

WATER BALANCE AND SPATIAL DISTRIBUTION OF AN ANURAN COMMUNITY FROM BRAZIL

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ABSTRACT: Terrestrial amphibians may dehydrate when exposed to low humidity, representing an important factor affecting spatial distribution and community composition. In this study we investigated whether rates of dehydration and rehydration are able to explain the spatial distribution of an anuran community in a Restinga environment at the northern coast of the State of Bahia, Brazil, represented by 11 species distributed in 27 sample units. The environmental data set containing 20 variables was reduced to a few synthetic axes by principal component analysis (PCA). Physiological variables measured were rates of dehydration, rehydration from water, and rehydration from a neutral substrate. Multiple regression analyses were used to test the null hypothesis of no association between the environmental data set (synthetic axes of PCA) and each axis representative of a physiological variable, which was rejected ($P < 0.001$). Of 15 possible partial regressions only rehydration rate from neutral substrate vs. PC1 and PC2, rehydration rate from water vs. PC1, and dehydration rate vs. PC2 were significant. Our analysis was influenced by a gradient between two different groups of sample units: a beach area with high density of bromeliads and an environment without bodies of water with low density of bromeliads. Species of very specific natural history and morphological characters occur in these environments: *Phyllodytes melanomystax* and *Scinax auratus*, species frequently occurring in terrestrial bromeliads, and *Ischnocnema paulodutraei*, common along the northern coast of Bahia and usually found in forest remnants within environments with low number of bodies of water. In dry environments species with lower rates of dehydration were dominant, whereas species showing greater rates of dehydration were found predominantly in microhabitats with greater moisture or abundance of bodies of water.

Key words: Amphibia; Dehydration; Evaporative water loss; Habitat; Rehydration; Water absorption; Water balance

HUMIDITY is one of the main factors affecting the spatial distribution of amphibians (Toft, 1982; Duellman and Trueb, 1986; Giaretta et al., 1997; Bastazini et al., 2007). The association between species distribution and humidity gradients may be related to variations in dehydration tolerance, since species showing low rates of dehydration are in general more terrestrial than species that show greater dehydration rates (Shoemaker et al., 1992; Hillman et al., 2009). Among

amphibians several adaptations can be found to increase dehydration tolerance, including morphological (e.g., cocoon formation to reduce evaporative water loss [EWL]), physiological (e.g., absorption of water from the bladder), and behavioral (e.g., water-conserving posture) adaptations (Wells, 2007). The rate of rehydration, which is greater than the dehydration rate (Burggren and Vitalis, 2005), may also play an important role in amphibian water regulation. Pelvic patches on the ventral body surface are specialized in absorbing large amounts of water in a short period of time (Hillman et al., 2009). Navas et al. (2004) considered the possibility that a smoother, thinner, and more vascularized skin is able to promote more efficiently the uptake of water in frogs from a Brazilian semiarid region

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(Caatinga environment) and, in this case, signaled for rehydration as a key process in water balance.

It is well known that terrestrial animals show a correlation between EWL and humidity. Among vertebrates, reptiles, birds, and mammals inhabiting different habitats possess varying rates of EWL (Hillman et al., 1979; Kattan and Lillywhite, 1989; Eynan and Dmi'el, 1993; Dmi'el et al., 1997; Tieleman et al., 2003; Muñoz-Garcia and Williams, 2005). In amphibians, relationships between water economy and habitat have been studied for more than 100 yr (see Jørgensen, 1997, for review) and recently Hillman et al. (2009), using mostly morphological characters, categorized amphibians according to their habitat use. To date, however, no studies have investigated the rates of dehydration and rehydration in anurans as explanatory variables for patterns of spatial distribution of species within a single amphibian community.

This study aims to test whether rates of dehydration and rehydration are able to explain the spatial distribution of an anuran community. The anuran community studied here was investigated by Bastazini et al. (2007), who found that the distribution of anurans can be explained by changes in the plant community along with variation in soil moisture. These variables influence directly the amount of humid microhabitats available for anurans and therefore influence the distribution of animals within this environmental gradient. Physiological parameters of water balance in the community studied by Bastazini et al. (2007), however, have not been tested.

MATERIALS AND METHODS

Characterization of the Study Sites and Anuran Community Structure

We studied a community of anurans from a contiguous Restinga landscape in the municipality of Mata de São João, State of Bahia, Brazil, the same community investigated by Bastazini et al. (2007). The Restingas are in the Atlantic Forest biome and are comprised of coastal sand dune habitats covered with herbaceous and shrubby vegetation, which is common along the Brazilian coast (Suguio and

Tessler, 1984). The sampling sites included two private areas: "Reserva Sapiranga" and a property in Praia do Forte. The Reserva Sapiranga (12°33'S, 38°02'W, datum = SAD69, 20 m above sea level) includes 500 ha covered by arboreal vegetation, containing bodies of water (springs, streams, rivers, lakes, and ponds) and areas without bodies of water and with low number of bromeliads, as well as sandy areas without bodies of water and with high density of terrestrial bromeliads. The Restinga formation from Praia do Forte (12°34'S; 38°00'W, 10 m above sea level) is characterized by sandy soil covered by many shrubs and terrestrial bromeliads (> 100 bromeliads per sampling plot), and it is continuous with a gallery forest that surrounds a lake formed by the freshwater River Timeantube, the latter filled with emergent vegetation. Both Reserva Sapiranga and Praia do Forte are included in the state environmental protected area APA Litoral Norte.

