Maya Romano Maia

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microambientes e sua relevância para a
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Orientador: Fernando Ribeiro Gomes

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DEDICATÓRIA

Este trabalho é dedicado à minha mãe!

EPÍGRAFE

The world always seems brighter when you've
just made something that wasn't there before.

Neil Gaiman

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INTRODUÇÃO GERAL

Atualmente, têm se discutido cada vez mais como variáveis comportamentais se integram com as fisiológicas e como essa integração pode acarretar em um tamponamento comportamental dos desafios ambientais, reduzindo a relevância de variantes fisiológicas sobre o valor adaptativo (Cooke et al., 2013; Murren, 2012). No caso de animais terrestres, a integração entre as variáveis comportamentais e fisiológicas pode ser essencial dado à complexidade física dos seus ambientes (Dawson, 2005). A complexidade física gera uma variação micro ambiental marcante que, aliada à mobilidade dos animais e a especialização sensorial, possibilitam a busca ativa de situações ambientais ideais, sendo assim importantes mecanismos de homeostase (Bartholomew, 1964).

Neste campo, a fisiologia evolutiva fez grandes contribuições no contexto de termorregulação comportamental (e.g. Bauwens et al, 1995; Adolph, 1990; Huey and Bennet, 1987), focando no uso de lagartos heliotérmicos como modelo de comparação. Em contraposição, este enfoque difundiu a idéia de que animais noturnos teriam, teoricamente, uma capacidade de ajuste da termorregulação por seleção de microambientes pequena a nula. Com os estudos de hidrorregulação em anuros, demonstrou se que mesmo em animais de hábito noturno,

mecanismos comportamentais e fisiológicos ocorrem na captação, conservação e na detecção de água, tamponando os o risco de desidratação. Contudo, pouco se sabe sobre como a seleção natural atuou sobre a interação desses mecanismos de homeostase hídrica no ambiente natural.

Buscamos mostrar neste trabalho a seleção de microambientes usados como sítios de vocalização por anuros noturnos como um forte componente da homeostase hídrica e, portanto, uma possível via de tamponamento comportamental em animais noturnos. Sendo assim, poderíamos encontrar diferentes interações fenotípicas entre características comportamentais e fisiológicas associadas ao balanço hídrico. Isso ocorreria, por exemplo, em espécies com alta eficiência em encontrar água, o que possibilitaria selecionar os microambientes mais propícios e permitiria que apresentassem baixa resistência a perda de água por evaporação (RPAE). No contexto antagônico, espécies vivendo em ambientes mais instáveis, em conjunto com a baixa eficiência na detecção de umidade, estariam mais sujeitos às forças seletivas sobre a fisiologia, como a RPAE.

É possível que as variações diárias e anuais de temperatura, umidade e pluviosidade sejam pressões seletivas relevantes para a evolução em anuros de atividade noturna, porém, o equilíbrio de ajustes comportamentais e fisiológicos pode ser diferenciado quando diferentes

espécies são consideradas. Como resultado, espera-se encontrar coadaptação nas características ecológicas, fisiológicas e comportamentais que influenciam o balanço hídrico dos anfíbios, sendo elas: (1) dependência da atividade reprodutiva da temporada de chuvas; (2) ocupação de microambientes, que variam em termos de temperatura e potencial de desidratação ao longo da temporada reprodutiva; (3) variação em RPEA e (4) variação na eficiência na detecção de umidade.

INTRODUCTION

Due to their high skin permeability, most amphibians feature high rates of water loss by evaporation when compared to other tetrapods (Withers et al. 1984; Wygoda, 1984). Therefore, the amount and seasonality of water availability in the environment represents an important limitation to the environmental occupation of this group, concentrating the richness of amphibian species towards the equator, characterized by predictable and intense rainfalls (Buckley and Jetz, 2007; Shoemaker et al., 1992). However, the evolution of physiology and behavior integrated as a phenotype improved in water balance allowed amphibians to have a worldwide distribution, except for Polar Regions, Greenland and oceanic islands (Bentley, 1966). Even semi-arid regions such as the Brazilian Caatinga display high amphibian richness counting about 80 anuran species (IBAMA, 2013). The evolution of physiological and behavioral traits related to water balance occurred together and in association with diversity in the length and degree of seasonality of the reproductive season, which does not necessarily peak at the rainiest or warmest season of year (Duellman and Trueb, 1986). As a result of the evolutionary processes associated to these variables, anurans show a complex interspecific micro-habitat selection according to climatic and water source conditions and the

microenvironmental proprieties that enhance or protects against these conditions. Moreover the variety of reproductive season lengths is also a result of the evolutionary process and can be depended or not to rainfall occurrence. In order to understand how micro-habitats and reproductive activity patterns are combined, specific physiological and behavior traits, among environmental conditions ought to be taken in account.

Among interspecific physiological strategies against dehydration studied over the past decades in the literature, are traits related to mechanisms towards waterproofing the skin, the ability to dramatically reduce glomerular filtration rate, urinary bladder's ability to store water and the tolerance to dehydration (Shoemaker et al., 1992). Treefrogs in particular have higher resistance to water loss (RWL) in comparison to non arboreal amphibians (Young et al., 2005; Withers et al., 1984; Wygoda, 1984), hypothesized as a result of the selective pressure of higher exposure to increased levels of radiation, temperature and convection of the arboreal habit (e.g., McClanahan and Shoemaker, 1987).

Behavioral mechanisms related to water detection and search are also crucial to water balance, but have not been explored in much detail. By detecting water, through exploratory behavior, unfavorable external conditions can be buffered, providing retreat sites for water conservation. The activity towards searching behavior occur in early desiccation stages,

in which, instead of investing towards moisture-conserving mechanisms, the animal adopt a strategy to regain water balance (Heatwole and Newby, 1972). Due to the higher risks of dehydration in leaving water conservation posture, the strategy implicates their level of dehydration, the distance of the water source and their efficiency in finding water. In this case, the efficiency concept involves sensory ability, the number of attempts and the time taken to accomplish this task.

Therefore, to understand spatio-temporal distribution in a wild community of hylid frogs our central aim is to test whether: 1) Species of anurans diverge in exploratory behavior and; 2) Verify if RWL and water search strategy are linked to microenvironment and temporal occupation in the context of water balance during anurans reproductive activity.

METHODOLOGY

Study animals

Males of five species of treefrog (Hylidae) from the Atlantic rain forest were included in the present study: *Hypsiboas albopunctatus*, *H. bischoffi*, *S. hayii*, *H. bandeirantes* and *Scinax crospedospilus* (Figure1).

We chose these species because, despite being phylogenetically related, they display large differences in micro-habitat occupation during reproductive activity and in temporal patterns of reproduction. *Hypsiboas bandeirantes* and *Scinax hayii* are found calling all year round, with larger aggregations observed in the rainy season (Bertolluci and Rodrigues, 2002). *Hypsiboas albopunctatus* and *H. bischoffi* are known to have very short reproductive seasons (Bertolluci and Rodrigues, 2002), with vocal activity probably triggered by heavy rains. *Scinax crospedospilus* do not reproduce all year, but presents a relatively large reproductive season from September till March (Bertolluci and Rodrigues, 2002).

