. We re-tested the best-fitting model for each of the 100 new datasets, recording whether p values were >0.1 and <0.01. This is highly conservative as the pattern of occupation likely seems to follow the choice rules detected in the preference analysis noted above, rather than a random allocation. Water volume and debris p values were <0.01 in 100% of scenarios, but inclination and diameter had p values >0.1 in 60% and 66% of scenarios respectively. This latter pattern is unsurprising, given the weak patterns observed for inclination and diameter in the original model. Therefore, overall the importance of inclination and diameter is likely to be weak.

Discussion

The dynamic scenario of anuran bromeliad-dwellers

The use of phytotelmata as reproductive microhabitats probably evolved due to predation and competition selection pressure present in larger water bodies (Magnusson and Hero 1991; Rieger, Binckley and Resetarits 2004). However, bromeliads may be considered a more unstable microhabitat since they tend to modify the storage water in their rosettes by absorbing nutrients (Lopez et al. 2009), even from animal feces (Romero et al. 2010). Given that, the use of these phytotelmata demanded morpho-physiological and morpho-ecological adaptations of larvae to a food-limited environment as endotrophic tadpoles (e.g. *Syncope antenori*, Krügel and Richter 1995) or oophagic tadpoles (e.g. *Osteopilus ocellatus,* Lannoo, Townsend and Wassersug 1987) or even, cannibalistic larvae (e.g. *Ranitomeya ventrimaculata*, Summers 1999), as wells as the evolution of diverse reproductive strategies in anurans such as small clutch size (Langone et al. 2008), split clutch (e.g. *Scinax perpusillus*, Alves-Silva and Silva 2009) and parental care (Weygoldt 1980).

In the case of *A. arapapa*, the choice of a bromeliad for calling activity implies simultaneously, the choice of oviposition site, since both events occur in the same bromeliad (Chapter Two). Therefore, adequate characteristics of a chosen plant may be a determinant in the reproductive success of individuals. Hence, one of our expectations was that pH could be a crucial variable to calling site selection of *A. arapapa*, since it may influence the larval development, as previously observed for other amphibian larvae (e.g. *Rana sylvaticus,* Berven and

Grudzien 1990; *Ambystoma maculatum*, Sadinski and Dunson 1992). However, pH did not interfere in the bromeliad choice, probably because it did exhibit a short variation among the measured plants' water volume, commonly found between bromeliads (e.g. Laessle 1961; Eterovick 1999; Teixeira, Mili e Rodder 2006), being, in most of the cases, above 4, which implies that the species should present a larval pre-adaptation to occupy these habitats.

We observed that bromeliads with higher volumes of water are preferred to lower volumes and there is an apparent threshold of avoidance of very low volumes. The model assumption is that frogs choose bromeliads on the basis of an absolute preference for a certain water volume, rather than choosing whichever bromeliad is more water-filled on a particular day. This assumption may not be completely supported, but the strength of the response to volume suggests that violating the assumption will not alter the general (and biologically logical) conclusion that low water volumes are unacceptable to this amphibian. In addition, higher water volume is probably related to the larval development, since *A. arapapa* presents aquatic larval phase. Preference for phytotelmata with higher water volume was also observed for *Ranitomeya biolat* which oviposits in bamboos (von May et al. 2009) but our study is the first to also observe this preference for an anuran specie that uses bromeliads as both a calling and oviposition site. Low-debris plants were preferred, implying another possible threshold response of complete avoidance of high-debris plants by calling males of *Aparasphenodon arapapa*. Debris may reduce evaporation levels inside units of bromeliads or prevent free water passage when it rains- completely opposite effects. Considering that (1) the individuals need free passage throughout the plant and (2) bromeliads are naturally found in environmental conditions in which there is a shortage of water, that is sandy soils, it is not surprising that a low amount of debris and higher water volumes are important to *A. arapapa*.

We also expected that bromeliad inclination and minimum diameter were important in calling site selection of *A. arapapa*. We found a slight preference for mildly inclined plants and smaller diameters. However, these first two variables did not seem as important as water volume and amount of debris. Even though the preference for an inclined plant is clear in a single-variable analysis, the tendency for water volume to increase in more inclined plants makes slope preferences hard to interpret within a multivariate set of choices. Possibly, larger plants simply incline more on average. At the same time, it is possible that the bromeliad's architecture and structure variables interact directly and indirectly in a certain way due to alometrical connections. Consequently, it is difficult to tell whether a true preference for inclined plants exists, or whether it is a colinearity artifact stemming from a preference for high volume. The inverse interpretation that frogs ignore water volumes and are in fact responding only to plant inclination, seems considerably less likely biologically. If a preference for inclined plants does exist, it may reflect ease of accessibility. Therefore, the observed effect of diameter and inclination may be a sub-product of the influence of water or debris accumulation. Interestingly, at the extreme, plants with very high volume were not used unless they were inclined. Thus, besides the great diversity of bromeliad species, there is still a variety in the combination of crossing and connecting abiotic and biotic influences that are modified through time performing a dynamic scenario of microhabitats available.

Bromeliad-dwelling frogs and conservation implications

Among bromeliad-dwellers, there are still gaps in knowledge regarding the factors that are desirable in a breeding site and how it might vary among bromeliad species-dwellers. Eterovick (1999) observed that *Phyllodytes luteolus* showed, for one of the bromeliad species of the study, a slight preference for large-sized and deeper bromeliad units and lower water pH. Males of *Scinax perpusillus* chose larger, clustered bromeliads with slightly acidic waters (Oliveira and Navas 2004). A sandbank anuran community study revealed that the species preferred bromeliads located at the edges of scrub patches, exposed to the sun (Silva, Carvalho and Bittencourt-Silva 2011). In light of these studies, the non-random usage of bromeliads is clear. Species-preference for distinct bromeliad characteristics exists, but those preferences are not necessarily the same for different species.

The lack of a general hypothesis of reproductive microhabitat selection leads us to propose patterns to specific groups of species and not a general view for the bromeliad breeders (see Peixoto 1995). Here we propose some pillars: (1) bromeliads are a dynamic resource in space and time; (2) the choice varies among the species, as the evaluation of the desirable quality (chemical or physical) of the microhabitat according to some species, or even individuals, idiosyncrasies. However, we still may question: “What are the patterns of characteristics of interest?”, and also “Which biological consequences of using bromeliads as reproductive sites are influencing anuran populations?”. We suggest that the great behavioral and biological constraints will guide these answers, but only after efforts to study these anurans life cycle species have been done it will be possible to describe patterns that fit for all bromeliad-dwellers, and with that, idiosyncrasies may dilute.

As previously suggested by Alves-Silva and Silva (2009), the high frequency in which bromeliads are used by anurans, especially those species that have a specific and complex life cycle associated to these phytotelmata, lead us to reinforce the importance of conservation plans to protect these plants and their environment and therefore conserve the anuran species diversity associated with these microhabitats.

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Appendix

Table 1. Characteristics of bromeliads with *Aparasphenodon arapapa* males (Presence) and without (Absence) according to the systematic sample. MD = minimum diameter (cm); ML = maximum central tank length (cm). Volume is in milliliters.

		MD	ML	Volume (ml)	pH
Bromeliads of Presence	Mean ±SD	2.69±0.76	17.96±4.95	20.77±11.59	4.19±0.69
	Range	1.5 - 5	9 -30	0.5 - 60	3 - 7
	N	72	72	72	58
Bromeliads of Absence	Mean± SD	2.84±1.03	14.62±6.90	6.33±10.43	4.33±0.60
	Range	0.8 – 7.5	6 - 57	0 - 80	3.32 – 5.8
	N	165	165	165	67

Table 2. Bromeliads’ characteristics frequency with *Aparasphenodon arapapa* males (Presence) and without (Absence) according to the systematic sample. Values in parentheses show the number of plants in each category. 0= no debris/upright plants; 1 = small amount of debris/slightly inclined plants; 2 = large amount of debris but not enough to prevent an individual of the anuran from passing through the central tank/ heavily inclined; 3 = very large amount of debris with no passage free through central tank/ it is not a category to “Inclination”.

		0		**1**		**2**		**3**		**Total**
Bromeliads of Presence	Debris	72.22%	(52)	22.22%	(16)	4.10%	(3)	1.38%	(1)	72
	Inclination	44.44%	(32)	48.61%	(35)	6.94%	(5)	-	-	72
Bromeliads of Absence	Debris	26.06%	(43)	24.24%	(40)	20%	(33)	29.69%	(49)	165
	Inclination	63.03%	(104)	29.69%	(49)	7.20%	(12)	-	-	165

Table 3. Regression models tested to explain bromeliad choice by calling males of *Aparasphenodon arapapa*, with Un-Biased Risk EstimatoR (UBRE) and Akaike Information Criterion (AIC) scores.