Since we studied the same anuran community analyzed by Bastazini et al. (2007) and performed the same statistical analyses of community structure, though using a lower number of species, we briefly summarize their methodology herein. Bastazini et al. (2007) sampled 30 plots (P1–P30), measuring 60 × 25 m each, distributed in the Reserva Sapiranga and a coastal area nearby Praia do Forte. They measured 20 environmental variables used in full in the present research: (1) soil moisture, (2) air moisture, (3) air temperature, (4) estimated percentage of water cover in the sample unit (SU), (5) kind of body of water in the SU, (6) maximum width of the water body, (7) maximum depth of the water body, (8) percentage of leaf litter, (9–12) percentage of stratum volume covered by leaves (0–5 m, 6–10 m, 11–15 m, and > 15 m), (13) density of trees, (14) number of terrestrial bromeliads, (15) number of epiphytic bromeliads, (16) bromeliad mean height, (17) bromeliad mean diameter, (18) percentage of bromeliads filled with leaf litter, (19) percentage of bromeliads containing water, and (20) percentage of terrestrial bromeliads in direct sunlight.

Species Studied

Between October 2007 and September 2008, of 30 anuran species found by Bastazini

TABLE 1.—Anuran species used in the analyses. Given are number of specimens used, body mass, surface area (means \pm standard deviations), and the prevailing physiognomy inhabited by each species in the study area following Bastazini et al. (2007).

Species	<i>n</i>	Body mass (g)	Surface area (cm ²)	Prevailing physiognomy
<i>Dendropsophus branneri</i> (Cochran, 1948)	10	0.31 \pm 0.03	3.42 \pm 0.60	Temporary ponds or permanent lakes
<i>Dendropsophus decipiens</i> (Lutz, 1925)	10	0.27 \pm 0.07	3.49 \pm 0.80	Arboreal vegetation with temporary ponds or permanent lakes
<i>Dendropsophus minutus</i> (Peters, 1872)	10	0.76 \pm 0.11	6.95 \pm 1.13	Temporary ponds or permanent lakes
<i>Ischnocnema paulodutraii</i> (Bokermann, 1975)	10	0.86 \pm 0.25	8.71 \pm 3.40	Arboreal vegetation with or without bodies of water on the ground
<i>Leptodactylus natalensis</i> (Lutz, 1930)	8	5.46 \pm 1.68	26.66 \pm 5.33	Arboreal vegetation with rivers, springs, streams, or lakes
<i>Leptodactylus macrosternum</i> (Miranda-Ribeiro, 1926)	10	36.92 \pm 11.08	96.20 \pm 18.71	Arboreal vegetation with rivers, springs, streams, or lakes
<i>Phyllodytes melanomystax</i> (Caramaschi, Silva and Brito-Pereira, 1992)	10	1.0 \pm 0.1	10.15 \pm 1.09	Beach zone (terrestrial bromeliads)
<i>Rhinella jimi</i> (Stevaux, 2002)	10	341.54 \pm 137.88	414.64 \pm 109.25	Forest borders and open habitats
<i>Scinax auratus</i> (Wied-Neuwied, 1821)	10	0.79 \pm 0.07	7.92 \pm 0.86	Beach zone (terrestrial bromeliads) and permanent lakes
<i>Scinax eurydice</i> (Bokermann, 1968)	8	7.38 \pm 0.68	39.27 \pm 2.51	Arboreal vegetation with rivers, springs, or streams
<i>Scinax</i> sp. (<i>S. ruber</i> species group)	10	2.07 \pm 0.54	14.67 \pm 2.98	Beach zone (terrestrial bromeliads)

et al. (2007), we were able to collect 11 species in sufficient numbers to perform our physiological experiments (Table 1).

Only visibly healthy animals were collected (license numbers 13001–1 and 2239548, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis), stored in plastic bags, and transported to the Laboratory of Animal Physiology, Federal University of Bahia. The specimens were kept for 7 consecutive days in a terrarium containing a petri dish with water ad libitum and another with moistened paper towel. No food was offered during the laboratory experiment. After the experiments, all animals were deposited in the Zoology Museum of the Federal University of Bahia. Collection and handling of the animals followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research as revised by the Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists.

Data Collection

To measure the rate of EWL of the animals, we assembled a system containing 10 cham-

bers made of plastic bottles of 500 mL (6.3-cm diameter by 18.5-cm length) with an entrance and an exit for air at the extremities. Inside the bottles we placed the frog inside a cage made of plastic with a 2-mm mesh, so that the entire body surface of the frog resting on this surface would be exposed to the airflow (EWL). The specimens of *Rhinella jimi* were kept in chambers with a diameter of 19.4 cm and 15 cm in height. To measure EWL of individuals, each animal, 24 h after its capture, was cleaned, dried using paper towel, and weighed on a semianalytical scale (± 0.01 g, Shimadzu), and then placed inside an experimental chamber. The animals were weighed while inside the cage and afterward the weight of the empty cage was subtracted to obtain the weight of the animal. Using an OXIBOLHA pump, room air was pumped through a column of silica gel to remove humidity. The dry air was directed by silicone tubing toward the experimental chambers, receiving the dried air at its narrower end, passing along the animal, and leaving the chamber at its rear end. Airflow rate was maintained at 110.0 ± 5.0 mL/min and controlled before and after the dehydration process using a mass flow

meter (TR-SSI SABLE SYSTEMS). Larger animals were weighed every 60 min, whereas smaller animals (body mass < 0.30 g) were weighed every 20 min. The dehydration was interrupted when an animal had lost 20% of its initial body mass (Burggren and Vitalis, 2005; Cartledge et al., 2006), or after 7 h of dehydration. No animal died during the dehydration procedures, and a loss of 20% body mass due to dehydration is easily handled by many amphibians (e.g., Churchill and Storey, 1995). Our data on surface-area-related water loss are well within the range reported for other anuran species (e.g., Withers et al., 1984; Barbeau and Lillywhite, 2005; Tattersall et al., 2006; Young et al., 2006). We considered also measuring EWL using an agar model. Although an agar model would provide a comparison with our EWL measurements on living animals, the model would not accurately represent key aspects of the animal's morphology and physiology, or reflect water balance of the entire animal (Navas et al., 2004) within its ecological context.