Collecting site

We collected data on microhabitat occupation and environmental conditions during reproductive activity for these five species of treefrogs in the Estação Biológica de Boracéia (EBB), Salesópolis, SP, Brasil, from

11/2012 to 10/2013, for five days a month, between 1900h and 2300h. We collected individual males of *Hypsiboas bischoffi* (N = 14), *H. bandeirantes* (N = 16), *Scinax hayii* (N = 19) and *S. crospedospilus* (N = 12) in the Quarry Pond - EBB (23°38'17.01"S 45°50'25.28"O), and we collected males of *Hypsiboas albopunctatus* (N = 14) in the Lake of the Mound - EBB (23°38'46.29"S 45°51'39.43"O) to perform experiments in the laboratory.

Field data

We measured daily mean temperature and relative humidity (RH) by setting a HOBO® Data Logger two meters high on the edge of the quarry pond. We measured rainfall in mm with the use of a rain gauge installed at the quarry pond. We also used rainfall measurements to classify daily rain intensity (a class variable ranging from 0 to 3) and to count the number of days from the last rain.

We characterized the microenvironment chosen by individuals of different species in calling activity based on (1) vegetation cover (presence or absence), (2) distance from the nearest water body (height and distance in centimeters), (3) the relative humidity of the microenvironment at that moment, (4) the difference between mean relative humidity of the day and relative humidity of the microenvironment, (5) the thermal envelope

experienced by the anurans in the calling sites, (6) the operative temperature during calling activity, and (7) the dehydration potential.

We estimated the dehydration potential of the microenvironments by placing fully hydrated plaster models, with size and shape compatible with the species studied, in the exact location where individuals were found calling (Tracy et al. 2007). We weighed the plaster models and left in the microenvironments during the periods of calling activity. At the end of this period, we weighed the plaster models again, and estimated the rates of water loss by subtracting the final model weight from the initial values and by dividing that by time of exposition in hours. We collected these data only during nights with no precipitation.

To measure the full range of temperatures available to the frog community, we estimated this thermal envelope by subtracting the temperature experienced by a fully permeable treefrog model from an impermeable one. We placed pairs of permeable and waterproof plaster models in the calling sites, according to Rowley and Alford (2010). To confer high resistance to water loss, we coated the models with thermoplastic rubber, PLASTI FILM®. We treated permeable models with the same coating only on the ventral surface to avoid water exchange with the substrate. We placed temperature sensors inside the plaster models to determine the maximum and minimum temperatures of the model in each

microenvironment. We coupled these sensors to HOBO® Data Logger programmed to record temperature every 5 minutes. We estimated operative temperature data by measuring the body surface temperature of individuals during calling activity with an infrared thermometer (IT- 860, Instrutherm).

To estimate the level of reproduction dependence to wet season, we conducted an audio strip procedure, quantifying the number of calling males in the chorus each night, according to Heyer (1994). As described hereinabove, we conducted rainfall measurements for each day of field trip by using a rain gauge placed in the site location. To characterize the patterns of calling activity by month, for each species, we calculated the mean of calling males from the five-day estimates. We conducted the same procedure to estimate the rainfall for each month. Therefore, we calculated the difference between percentage of calling males during the four months of highest rainfall and the quarter of highest drought. Species characterized by reproductive season unrelated to rainfall tended to lower index values, approaching to zero. Otherwise, species characterized by reproductive season peaks related to highest rainfalls, showed higher and positive index values.

Laboratory maintenance conditions

We brought individuals of each species to the laboratory and maintained individually in plastic containers provided with clean water and a place for refuge, at natural temperature and natural light cycles. We fed the animals three times a week with domestic flies and small cockroaches bred in captivity. Ethical approval for the study was given by Comissão de Ética em Uso de Animais (CEUA) from Instituto de Biociências, Universidade de São Paulo (161/2012).

Water search behavior experiment

To test for intra and interspecific differences in exploratory behavior, we presented individuals from each species to a challenging situation of water search when slightly dehydrated. We individually tested the animals in a multiple choice labyrinth consisting of a central arena (35 cm diameter) and eight arms (13 cm in length) (Figure 2-3). We made the labyrinth of transparent cellular corrugated plastic and illuminated from below with a red lamp (26W) located centrally at 110 cm distance to allow observers to locate animals but avoid any illumination effect (Boycott, 1964). We placed a transparent acrylic on the top of the labyrinth, allowing a clear view of the interior and preventing the animal from escaping. We placed phenolic floral foam cubes at the arms ends, with only one of them,

randomly selected in every test, soaked in water. The trials were video-recorded by a security camera CCTV 480 TVL with infrared LEDs, connected to a *H.264 Network Digital Video Recorder*. We placed a video camera 170 cm above the labyrinth and tests were recorded continuously from 18:00 h to 6:00 h. We numbered the arms for reference to align the labyrinth to the video camera. The experiments occurred inside an acclimated room (FITOTRON 011 – Eletrolab) with controlled temperature (21 ± 1 °C) and relative humidity ($50\% \pm 5$ RH).

Previously to each test, we dehydrated the animals until they reached 85% of the standard body mass (hydration level of 100%) measured at the beginning of the experiment. We dehydrated individuals by using a plastic wind tunnel (5 cm x 5 cm x 20 cm) connected to an electric fan set to 2.9 m/s for larger species (*Hypsiboas albopunctatus*, *H. bischoffi* and *Scinax hayii*) and to 2.2 m/s for the smaller ones (*Hypsiboas bandeirantes* and *S. crospedospilus*). We restricted the treefrogs within the wind tunnel to the opposite end of the electric fan by using a fishnet fabric. By using this protocol, we monitored the mass during dehydration and we discontinued the process when body mass were equivalent to 85% of the initial mass. All individuals achieved the experimental hydration level in about 30 to 60 min. Once dehydrated, we placed the animals at the centre of the labyrinth, always oriented in the same direction (towards the arm end n°1), inside an

aluminium cage (hemisphere, 8 cm in diameter) with the opening facing down, for 15 minutes. At 18:00 h, the experiment began by removing the aluminium cage. At the end of the experiment, we weighed the animals again to see if they rehydrated overnight. We tested some individuals from each species twice to identify intra-individual consistency of behavioral patterns. We measured the following variables: 1) Number of trials (number of attempts in dry retreat sites until success); 2) Time to succeed (time to find the humid retreat site in hours); 3) General activity (number of trials made per hour), and 4) Residence time (the time spent in moist site). We registered the selection of humid retreat site only if residence time exceeded the time required for rehydration from the water surface of at least 10% of body mass. We estimated this time from published rates of rehydration of different species of anurans (Titon Junior 2010), based on the regression of body mass gain by time.

RWL data

We took data on resistance to evaporative water loss for each species from Titon Junior (2010; Table 1). RWL data for these species were collected using individual males from the same locality of our study.

Statistical Analysis

Water search behavior experiment

We compared standard body mass to mass after the dehydration protocol in order to confirm that the animals were dehydrated immediately before they entered the labyrinth. Additionally, we compared the standard body mass to body mass at the end of the experiment, to confirm that animals were completely rehydrated by that time. We performed these comparisons through paired Wilcoxon ranking tests (Zar, 1996).

Descriptive statistics of the variables measured in water search behavior experiment for the species included in this study are shown in Table 2. We performed generalized linear models (GLM) ANOVA to compare the variables: “Number of trials”, “Time to succeed”, “General activity” and “Residence time” between species, followed by Tukey's multiple comparison tests. The variables “General activity”, “Time to succeed” and “Residence time” are related to measurements of time until the first instance of an event, given a constant rate of occurrence, so values cannot be negative. In this way, we used a gamma distribution and a log link function, given that this inverse link is the natural one for gamma distributions in GLM (Zuur et al., 2009; McCullagh and Nelder, 1989). We used generalized linear models with a Poisson distribution for modeling “Number of trials” trait, since it is a count data.