	MODEL TERMS	**UBRE**	**AIC**
1	Volume, debris, inclination, diameter	-0.49296	104.1894
2	Volume, debris, inclination, diameter, volume x debris	-0.4904	101.4683
3	Volume, debris, diameter	-0.48874	104.8086
4	Volume, debris, inclination	-0.48756	104.4694
5	Volume, debris	-0.47451	107.7255
6	Volume, diameter, (inclination and debris as categorical)	-0.47393	107.8439
7	Volume, debris, volume x debris	-0.47069	108.5093

Table 4. The best-fitting model explaining bromeliad choice by calling males of *Aparasphenodon arapapa* at the Reserve Boa União, state of Bahia, municipality of Ilhéus, Brazil. LVOL = water volume cube root transformed and standardized

Parametric coefficients			
Estimate (Intercept)	**Std. Error**	**z-value**	**Pr(>|z|)**
Debris -1.6055	0.4119	-3.897	9.72e-05
Inclination 0.5103	0.3019	1.690	0.090962
Minimum diameter -0.5394	0.3118	-1.730	0.083616
	Ref.df	**Chi.sq**	**p-value**
s(LVOL) edf	4.003	24.56	6.19e-05
3.171			

Figure headings

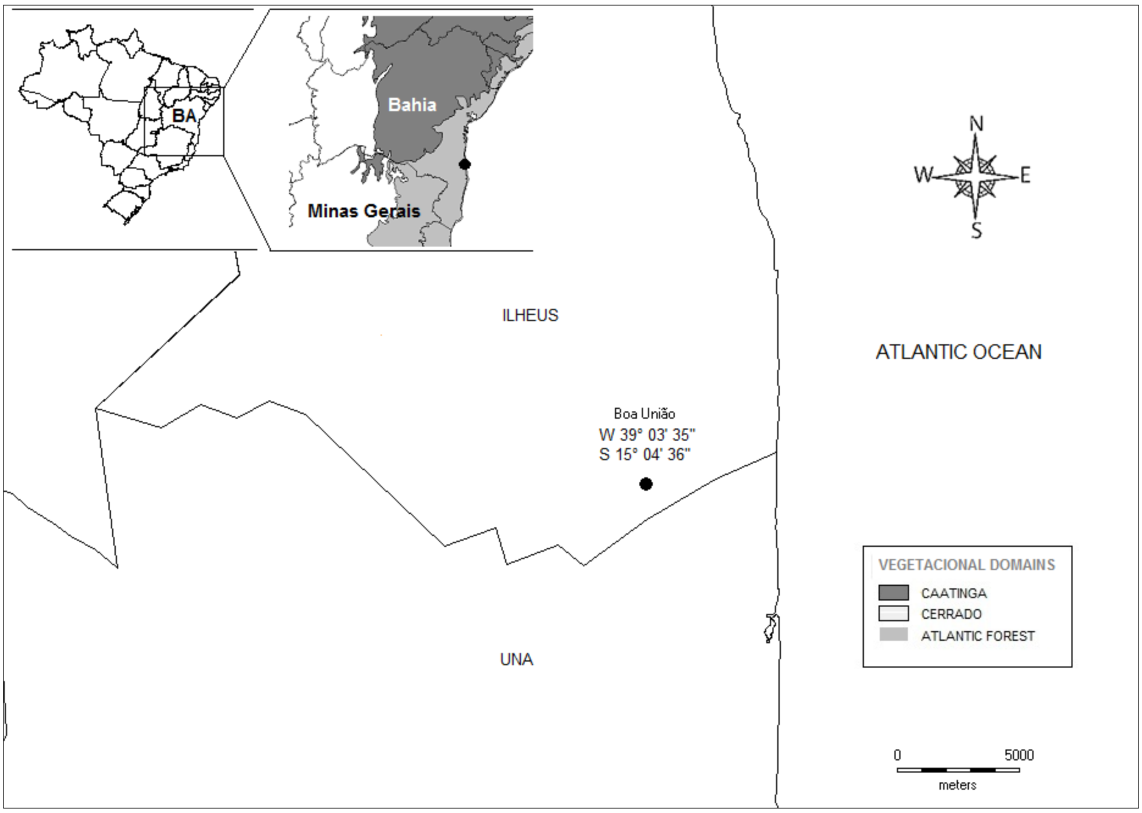
Figure 1. Map of south portion of Bahia, Brazil with study area appointed.

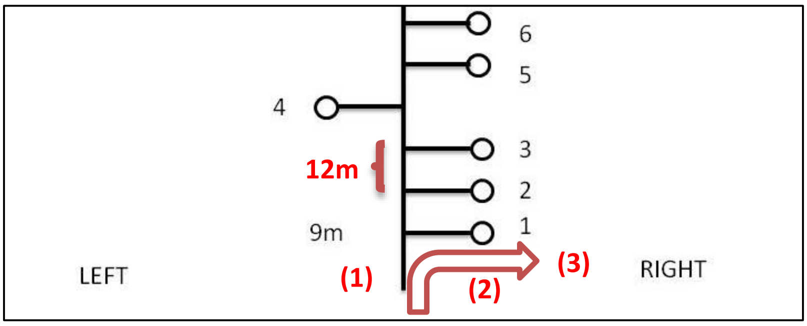
Figure 2. Esquematic view of the sorted plots along the trail. Sorted: (1) Position of the first plot (1 to 10 first meters); (2) Direction (left/right); (3) Distance from trail + 5m (1 to 10m); Distance to one plot to another along the trail = 12m.

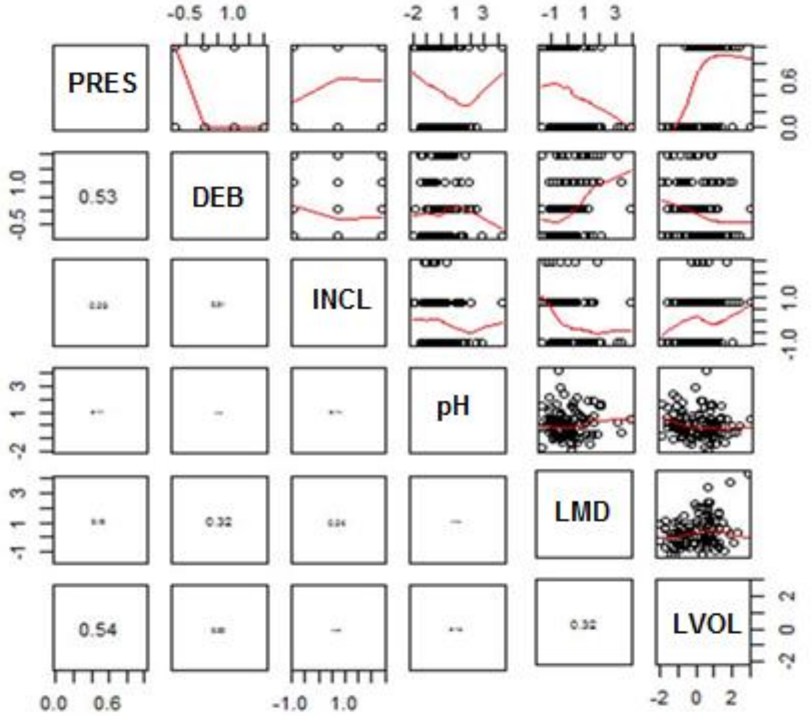
Figure 3. Covariate patterns of bromeliads' variables. Legend: Pres= Presence; Deb = debris; Incl = inclination; pH = ; LMD = log Minimum diameter; LVOL = water volume cube root transformed and standardized.

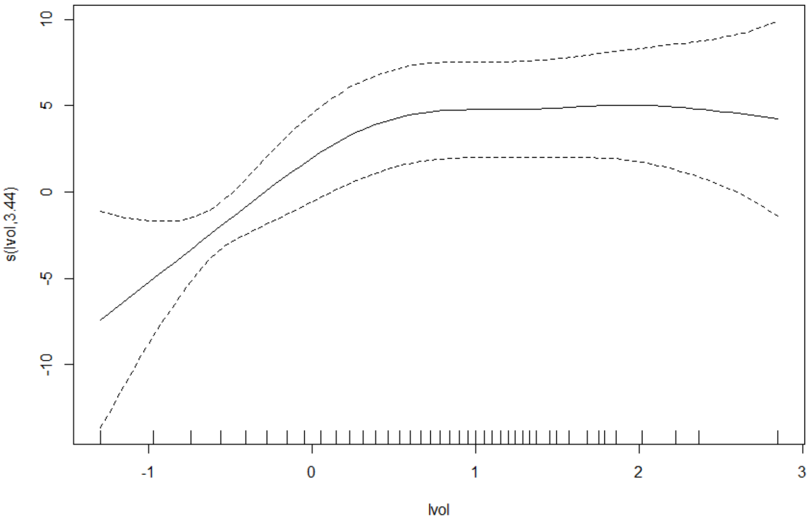
Figure 4. The non-linear use response to water volume (lvol, cube root transformed and standardized). The rug at the bottom shows the location of individual use observations.