Immediately after dehydration, the rate of rehydration (Re) was determined using two substrates: water and a neutral substrate. Half the animals, one at a time, was placed into a container containing distilled water (Re_{H_2O}), immersing the ventral half of the animals. In two 30-min intervals, the animals were removed, dried on a paper towel, and weighed. The other half of the animals was subjected to rehydration from a neutral substrate (Re_{Sub}), where a piece of foam (6.6-cm thick) was wetted with 1 L of distilled water and kept in recipients 16.4 cm in diameter. After 30 and 60 min, the animals were removed from the substrate, dried on a paper towel, and weighed. Two days after the first dehydration, the animals were dehydrated again following the same protocol, but rehydrated using the substrate not used during the first rehydration.

The total surface area of each animal, or only one-third of this, was used to standardize the rates of dehydration and rehydration, respectively (Table 1). After the experiments, the animals were anesthetized and killed using lidocaine chlorhydrate, fixed in formalin (10%), and their integument removed (except

hands and feet). The integument was stained in methylene blue, cut into pieces that would lay out on a flat surface, and photographed at 5-megapixel resolutions using a Sunfire Z50 digital camera at a distance of 17.5 cm. The total surface area of a specimen was determined from the digital photographs, transforming the pictures into black and white pixels using the program PhotoPaint®, adjusting visually the tonal “threshold” of each colored photograph. We calculated the percentage of black pixels representing integument. Black and white photographs were saved in TARGA format, and then we used an ad hoc developed program, “area,” for calculating the total number of black pixels in each photograph (Camacho and Rocha, 2010).

Data Treatment

To calculate EWL, we used measures of initial (M_{BI}) and final body mass (M_{BF}) in micrograms, time, and surface area: $EWL = (M_{BI} - M_{BF}) \text{ min}^{-1} \text{ cm}^{-2}$. To calculate Re_{H_2O} and Re_{Sub} , the values of initial body mass (M_{BI}) and final (M_{BF}) were standardized by time and surface area: Re_{H_2O} or $Re_{Sub} = (M_{BI} - M_{BF}) \text{ min}^{-1} \text{ cm}^{-2}$. For specimens that were weighed more than once during dehydration and rehydration the average rates of dehydration and rehydration were calculated ($\text{mg H}_2\text{O min}^{-1} \text{ cm}^{-2}$).

To test for phylogenetic signal in our physiological data we used the PDAP package for Mesquite (Maddison and Maddison, 2010; Midford et al., 2010). A phylogenetic tree (Appendix 1) was constructed using data published by Faivovich (2002), Faivovich et al. (2005), Frost et al. (2006), and Frost (2011), applying the branch length method of Grafen (1989). Running the PDAP diagnostic chart on log-transformed values of mean body mass vs. log-transformed values of mean dehydration or log-transformed values of mean rehydration, three different regressions of contrasts through the origin were generated.

To analyze the physiological data we tested the null hypotheses (H_0) of absence of difference between EWL on the first and second days of dehydration, and between rehydration from water and neutral substrate, by the paired *t*-test ($H_0 = \text{the mean of the}$

difference between variables is zero). The null hypotheses of absence of association between body mass (independent variable) with rehydration from water and neutral substrate (dependent variables), and between the former with dehydration (dependent variable) were tested by linear regression analyses. The H_0 of no difference between the responses of rehydration from water and from neutral substrate (dependent variables) in relation to variations in body mass (independent variable) was tested by comparing regression lines using the F -test (Sokal and Rohlf, 1981; Vanzolini, 1993). All tests were two-tailed, and performed on log-transformed data. We tested the assumptions of normality and homoscedasticity for linear comparisons with the Shapiro–Wilk’s test and the Levene’s test, respectively, and found that they were not violated in any comparison. The critical value of significance was set to $P \leq 0.05$, and was corrected by the Bonferroni method (Bland, 2004) when the same set of data was used in comparisons.

To test the assumption that the pattern of spatial distribution of the anuran community obtained by Bastazini et al. (2007) may be represented by only 11 of the 30 species sampled originally, we prepared a data matrix of 30 lines (plots) vs. 11 columns (species), called matrix of abundance (matrix A), representing the dependent variable of analysis. We produced a transformed abundance matrix, calculated from matrix A by dividing each cell by the line sum to reduce differences in weight of SUs in analyses. We used the nonmetric multidimensional scaling method (NMS), an ordination technique, to seek and display the strongest anuran community structure using only the anuran species data set (matrix B; McCune and Grace, 2002). We selected only one dimension (ordination axis) to the NMS solution, and used the Sorensen distance measure. To avoid the local minima problem, we applied 50 starting configurations, using as stability criteria the instability value of 0.0005, 20 iterations to evaluate the stability of the solution, and 500 as the maximum number of iterations. The Monte Carlo test was used to evaluate whether NMS extracted a stronger axis than expected by chance. The proportion of variance represent-

ed by the NMS axis, on the basis of the correlation between distance in the ordination space (Euclidian distance) and distance in the original space (Sorensen distance), was obtained by the standardized Mantel test (r). The null hypothesis of no association between the NMS axis obtained in Bastazini et al. (2007) and the NMS axis obtained here was tested by the Pearson correlation coefficient (r ; Sokal and Rohlf, 1981). Assumptions of normality and homoscedasticity were tested with the Shapiro–Wilk’s test and the Levene’s test, respectively, and were not violated. The critical value of significance was set to $P \leq 0.05$.