We used the variable “General activity” to test for intra and interspecific differences in exploratory behavior because it resumes two major factors in the search for water: “Time to succeed” and “Number of trials”. Additionally, this variable allowed distinguishing a dichotomous response pattern in prior analyses.

We included all animals that underwent the test twice in analysis for activity repeatability tested by Spearman’s correlation test, followed by a linear regression model. This analysis included 9 individuals of *Hypsiboas albopunctatus*, 8 *H. bischoffi*, 6 *H. bandeirantes*, 4 *Scinax crospedospilus* and 13 *S. hayii*. We used data from the first test for all these individuals for the other analyses. General analyses did not include data from three individuals (one *Hypsiboas albopunctatus*, one *Scinax crospedospilus*, and one *S. hayii*) that did not find the humid retreat site within the period of 12 hours.

We tested the association between the reproduction dependence to wet season with behavioral traits from the labyrinth (“Time to succeed” and “Number of trials”). We calculated the reproduction dependence to wet season as the difference between percentage of calling males during the four months of highest rainfall and the quarter of highest drought. The calculated difference was tested for any correlation with both behavioral traits by Pearson’s correlation test. We also tested for correlations between

mean number of calling males per day, mean rainfall of a day, environmental relative humidity and microenvironmental relative humidity by Spearman's correlation test. We performed all statistical tests described above with the program R© v. 3.0.1 (R Development Core Team, 2013). The significance level (P) was 0.05.

Discriminant models

We performed Linear Discriminant Analyses (LDA) with SPSS 20 software (SPSS 2011) to understand the possible relationships of interspecific variation in RWL and water search strategy with microenvironmental occupation and climatic conditions during reproductive activity. The LDA generates a linear discriminant function that maximizes the ratio of between-groups to within-groups sums of squares (SPSS 2010). In the discriminant function, explanatory variables are input simultaneously and are represented as scale-dependent coefficients that can be standardized to compare their relative loads.

In this analysis, the RWL data consisted of the five means of RWL, measured for each species by Titon Junior (2010), and behavioral data consisted of three categories which involved the two opposite strategies and an intermediate one, assigned to a species that did not present a clear trend to any of the two categories. The environmental data consisted of:

reproduction dependence to wet season; the rain intensity; n° of days from the last rain; rainfall in mm; mean temperature of the day; mean relative humidity of the day; presence or absence of vegetation coverage; operative temperature; relative humidity of the microenvironment; water distance; height of the water level; dehydration potential of the microenvironment; thermal envelope; and difference between mean overall relative humidity and mean microenvironmental relative humidity.

RESULTS

Behavior experiment

Animals underwent body mass loss after the dehydration protocol (Wilcoxon rank sum test: $V = 3286$, $P < 0.05$), and didn't presented different masses before the experiment (standard mass) and after rehydration during the experiment (Wilcoxon rank sum test: $V = 2707$, $P = 0.91$).

Some species displayed more activity than others ("General activity", GLM ANOVA: $F_{4,70} = 9.40$, $P < 0.005$), took longer to find the humid arm of the labyrinth ("Time to succeed", GLM ANOVA: $F_{4,70} = 4.72$, $P < 0.005$) and required more attempts ("Number of trials", ANOVA, Poisson glm $\chi^2 = 30.49$, $P < 0.001$), but none of the species differ in the time spent inside the correct arm, usually remaining there till the end of the experiment. *Hypsiboas albopunctatus* was the least active species, exhibiting the lowest "General activity" and "Number of trials" when compared to *H. bandeirantes*, *Scinax crospedospilus* and *S. hayii* (Tukey HSD, $P \leq 0.05$; Table 2), followed by *H. bischoffi* that did not differ from *H. albopunctatus*. Individuals of *Scinax crospedospilus* showed almost twice as high as the attempts showed by individuals of *Hypsiboas albopunctatus* (Table 2). The fastest species to reach the moist arm was

Scinax crospedospilus, with average time of 0.71 ± 0.62 (“Time to succeed”, Tukey HSD, $P \leq 0.05$; Table 2).

Standard deviation of “General activity” variable indicated high variance to species *Hypsiboas bandeirantes* followed by *Scinax crospedospilus* and *S. hayii*, suggesting changes in effort with time. *Hypsiboas bischoffi* and *H. albopunctatus* showed reduced variance, meaning that these species maintained constant effort (Table 2; Figure 4). “General activity” variable allowed distinguishing a dichotomous response pattern in prior analyses (Figure 5). The first strategies represented by species *Hypsiboas albopunctatus* e *H. bischoffi* indicates more time to succeed but, on the other hand, less number of trials. The second strategy represented by species *Scinax crospedospilus* and *S. hayii* indicates less time to succeed, but with more number of trials. The first one is characterized by maintaining a water conservation posture and does not move randomly and we called it the “direct strategy”. The second group of treefrogs seems to find water by random trial, and as a consequence ends up to find the water source quicker. We call this the “energetic erratic strategy”. Although they find water in less time, the higher levels of locomotion are probably associated to higher rates of water loss (Heatwole et. al 1969).

Activity was considered as a repeatable trait, with significant correlation between first and second experiment (Spearman's $r = 0.65$; $P < 0.001$; Figure 6). Regarding to water search strategy, 78% of the individuals were consistently classified in strategy between first and second trial in the exploratory behavior for water search. Almost all species had an uneven distribution of strategies. *Hypsiboas albopunctatus* and *H. bischoffi* demonstrated a clear trend towards direct strategy, which we scored as -1. On the opposite, *Scinax crospedospilus* and *S. hayii* could be easily defined as energetic and erratic in exploration behavior, scored as +1. The exception was *H. bandeirantes*, which had an almost even frequency of individuals classified as direct and energetic erratic. For this reason, we classified this species as intermediate one, scored as 0 (Table 3).

Estimate of reproduction dependence to wet season

The temporal patterns from the most relevant variables for this study, such as mean rainfall of the day, environmental and microenvironmental relative humidity are shown in the graphs below (Figure 7). The quarter with the highest rainfall was February-May, accounting for 14.19% of the annual rainfall (Figure 8; Table 4). The driest season was in June-September quarter, representing 2.91% of total rainfall (Figure 8; Table 4). The species with higher association between reproductive activity and rainfall was *H. albopunctatus*, followed by the other species from genus

Hypsiboas (Table 4). The species from genus *Scinax* showed low association between reproductive activity and rainfall (Table 4). The interspecific variation in level of dependency of reproductive season to rainfall is negatively associated to “Number of trials” (Pearson’s $r = 0.86$; $P < 0.001$; Figure 9) and positively associated to “Time to succeed” (Pearson’s $r = -0.99$; $P < 0.001$; Figure 10).

Discriminant functions

The discriminant analysis which uses the five species numbered from 1 to 5 as grouping variable showed that the first discriminant function had only 53.5% of accuracy (Figure 11A, Table 5). The first function, with the most discriminant power, presented the highest loads in mean temperature, relative humidity of the microenvironment, distance of the calling site from water and the presence of vegetation cover. In this function, cross-validated classification showed a rate of 54.8% effect in predicting the actual results.