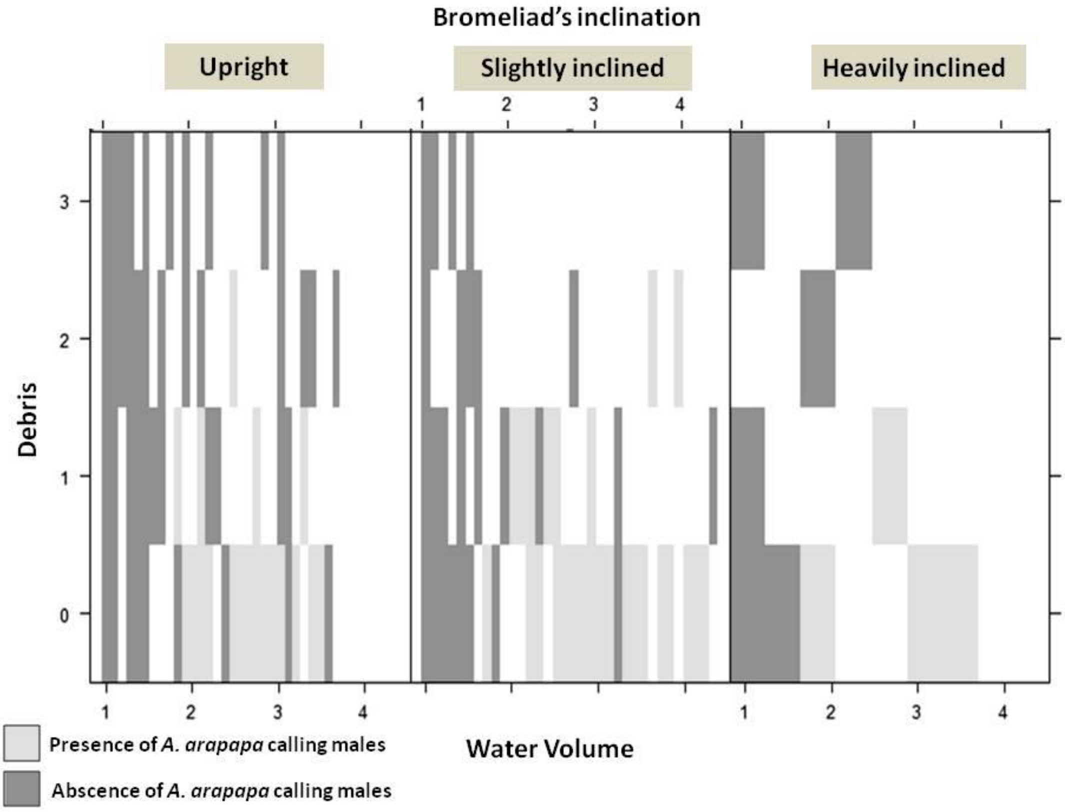
Figure 5. The area of the universe of habitat variables combinations associated with a use record. Light gray = presences, dark gray = absences.


BA
Bahia
Minas Gerais
ILHEUS
N
W
E
S
ATLANTIC OCEAN
Boa União
W 39° 03' 35"
S 15° 04' 36"
VEGETACIONAL DOMAINS
CAATINGA
CERRADO
ATLANTIC FOREST
UNA
0
5000
meters


6
5
4
3
12m
2
1
9m
LEFT
(1)
(2)
(3)
RIGHT


PRES
DEB
INCL
pH
LMD
LVOL
0.53
0.32
0.54
0.32
-0.5
1.0
-2
1
3
-1
1
3
0.0
0.6
-1.0
1.0
-2
0
2


s(lvol,3.44)
lvol
10
5
0
-5
-10
-1
0
1
2
3


Bromeliad's inclination
Upright
Slightly inclined
Heavily inclined
Debris
3
2
1
0
1
2
3
4
Water Volume
Presence of *A. arapapa* calling males
Abscence of *A. arapapa* calling males

3. Chapter Two:

THE REPRODUCTIVE BIOLOGY OF THE PHYTOTELM-BREEDER *Aparasphenodon arapapa* PIMENTA, NAPOLI AND HADDAD, 2009 (ANURA: HYLIDAE)

Amanda S. F. Lantyer-Silva, Mirco Solé, Juliana Zina

Abstract

The genus *Aparasphenodon* is poorly studied and here we present comments on the reproductive biology of the species *Aparasphenodon arapapa*. Our observations indicated that *A. arapapa* is a prolonged breeder, reproducing throughout the year using bromeliads as calling and breeding site. The tadpoles complete their development inside the plants. The bromeliads may be also used by males, females and juveniles as a shelter. Herein we also describe the courtship behavior and the parental care of the species with an evidence of temporally monogamy. We review some behavioral concepts and propose new terminologies and one new reproductive mode.

Introduction

Species continue to be described each year in Brazil (Cruz, Napoli and Fonseca 2008; Pimenta, Napoli and Haddad 2009; Napoli et al. 2011; Lourenço-de-Moraes, Solé and Toledo 2012). However, natural history studies are still scarce for the vast majority of anurans species, and new information about some long time described species are only available nowadays (e.g. Silva and Juncá 2006; Ruas et al. 2012; Dias, Lourenço-de-Moraes and Solé 2012). Nevertheless, studies on natural history are specially important and relevant to understand interactions between individuals and their environment, being crucial for conservation action plans and phylogenetic studies (Wells 2007; Gomez-Mestre, Pyron and Wiens 2012).

Species that exhibit specialized reproduction, including complex courtship, use of alternative reproductive sites and specialized reproductive modes, are still understudied, although they might represent sets of novel adaptations to occupy new sites and new niches. One of the factors that may be pointed out as responsible for the few natural history studies of those species is their low representativity in anuran communities. Among those anuran breeder specialists are the phytotelm-breeders (*phyto*=plant, *telmos*=ponds) that depend on water filled plants. They exhibit the most complex and surprising adaptations to reproduce in these plants (e.g. complex arrangements of parental care (Gomez-Mestre, Pyron and Wiens 2012)). Among 913 species registered in Brazil (Moser 2010; Segalla et al. 2012) only 38 use phytotelmata as breeding sites.

The evolutionary and ecological mechanisms of that animal-plant association are just beginning to rise in understanding (Lehtinen, Lanoo and Wassersug 2004; Brown, Morales and Summers 2010; Gomez-Mestre, Pyron and Wiens 2012). It is known that phytotelm-breeders had to evolve adaptations to phytotelmata constraints as low dissolved oxygen levels, risk of desiccation and unpredictable food availability (Lehtinen, Lanoo and Wassersug 2004). Additionally, competition may be stronger in an intra-specific way (Lehtinen 2004; Lin, Lehtinen and Kam 2008). However, predation pressure is less intensive in units of phytotelmata (Kitching 2001).

Bromeliads are the most conspicuous phytotelmata in Coastal regions in Brazil. For some anuran species that uses bromeliads as microhabitats a few adaptations were observed as: small clutch size (e.g. *Ranitomeya biolat* von May et al. 2009), endotrophic tadpoles (e.g. *Syncope antenori*, Krügel and Richter 1995), oophagy (Lannoo et al. 1987), cannibalistic larvae (e.g. *Ranitomeya ventrimaculata*, Summers 1999), split clutch (e.g. *Scinax perpusillus*, Alves-Silva and Silva 2009), and parental care (e.g. *Dendrobates pumilio,* Weygoldt 1980). Among bromeliad-dwellers, some species exhibit morphological adaptations for the use of this microhabitat, such as species of the genus *Aparasphenodon* that presents a hiper-ossified cranium (Mertens 1950; Pombal 1993; Mesquita, Costa and Zatz 2004) useful to reduce water loss by evaporation through the mechanism of sealing the bromeliads with its head displaying phragmotic behavior (Andrade and Abe 1997).

The genus *Aparasphenodon* comprises four species (*A. arapapa, A. bokermanni, A. brunoi, A. venezolanus*) distributed in the coastal region of Brazil

(Bahia to São Paulo) and upper Orinoco basin to Venezuela and Colombia (Frost 2013). Few studies are available for the species of this genus. *Aparasphenodon brunoi* and *Aparasphenodon bokermanni* have been registered in community studies and some ecological and physiological aspects were documented (Teixeira, Schneider and Almeida 2002; Mesquita, Costa and Zatz 2004; Bertoluci et al. 2007; Vilela, Brassaloti and Bertoluci 2011). Meanwhile, *Aparasphenodon arapapa* was the last described species of the genus and it was considered a bromelicolous anuran that inhabits only "restinga" areas (Pimenta, Napoli and Haddad 2009). The advertisement call and tadpole of this species is about to be published (Lourenço-de-Moraes et al. in press). Herein we present the habitat use and some reproductive aspects of *Aparasphenodon arapapa* including the courtship behavior. As a consequence of its specialized and stereotyped behavior in comparison with what is known for a great set of anuran species, also in this study, we propose new nomenclatures and a new reproductive mode.

