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Full Length Research Paper

Acidity of tank bromeliad water in a cloud forest, Cusuco National Park, Honduras

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Phytotelmata are plant-held water bodies housing complex aquatic invertebrate communities drawing attention for their suitability as breeding sites for disease bearing mosquitoes, and the unique fauna occurring in these habitats. Despite the human and scientific relevance, relatively little information is available on the water characteristics in these habitats and these scarce references consist only of isolated point measurements. To begin filling this knowledge gap, we collected high resolution data series of the acidity and temperature of tank water from bromeliads in the cloud forest of Cusuco National Park, Honduras. Average bromeliad water remained acidic for the duration of this study and fluctuated between 4.3 ± 0.1 and 5.5 ± 0.5. Extreme pH values measured as high as 9.3 and as low as 3.3. Water temperature varied between 14.8 ± 0.2°C and 19.2 ± 0.2°C. We found strong diel fluctuations in water condition increasing in maximum and minimum values together with an increase in acidity as the water evaporated. The variation in water temperature and pH were both strongly correlated with the size (total weight) of the bromeliad. The presence of highly unstable environments with significant variation between neighboring plants is a potential crucial element driving aquatic animal community structure in these aquatic habitats.

Key word: Phytotelmata, bromeliads, maximum, minimum, mosquitoes

INTRODUCTION

Bromeliaceae is a predominantly neotropical plant family that consists primarily of epiphytes, growing on a wide range of substrates. A large number of bromeliad species have leaves organized in rosettes that overlap tightly and form a concave "tank" which collects rainwater and organic material such as plant debris and dead invertebrates. The organic material that falls into these water bodies provides the main source of food for these plants as it begins to rot and decompose, unlocking important nutrients. The activity of aquatic invertebrates present in these water bodies often further reduces the size of this organic material. These aquatic habitats represent miniature ecosystems and are well known for the presence of complex animal communities (Kitching, 2000; Greeney, 2001), consisting mainly of aquatic invertebrates (Frank and Lounibos, 2009) but also providing breeding sites for many species of frogs and salamanders (Wells, 2007).

There has always been a certain interest in phytotelmata (= plant held water bodies), partly because of the unexpected high diversity of species adapted to these habitats, but also as breeding sites for disease (for example, dengue and yellow fever) bearing mosquitoes such as *Aedes aegypti* (Linnaeus). More recently, these habitats have also drawn attention as model systems for ecological and evolutionary research (Srivastava et al., 2004). Despite this human and scientific value, basic water characteristics and the extreme values that these variables can reach are still largely unknown. The first significant limnological study on bromeliad water was completed by Laessle (1961) characterizing the aquatic habitat, including the pH and associated macro-invertebrate fauna in Jamaican bromeliads. Laessle recorded environmental variables in 75 bromeliads throughout Jamaica and provided herewith a robust glimpse into these habitats. Data however was only

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Table 1. Literature review summary of previous bromeliad water investigations for pH conditions.

Study	Year	Bromeliad	#	M	Max	Min	AVRG	STDEV	Comments	Country
Laessle	1961		5		7	4				Jamaica
Benzing	1972	*Aechmea bracteata*			8.4	3.8	4.6			
Eterovick	1999	*Aechmea saxicola*	3	1			4.8	0.3	no frogs	Brazil
		Aechmea victoriana	7	1			5.2	0.6	no frogs	
		Vriesea neoglutinosa	11	1			5.3	1.3	no frogs	
		Unidentified sp.	9	1			6	0	no frogs	
De Oliveira and Nava	2004	*Vriesea* sp. and *Nidularium* sp.	64		6.5		4.6	0.4	no frogs	
							5.5	1.4	with frogs	
Janetzky	2005	*Aechmea paniculigera*	74	1	6.9	4.3	6	0.4	dry season	Jamaica
		Hohenbergia sp.		1			5.8	0.3	rainy season	
Souza et al.	2006	*Nidularium cruenta*	8	1			4.6	0.8	in the sun	Brazil
		Nidularium nidicaulis	8	1			5.6	0.9	in the sun	
		Nidularium cruenta	8	1			5.5	0.4	in the shade	
Torreias et al.	2008	*Guzmania brasiliensis*	12	1			4	0.1	April	Brazil
		Guzmania brasiliensis	12	1			6	0.6	September	
Lopez et al.	2009	*Aechmea nudicaulis*	8	5			5	0.8	rinsed	Brazil