The discriminant analysis that used RWL as the grouping variable gathered the values from the species *H. bischoffi* and *H. bandeirantes* to the same group. Accordingly, the first discriminant function discriminated RWL at 89.4% accuracy, based mainly on reproduction dependence to wet season as the environmental discriminator (Figure 11B, Table 6). The second discriminate function explained 6.6% of the variance, and

differenced mainly *S. crospedospilus* from the other ones (Figure 11B). This second function showed higher loads for the variables “relative humidity of the microenvironment”, “water distance”, “height of the water level”, “mean relative humidity of the day” and “thermal envelope” (Table 6). Cross-validated classification showed a classification rate of 94.5%.

Given the extremely high discriminative power of the variable that associates reproductive activity to patterns of rainfall, we ran again the analysis for RWL without this variable, to understand better the possible association of interspecific RWL with variables more finely tuned to the microenvironment of calling activity. When we removed “reproduction dependence to wet season”, the discriminant function had lost much of the power, having only 50.1% of accuracy and 74% of classification rate in cross-validated test (Figure 12A, Table 7). From the explanatory variables, the ones that stand out in the first function are “water distance”, “height of the water level”, “mean relative humidity of the day”, “relative humidity of the microenvironment” and “thermal envelope”, a very similar result of the second function of the discriminant analysis described above. Again, *S. crospedospilus* showed higher distances towards water but a greater dependence of higher relative humidity levels, both in large scales and in microenvironmental scale (Table 8). *S. crospedospilus* also experience lower thermal envelopes (Table 8). The two species with the highest RWL

(values around 5) show very different relationships with the environmental variables. *Hypsiboas bischoffi* (RWL = 5.6) show reproductive season more related with rainfall and higher levels of RH% than *H. bandeirantes* (RWL = 5.46; Table 9).

The discriminant analysis using water search strategies as the grouping variable produced similar results from the analysis of RWL, but with more definitive separation of groups (96.24% correct classification) (Figure 11C, Table 10). The environmental variable that provides the best discrimination among groups was also “reproduction dependence to wet season”, presenting much higher values for species that adopt direct search strategy (Table 10). This model was extremely accurate in predicting the actual results, with cross-validated results of 100%. The removal of “reproduction dependence to wet season” variable generated less powerful discriminant functions (Figure 12B, Table 11), but have highlighted the importance of other variables in the background of the first analysis. The first function discriminated mainly *H. bandeirantes* from the species with more definite strategy classes (Figure 12B), and this discrimination was based on rain intensity and variables associated to temperature (Table 11). *Hypsiboas bandeirantes* showed lower values of rain intensity and temperatures during reproduction than species with direct and energetic erratic strategies (Table 12). The second function discriminated mainly

energetic erratic from direct strategy groups (Figure 12B), and almost all variables with impact in the function are related to the presence of water: "rainfall in mm", "dehydration potential of the microenvironment", "mean relative humidity of the day", "water distance "and "height of the water level". Variables "rainfall in mm" and "mean relative humidity of the day" means were higher in species of energetic erratic strategy and variables "water distance" and "height of the water level" were comparatively lower (Table 12).

DISCUSSION

According to several observations in other phylogenetic groups and behavioral contexts (Wilson et al.1994), we found that treefrogs also present interspecific variation in reaction to challenging situations. Moreover, the interspecific variation found in water search strategy showed a very consistent pattern of association to microenvironment choice characterized by differences in water availability during reproductive activity. Species with direct strategy, like *Hypsiboas albopunctatus* and *H. bischoffi*, showed lower activity levels, maintaining water conserving posture for longer times and requiring less trials to find the water source. Otherwise, the energetic erratic strategy group of treefrogs (*Scinax crospedospilus* and *S. hayii*) presented higher levels of locomotor activity and seemed to find water by random trial. As a consequence, energetic erratic species end up finding the water source comparatively sooner.

Suboptimal behaviors occur in many cases by proximate constraints. For example, animals that present excessive activity behave inappropriately when predators are present and strong selection that reinforce high feeding voracity may lead to excessive sexual cannibalism (Brodin and Johansson, 2004; Arnqvist and Henriksson, 1997). In the case of the present study, direct strategy seems to benefit from the water economy associated to the

maintenance of the highly permeable ventral surface protected from exposure to air (Pough et al., 1983). Energetic erratic species, on the other hand, increase the exposure of the ventral surface to air when moving randomly, thus are probably subjected to higher rates of water loss when searching intensely for water sources (Heatwole et al. 1969). The functional trade-off associated with the locomotion to search for water sources and the higher rates of water loss associated to exposure of the ventral surface certainly restraint adaptive evolution. Either way, behavioral correlations can be adaptive; that is, they can reflect alternative behavioral strategies for coping with a variable environment (Hedrick, 2000; Koolhaas, 1999).

To understand how water search strategy differences occur in a natural population, both genetics and environment should be taken into consideration (Dingemanse and Réale, 2005). Although we have not measured the heritability and the impact in terms of *fitness* of the traits measured herein, we can hypothesize a bit about the environmental conditions that lead to strategies variations in the search for water found in these populations. In the case of species with direct strategy, *Hypsiboas albopunctatus* and *H. bischoffi*, the low locomotor activity associated with the water conserving posture when dehydrated might have been selected in environments characterized by episodic but frequent rain events during the

reproductive season. Energetic erratic strategy, otherwise, might have evolved in stable environments, with constant and high water availability during the reproductive season. The maintenance of high locomotor activity levels when dehydrated would be possible especially in environments with high availability of water in the soil and temporary ponds because it would reduce the risk of death by dehydration associated to the accelerated rates of evaporative water loss during locomotion. However, energetic erratic species (*Scinax crospedospilus* and *S. hayii*) were characterized by the lowest levels of association with rainy season, unlike species with direct strategy (*H. albopunctatus* and *H. bischoffi*), that show a breeding season coincident with the peak of annual rainfall. Interestingly, the dependence of *Scinax crospedospilus* and *S. hayii* energetic erratic strategy to a high availability of water is suggested in the second function of discriminant analysis of behavior categories without the variable “reproduction dependence to wet season”. This function showed an association of the energetic erratic search strategy to a higher daily relative humidity, lower dehydration potential of the microenvironment and shorter distances from water sources when compared to direct search strategy.

These results suggest that although the energetic erratic species of *Scinax* maintain their main reproductive activity uncoordinated with the

peak of rain season, they tend to opportunistically call in nights of high rain probability and intensity. In fact, species of *Scinax* from the *S. ruber* group have been frequently described in the literature as opportunist breeders, with a relatively long reproductive season (Bertolucci, 2002). In this way, our data suggest that the direct strategy might have coevolved with a reproductive pattern more associated to the maintenance of a more constant calling behavior coincident with the peak of rain season, while the energetic erratic strategy might have coevolved with opportunistic calling activity triggered by rain events, independently of the peak of rain season. The inclusion of more species is necessary to test this hypothesis properly.

The intermediate behavioral classification of *Hypsiboas bandeirantes* reflect the fact that this species harbors high variation in behavior types, given that almost half of the individuals within this species were classified both as direct and energetic erratic strategists. This configuration could be selected in unpredictable environments in cases of individuals exhibiting limited plasticity for the trait and a poor ability to cope with environmental change, given that the maintenance of substantial variation in behavioral types would result in lower population fluctuation around equilibrium (Bolnick et al., 2003).