Material and Methods

Study area

Our study was conducted in the Reserve Boa União (15º04'S, 39º03'W, 95 m a.s.l.) located in the municipality of Ilhéus, southern Bahia, Brazil (Figure 1). The area is inside a phytophysignomie of Atlantic Forest domain, nominated as Mussununga Forest, in which vegetational traits resemble sandy coastal plain of arboreal 'restingas' (short trees and sandy soil) (*sensu* Thomas 2003). The local climate is tropical wet (Af, according to Köppen 1936); warm and humid with no dry season defined.

Data Collection and Data analysis

From November 2011 to October 2012 we visited the reserve approximately weekly. Individuals were sampled by visual and auditory search along a trail of 300m. The observations began after sunset (approximately 18:00h) and finished until the end of males vocal activities' or at 24:00h. Focal animal and all occurrence samplings were used for behavioral records (Altmann 1974).

Males were sexed solely by calling activity, because vocal sac is undistinguishable. Females were sexed when visually gravid (Figure 2) or when they were seen mating with a male in courtship. The highest number of males in calling activity at one point of the trail was registered at least twice each field trip between 18:00 and 24:00h. All individuals found were measured (with a caliper of 0.01 cm precision) and weighted (with a field scale of 0.1 g precision). Toe-clipping followed Waichman (1992) and individuals were released in the same site where they were found. Operational Sex Ratio was calculated from the mean number of females divided by males each day (Kvarnemo and Ahnesjö. 1996.). To test the null hypothesis of no difference of the snout-vent length (SVL) and body mass between males and females we conducted a Mann-Whitney test. Mean occurrence frequency of males and females by night were obtained throughout the division of the total number of individuals registered in the area along the whole study period by the number of field trips. To verify whether the mean number of calling males *per* month differed along the year we applied Kruskal-Wallis. To evaluate whether male calling activity was associated to each environmental variable (rainfall, temperature, humidity) we used the Spearman Correlation test. Temperature and humidity maximum values were recorded with a thermohygrometer at least twice each field trip. We obtained rainfall data from a local station in the most near municipality, Una, distant about 22 km from the study area (except for November, December 2011 and October 2012 that did not enter in the analysis). We carried out statistical analyses using R Core Team for Windows version 2.15.2 (2012) and a significance level of 5% to reject null hypotheses.

We collected two clutches and eggs were counted. Twenty-four eggs were measured (with a caliper of 0.01 cm precision) from three different clutches. We followed the development of tadpoles of *A. arapapa* in one bromeliad and to facilitate observations we categorized development stages of Gosner (1960) in: early (19 to 30), intermediate (30 to 36) and advanced (37 to 46). Two males and two females were collected as voucher specimens and deposited at Zoological Collection of Universidade de Estadual Santa Cruz – Herpetological section (adults: MZUESC 11084-11089/ tadpoles: MZUESC 9097–98; 10354) under the collecting permit provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) to MS (13708-1).

Results

1.Adults

Males of *Aparasphenodon arapapa* had a mean SVL of 45.4mm (SD=2mm; range=41.2-50.2mm; n=73) and a mean body mass of 3.62g (SD=4.3g; range=2.6-4.5g; n=73); females had a mean SVL of 53.3 mm (SD=2.4; range= 50.0- 60.0mm; n=23) and a mean body mass of 5.5g (SD=0.75;range=4.0-7.0g; n=23). There was a significant difference between SVL of both sexes (W = 1679, p-value < 0.001) and body mass (W = 1657.5, p-value < 0.001) (Figure 3).

2. Eggs and tadpoles

Along the study period we registered 34 bromeliads with tadpoles and 11 with *A. arapapa* eggs. Tadpoles and eggs registered along the study period are presented in Table 1. At least 64.7% of bromeliads that contained tadpoles and 66.7% of bromeliads that contained eggs were also occupied exclusively by a male of *A. arapapa*. We observed that tadpoles ate eggs until advanced stages of development (Figure 4). We also found tadpoles in different Gosner's stages in the same bromeliads. Eggs' animal pole color is dark gray with diameter varying from 0.50 to 0.97mm (mean ±SD : 0.78 ±0.14; N=24) (Figure 5).

In first attempts to follow the development of tadpoles inside bromeliads we tried removing the tadpoles and adults to perform measurements and identify the adult individuals. However, when a bromeliad was disturbed, the male inside of it always dropped and hid the tadpoles below it, blocking their passage to the top, making the process to access individuals much more difficult. Also, probably this method affected the permanence of adults in bromeliads and must have consequently affected tadpoles' survivorship, since at the third visit, using this method, we no longer registered tadpoles and adults. Hence, we decided to just count visually the number of individuals, intervening only at an initial identification of adults whom were in the bromeliad.

On September 13th 2012, we observed a bromeliad with a male. Five days later, we went back and found tadpoles in it (with just over 20mm of total length) in the early stages of development (Figure 6A). Ten days after that, we revisited the bromeliad and there were 3 tadpoles in similar early stages (approximately 30mm of total length) (Figure 6B). We revisited the bromeliad during four more fields surveys (9/30, 10/03, 10/17, 10/21). In the penultimate visit, the same male and also a female were registered in courtship (Figure 6C). After oviposition, as soon as the female left the bromeliad (moment when we collected to identify), we observed that the tadpoles (intermediate Gosner's stages) had eaten eggs. On the last visit, we registered the male and there were only two tadpoles in advanced Gosner's stages.

3. Habitat use, Population dynamics and Reproductive activity

We registered males calling and sheltering only inside bromeliads, in the central tank (Figure 7A) or lateral axils (Figure 7B). They were also found perched in branches next to bromeliads, not in calling activity. Females (n=17) and juveniles (n=21) were also found perched in adjacent vegetation between 24cm to 1.60m of the ground (this highest record correspond to a female found perched in a trunk). A female registered in a branch, after being accidentally disturbed, was observed sheltering inside a large bromeliad full of debris. Some juveniles were seen inside epiphytic small bromeliads.

We observed individuals of other species (*Phyllodytes tuberculosus, Bokermannohyla lucianae, Pristimantis* sp.) and few *A. arapapa* males (n=3) in calling activity inside bromeliads previously sampled and tagged for another individual of *A. arapapa*. We did not observe agonistic interactions between *A. arapapa* and other species. Likely, tadpoles of other species were not registered inside bromeliads with *A. arapapa* tadpoles.

We marked 80 males, 46 of which were recaptured at least once in calling activity. Few males (n=6) were found in the same bromeliad where they had already been captured days or months later (Table 2). Twenty one females were marked and four were recaptured. We could not access females displacement, since the majority were found perched in branches, not inside a bromeliad (that we had been marking and locating). For 11 recaptured males it was possible to access their displacement between bromeliads used as calling site. The mean distance of bromeliads used by the same males was 3.27 ± 3.62 meters (n= 16; range= 0.45-12m). The individual males' distances and directions of displacement among bromeliads are presented in Figure 8 and the time interval between recaptures in Table 3.

The longest distance registered between males in vocal interaction was 8.30m and the shortest was 0.59m (Figure 9). No satellite males were observed. However, in one occasion we registered two previously marked *A. arapapa* males in the same bromeliad. The Male 1, which was previously recorded in a bromeliad located at 74 cm from the observed bromeliad, was in calling activity above other male (Male 2) that remained quiet. The call was very similar to the advertisement call, but it was emitted at lower note intervals.

The operational sex ratio mean was 0.21 (SD=0.38; n=43; range=0-2). Mean frequency of occurrence of males was 2.60 individuals *per* night while females presented mean occurrence frequency of 0.47 individuals *per* night. Individuals usually began to call between 5p.m. and 6p.m, independently of sunset hour. The activity peak was between 7p.m. and 9p.m (Figure 10). Males were found in calling activity all over the year, but varied in abundance among nights. In two nights of heavy rain we observed that males did not stop calling. The number of calling males was not correlated with monthly rainfall (r=-0.031; p= 0.9368), mean monthly air temperature (r=-0.059; p=0.8545), and humidity (r=0.234; p=0.463). Calling activity of *A. arapapa* lasted all year and there were no significant differences between the mean number of males calling by month (Kruskal-Wallis chi-squared= 11; p=0.4433). Courtships were observed in four months during study period, while tadpoles and clutches were not registered only on June and July 2012 (Table 4).

4. Courtship and spawning

We recorded five courtships of *A. arapapa*, all of them occurred inside bromeliads' central tank. One courtship was followed from the beginning until the end, and last three hours, approximately. The description of the first mating recorded is given thereby (Figure 11):

(1) Initially the female was perched on one of the leaves of a bromeliad adjacent to a calling male. When she jumped to a leaf of the bromeliad in which the male was calling (Figure 11A), he stopped calling and both remained still for a few moments;

(2) After this period of silence, the male returned to calling activity, emitting a very similar call to the advertisement call, but with a higher note repetition rate. The female moved backward slowly to the central tank with the head turned to the opening of the central tank (Figure 11B);

(3) Immediately after that, the male emitted three calls at lower intensity positioning himself behind the female with his head in touch with the female's head, grasping the female by the axilla, in amplexus position (Figure 11C);

(4) After about 50 minutes, the female began performing concave arching of head and body together against the bromeliad. The male followed her movements (Figure 11D);

(5) During the last 20 minutes, the female did at least 13 concave arching movements, the longest lasting 24 seconds and the fastest, 6 seconds; between one arching movement and another, the male emitted two low intensity calls and then the female made one more arching movement;

(6) Few minutes later the female moved upward, away from the male, up to the central tank of the bromeliad (Figure 10E). From that moment forward they were not in amplexus anymore; The male remained inside the central tank, and after few minutes resumed calling activity.