collected once or twice during a single diurnal period giving little insight in diel variability, extreme values and long-term fluctuations. Another major contribution to the field was the work from Benzing et al. (1972), continuing the study from Laessle (1961) and also seeking whether the observed acidic water conditions were caused by the plant itself or by tank components. Benzing and colleagues used one bromeliad species *Achmea bracteata* (Swartz) and compared water chemistry between rinsed plants filled with distilled water and those with only certain tank components added such as oak leaf litter, algae (*Spirogyra* sp.) or aquatic invertebrates. Four measurements every 24 h were performed, providing the first insight into diurnal fluctuations. Extreme pH values in these experimental settings were 3.8 and 8.4. In addition to these two major contributions, isolated information on the acidity of bromeliad water can be found scattered across the literature, which we have summarized in Table 1. The data presented in these publications are primarily single point measurements; the same plants were seldom revisited for multiple readings.

In small water bodies with unstable environmental characteristics, inhabitants are required to possess a high degree of tolerance to variability and only those able to withstand the most extreme values will persist (Williams, 2006). Therefore in bromeliads, pH may serve as a keystone community structuring factor. Since it is the occasional extreme condition that could express a greater effect upon long-term survival than the average condition, single point measurements are of limited value in ecological studies. In order to create a thorough understanding of the conditions in these aquatic habitats, a substantial number of measurements must be collected from the same locations over an extended period of time. High-resolution environmental characterization is an integral component of understanding the ecology of the organisms living in a particular habitat. Previously, the researcher had to manually record each measurement, making the collection of standardized long-term data extremely labour-intensive and which has resulted in the production of incomplete snapshots, particularly for small and variable environments. Today, the availability of a variety of electronic remote data loggers has made it possible for scientists to establish standardized long-term environmental studies able to provide a kaleidoscope of important ecological environmental data. Demonstrating this point, we collected high resolution data on the pH and water temperature of tank bromeliads in the cloud forest of Cusuco National Park, Honduras by using arrays of programmed data loggers. At a variety of locations across the forest, we measured water temperature and pH values in 30 min intervals over a period of four to 28 days. In this paper we present the average and extreme values and illustrate the fluctuations observed during 1,486 h of sampling. To gain some insight in the factors affecting variation in pH, we explored the effect of bromeliad size, attachment height on the tree (from ground level to 34 m) and precipitation on the microenvironment in these bromeliads.

MATERIALS AND METHODS

Data was collected from 12 July to 9 August, 2009 in Cusuco National Park (CNP), Honduras. CNP is situated in north-western Honduras, within the Meréndon mountain range. The core zone of the park consists of lower montane tropical rain forest (both primary and secondary), with patches of primary cloud forest and upper montane rain forest at the higher regions of the park (up to 2245 m), characterized by high densities of bromeliads. We collected information on water temperature and pH in bromeliads at multiple locations in the Park. The bromeliads sampled were *Tillandsia guatemalensis* LB Smith and *Catopsis* sp. identified according to the Flora of Nicaragua (Stevens et al., 2001). Each data logger (PHTEMP101, Madgetech, Inc.) was affixed with two independent probes; one for temperature (RTDS-4-3/16-6-36, Madgetech, Inc.) and the other for pH (pH1, Madgetech, Inc.) (Figure 5). Data loggers were programmed to record both values in 30 min intervals. The bromeliads sampled were separated into three study groups: “tree”, “cluster” and “time series”. Information on the number of measurements, length of recording time and the number of bromeliads monitored in each group is presented in Table 2.

The "tree" dataset comprised data loggers placed at three height intervals (low-mid-high) on nine trees equally divided into three clusters in the Park. The low heights encompassed those between the forest floor and 5 m above the ground. The mid heights were those between 5 and 15 m up and the highest level included everything above 15 m. At each level a data logger to measure pH and water temperature in the bromeliad was placed. Each assemblage included three trees located within 30 m of one another at the three different sites across the Park. These sites were classified as Cantiles Camp (CA) (N15 30.791, W88 14.500, 1828 m elevation), Cortecito Camp (CO) (N15 31.392, W88 16.965, 1397 m elevation) and Base Camp (BC) (N15 29.674, W88 12.813, 1546 m elevation). The data loggers in BC were set up in three *Pinus* sp. trees (Pinacea) in old secondary mixed forest. In CA and CO, data loggers were placed in Liquidambar trees (Altingiaceae) within undisturbed primary broadleaf. The highest bromeliad sampled measured 34.1 m above the ground and was located in one of the CO trees included in the "tree" dataset. All data loggers were affixed at height by using the double rope climbing technique.