Interestingly, interspecific variation in the physiological variable, the RWL, can also be discriminated on the basis on environmental variables.

Moreover, in the same way that “reproduction dependence to wet season” was the major factor discriminating among water search strategies, it also discriminated efficiently interspecific variation in RWL. It is notable that when considered only the species exhibiting lower RWL values, we can observe a positive association between RWL and reproduction dependence to wet season (*H. albopunctatus* < *S. crospedospilus* < *S. hayii*). This result suggests the possibility of an adaptive role of RWL determining the level of association between reproductive activity with occurrence of rainfall. When we excluded the variable “reproduction dependence to wet season” from the discriminant analysis, the first discriminant function highlighted a high discriminant power of microenvironmental relative humidity for RWL, together with distance from the water and mean relative humidity of the day. This function particularly showed that *S. crospedospilus*, the species showing the second lower RWL, despite presenting greater distance from a water body, called at conditions of particularly high relative humidity at different scales, highlighting its need for increased air humidity. Lower “thermal envelope” also indicates that, for reproducing in the spring, *S. crospedospilus*’ microhabitats have comparatively low temperatures. Given that the species with comparatively higher RWL, *H. bandeirantes* and *H. bischoffi*, are closely related (Caramaschi et al., 2013; Faivovich et al., 2005), but display very different patterns of reproduction

dependence to wet season, the patterns of association with environmental characteristics are less clear.

According to Titon Junior (2010), RWL display phylogenetic signal in a broader comparative analysis of anurans from the Atlantic forest. In this way, it is possible that both phylogenetic inheritance and adaptation play important roles in the evolutionary history of RWL and its association to temporal patterns of reproduction. The investigation of these associations in more species of Hylids, mainly within the genus *Hypsiboas*, would be important to understand better these patterns of variation and their evolutionary history.

In conclusion, our results show that species of treefrogs from the same locality in the Atlantic forest differ in water search strategy and RWL. The interspecific variation in strategies and RWL can be highly discriminated by their differences in time and microenvironment conditions of reproduction. The direct strategy pattern of water search behavior, associated with maintenance of water conservative posture when dehydrated for longer time, lower rates of locomotion and more precise detection of water sources, is found mainly in species that concentrate their reproductive activity around the peak of rain season. This species seem to rely on maintenance of calling behavior even in dry nights, given that the probability of rain events is high during this time of the year. Otherwise,

the energetic erratic pattern of water search behavior, associated with high rates of locomotion when dehydrated and random search for water sources, is found in species characterized by more extensive reproductive seasons, and opportunistic calling behavior triggered only by rain events. The only species showing high frequency of both direct and energetic erratic individuals reproduce all year round, and the maintenance of this behaviorally diverse population might be relevant to this extensive breeding season. Interspecific variation in RWL seems to reflect both adaptation to environmental water availability and phylogenetic inheritance, particularly with species characterized by low RWL tending to call at periods of high water availability. Summarizing, even though all animals included in this study belongs to the same family (Hylidae), with similar physiological constraints and syntopically living in an Atlantic rain forest, it was possible to discriminate their occupational pattern by small differences in skin resistance to water loss and by different strategies in the search for water.

ABSTRACT

A longstanding question in ecophysiology is how behavior buffers environmental challenges in animals. Amphibians lose water at high rates through skin and assume risks when searching for water. We tested if five species of treefrogs differ in water search strategy and whether differences in physiology and in behavior would correlate with temporal distribution and microhabitat choice for reproductive activity. We classified strategies as direct or energetic erratic according to individuals' activity finding water in a maze. Temporal distribution and microenvironmental conditions related to water availability were recorded associated to males reproductive activity during a year in a same pond. We observed high interspecific differences in treefrogs activity in search for water. Species from genus *Hypsiboas* demonstrated a clear trend towards direct strategy, taking longer to find water source but being more accurate. Species from *Scinax* genus were energetic erratic, finding water source sooner by an erratic-active search. *Hypsibos bandeirantes*, however, had a generalist behavior, exhibiting both strategies. Discriminant analysis indicated that water search strategies had higher discriminative power when associated to temporal distribution of reproductive activity (96.24% correct classification, 100% cross-validated results), with higher dependence of species from direct search strategy to wet season. Within microhabitat variables, energetic erratic strategies could be differentiated by occupying microhabitats with higher humidity. These results suggest that while species with the direct strategy concentrate activity at the wet season peak regardless of microenvironment conditions, energetic erratic species opportunistically call when UR% is very high, independent of the season. Interspecific

variation in the physiological variable, the skin resistance to water loss (RWL), can also be discriminated on the basis on temporal distribution (89.4% correct classification, 94.5%. cross-validated results). The lower RWL values (*H. albopunctatus* < *S. crospedospilus* < *S. hayii*) are directly proportional to their dependence to wet season and seems to reflect both adaptation to environmental water availability and phylogenetic inheritance, particularly with species characterized by low RWL tending to call at periods of high water availability. Tests on more species are necessary to confirm this pattern.

RESUMO

Desde o início a ecofisiologia tenta entender como o comportamento dos animais tampona os desafios ambientais. Anfíbios perdem água através da pele em taxas elevadas e assumem riscos quando buscam por água. Testamos se cinco espécies de anfíbios arborícolas diferem em na adoção de estratégias na busca de água e se diferenças interespecíficas na fisiologia e no comportamento se correlacionam com distribuição temporal e escolha microhabitat na atividade reprodutiva. Estratégias de busca de água foram classificadas como direta ou energética errática de acordo com a atividade de busca de água dos indivíduos em um labirinto. Foram registradas, também, a atividade reprodutiva dos machos durante um ano e sua associação com condições microambientais e de distribuição temporal relacionadas com a disponibilidade de água em um mesmo charco. Observamos grandes diferenças interespecíficas na atividade de busca de água. As espécies do gênero *Hypsiboas* demonstraram uma clara tendência para a estratégia direta, levando mais tempo para encontrar a fonte de água, em contraposição foram mais precisas. A estratégia das espécies do gênero *Scinax* foi classificada como energética errática, pois costumavam encontrar a fonte de água mais rápido por meio de uma busca ativa e errática. A espécie *Hypsiboas bandeirantes*, no entanto, tem um comportamento generalista, exibindo ambas as estratégias. A análise discriminante indicou que a estratégia tinha maior poder discriminativo quando associado à distribuição temporal da atividade reprodutiva (96.24 % de classificação correta, 100% de confiabilidade na validação cruzada), sendo que espécies tímidas são mais dependentes da temporada de chuva. Dentro de variáveis de microhábitat, a estratégia energética errática poderia ser diferenciadas por ocupar microhabitats com maior umidade relativa.

Estes resultados sugerem que, embora as espécies de estratégia direta concentrem sua atividade no pico da estação chuvosa, elas são independentes das condições do microambiente. Por outro lado, espécies energéticas erráticas se reproduzem quando a umidade relativa é muito alta, independente da temporada. A variação interespecífica na variável fisiológica de resistência da pele à perda de água (RPA) também pode ser discriminada com base na distribuição temporal (89.4 % de classificação correta, 94.5 % de confiabilidade na validação cruzada). Os valores mais baixos de RPA (*H. albopunctatus* < *S. crospedospilus* < *S. hayii*) são diretamente proporcionais à dependência a estação chuvosa e parece refletir tanto a adaptação à disponibilidade de água no ambiente quanto a herança filogenética, particularmente em espécies caracterizadas por baixo RPAE que tende a vocalizar em períodos de alta disponibilidade de água. Testes em mais espécies serão necessários para confirmar esses padrões.