To test whether rates of dehydration and rehydration are able to explain the spatial distribution of the anuran community, four matrices were produced: three matrices with 27 lines (plots) vs. 1 column (physiological variables), representing the dependent variables of the analysis (matrices D, E, F), and a matrix of 27 lines (plots) vs. 20 columns (environmental variables) representing the independent variables of the analysis (matrix G). Three plots (P22, P25, P27) of the 30 original SUs used by Bastazini et al. (2007) were discarded because they did not contain specimens of the 11 species used in this study. Each cell value of a physiological variable in matrices D–F (dehydration, rehydration from neutral substrate, and rehydration from water, respectively) was the average value of the respective variable in the plot (average value of the plot—AVP). To calculate the AVP we followed three steps. In the first step, we calculated a standard value for each physiological variable per anuran species, namely species average standard (SAS), computed as the arithmetic mean of the values obtained for each species. We combined the data from the first and second dehydration calculating a single value of EWL of each specimen from the arithmetic mean of the values of EWL. In the next step, we calculated the partial value of the plot (PVP) by multiplying, for each plot, the number of specimens of each species found by Bastazini et al. (2007) by the respective SAS. The third and last step was the computation of the AVP. The AVP was the sum of the resulting PVP values of a same plot divided by the total number of specimens of

TABLE 2.—Measurement data for 11 anuran species used in this study. EWL first day and second day = evaporative water loss during the first and second days of treatment, respectively; Re_{sub} and Re_{H_2O} , rehydration from a neutral substrate and from water, respectively. Data are presented as mean \pm SD.

Species	EWL first day (mg min ⁻¹)	EWL second day (mg min ⁻¹)	Re_{sub} (mg min ⁻¹)	Re_{H_2O} (mg min ⁻¹)	EWL first day (mg min ⁻¹ cm ⁻²)	EWL second day (mg min ⁻¹ cm ⁻²)	Re_{sub} (mg min ⁻¹ cm ⁻²)	Re_{H_2O} (mg min ⁻¹ cm ⁻²)
<i>Dendropsophus branneri</i>	1.69 \pm 0.35	1.31 \pm 0.30	3.00 \pm 2.72	4.73 \pm 2.46	0.45 \pm 0.11	0.37 \pm 0.10	2.77 \pm 2.34	3.97 \pm 1.91
<i>Dendropsophus decipiens</i>	1.07 \pm 0.28	1.38 \pm 0.63	2.45 \pm 1.40	3.10 \pm 2.51	0.30 \pm 0.07	0.41 \pm 0.23	2.21 \pm 1.35	2.59 \pm 1.93
<i>Dendropsophus minutus</i>	1.52 \pm 0.52	1.73 \pm 0.64	1.67 \pm 0.87	4.11 \pm 1.88	0.22 \pm 0.07	0.26 \pm 0.13	0.73 \pm 0.40	1.78 \pm 0.69
<i>Ischnocnema paulodutraii</i>	2.58 \pm 0.85	2.13 \pm 0.62	2.60 \pm 1.73	5.50 \pm 2.32	0.27 \pm 0.07	0.24 \pm 0.08	0.86 \pm 0.44	1.94 \pm 0.83
<i>Leptodactylus natalensis</i>	3.74 \pm 0.24	3.33 \pm 0.29	9.33 \pm 5.53	23.62 \pm 6.70	0.12 \pm 0.01	0.11 \pm 0.01	0.87 \pm 0.51	2.21 \pm 0.62
<i>Leptodactylus macrosternum</i>	6.18 \pm 0.42	5.82 \pm 0.55	11.97 \pm 5.27	55.62 \pm 26.76	0.07 \pm 0.01	0.06 \pm 0.01	0.39 \pm 0.22	1.68 \pm 0.64
<i>Phyllodytes melanomystax</i>	3.17 \pm 0.65	2.38 \pm 0.49	3.62 \pm 3.80	3.32 \pm 0.95	0.31 \pm 0.08	0.23 \pm 0.06	1.11 \pm 1.16	0.99 \pm 0.30
<i>Rhinella jimi</i>	10.50 \pm 0.80	9.25 \pm 0.58	33.64 \pm 34.10	123.88 \pm 69.08	0.02 \pm 0.00	0.02 \pm 0.00	0.23 \pm 0.19	1.00 \pm 0.75
<i>Scinax auratus</i>	1.13 \pm 0.16	1.08 \pm 0.10	1.85 \pm 0.87	2.63 \pm 0.95	0.14 \pm 0.02	0.14 \pm 0.03	0.75 \pm 0.35	1.02 \pm 0.37
<i>Scinax euridice</i>	2.61 \pm 0.24	2.83 \pm 0.21	8.73 \pm 8.05	33.29 \pm 12.02	0.07 \pm 0.01	0.08 \pm 0.00	0.68 \pm 0.65	2.51 \pm 0.80
<i>Scinax</i> sp. (<i>S. ruber</i> species group)	2.07 \pm 0.32	2.01 \pm 0.54	4.40 \pm 1.95	10.22 \pm 2.72	0.14 \pm 0.03	0.13 \pm 0.04	0.89 \pm 0.34	2.08 \pm 0.37

the plot, thereby giving greater weight to the species most numerous in the plot. An ordination axis for each physiological variable was obtained by ordering the cells (plots) in a crescent way from left to right.