The "cluster" dataset is comprised of an assemblage of five bromeliads located in very close proximity to one another and all within 2.5 m of the ground. This was replicated in two locations. The first cluster was close to BC on the top of a small hill (N15 30.130, W88 12.716) in disturbed secondary forest with dense undergrowth and a patchy distribution of bromeliads; the second was close to CA on a mountain ridge at 1979 m altitude (N15 31.124, W88 14.675) in pristine broadleaf forest with high bromeliad density. The "time" series was produced by a single bromeliad at CA situated in a *Liquidamber* tree.

Analyses were performed on data only from those loggers which produced reliable measurements. Data logger output was evaluated based on two criteria. First, all off-scale readings (pH > 14) or excessively high temperatures (> 200°C), likely produced by electronic interruption of the probes, resulted in the exclusion of the entire data set collected by that particular data logger. Second, before and after the deployment of data loggers, calibration was assessed by exposing the probes to pH 4.01 buffer solution at ambient air temperature for approximately two hours. If the probes failed to produce accurate readings, the data set produced by those loggers was also excluded from analyses. This screening process resulted in the exclusion of 11 of the 40 data sets produced (Table 2).

During this study, we also collected information on the attachment height of the bromeliad on the tree and the plant weight. To determine the weight of the plant, the bromeliad was dismantled leaf by leaf and then all leaves were washed to remove debris. A 200 g PESOLA scale was used to measure the weight of several leaves together and then combined to determine the total weight of the plant. Precipitation was measured nearby the "tree" study sites (BC, CO and CA) with a pluviometer and this daily precipitation is indicated in each graph with a bar chart. The height of the bar represents the relative volume of precipitation captured during that particular data collection interval.

From the selected data, we calculated averages, maximum and minimum values for both pH and water temperature. In the analyses we used standard deviation of the averages as a measure for variation. We explored patterns and extreme readings in the datasets by correlating the environmental data with height of the bromeliad on the tree and size (weight) of the bromeliad. We also compared variation with patterns of precipitation. Data was examined visually using MadgeTech 2.00.74 software and graphs were made with SIGMAPLOT 9.0. Data exploration and statistics was performed in STATISTICA 9.0 (Statsoft, Inc, 2009). We used the nonparametric Kruskall Wallis tests to consider the variation in measured environmental param with the different height characteristics. General Regression Models (GRM) were used to test correlations between variables. We also tested for the presence of a significant relationship between the environmental factors and the attachment height after removing the variance in the dataset caused by the size of the bromeliad. We here fore applied a linear constrained multivariate analysis (RDA) CANOCO 4.5 (Ter Braak and Smilauer, 1998), with species data (environmental factors) centered and standardized, bromeliad size as covariable and 999 Monte Carlo permutations.

RESULTS

The recorded values for water temperature and pH in the bromeliads are presented in Table 3. The average pH in the bromeliads always remained acidic and fluctuated between 4.3 ± 0.1 and 5.5 ± 0.5. Extreme pH values recorded measured 9.3 in a bromeliad in BC (Cluster dataset) and 3.3 in a bromeliad at the top of a tree at BC (Tree dataset). The average water temperature observed in the bromeliads was between 14.8 ± 0.2°C and 19.2 ± 0.2°C (Table 3). The maximum water temperature recorded was 31°C, but normally fluctuated between 17.2 ± 0.6°C and 20.7 ± 0.4°C (Table 3). Minimum water temperature measured was 13.8°C, and more commonly remained between 13.5 ± 0.2°C and 17.4 ± 0.3°C.

The measurements for pH and water temperature

Table 2. Bromeliad locations and dataset characteristics. Information on the location, study site elevation, name of data series, total number of bromeliads monitored and the number used in the analyses (in brackets), length of the recording time and the number of measurements collected are presented.