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LEGENDAS DAS FIGURAS

Figure 1. Species studied, in order: *Hypsiboas albopunctatus*, *H. bischoffi*, *Scinax hayii*, *H. bandeirantes*, *S. crospedospilus*. Photos taken by Agustín Camacho.

Figure 2. Schematic picture of the labyrinth

Figure 3. Labyrinth image captured by video camera during water search test.

Figure 4. Scatterplot representing Activity in the 1st experiment as a function of Activity in the 2nd experiment in all species.

Figure 5. Individual measurements of “General Activity” for each species.

Figure 6. Animal’s general activity, by relating “Time to succeed” in hours with the “Number of trials”. The lines are merely illustrative and represent the linear trend, derived from regression lines, of the distribution of points for each species.

Figure 7. Mean relative humidity in two scales and mean rainfall of the days in which each species reproductive activity occurred.

Figure 8. Average of calling males per day and average rainfall per day in each month.

Figure 9. Plot showing the relationship between reproduction dependence to wet season and “Number of trials”. This relationship suggest a linear trend, as derived from regression lines. However, given the small number of species represented in the plot, these trends may not be representative in broader analysis.

Figure 10. Plot showing the relationship between the reproduction dependence to wet season and “Time to succeed”. This relationship suggest a linear trend, as derived from regression lines. However, given the small number of species represented in the plot, these trends may not be representative in broader analysis.

Figure 11. Scatterplots of the discriminant scores from the three linear discriminant analyses (LDA) of the original 13 environmental variables and the grouping variables of: A) species (1, 2, 3, 4, 5); B) resistance to water loss (RWL; 2, 3, 4, 5) and C) water search strategy (-1, 0, +1). Correlations

of the variables with each discriminant function can be found in Tables 3, 4 and 7. Each pool is denoted by a color representing its category in the grouping variable, in the second plot, 2, 3, 4 groups represents *H. albopunctatus*, *S. crospeospilus*, *S. hayii*, respectively and group 5 represents both *H. bischoffi* and *H. bandeirantes*. On the third plot: -1 (direct strategy), 0 (intermediate), 1 (energetic erratic strategy).

Figure 12. Scatterplots of the discriminant scores from linear discriminant analyses (LDA) without “reproduction dependence to wet season” variable for the grouping variables of: A) resistance to water loss (RWL; 2, 3, 4, 5) and B) water search strategy (-1, 0, +1). Correlations of the variables with each discriminant function can be found in Tables 5 and 8. Each pool is denoted by a color representing its category in the grouping variable, in the first plot, 2, 3, 4 groups represents *H. albopunctatus*, *S. crospeospilus*, *S. hayii*, respectively and group 5 represents both *H. bischoffi* and *H. bandeirantes*. On the second plot: -1 (direct strategy), 0 (intermediate), 1 (energetic erratic strategy).

FIGURAS

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

A)

H. albopunctatus

B)

H. bischoffi

C)

H. bandeirantes

D)

S. hayii

E)

S. crospedospilus

Figure 4

Figure 5

Figure 6

Figure 7

A)

B)

C)

Figure 8

A)

B)

Figure 9

LEGENDAS DAS TABELAS

Table 1. Descriptive analysis of each species resistance to water loss (RWL) measured by Titon Junior (2010).

Table 2. Descriptive analysis of average efficiency measures in detecting humidity $\pm$ SD.

Table 3. Descriptive analysis of activity in the search for water and number of individuals classified in each water search strategy for five species of Hylidae.

Table 4. Descriptive analysis of species “n° of trials” and “time to succeed” traits. Reproduction dependence to wet season is the difference between percentage of calling males in the rain season and in drought season.

Table 5. Standardized canonical discriminant function coefficients for species and their structure matrix. *represents the largest absolute correlation between each variable and any discriminant function.

Table 6. Standardized canonical discriminant function coefficients for Resistance to Water Loss (RWL) and their structure matrix.*represents the

largest absolute correlation between each variable and any discriminant function.

Table 7. Standardized canonical discriminant function coefficients for Resistance to Water Loss (RWL) without “reproduction dependence to wet season” variable and their structure matrix.*represents the largest absolute correlation between each variable and any discriminant function.

Table 8. Means and standard deviations of environmental variables for each Resistance to Water Loss (RWL) group used in the analysis.

Table 9. Means and standard deviations of environmental variables for Resistance to Water Loss (RWL) of species *H. bischoffi* and *H. bandeirantes*, both represented as group 5 in the analysis.

Table 10. .Standardized canonical discriminant function coefficients for water search strategy and their structure matrix.*represents the largest absolute correlation between each variable and any discriminant function.

Table 11. . Standardized canonical discriminant function coefficients for water search strategy without “reproduction dependence to wet season” and

their structure matrix.*represents the largest absolute correlation between each variable and any discriminant function.

Table 12. Means and standard deviations of environmental variables for each water search strategy.

TABELAS

Species	RWL
<i>H. albopunctatus</i>	2.54 ± 0.72
<i>H. bischoffi</i>	5.60 ± 1.57
<i>H. bandeirantes</i>	5.46 ± 1.86
<i>S. crospedospilus</i>	3.13 ± 0.01
<i>S. hayii</i>	4.42 ± 0.74

Table 1

Species	mass (g)	activity (trials/h)	n° of trials	Time to succeed (h)	residence time (h)
<i>H. albopunctatus</i> (14)	8.15 ± 1.05	1.52 ± 2.44	3.36 ± 3.13	4.60 ± 2.86	6.52 ± 3.43
<i>H. bischoffi</i> (14)	3.67 ± 0.28	3.49 ± 2.99	4.21 ± 2.39	3.56 ± 3.57	5.94 ± 4.38
<i>H. bandeirantes</i> (16)	1.06 ± 0.20	14.64 ± 17.35	5.31 ± 5.99	2.22 ± 3.05	8.92 ± 3.17
<i>S. crospedospilus</i> (12)	1.58 ± 0.21	11.70 ± 7.26	6.58 ± 5.14	0.71 ± 0.62	5.88 ± 4.90
<i>S. hayii</i> (19)	3.82 ± 0.57	5.61 ± 4.19	6.47 ± 6.19	2.47 ± 2.78	7.21 ± 4.25

Table 2

Species	mass (g)	activity (trials/h)	Energetic Erratic Strategy (n)	Energetic Erratic Strategy (%)	Direct Strategy (n)	Direct Strategy (%)
<i>H. albopunctatus</i> (14)	8.15 ± 1.05	1.52 ± 2.44	1	0.07	13	0.93
<i>H. bischoffi</i> (14)	3.67 ± 0.28	3.49 ± 2.99	2	0.14	12	0.86
<i>H. bandeirantes</i> (16)	1.06 ± 0.20	14.64 ± 17.35	7	0.44	9	0.56
<i>S. crospedospilus</i> (12)	1.58 ± 0.21	11.70 ± 7.26	8	0.67	4	0.33
<i>S. hayii</i> (19)	3.82 ± 0.57	5.61 ± 4.19	13	0.68	6	0.32