We used principal component analysis (PCA) to reduce the environmental data set (matrix G) to a smaller number of orthogonal synthetic variables that represent most of the original information. The principal components were obtained from a correlation matrix among characters, and those with eigenvalues larger than 1.0 were rotated to a new Varimax solution (McCune and Grace, 2002). The null hypothesis of absence of association between the environmental data set (principal components) and the anuran community composition represented by each physiological variable (dependent variables) was tested by multiple regression analysis (Sokal and Rohlf, 1981), and the coefficient of determination (R^2) indicated how much of the variability of a physiological variable can be explained by its relationship to the environmental data set. The α level ($P \leq 0.05$) was Bonferroni corrected (Bland, 2004) for multiple and partial regression analyses developed with the same set of subjects.

RESULTS

Testing for phylogenetic signal within our dehydration and rehydration data, the PDAP regression of contrasts resulted in significant two-tailed P -values of 0.00001 for $\log-M_B$ against \log -EWL and $P = 0.007$ for $\log-M_B$ against $\log-RE_{sub}$, whereas the regression of contrasts of $\log-M_B$ against $\log-Re_{H_2O}$ was not significant ($P = 0.87$).

The mean values of EWL as well as Re_{H_2O} and Re_{sub} for each anuran species are presented in Table 2. The paired t -test for first and second days of dehydration was not significant ($t = 0.76$, $n = 11$, $P < 0.47$), not rejecting the null hypothesis of absence of difference for the EWL between the first and second days of treatment. These results led us to assume that the relationships are the same (first vs. second day of treatment), and therefore we combined data from the first and second dehydrations. The linear regression of the overall dehydration rate (dependent variable) on body mass (independent

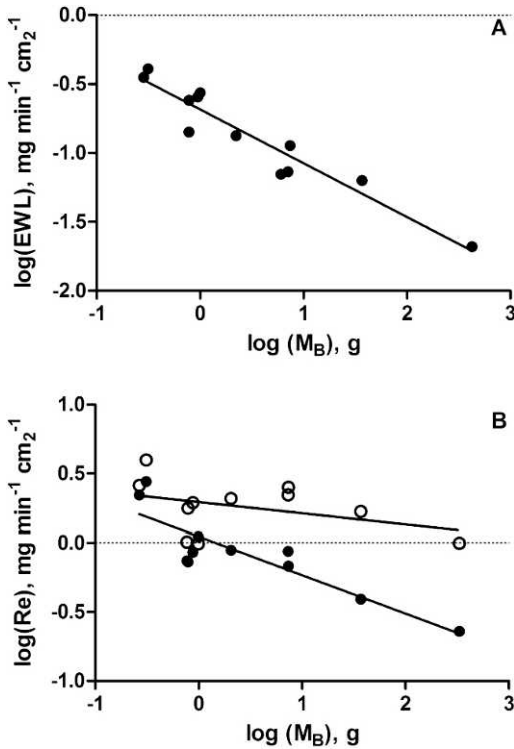


FIG. 1.—(A) Linear regression of evaporative water loss (EWL) of anuran species studied. (B) Linear regressions of rates of rehydration from water (Re_{H₂O}, open circles) and from a neutral substrate (Re_{Sub}, filled circles).

variable) was highly significant ($F_{1,9} = 22.71$, $r = -0.84$, $P < 0.001$), rejecting the H_0 of absence of dependence of the former on the latter, and denoting a higher dehydration rate in smaller anurans (Fig. 1A).

The paired t -test for Re_{H₂O} and Re_{Sub} was significant ($t = 4.78$, $n = 11$, $P < 0.0007$), rejecting the null hypothesis of absence of difference between the rehydration substrates. This result led us to analyze these variables separately. The linear regression of Re_{Sub} on body mass was significant ($F_{1,9} = 30.29$, $r = -0.82$, $P < 0.0004$), rejecting the H_0 of no dependence of the former on the latter, and denoting a higher rehydration rate of small-sized anurans on a neutral substrate. On the other hand, the regression of Re_{H₂O} on body mass was not significant ($F_{1,9} = 1.67$, $r = -0.39$, $P < 0.23$), not rejecting the H_0 of no dependence of the former on the latter, which did not permit us to make further compari-

sons between regression lines to test the H_0 of absence of difference between the response of Re_{Sub} and Re_{H₂O} in relation to body mass (Fig. 1B).

The NMS axis was able to express structure in the community. The stress associated with NMS axis was 20.3; the variance represented by the NMS axis explained 71% of the variance in the original multidimensional space (Mantel test: $r = 0.84$, $P < 0.001$). The Monte Carlo test resulted in a probability of 0.02, indicating that the NMS extracted a stronger axis than expected by chance. The NMS axis ordered the anuran community through an environmental gradient that reflects the same sequence of physiognomies obtained by Bastazini et al. (2007): (1) beach zone, characterized by sandy soil covered by many shrubs and terrestrial bromeliads; (2) arboreal vegetation with temporary ponds or permanent lakes; (3) arboreal vegetation with rivers, springs, or streams; and (4) arboreal vegetation without bodies of water on the ground. The premise that the 11 anuran species used herein as attributes are able to adequately represent the structure of the original data (30 species in Bastazini et al., 2007) was tested by the Pearson product-moment correlation coefficient, and was accepted ($R^2 = 0.94$, $n = 27$, $P < 0.0001$).