Location	Elevation (m)	Data series	#	Time	Measurements
Basecamp (BC)	1641	Tree set	9(7)	7 days	338
Cantiles (CA)	1831	Tree set	9(6)	7 days 23 hours	383
El Cortecito (CO)	1493	Tree set	9(6)	3 days 20 hours	185
Basecamp (BC)	1623	Cluster	5(5)	10 days 22.5 hours	526
Cantiles (CA)	1979	Cluster	5(4)	4 days 1 hour	195
Cantiles (CA)	1831	Time series	3(1)	28 days 4 hours	1353

throughout the study period are visually represented in Figures 1 to 3 ("Tree" dataset) and Figure 4 ("Cluster" dataset and "Time" dataset). For the "Tree" dataset, the figures are grouped per camp; BC (Figure 1), CA (Figure 2) and CO (Figure 3). In each figure, the three graphs each represent a relative attachment height of the bromeliads on the trees studied, the uppermost one being the highest in the tree.

Vertical size (total weight) distribution of bromeliads was unequal on the tree, with the biggest bromeliads located near the midsection (Figure 5). These bromeliads were significantly larger than plants closer to the ground ($p = 0.019$, $H = 2$, $N = 20$) or the canopy ($p = 0.025$ $H = 2$, $N = 20$). Water temperature variability showed an inverse relationship with bromeliad size (Table 4). As bromeliads decreased in size, their water also became more acidic (Table 4). In this study, average water temperature was not correlated with bromeliad size.

The correlation between temperature and pH readings with tree attachment height was analyzed both as a continuous and categorical (three class) variable. Analysis revealed no correlation or difference in values between categories (Table 4). As such, we were unable to detect a correlation between bromeliad attachment height and water condition after removing the variation attributed to the bromeliad’s size (Table 5).

DISCUSSION

Average water condition in the studied bromeliads always remained acidic, dropping as low as pH 3.3 with occasional fluctuations reaching as high as 9.3, but with large differences in pH between bromeliads in close proximity to one another.

Based on the environmental contrasts between the upper canopy and forest floor, partly due to sun and wind exposure, we expected to find considerable differences in the environmental conditions of bromeliad water. However, we were unable to detect any relationship between bromeliad attachment height and the measured or derived variables considered. The greatest variation in the dataset was predominately dictated by the size of the bromeliad rather than attachment height. There was a strong negative correlation between water temperature variability and bromeliad size, with diel fluctuations reducing as the bromeliad increases in size. This is to be expected, as a larger volume of water should possess an increased buffering capacity against changes in air temperature. In addition, the higher number and greater leaf size of larger plants may function as a physical shield and additionally buffering the plants' contents from changing weather conditions. Larger bromeliads are usually older and it is also possible that these plants have reached a greater balance in the composition of their micro-organismal communities.

Many factors contribute to the pH of bromeliad water (Benzing et al., 1972). The most important are thought to be the microorganisms inhabiting the bromeliad tank, the organic material which falls into the tank, and the activity of the plant itself, but little is known about their relative influence and how their contributions change over time. Information about the microorganisms in bromeliads is scant; few studies have elucidated microbial diversity and processes within phytotelmata, although they are expected to play an important role. Bromeliad waters are rich in nutrients and provide a suitable environment for the development of complex communities of aquatic microorganisms including bacteria (Rivera et al., 1988), yeasts (Hagler et al., 1993; Landell et al., 2009), algae (Bermudes and Benzing,