(7) The female then left the bromeliad after 35 minutes holding still. And the male remained inside the bromeliad with eggs.

From the moment they were sighted, the other four courtships showed basically the same pattern, but there was a previously unobserved behavior in the first courtship. In the second event we observed that a male in amplexus with a female ducked his head up quickly a few times, making it look like a slight hit to the female's head, during step 5.

Two of the courtships that we observed partially, were performed by the same couple in a very short period of time (01/24/2012 and 02/04/2012). At the first time we observed the couple laying eggs inside the bromeliad. Eleven days later we returned to the same bromeliad and there were tadpoles and the same male within the plant. Later on that day, the male was in amplexus with the same female from before. After around two hours the tadpoles presented eggs in theirs digestive system.

Discussion

1.Adults

We found statistical difference between sexes in *A. arapapa*, females being larger than males. At least 90% of anurans present sexual dimorphism (Shine 1979) with females being larger than males. Since Charles Darwin's sexual selection theory, three hypothesis have been proposed to explain this morphological pattern between sexes: (1) Females are larger because this characteristic allowed them to produce and store more eggs, since there is a correlation between SVL and clutch size, already observed for many anuran species (Duellman and Trueb 1986); (2) Males larger than females are a common characteristic among territorial species, since this feature arise their chances during physical combats (Lee 1986; Tsuji 2004); (3) The presence of dimorphism can be a mechanism for reducing intersexual competition for resources as food or shelter (see the review by Shine 1989). Since we did not find any male-male aggressive behavior and the sexual dimorphism is pronounced, the larger size of females is probably related to clutch size (Duellman and Trueb 1986) or even, female parental care ("trophic eggs", see below).

2. Eggs and Tadpoles

Phytotelm-breeders clutch size varies between 1 to 4.336 eggs, *Ranitomeya imitator* and *Trachycephalus resinifictrix*, respectively. Nevertheless, only seven species, in 53, for which the clutch size is available, present more than 100 eggs *per* clutch (Moser 2010). Clutch size of *A. arapapa* is greater than that of other phytotelm-breeders, as all species from genus *Flectonotus, Dendrobates* and *Phyllodytes*, that present less than 30 eggs per clutch (Moser 2010). Notwithstanding, we observed when trying to follow tadpoles development, that few of them would survive until metamorphosis. Couples of *Scinax perpusillus* split their clutches in different axils of bromeliads or even through different bromeliads (Alves-Silva and Silva 2009); this strategy may succeed to species with few eggs per clutch since it reduces individual chances of predation and also competition for resources inside the same plant. *Aparasphenodon arapapa* presents a maximum clutch size registered of 35 eggs and this split strategy could be successful, however the displacement of the couple between bromeliads seems impractical once with its medium body size it would expose them to predation. In addition, clutch split to *A. arapapa* could invalidate males' choice for a bromeliad with adequate quality to oviposition. Instead of clutch split we observed parental care, also an investment to offspring success (Townsend 1989).

Body size of tadpoles of *A. arapapa* species is large (Lourenço-de-Moraes et al. in press) and this fact must enhance competition for resources; this is probably the reason why just few bromeliads were seen with more than two tadpoles in advanced development stages. Indeed, the reduction of the number of tadpoles was observed to be in early stages but we do not know the mortality rates. For *Ranitomeya ventrimaculata* most of *Heliconia* pools, independent of the number of tadpoles deposited, end with only one tadpole that survives through cannibalism strategy (Summers 1999). Teixeira et al. (2006) estimated that approximately 92.4% of *Scinax perpusillus* tadpoles in a saxicolous community of bromeliads would not survive until adulthood. The most likely causes of this high mortality rates pointed by the authors were: predation, cannibalism and food shortage. For *A. arapapa* and also for other phytotelmata breeders predation hardly would be a selective pressure in this environment, since predation is relatively low in phytotelmata compared to other frogs breeding sites (Summers 1999; Kitching 2001). Additionally, why a predator would choose eat only some tadpoles and not all of them? The buccal morphology of tadpoles does not support the second possibility; cannibalism (Lourenço-de-Moraes et al. in press). Therefore, the most likely hypothesis that explains the higher tadpole mortality would be a shortage of food, evidenced by parental care to provide eggs as food for tadpoles. Still, we recommend further studies since it is of fundamental importance to understand the mortality rates of tadpoles to determine the reproductive success of individuals and comprehend population dynamics.

Egg size is known as a more conservative reproductive characteristic that varies relatively little among congeneric species when compared to clutch size (Duellman and Trueb 1986). However, information on egg size from other *Aparasphenodon* species are not available. When compared to *Osteopilus ocellatus*

(Jungfer and Weygoldt 1999), another bromeligen species with biparental care that provides eggs to their tadpoles, *A. arapapa* presents a smaller egg size. Summers et al. (2007) revealed a non-significant relationship between egg size and phytotelmata breeding, however they used a small sample size, since there is little comparative data of phytotelm anuran breeders, specially tropical ones. Concerning development rate, the Amazonian species *Osteocephalus oophagus* presents parental care and provides fertilized eggs (Jungfer and Weygoldt 1999) to tadpoles that become metamorphs between 27 to 50 days. *Kurixalus eiffingeri* exhibit parental care with unfertilized eggs provided by female, completing their development in 40 to 78 days after hatchling (Lin and Kam 2008). At the same time, species that breed in temporary ponds in arid regions show rapid development which may take 13 to 18 days to metamorphosis (Duellman and Trueb 1986). A great part of Hylids present a long growing and developmental rate (Richardson 2002) despite the natural plasticity in rate of tadpoles' development of any species due to environmental and/or intrinsic factors (Rose 2005). When we followed tadpoles development, it is possible that one of them in advanced stage of development had metamorphosed before the penultimate visit and this implies that *A. arapapa* tadpoles have a relatively short development rate that, in addition to exotrophic food supply, must have been fundamental to occupy this niche less explored by anurans, the phytotelmata.

The use of the term "oophagy" for any type of predation of fertilized (e.g. *Osteocephalus oophagus* and *Trachycephalus resinifictrix*) and unfertilized eggs (e.g. *Anotheca spinosa* and *Kurixalus eiffingeri*) in anurans, is also widely common in the literature to other groups of animals, such as fish (Gilmore 1993), reptiles (Mullin 1996), birds (Burger 1980), mammals (Estrada, Rivera and Coates-Estrada 2002), molluscs (Coelho, Malaquias and Calado 2006), and insects (Kudo and Nakahira 2004). In general, species that exhibit an oviposition behavior of unfertilized eggs to feed their tadpoles (e.g. *Osteopilus ocellatus, Leptodactylus labyrinthicus*), are considered as species that lay 'trophic eggs' (Lin and Kam 2008; Prado et al. 2005; Jungfer and Weygoldt 1999). However, even fertilized eggs can be used to feed tadpoles, which is known in literature as 'predation of embryos' or 'cannibalism' (São Pedro et al. 2008; Summers 1999). However, in this latter case the eggs are also trophic and in the first case, the use of the term "egg" may also lead to an inaccuracy, since it is called ‘egg’, the union between the male and female gametes. Thus, we propose that the behavior expressed in the first case (species that lay unfertilized eggs to feed their tadpoles) should be termed as ‘ovumphagy’; while only the second case should be considered as ‘oophagy’.

3. Habitat use, Population dynamics and Reproductive activity

Males, females and juveniles of *A. arapapa* were registered using bromeliads as a shelter and adjacent vegetation as foraging areas. We also observed this behavior in other bromeliad-breeders as *Phyllodytes melanomystax* and *P. luteolus*. In the study area the number of bromeliads is high and the density of *A. arapapa* males in calling activity was relatively low, when compared to other species that usesother water bodies for reproduction (e.g. *Phyllomedusa nordestina*, Vilaça, Silva and Solé, 2011). At the same time, we observed a spatial segregation of anuran community in the study area, we either registered a temporal partition of some few terrestrial bromeliads by different individuals of *A. arapapa* and other species. This occupation pattern may prevent spatial intra and interspecific competition inside terrestrial bromeliads that are more accessible than epiphytic ones to some anurans species. In fact, we did not observed tadpoles of different species in syncronopatry. However, it had been weighted as a possibility since Peixoto (1995) registered *Crossodactylus* sp. and *Scinax* sp. larvae in a same bromeliad.