The PCA applied to 27 plots (objects) and 20 environmental variables (attributes) generated five principal components with eigenvalues greater than 1.0 (Table 3). They represented 80.8% of the total variance after Varimax solution, and the overall result coincided with that of Bastazini et al. (2007) using 30 plots. The PC1 accounted for 39.3% and presented high loadings (≥ 0.7) on the following variables, in decreasing order: (1) percentage of stratum volume covered by leaves 6–10 m, (2) percentage of leaf litter, (3) percentage of terrestrial bromeliads in direct sunlight, (4) number of terrestrial bromeliads, and (5) soil moisture; for PC2 (18.8%): (1) bromeliad mean height, (2) bromeliad mean diameter, (3) maximum width of the water body, and (4) maximum depth of the water body; for PC3 (9.9%): stratum volume covered by leaves > 15 m; for PC4 (7.2%): air temperature; and for PC5 (5.6%): percentage of stratum volume covered by leaves 0–5 m.

TABLE 3.—Loadings from the principal component analysis (after Varimax rotation) for 20 environmental variables of 27 combined sample units from the municipality of Mata de São João, state of Bahia, Brazil. Loadings ≥ 0.7 are in bold.

Environmental variables	PC1	PC2	PC3	PC4	PC5
% stratum volume covered by leaves: 6–10 m	0.91	0.14	0.10	0.23	0.17
% leaf litter	0.90	0.12	0.20	0.11	0.19
% terrestrial bromeliads in direct sunlight	-0.89	-0.17	0.16	0.06	-0.01
Number of terrestrial bromeliads	-0.86	-0.18	-0.13	-0.07	0.02
Soil moisture	0.84	0.23	-0.09	-0.21	-0.12
Bromeliad mean diameter	0.09	0.89	0.12	0.09	0.10
Bromeliad mean height	-0.20	0.89	0.16	0.10	0.11
Maximum width of the water body	0.41	0.79	0.16	0.09	0.08
Maximum depth of the water body	0.39	0.76	-0.09	0.08	0.18
% stratum volume covered by leaves: >15 m	0.15	0.20	0.88	0.13	0.02
Air temperature	0.19	0.11	0.06	0.93	-0.12
% stratum volume covered by leaves: 0–5 m	0.12	0.33	0.10	-0.15	0.84
Density of trees	0.67	-0.19	0.22	0.07	0.52
% bromeliads containing water	-0.63	-0.04	-0.14	0.26	0.22
% stratum volume covered by leaves: 11–15 m	0.54	0.07	0.63	-0.13	0.25
Kind of body of water in the sample unit	0.52	0.62	-0.23	0.26	0.08
Air moisture	0.45	-0.09	-0.69	-0.23	-0.19
% water cover in the sample unit	0.36	0.61	0.42	0.02	-0.11
Number of epiphytic bromeliads	0.31	0.60	0.43	0.05	-0.19
% bromeliads filled with leaf litter	-0.28	0.41	0.29	0.67	0.04
% total variance	39.30	18.85	9.88	7.16	5.57
Eigenvalues	7.86	3.77	1.98	1.43	1.11

The null hypotheses of no association between the environmental data set (independent variables: principal components with eigenvalues ≥ 1.0) and the community structure represented by each physiological variable (dependent variables) were tested using multiple regression analyses, which were strongly significant for Re_{H_2O} ($F_{5,21} = 13.64$, $R^2 = 0.76$, $P < 0.00003$), Re_{Sub} ($F_{5,21} = 10.58$, $R^2 = 0.72$, $P < 0.00012$), and rate of dehydration ($F_{5,21} = 5.6821$, $R^2 = 0.54$, $P < 0.005$; P -values Bonferroni corrected). Nonetheless, of 15 possible partial regressions only four were significant (P -values Bonferroni corrected): Re_{Sub} vs. PC1 and PC2 ($F_{1,25} = 42.66$, $r = -0.79$, $P < 0.0001$; $F_{1,25} = 16.40$; $r = -0.63$, $P < 0.002$; respectively), Re_{H_2O} vs. PC1 ($F_{1,25} = 74.99$, $r = 0.86$, $P < 0.0001$), and dehydration rate vs. PC2 ($F_{1,25} = 23.93$, $r = -0.70$, $P < 0.0002$; Fig. 2).

DISCUSSION

Our results showed that EWL and Re_{Sub} contain phylogenetic signal when plotted against M_B , indicating that the rate of dehydration and the capacity to reabsorb water may vary among different taxa, possibly due to variations in skin resistance to dehy-

dration (Wells, 2007) or due to differences in size of the pelvic patch or number of aquaporins in the skin (Ogushi et al., 2010), respectively. Re_{H_2O} , on the other hand, did not reveal such a correlation, which could be caused by the small number of species analyzed. Since Blomberg et al. (2003) showed that the statistical power of a permutation test for detecting phylogenetic signal increases with the number of species analyzed, and that statistical power is largest in studies using 20 or more species, our results have to be treated cautiously and we therefore limit our discussion to our ecological and physiological findings and refrain from discussing phylogenetic influences on dehydration or rehydration until a larger set of data becomes available.