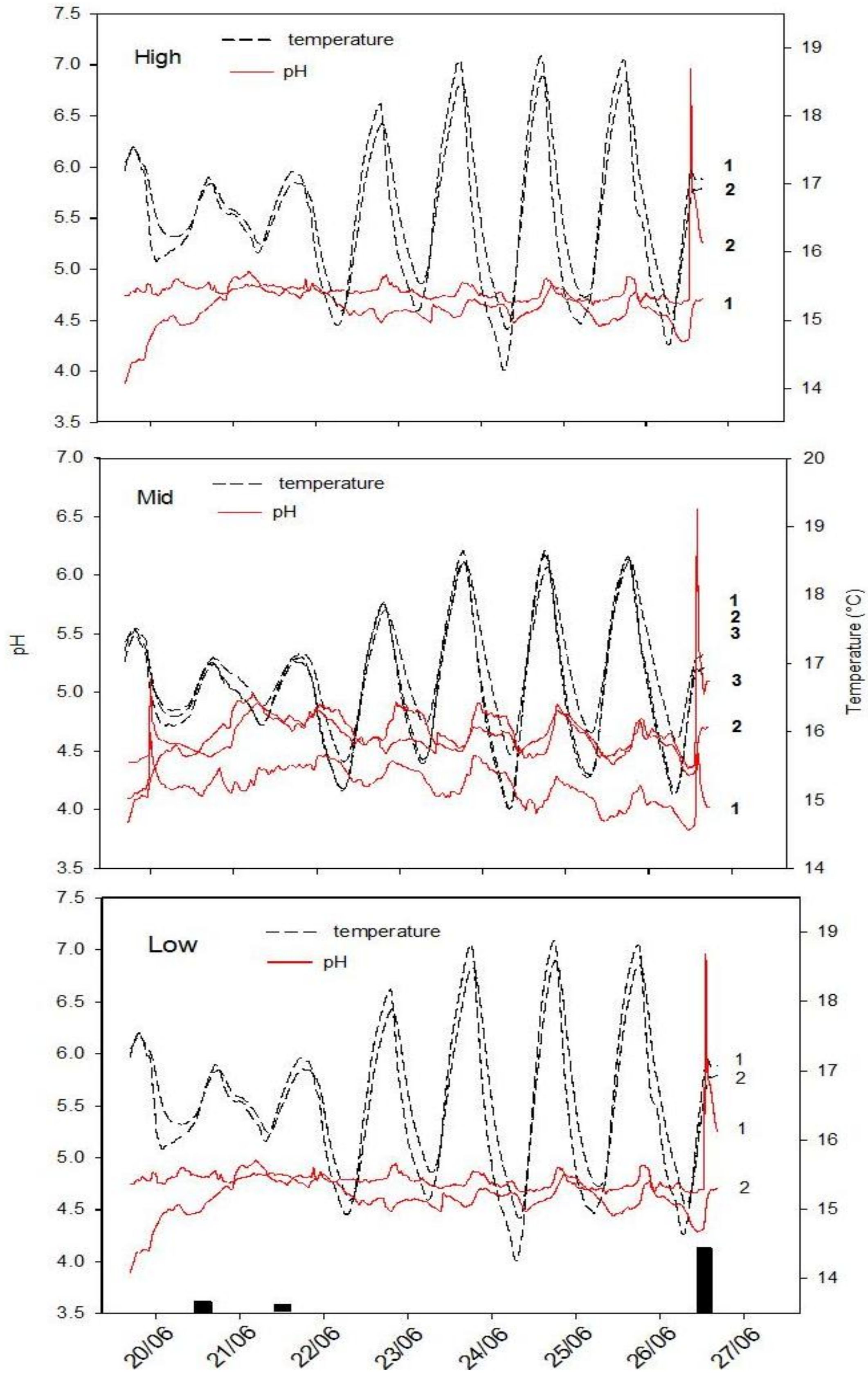

Figure 1. Water temperature and pH measured in bromeliads at BC which were part of the "tree" series. Numbers link the temperature and pH in the individual bromeliads. Black bars give a relative indication of precipitation during the data collection.