Table 3

Species	n° of trials	time to succeed (h)	reproduction dependence to wet season
<i>H. albopunctatus</i>	3.36 ± 3.13 (14)	4.60 ± 2.86 (14)	53.33
<i>H. bischoffi</i>	4.21 ± 2.39 (14)	3.56 ± 3.57 (14)	36.21
<i>H. bandeirantes</i>	5.31 ± 5.99 (16)	2.22 ± 3.05 (16)	10.80
<i>S. crospedospilus</i>	6.58 ± 5.14 (12)	0.71 ± 0.62 (12)	- 4.00
<i>S. hayii</i>	6.47 ± 6.19 (19)	2.47 ± 2.78 (19)	- 7.05

Table 1

species

Variable	Function 1		Function 2	
	Coefficient	Structure matrix	Coefficient	Structure matrix
relative humidity of the microenvironment (%)	0.522	0.635*	-0.460	-.108
mean temperature of the day (°C)	0.820	0.438*	-1.468	.388
water distance (cm)	0.383	0.360*	-0.394	-.282
mean relative humidity of the day (%)	0.301	0.238*	0.071	-.121
rain intensity (rank from 0 to 3)	0.021	0.362	0.964	0.517*
operative temperature (°C)	-0.276	0.387	2.075	0.440*
rainfall (mm)	-0.052	0.051	0.088	.209
vegetation coverage (presence or absence)	0.388	0.346	0.144	.082
n° of days from the last rain	0.032	-0.160	0.415	-.143
dehydration potential of the microenvironment (%/h)	-0.150	-0.144	0.139	-.003
height of the water level (cm)	0.145	0.231	-0.209	-.174
thermal envelope (°C)	-0.113	-0.152	0.069	-.015

Table 2

RWL + reproduction dependence to wet season

Variable	Function 1		Function 2	
	Coefficient	Structure matrix	Coefficient	Structure matrix
reproduction dependence to wet season	-1.271	-0.761*	0.043	0.337
relative humidity of the microenvironment (%)	0.507	0.052	0.501	0.511*
water distance (cm)	0.099	-0.016	0.524	0.498*
height of the water level (cm)	-0.106	-0.034	0.354	0.378*
mean relative humidity of the day (%)	0.235	0.049	0.144	0.274*
thermal envelope (°C)	0.055	-0.002	-0.217	-0.167*
rain intensity (rank from 0 to 3)	0.059	-0.015	-0.429	0.155
mean temperature of the day (°C)	-0.148	-0.073	1.866	0.258
operative temperature (°C)	0.558	-0.076	-1.747	0.182
vegetation coverage (presence or absence)	0.273	0.071	0.206	0.299
rainfall (mm)	0.128	0.067	-0.180	-0.066
dehydration potential of the microenvironment (%/h)	0.137	-0.061	-0.366	-0.166
n° of days from the last rain	0.157	0.036	-0.248	-0.112

Table 3

RWL - reproduction dependence to wet season

Variable	Function 1		Function 2	
	Coefficient	Structure matrix	Coefficient	Structure matrix
relative humidity of the microenvironment (%)	0.592	0.531 [*]	-0.435	0.138
water distance (cm)	0.472	0.471 [*]	-0.266	-0.056
height of the water level (cm)	0.342	0.341 [*]	0.066	0.043
mean relative humidity of the day (%)	0.174	0.301 [*]	0.140	0.064
thermal envelope (°C)	-0.209	-0.161 [*]	-0.116	-0.141
rain intensity (rank from 0 to 3)	-0.529	0.132	0.797	0.679 [*]
mean temperature of the day (°C)	1.824	0.192	-0.619	0.461 [*]
operative temperature (°C)	-1.731	0.116	1.372	0.457 [*]
rainfall (mm)	-0.072	-0.019	0.106	0.283
vegetation coverage (presence or absence)	0.268	0.337	0.343	0.362
dehydration potential of the microenvironment (%/h)	-0.356	-0.203	-0.200	-0.224
n° of days from the last rain	-0.217	-0.080	0.336	-0.192

Table 7

	RWL									
	2.54 (<i>H. albopunctatus</i>)		3.13 (<i>S. crospeidospilus</i>)		4.42 (<i>S. hayii</i>)		(H. bandeirantes + H. bischoffi)		Total	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
rain intensity (rank from 0 to 3)	1.47	0.83	1.25	0.96	1.14	1.04	0.31	0.64	0.85	0.95
n° of days from the last rain	0.20	0.41	0.50	1.00	1.14	2.44	1.25	1.80	0.96	1.84
rainfall (mm)	1.48	2.52	3.00	3.46	6.96	13.22	0.25	1.02	2.68	7.85
mean temperature of the day (°C)	19.06	1.77	18.18	1.21	15.70	3.28	14.97	3.58	16.20	3.46
mean relative humidity of the day (%)	88.18	5.65	94.52	1.53	89.11	3.76	88.01	3.68	88.73	4.30
vegetation coverage (presence or absence)	0.33	0.49	1.00	0.00	0.50	0.51	0.13	0.34	0.33	0.47
operative temperature (°C)	21.07	2.58	18.75	1.71	17.14	4.00	16.22	4.22	17.63	4.15
relative humidity of the microenvironment (%)	76.36	5.35	90.40	5.50	76.42	5.65	74.67	6.16	76.41	6.69
water distance (cm)	149.53	124.31	334.75	120.91	80.03	81.36	123.81	104.30	127.46	115.80
height of the water level (cm)	59.80	25.92	93.75	13.94	35.53	32.24	45.34	36.75	48.01	34.92
dehydration potential of the microenvironment (%/h)	0.37	0.60	-0.18	0.14	0.15	0.34	0.48	0.57	0.32	0.53
thermal envelope (°C)	-1.54	8.37	-4.52	8.92	-0.57	2.94	0.41	5.29	-0.55	5.74
(overall RH) - (microenvironmental RH) (%)	-11.82	5.81	-4.12	5.50	-12.69	6.19	-13.34	6.65	-12.33	6.51
reproduction dependence to wet season	53.33	0.00	-4.00	0.00	-7.05	0.00	16.36	10.67	15.79	22.89

Table 8.

	RWL			
	5.46 (<i>H. bandeirantes</i>)		5.6 (<i>H. bischoffi</i>)	
	Mean	Std. Deviation	Mean	Std. Deviation
rain intensity (rank from 0 to 3)	0.12	0.44	1.00	0.82
n° of days from the last rain	1.48	1.94	0.43	0.79
rainfall (mm)	0.12	0.60	0.71	1.89
mean temperature of the day (°C)	13.91	3.27	18.73	1.51
mean relative humidity of the day (%)	87.73	3.61	89.01	4.02
vegetation coverage (presence or absence)	0.08	0.28	0.29	0.49
operative temperature (°C)	14.92	3.63	20.86	2.67
relative humidity of the microenvironment (%)	72.45	4.69	82.60	3.81
water distance (cm)	110.92	84.81	169.86	155.79
height of the water level (cm)	45.60	40.26	44.43	22.23
dehydration potential of the microenvironment (%/h)	0.45	0.51	0.60	0.79
thermal envelope (°C)	0.60	3.01	-0.29	10.37
(overall RH) - (microenvironmental RH) (%)	-15.28	6.01	-6.41	3.47
reproduction dependence to wet season	10.80	0.00	36.21	0.00