The rate of dehydration showed a positive association with PC2 (Fig. 2A), in which the variables bromeliad height, bromeliad diameter, maximum width, and maximum depth of the bodies of water were the environmental variables with the highest contributions (Table 3). The plots with the lowest rates of dehydration were those found in environments without bodies of water and low density of bromeliads, consequently of low humidity. The most abundant species in these plots was

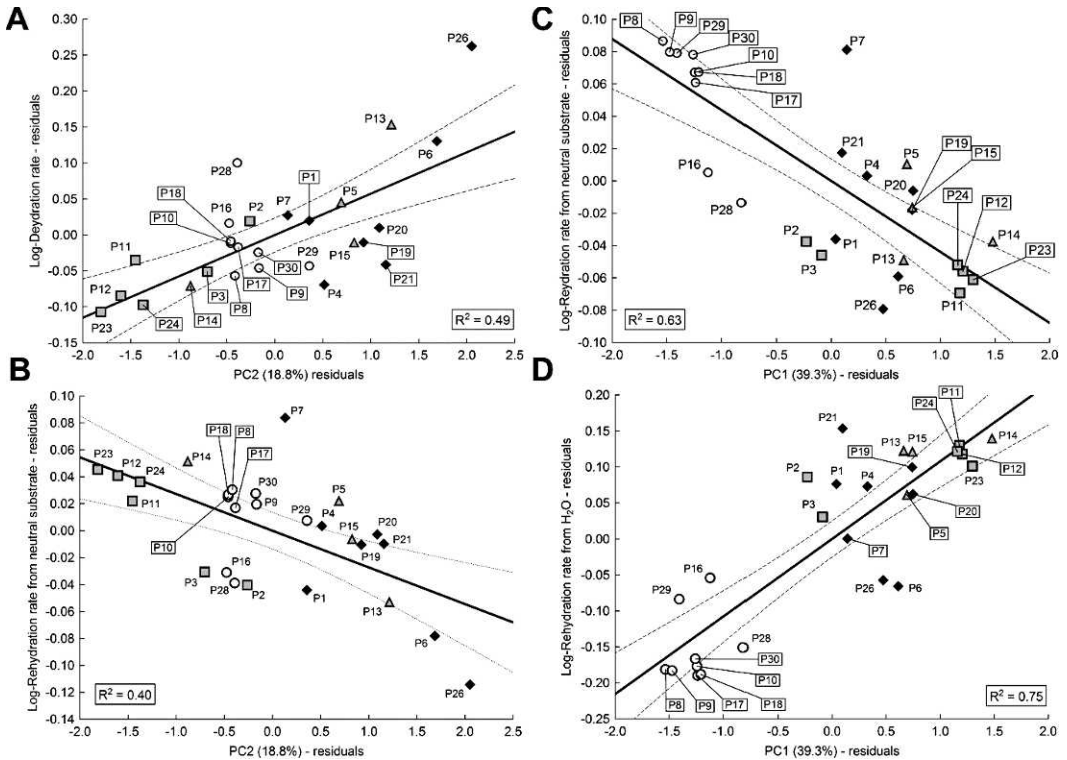


FIG. 2.—Projections of partial regression of dependent physiological variables on principal component (PC)2 (A and B) and PC1 (C and D). Symbols represent types of environments: open circle, beach zone characterized by sandy soil covered by many shrubs and terrestrial bromeliads; closed diamond, arboreal vegetation with temporary ponds or permanent lakes; gray triangle, arboreal vegetation with rivers, springs, or streams; gray square, arboreal vegetation without bodies of water on the ground.

Ischnocnema paulodutrai ($n = 79$; referred as *I. ramagii* in Bastazini et al., 2007), a common species usually found in forest remnants of the northern coast of Bahia State within environments with few bodies of water (M. F. Napoli, pers. obs.). As other Terrarana, this species undergoes direct development and terrestrial breeding (Hedges et al., 2008; Napoli et al., 2009). This mode of reproduction allows these species to breed in environments deprived of water bodies (Xavier and Napoli, 2011), which, in turn, combined with its low dehydration rates, could explain, in part, its ability to occupy distinct types of environments. The second group of plots showing the lowest rates of dehydration was that of the beach area (sandy soil, absence of bodies of water, and high density of terrestrial bromeliads). These plots were dominated by *Phyllocladus melanomystax* and *Scinax auratus*,

species restricted to bromeliads in this dry environment. Animals found in plots located in more humid environments showed higher rates of dehydration. We can assume that in dry environments species with lower rates of dehydration were dominant, whereas species showing greater rates of dehydration were found predominantly in microhabitats with greater moisture or abundance of bodies of water.

Rehydration rate was greater than dehydration rate, which has been noted previously (Burggren and Vitalis, 2005). In general, rehydration from the water was so fast that the animal recovered the body mass lost during dehydration in 30 min of rehydration (most of the animals in this study) and some animals tried to get out of the water at the end of the rehydration period. Rehydration rate showed clear physiological gradients both with

PC1 and PC2 (Fig. 2B–D). The environmental variables with the greatest contribution to PC1 are, like the most important environmental variables from PC2, all directly or indirectly related to environmental humidity (Table 3). Decreasing rates of rehydration from neutral substrate and increasing rates of rehydration from water were obtained from the beach area to fully forested areas: beach (open habitats of sandy soil with terrestrial bromeliads as the only water supply), open habitats of lentic environments bordered by forests, lotic environments inside forests, and forests without bodies of water (Figs. 2C,D). In the beach zone of shrubby vegetation with direct sunlight terrestrial bromeliads are the unique source of water supply to maintain the minimum physiological requirements of an anuran. These bromeliads may contain abundant water during rainy seasons, but very often only retain moisture between their leaves. In this kind of microhabitat, the capacity to rapidly absorb water will benefit an anuran, and species with these capacities could use this microhabitat as a permanent refuge or reproduction site. This statement agrees with Wells (2007), who affirmed that some amphibians possess adaptations that reduce EWL, whereas others are restricted to moist microhabitats, as they have little or no ability to regulate EWL. The plots of the beach zone were dominated by *P. melanomystax* (74 specimens) and *S. auratus* (13 specimens; Bastazini et al., 2007), the former always obtained from terrestrial bromeliads. Species of the genus *Phyllodytes* use bromeliads during their entire life cycle, reproducing and feeding inside the plant axils (bromeligen species sensu Peixoto, 1995), which explains the apparent restriction of *P. melanomystax* to the shrub *Restinga* of Praia do Forte (Bastazini et al., 2007). Our results pointed to the plots of beach zone as those with the highest rates of rehydration from a neutral substrate and with the lowest rates of rehydration from water, favoring animals that live in environments with high hydric stress, like the inhabitants of terrestrial bromeliads of the beach zone.