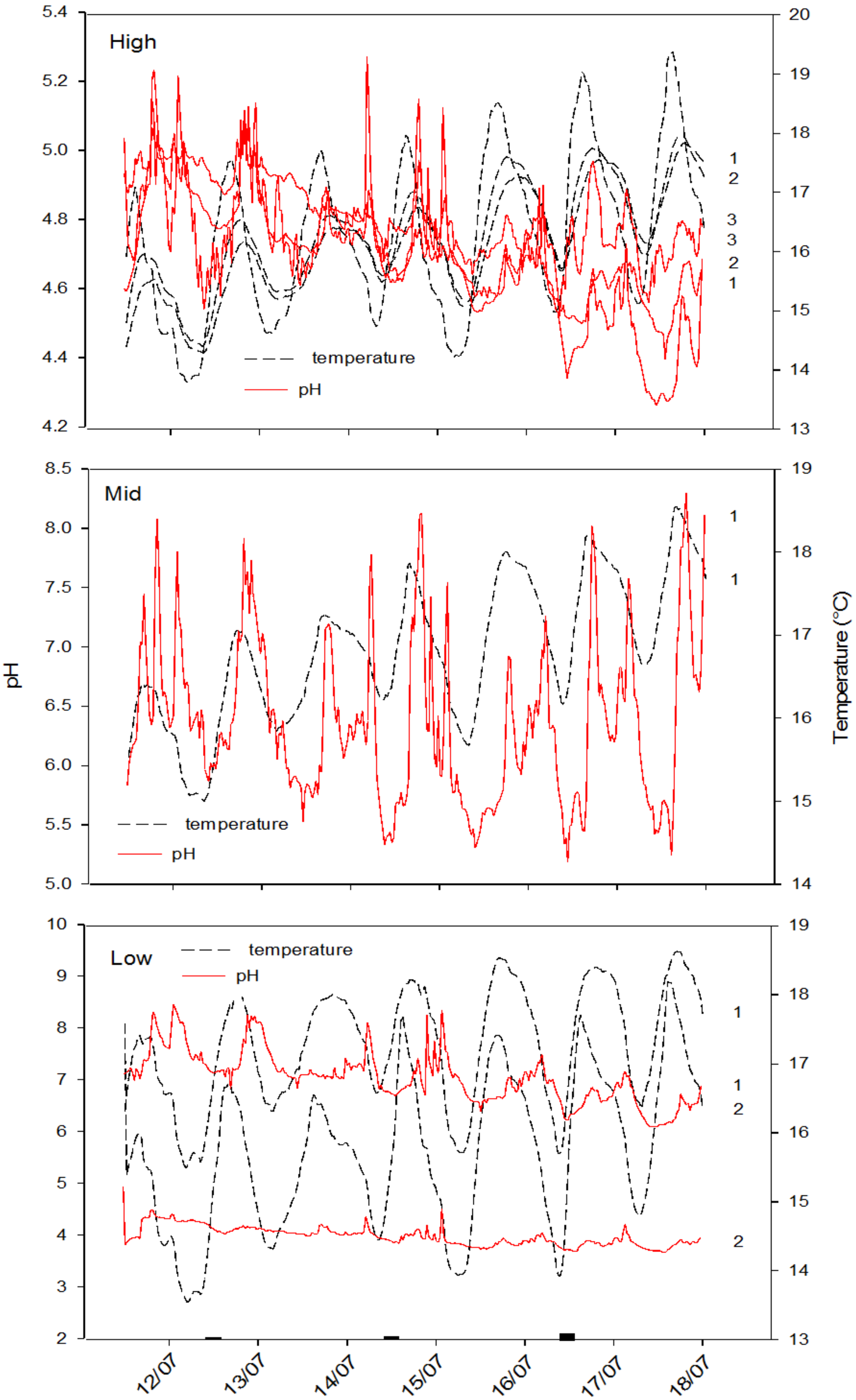

Figure 2. Water temperature and pH measured in bromeliads at CA which were part of the "tree" series. Numbers link the temperature and pH in the individual bromeliads. Black bars give a relative indication of precipitation during the data collection.