The philopatry is the tendency of some animals to return to specific locations to feed or breed (e.g. *Rana sylvatica,* Berven and Grudzien 1990), while territoriality is the spatial resource defense against intruders, especially intra-specific ones expressed by vocal activity and or agonist interactions (*e.g. Phyllomedusa nordestina,* Vilaça, Silva and Solé 2011). The displacement distances of *A. arapapa* males indicate philopatry. In fact, some individuals were registered distant less than a few centimeters from the first bromeliad tagged. However, the distances of males involved in vocal interaction were sometimes shorter than expected. Also, no agonistic interactions were observed among males. Nevertheless, it is possible that the territorial area, considered by *A. arapapa* males, is just the bromeliad unit indicated also by philopatry behavior. We have two hypotheses to explain the philopatry of *A. arapapa* concerning its bromeliad:

(1) one particular bromeliad is a chosen resource among others; it is the habitat for breeding (Chapter One) and also a shelter that presents favorable features to these activities. Therefore the individual of *A. arapapa* would remain in the same location until it is necessary to move, (2) *A. arapapa* presents parental care and the male has a territorial behavior related to one bromeliad, to make it possible that females return to mate and feed the tadpoles.

Although we observed a reduction of calling activity from February to May, *Aparasphenodon arapapa* may be considered a prolonged breeder (*sensu* Wells 2007). Moreover, males of *A. arapapa* typically presented the behaviors of prolonged breeding anuran species (Wells 1977); using vocalization to attract females and being territorialists. Additionally the species present low operation sex ratio, other typical characteristic of prolonged breeding season species (Wells 2007).

A great set of tropical anuran species presents reproduction positively associated to temperature, humidity and/or rainfall (Aichinger 1987; Duellman and Trueb 1986). We expected these variables to be likely important for *A. arapapa* though it is a one-year study and we do not have temporal replication. Nonetheless, the absence of correlation of calling activity with rainfall, humidity and temperature may be explained by the relative stability of these variables to the anuran species since (1) the microhabitat used by *A. arapapa* stores water and do not dry so easily as a temporary water body formed by a rainfall on a 'restinga' soil, (2) the proximity with the coast implies that the levels of humidity are relatively high all year round (3) the levels of temperature do not suffer abrupt changes as observed in other Brazilian regions.

4. Courtship and spawning

As observed by Duellman and Trueb (1986), most anurans display amplexus at or near oviposition site. In the case of *A. arapapa*, male and female amplexus were recorded only inside bromeliads and included concave arching of female and tactile stimuli of male's head to female's head. The first behavior tends to be related to peristaltic abdominal contractions moving ovules to oviducts of females, but these movements may not be perceived by a male that breeds in other water bodies. However, the amplexus of *A. arapapa* is limited by bromeliad walls and it is probable that males have a more accurate perception of oviposition by female demonstrated by tactile stimuli of males' head and low intensity calls in interval moments of females' concave arching body.

According to Wells (1977), females of prolonged breeders choose the males by acoustic characteristics of them, which seem to be the case of *A. arapapa.* Additionally, we did not observe female inspection behavior. For *Hyla femoralis*, a tree-frog that inhabits Pine flatwoods, experiments demonstrated that females identify habitats with and without predator and prefer to oviposit in sites with no predator (Rieger et al. 2004). In the species *Phrynobatrachus guineensis*, a tree-hole breeding frog, females inspect breeding sites before spawning (Rödel et al. 2004), behavior also observed for *Scinax perpusillus* females, a bromeliad-breeder (Alves-Silva and Silva 2009). In the view of a possible shortage of suitable breeding sites (see below) and a large investment in parental care, it is possible that (1) the choice for a male is more important than the choice for an oviposition site and therefore, the female is based on "vocalization quality" (Ryan 1980), (2) the vocalization of the male within the bromeliad indicates bromeliad characteristics of interest by acoustic effects (males of *Metaphrynella sundana* are capable to exploit resonance effects in tree-holes, Lardner and Lakim 2002), (3) the female mates with more than one male (polyandrous system), (4) the behavior of inspection does exist, but was not observed.

A same couple of *A. arapapa* formed in other occasion at the same bromeliad and in a short period of time, may indicate a temporary monogamy related to parental care. Only one anuran species, *Ranitomeya imitator*, is admittedly monogamous, a trait evolutionarily rare (Wittenberger 1980) and related to biparental care (Brown, Morales and Summers 2010). Among anurans species, male parental care is typically primitive and the evolution of maternal care must have been critical to the evolution of biparental care (Summers and Earn 1999). Noteworthy the vast majority of sophisticated parental care in anurans are presented by phytotelm-breeders as nest construction, egg attendance, egg transport, tadpole attendance, tadpole transport, tadpole feeding (Lehtinen and Nussbaum 2003). Males of *A. arapapa* remain in the bromeliad with eggs (egg attendance) and tadpoles (tadpole attendance). Females oviposit eggs that are fertilized by a male and serve as food to tadpoles (tadpole feeding).

5. Reproductive Mode

Anuran species present a great variety of reproductive modes among tetrapods (Haddad and Prado 2005; Wells 2007; Kühnel, Reinhard and Kupfer 2010). Reproductive modes are a set of reproductive characteristics that include oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling, and type of parental care, if any (Salthe and Duellman 1973). It is a system of classification created by scientists that allows discussion about reproductive features from an evolutionarily and behaviouristically approach. As observed by Wells (2007) the reproductive modes "(…) do not necessarily represent all the great variety of egg-laying strategies of anurans". Yet, we continuously review the list and each new set of reproductive characteristics discovered for other species must be include in the list.

Phytotelm breeding has evolved in over 100 species of anurans across a wide phylogenetic spectrum (Lehtinen et al. 2004). At least five reproductive modes are associated to phytotelmata in the last reviews of Wells (2007) and Haddad and Prado (2005). Regarding the reproductive mode of *A. arapapa,* it is not well fitted in none of the modes. *Aparasphenodon arapapa* exhibits biparental care, exotrophic larvae and oviposition inside terrestrial bromeliads (lateral axils and central tank), so we suggest that reproductive mode #6 in order to represent only "eggs and exotrophic tadpoles in water in tree holes or aerial plants", should be reformulated as "eggs and exotrophic tadpoles in water in phytotelmata without parental care" and propound the creation of a new reproductive mode #40 "eggs and exotrophic tadpoles in water in phytotelmata with parental care". Parental care implies in a variety of other behaviors and ecological adaptations previously described here and the population dynamics of these species is distinct from the others that present reproductive mode #6. For now, the new mode would harbor *A. arapapa, Osteopilus ocellatus, Kurixalus eiffingeri, Trachycephalus resinifictrix,* and *Anotheca spinosa.* In this sense all kinds of phytotelmata breeder would be contemplated.

6. Conservation remarks

Low fecundity species with high habitat specialization are most likely to suffer with populations declines (Bielby, Cunningham and Purvis 2006). Furthermore, more attention has been dedicated to specific reproductive modes once they answer differently to habitat disturbance and deserve a different conservation approach. As pointed out by Loyola et al. (2008) and Silva et al. (2012) conservation assessments for Atlantic forest should include aspects of amphibian life history. *Aparasphenodon arapapa* is an endemic species from the Atlantic forest, with a specific and rare reproductive mode and deserves pretty much attention to its populations and conservation status. Additionally, as a consequence of dwelling bromeliads, the anuran species are equally vulnerable to factors that affects bromeliads conservation, as land speculation on the costal region of Brazil (Rocha et al. 2007).

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Appendix

Table 1. Occurence of eggs, tadpole and adults inside bromeliads by months of study period. M = Males; F=Females; x = present, but not counted; Larval development stages of Gosner (1960) categorized in: early (19 to 30), intermediate (30 to 36) and advanced (37 to 46); adult equal letters equivalent to the same individuals registered. (*) Entire clutches.

Month	Adult	Sex	Snout-Vent Length (mm)	Eggs	Tadpoles	Gosner's stages
nov/11	A	M	46.70		22	early
dec/11	-	-	-		13	early
	-	-	-		3	2 advanced, 1 early
	B	M	not recorded		22	early
	C	M	not recorded	x	X	not recorded
	-	-	-		X	not recorded
	-	-	-		2	not recorded
	C	M	not recorded	1	1	not recorded
	D	M	43.20		1	early
jan/12	E	M	46.80	30	8	early
	-	-	-		2	intermediate
	-	-	-	15	1	Intermediate
	F	M	45.30		6	early
	G	M	44.55	2	9	early
	H	M	46.00	x	X	not recorded
	I + J	M+F	43.80 (M); 53.95 (F)	34*	10	early
feb/12	H	M	46.00		4	early
	C	M	46.85	6	1	early
	K + L	M+F	46.85 (M); 51.90 (F)	35*	2	early
mar/12	-	-	-		3	intermediate
apr/12	M	M	45.90 (M); 50.85 (F)		3	not recorded
may/12	G	M	46.70			-
aug/12	N	M	46.80		9	early
	O + P	M+F	45.40 (M); 50.55 (F)		12	early
	Q	M	45.90	3	3	not recorded
sep/12	R	M	47.80		9	early
	P	M	46.10		5	early
	R	M	45.20		2	early
	S	M	45.75		3	early
	T + U	M+F	49.80 (M); 60.55 (F)		5	various
oct/12	V	M	44.20		2	1 early, 1 intermediate
	X	M	46.65		8	early
	Y	M	47.80		4	intermediate
	W	M	44.95	X	1	Intermediate
	N + Z	M+F	46.05 (M); 54.05 (F)		3	advanced

Table 2. Males of *Aparasphendon arapapa* recaptured in the same bromeliad in a Mussununga of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil. M = months; D = days.