Regarding the relationship of rehydration rate from neutral substrate and the PC2 (Fig. 2B) we found the greatest rates of rehydration

in anurans from environments without bodies of water and with low densities of bromeliads (P11, P12, P23, P24), indicating a very low availability of free water. In such circumstances a great capacity to absorb water from humid substrates like moist soil would be of significant advantage. It would be very interesting to study the amount of aquaporins in the ventral region of these species, since Ogushi et al. (2010) demonstrated that the expression of aquaporins varies among amphibians, as well as regarding the study of Bentley and Heller (1964), which showed significant variation in neurohypophyseal hormones among amphibians, hormones necessary to control the action of aquaporins. In species from dry habitats, more aquaporins in the pelvic patch region or a larger pelvic patch area would increase the animals' ability to absorb water from a substrate, whereas in amphibians in direct contact with water fewer aquaporins are probably necessary to regulate water balance. This could partially explain the discrepancy found in our data, with rehydration from the neutral substrate being greatest in animals from the beach zone together with rehydration from water being lowest in the same animals. Since water over most of the year is scarce in this habitat, animals are well adapted to conditions of shortage of water. Once water is abundantly available, animals may not be able to cope appropriately with excess water and endocrinologically reduce water absorption. Morphological studies carried out on the same anuran community studied herein demonstrated that the system of lymph sacs showed significant variation among species (Browne, 2008). The author reported that *P. melanomystax* and *S. auratus* have proportionally the largest lateral lymph sacs of all species of the community, occupying 20% of the total area covered by lymph sacs. Large lateral lymph sacs could be a consequence of the body being flattened dorsoventrally (reducing the lateral size of the body and increasing the lymph sac surface). A body flattened dorsoventrally probably facilitates the intrusion of an individual between the bracts of bromeliads, allowing the large lateral lymph sacs to absorb and accumulate greater quantities of water. *Scinax auratus* is a typical species of beach

zones, often inhabiting marginal vegetation of permanent and temporary ponds, and the presence of this species does not appear to be the result of primary adaptations to a life in bromeliads or in environments with low soil humidity. However, morphological characteristics of this species like dorsoventral flattening and a large area of lateral lymph sacs may enable their survival in the bromeliad tank. Species of the genus *Phyllodytes*, on the other hand, possess additional features pointing to an evolutionary history related to bromeliads, developing specific life-history traits to a life in these plants, such as the deposition of eggs in each axil of a bromeliad, which reduces competition among neonates (Schneider and Teixeira, 2001). The flattened body and the area covered by lymph sacs in *Phyllodytes* probably evolved in aquatic environments different from bromeliads or could characterize an ancestral state. Genera belonging to the clade of *Phyllodytes* (e.g., *Trachycephalus* Tschudi, 1838; *Corythomantis* Boulenger, 1896, and *Aparasphenodon* Miranda-Ribeiro, 1920; Faivovich et al., 2005) consist of species that reproduce in aquatic environments, although they may use bromeliads and other similar microhabitats.

Morphological and physiological traits that lead to the reduction of EWL also will be useful to species that inhabit temporary ponds at open habitats or permanent ponds with great variability in water level during seasonal fluctuations. In the present study, species from plots within open areas of lentic environments bordered by forests presented intermediary rates of rehydration when compared with those from plots at the beach zone and full forested areas. In the same way, it will be useful for species that inhabit plots of full forested zones with permanent bodies of water or humid soils to present high rates of rehydration from water and low rates of rehydration from neutral substrates, as observed in the present study. It is important to notice that species that inhabit dry forested areas, like *I. paulodutraei*, showed a low rate of rehydration from a neutral substrate. Someone could argue that a high rate of rehydration from a neutral substrate will not be favorable to this species in a drier soil to absorb the available water. However, a high rehydration

rate would be worthless without any significant reduction in the rate of dehydration and indeed, plots at these environments presented the lowest dehydration rate, minimizing water loss. Nevertheless, when rehydrating from water, the same plots presented the highest rates of rehydration, indicating that the animals absorb greater quantities of water from the environment when water is abundantly available, suggesting plasticity regarding water absorption under different osmotic stresses.

Bastazini et al. (2007) identified a pattern of spatial distribution of species in the anuran community studied herein and identified five environmental variables able to explain part of the community structure. Browne (2008) investigated the spatial distribution of anurans in the same community and showed that the morphology of the lymph sacs can aid to explain some aspects of the species distribution. The study of physiological variables related to water balance in anurans, combined with the research mentioned above, led to the understanding of how extrinsic factors (environmental variables) may limit the spatial distribution of anurans over an environmental gradient by locally favoring species with certain morphological and physiological attributes. The integration of ecological, morphological, and physiological data is essential to answer questions like why some species can be found in a given environment and why an animal is dominant in an environment, as well as to indicate directions for future studies.

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APPENDIX