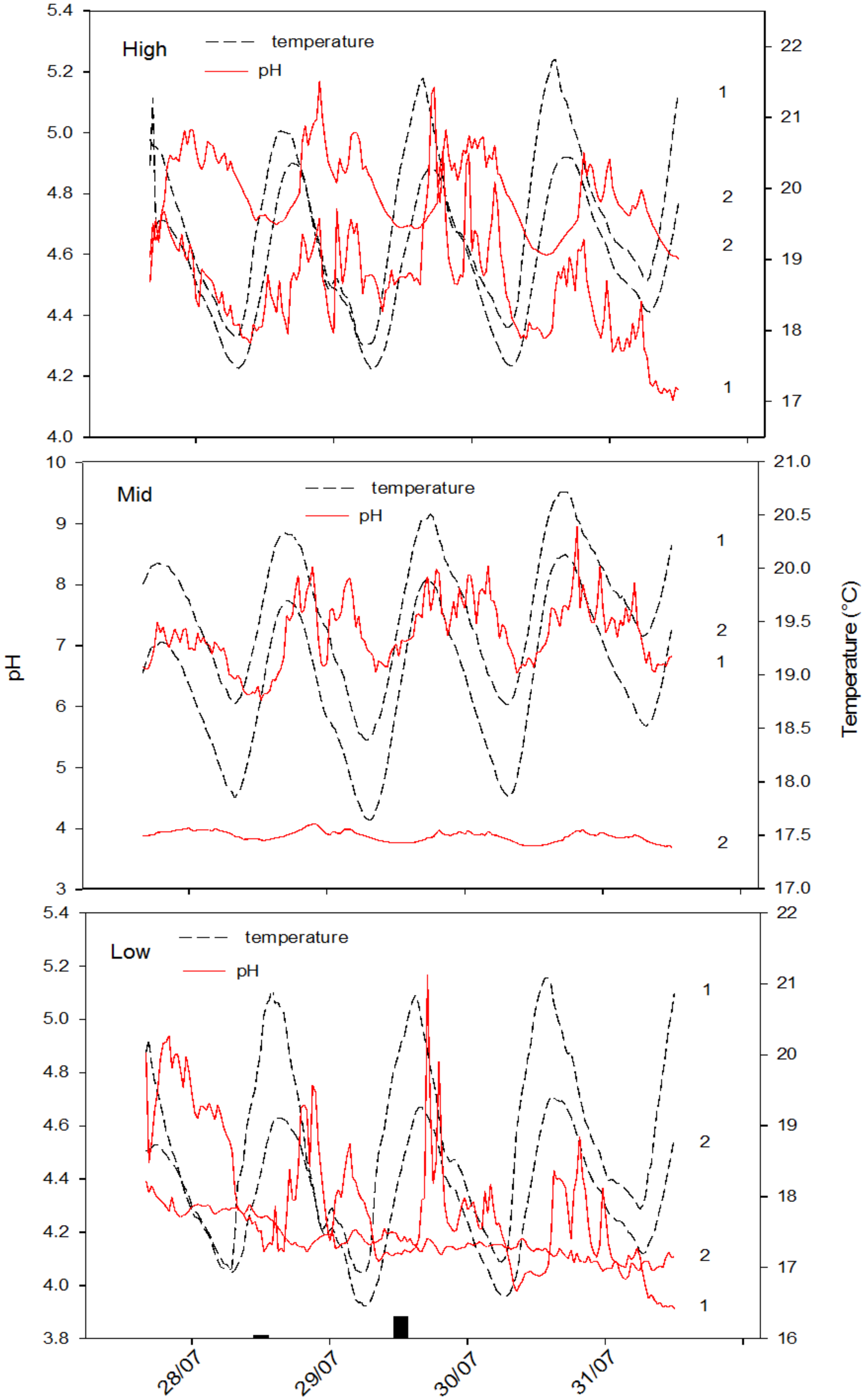

Figure 3. Water temperature and pH measured in bromeliads at CO which were part of the "tree" series.. Numbers link the temperature and pH in the individual bromeliads. Black bars give a relative indication of precipitation during the data collection.