Male	Bromeliad	Day interval between recapture
A	1	23D
B	2	9M 5D
C	3	2M 27D
D	4	4M 12D
E	5	12D
F	6	1M 11D

Table 3. Mean of maximum number of *Aparasphenodon arapapa* males in calling activity by collection day per month (MMM), mean of maximum temperature by collection day *per* month (MMT), mean of maximum relative humidity by collection day *per* month (MMR) and total rainfall during the study months (TRM) in a Mussununga of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil. (*) rainfall data in milliliters; (**) rainfall data for October, November 2011 and February 2012 not available.

Months/ Year	MMM	MMT (°C)	MMR (%)	TRM (ml)*
nov/2011	5.80	24.94	94.20	**
dec/2011	7.25	25.33	84.75	**
jan/2012	5.17	25.58	79.00	104.60
fev/2012	4.00	25.43	79.75	153.80
mar/2012	1.50	25.38	81.00	184.81
apr/2012	0.80	24.76	74.20	40.40
may/2012	0.75	23.68	75.75	194.00
jun/2012	2.00	23.60	80.00	150.00
jul/2012	2.50	22.78	76.75	96.20
aug/2012	5.00	22.50	82.25	210.60
sep/2012	3.25	22.85	74.75	51.80
oct/2012	6.60	24.52	73.00	**

Table 4. Individual displacement of *Aparasphendon arapapa* males registered in space and time between bromeliads used in a Mussununga of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil. D.I. = day interval; m = months; d = days; B1-B2 = bromeliad 1 distance to bromeliad 2 (same pattern to B2-B3 e B3-B4); Distance in meters. (*) Not present in Figure 8.

Male	**D.I. B1-B2**	**Distance(m)**	**D.I. B2-B3**	**Distance(m)**	**D.I. B3-B4**	**Distance(m)**
I	12d	**2.10**	-	**-**	-	**-**
II	8m 1d	**0.66**	-	**-**	-	**-**
III	1m 8d	**1.14**	-	**-**	-	**-**
IV	9 d	**2.26**	1m 20d	**2.65**	5 m 9d	**2.93**
V	6m 27d	**5.03**	1m 6d	**0.68**	2m 5d	**0.45**
VI	7d	**2.60**	1m 2d	**3.40**	-	**-**
VII	7d	**2.80**	-	**-**	-	**-**
VIII	7m 15d	**1.01**	-	**-**	-	**-**
IX	10m 8d	**0.74**	-	**-**	-	**-**
X*	29d	**12**	-	**-**	-	**-**
XI*	3m 11d	**12**	-	**-**	-	**-**

Figure headings

Figure 1 . Map of a South portion in state of Bahia. Study area appointed between the two municipalities' area of Ilhéus and Una, Brazil.

Figure 2. Gravid female (gonads apparent) of *Aparasphenodon arapapa* perched in a tree trunk; in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil.

Figure 3. Sexual dimorphism in snout-vent length in *Aparasphenodon arapapa;* female (left) and male (right).

Figure 4. Metamorph of *Aparasphenodon arapapa* with eggs inside digestive system appointed; in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil.

Figure 5. Egg of *Aparasphenodon arapapa*.

Figure 6. Tadpoles of *Aparasphenodon arapapa* and biparental care of adults. A) Male and tadpoles in bromeliad; B) Male and tadpoles in bromeliad; C) Male and female inside bromeliad.

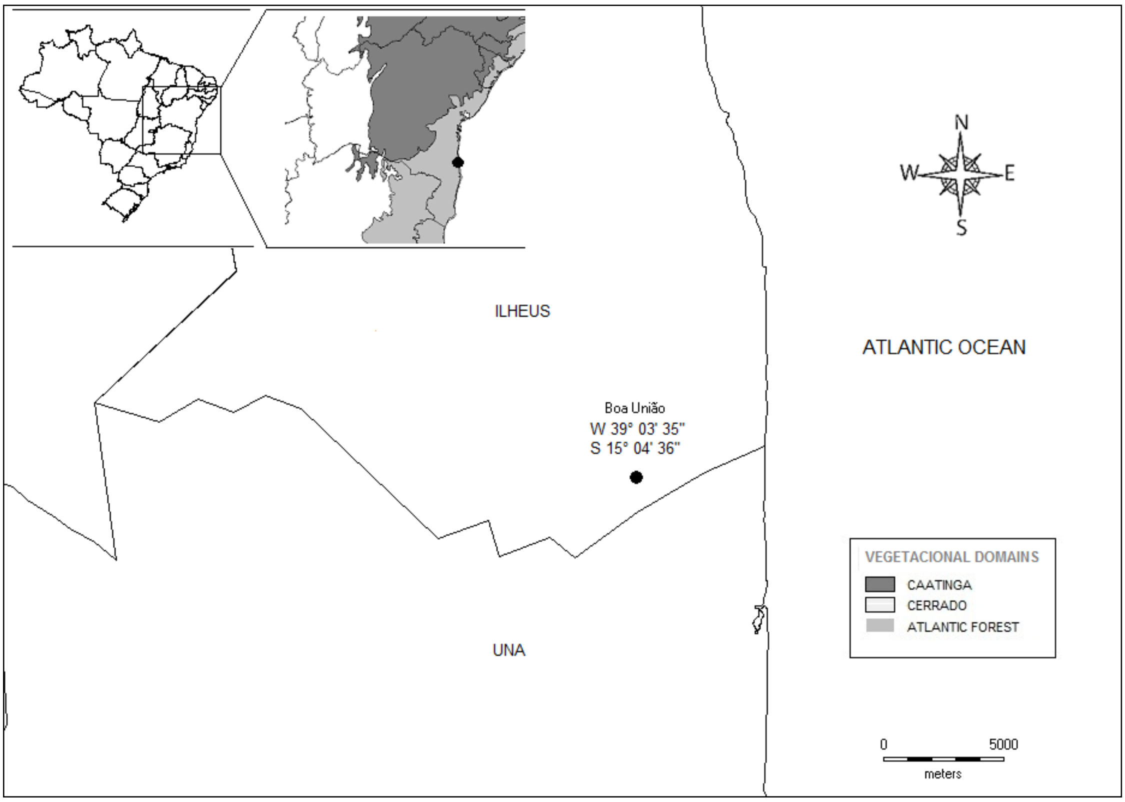
Figure 7. *Aparasphenodon arapapa* calling males (A) at central tank and (B) bromeliad's lateral axils; in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil.

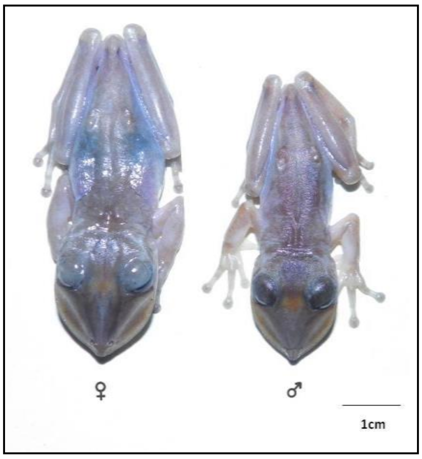
Figure 8. Individual displacement of *Aparasphenodon arapapa* males between bromeliads; in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil.

Figure 9. Maximum distance of *Aparasphenodon arapapa* calling males bromeliads; in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil.

Figure 10. Calling activity peak of *Aparasphenodon arapapa* males; in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil.

Figure 11. Courtship of *Aparasphenodon arapapa* in a Mussununga vegetation of Southern Atlantic Forest of Bahia, municipality of Ilhéus, Brazil. (A) Calling male inside a bromeliad; (B) Female's approach; (C) Female prepare to go down to the central tank of the bromeliad; (D) Male and female in axillar amplexus; (E) Concave arching of female followed by male and oviposition; (F) Female leave the bromeliad and male remain still with eggs ovipositioned.