Table 9

strategy + reproduction dependence to wet season

Variable	Function 1		Function 2	
	Coefficient	Structure matrix	Coefficient	Structure matrix
reproduction dependence to wet season	1.097	0.911*	-0.043	0.016
rain intensity (rank from 0 to 3)	-0.107	0.034	0.532	0.595*
mean temperature of the day (°C)	-0.380	0.092	-0.589	0.477*
operative temperature (°C)	0.329	0.095	1.337	0.468*
relative humidity of the microenvironment (%)	0.353	0.011	0.175	0.433*
vegetation coverage (presence or absence)	-0.039	-0.032	0.509	0.421*
rainfall (mm)	0.061	-0.043	0.255	0.270*
dehydration potential of the microenvironment (%/h)	0.287	0.045	-0.004	-0.205*
mean relative humidity of the day (%)	0.051	-0.021	0.319	0.178*
n° of days from the last rain	0.037	-0.038	0.387	-0.168*
thermal envelope (°C)	0.150	-0.004	-0.022	-0.134*
water distance (cm)	0.036	0.026	-0.170	0.076*
height of the water level (cm)	-0.221	0.023	-0.069	0.028*

Table 10

strategy - reproduction dependence to wet season

Variable	Function 1		Function 2	
	Coefficient	Structure matrix	Coefficient	Structure matrix
rain intensity (rank from 0 to 3)	0.653	0.601*	-0.605	0.027
mean temperature of the day (°C)	-0.602	0.578*	-0.067	-0.466
operative temperature (°C)	1.385	0.576*	-0.059	-0.499
relative humidity of the microenvironment (%)	0.127	0.416*	0.337	0.127
n° of days from the last rain	0.347	-0.212*	0.250	0.205
thermal envelope (°C)	0.000	-0.130*	-0.085	-0.036
rainfall (mm)	0.100	0.184	0.845	0.461*
vegetation coverage (presence or absence)	0.409	0.340	0.557	0.449*
dehydration potential of the microenvironment (%/h)	0.048	-0.122	-0.211	-0.443*
mean relative humidity of the day (%)	0.290	0.133	0.191	0.247*
water distance (cm)	-0.060	0.110	-0.580	-0.164*
height of the water level (cm)	-0.099	0.061	0.105	-0.164*

Table 11

	strategy							
	-1		0		1		Total	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
rain intensity (rank from 0 to 3)	1.32	0.84	0.12	0.44	1.15	1.01	0.85	0.95
n° of days from the last rain	0.27	0.55	1.48	1.94	1.04	2.27	0.96	1.84
rainfall (mm)	1.24	2.32	0.12	0.60	6.35	12.26	2.68	7.85
mean temperature of the day (°C)	18.96	1.66	13.91	3.27	16.08	3.17	16.20	3.46
mean relative humidity of the day (%)	88.44	5.11	87.73	3.61	89.94	4.01	88.73	4.30
vegetation coverage (presence or absence)	0.32	0.48	0.08	0.28	0.58	0.50	0.33	0.47
operative temperature (°C)	21.00	2.54	14.92	3.63	17.38	3.76	17.63	4.15
relative humidity of the microenvironment (%)	78.34	5.66	72.45	4.69	78.57	7.54	76.41	6.69
water distance (cm)	156.00	131.64	110.92	84.81	119.22	126.88	127.46	115.80
height of the water level (cm)	54.91	25.35	45.60	40.26	44.49	36.81	48.01	34.92
dehydration potential of the microenvironment (%/h)	0.45	0.65	0.45	0.51	0.10	0.34	0.32	0.53
thermal envelope (°C)	-1.14	8.82	0.60	3.01	-1.17	4.35	-0.55	5.74
(overall RH) - (microenvironmental RH) (%)	-10.10	5.71	-15.28	6.01	-11.37	6.77	-12.33	6.51
reproduction dependence to wet season	47.88	8.16	10.80	0.00	-6.58	1.12	15.79	22.89

Table 12

CONCLUSÃO GERAL

Neste trabalho, encontramos variação intra e interespecífica nos padrões de busca de água em cinco espécies de perereca da Mata Atlântica. A variação interespecífica em padrões de busca de água e resistência da pele à perda de água por evaporação encontra-se relacionada à diversidade de associação da concentração temporal da atividade reprodutiva com o pico de ocorrência anual de chuvas, bem como com a escolha de microambientes para a manutenção da atividade vocal.

As diferentes interações fenotípicas entre características comportamentais e fisiológicas encontradas e associadas ao balanço hídrico neste trabalho incluíram a estratégia de busca de água direta, a estratégia energética errática e as medidas de resistência cutânea à perda de água.

Espécies caracterizadas pelo padrão direto de busca de água (*H. albopunctatus* e *H. bischoffi*), associado à manutenção da postura de conservação de água por longos períodos, baixa taxa de deslocamento e detecção mais precisa de fontes de água, concentram sua atividade reprodutiva no pico da estação chuvosa. Essas espécies aparentemente apresentam comportamento de corte mesmo durante dias relativamente secos, dado que, apesar disso, a probabilidade de chuva nesta data do ano é alta.

No extremo oposto, o padrão comportamental energético errático de busca de água é associado com altas taxas de locomoção e busca aleatória de fontes de água quando desidratados. Essa estratégia é encontrada em espécies caracterizadas por temporadas reprodutivas mais extensas, e comportamento reprodutivo desencadeado somente por eventos de chuva.

A única espécie que mostra alta frequência dos dois padrões comportamentais de busca de água, direto e energético errático, se reproduz o ano todo. A manutenção dessa população estrategicamente diversa pode ser relevante para a temporada reprodutiva extensa que ela apresenta.

Assim, observou-se mais uma vez que mesmo em animais de hábito noturno, e ainda pertencentes à mesma família (Hylidae), com limites fisiológicos semelhantes e vivendo de forma sintópica na Mata Atlântica, é possível discriminar seus padrões ocupacionais por pequenas diferenças na resistência da pele à perda de água por evaporação e por diferentes estratégias na busca por água que afetam a hidrorregulação. Desta forma, pudemos fazer algumas suposições de como a seleção natural teria atuado na interação desses mecanismos de homeostase hídrica no ambiente natural.

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INSTITUTO DE BIOCIÊNCIAS

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São Paulo, 05 de setembro de 2012.

Prezado Senhor

Dirijo-me a V. Sa. para informar que a Comissão de Ética em Uso de Animais do IB (CEUA), em reunião realizada no dia 20 de agosto de 2012, **APROVOU** o Projeto “Variação intra e interespecífica na escolha de microambientes e sua relevância para a manutenção do balanço hídrico em anuros” - **Protocolo 161/2012**, de sua responsabilidade (Colaboradora: Maya Romano Maia).

Lembramos que, para utilização de animais silvestres, o pesquisador responsável deve, ainda, ter/portar as autorizações de coleta, transporte e manutenção emitidas pelos órgãos ambientais competentes.

O pesquisador responsável deverá comunicar à esta Comissão qualquer alteração e/ou intercorrência que houver durante a realização da pesquisa. Além disso, deverá ser encaminhado um breve relatório parcial, ou de finalização, a cada 2(dois) anos.

Atenciosamente,

Prof. Dra. Mariz Vainzof

Coordenadora da Comissão de Ética no
Uso de Animais do IB (CEUA)

Ilmo. Sr.

Prof. Dr. FERNANDO RIBEIRO GOMES

Departamento de Fisiologia do IBUSP.