N
W
E
S
ILHEUS
ATLANTIC OCEAN
Boa União
W 39° 03' 35"
S 15° 04' 36"
VEGETACIONAL DOMAINS
CAATINGA
CERRADO
ATLANTIC FOREST
UNA
0
5000
meters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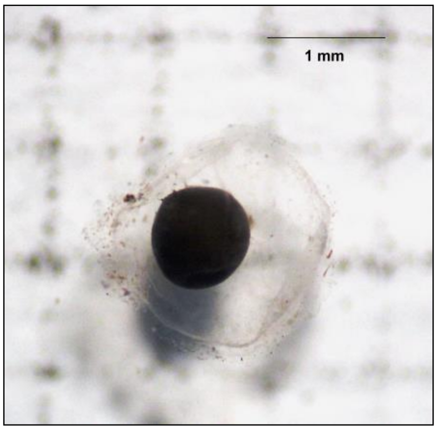
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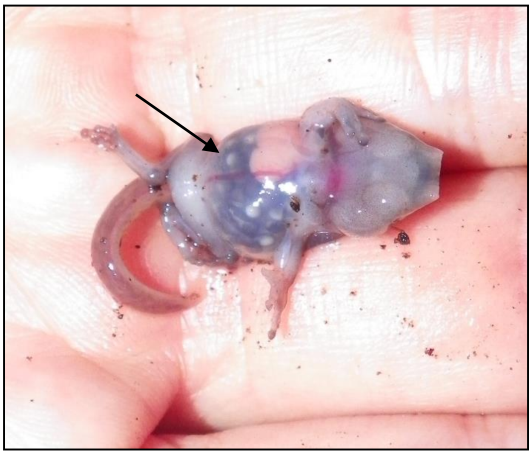
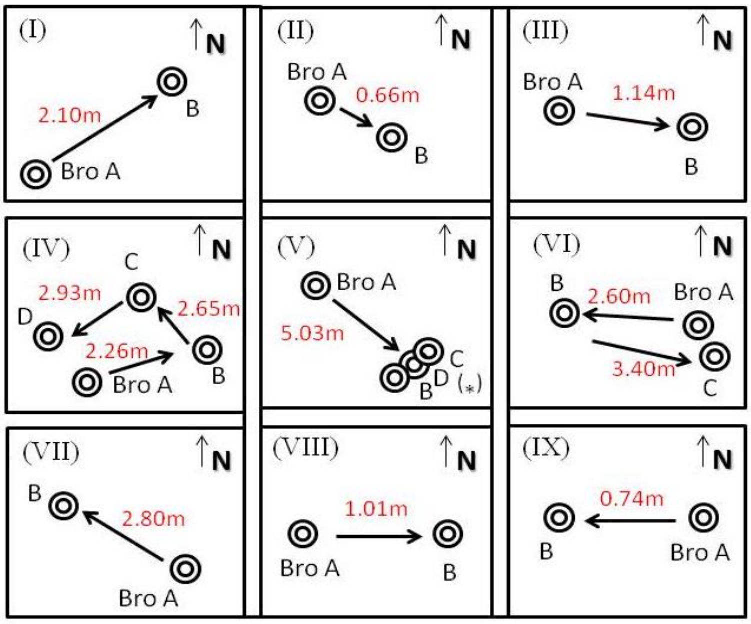



A
B
C


(A)
(B)


(I)
N
2.10m
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(IV)
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C
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2.65m
D
2.26m
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Bro A
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(VI)
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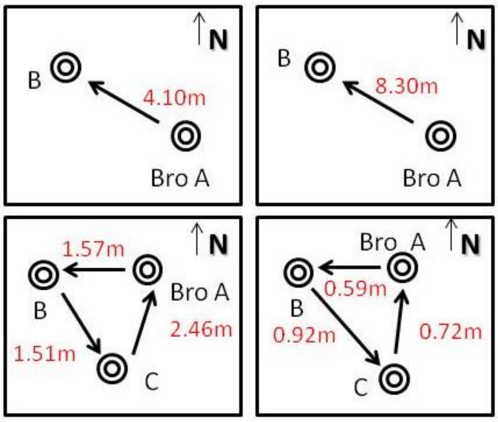
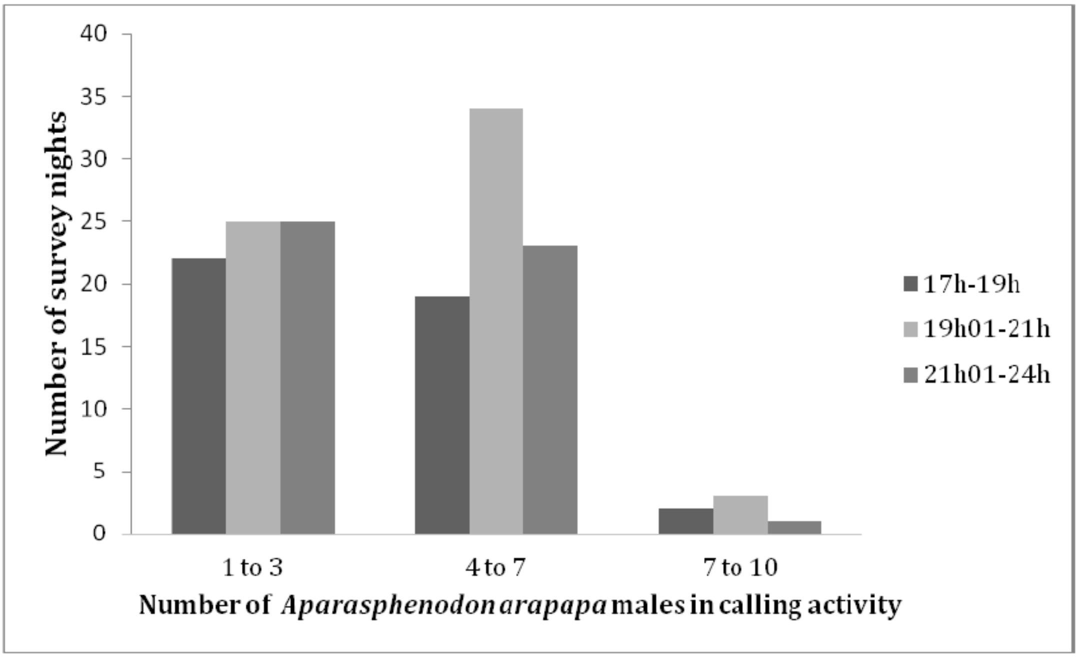
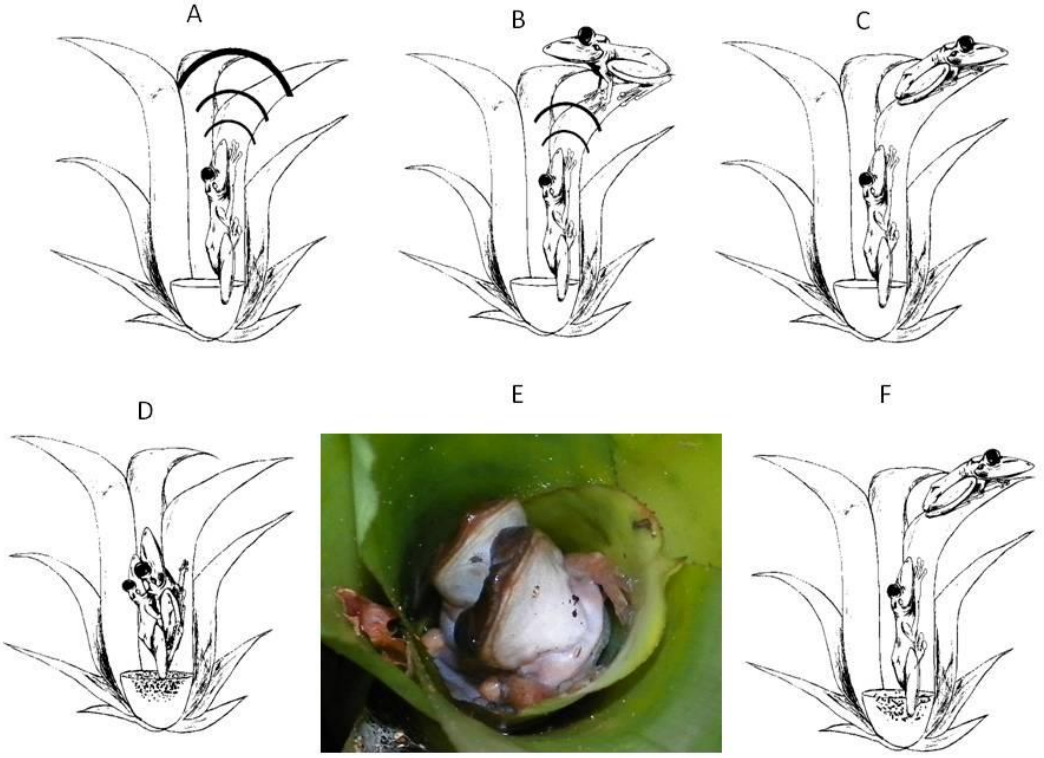

N
B
4.10m
Bro A
N
B
8.30m
Bro A
N
1.57m
B
Bro A
2.46m
1.51m
C
Bro A
N
0.59m
B
0.92m
0.72m
C


40
35
30
25
20
15
10
5
0
Number of survey nights
1 to 3
4 to 7
7 to 10
Number of *Aparasphenodon arapapa* males in calling activity
■ 17h-19h
■ 19h01-21h
■ 21h01-24h


A
B
C
D
E
F


IN ALTUM


PPG Ecologia da Conservação