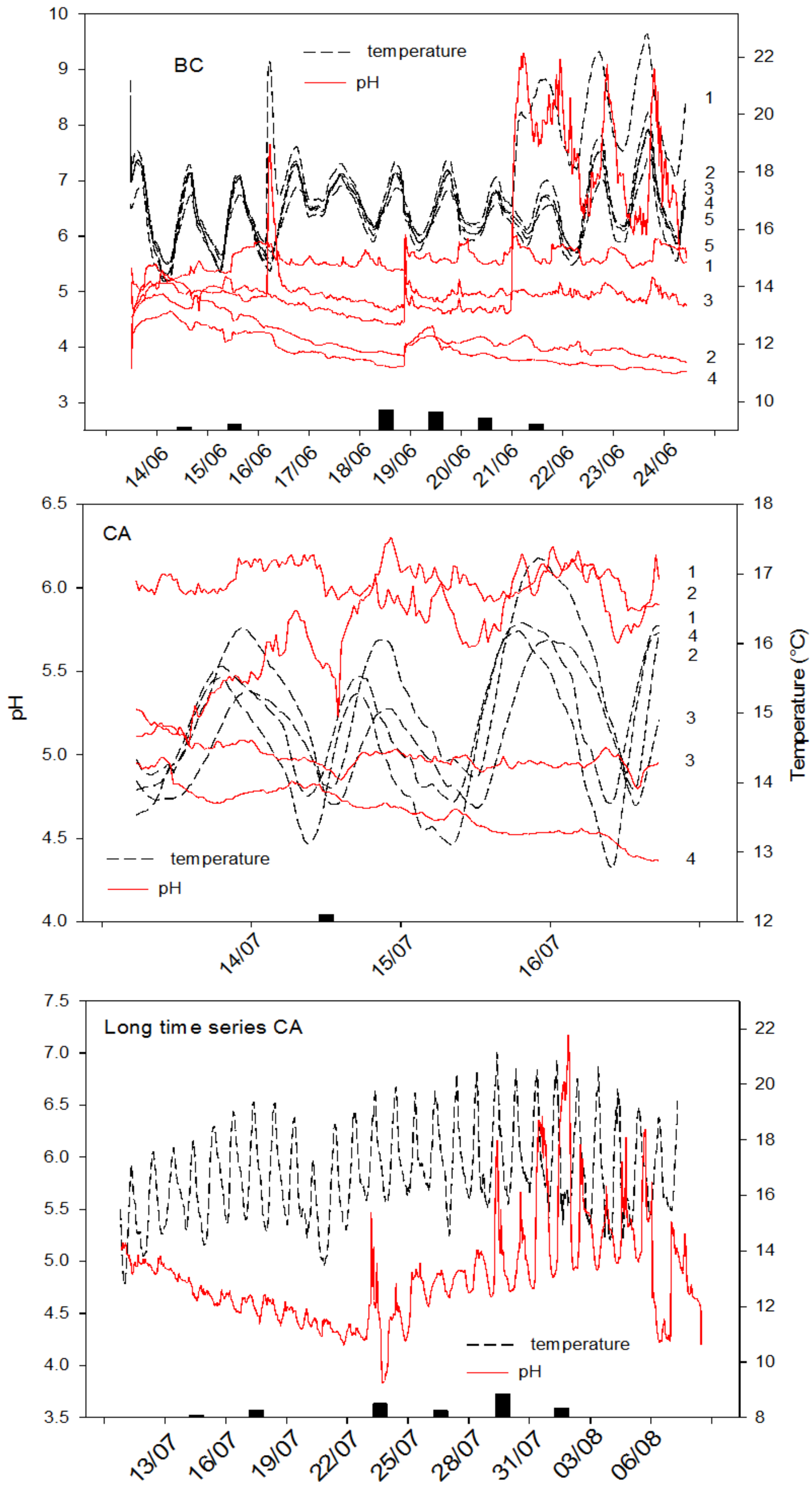

Figure 4. Water temperature and pH measured in bromeliads at BC and CA which formed the "cluster" series, along with the "time series" from a single bromeliad at CA. Numbers link the temperature and pH in the individual bromeliads. Black bars give a relative indication of precipitation during the data collection.

Figure 5. PHTEMP101 Data Logger *in situ* with pH and temperature probes extending into bromeliad tank (*Tillandsia guatemalensis*). The unit is sealed within a plastic bag to protect it from precipitation

1991) and diatoms (Lyra, 1971). Little is known on the relationship between the microorganisms in bromeliads and water acidity and to what extent this biota is affecting and/or being affected by this unique environment. For example, some studies suggest the importance of micro-organisms in sustaining high carbon dioxide and low oxygen levels in the water, as affected by the photo-synthetic activity of algae (Laessle, 1961). The location of a bromeliad determines the amount of organic debris and sunlight received, which in turn, affects the dominance of algae and the chemistry of the entire water column (Guimaraes-Souza et al., 2006). Furthermore, yeast transforms fermentable sugars into carbon dioxide, and it seems plausible that their presence may play a role in mediating the water acidity.

Meanwhile, bacterial communities appear to be sensi-tive to changes in bromeliad water pH (Haubrey et al., 2009, Goffredi et al., 2011) with water condition deter-mining the resident microbial community, but the bacteria themselves only modestly affecting their environment.

There have been strong indications that the plant itself plays a major role in the regulation of the pH of the tank water. In an experimental set up, bromeliads were washed, removed of all debris and organic material, and filled with pond water (Lopez et al., 2009). Over the course of 5 to 6 weeks, this water became acidic indicating that the plant itself might be a major source of acidity. The process may be similar to that which causes Western European bogs to become acidic. In these bogs *Sphagnum* mosses are the dominant vegetation. These mosses have a unique property, exchanging hydrogen (H+) cations on carboxyl groups for other cations in the water (Ca+, Mg+, K = Na+), hereby lowering the pH, and maintaining the typical bog at a pH below 4.5 (Clymo, 1963). The base of bromeliad leaf axils have specialized cells called trichomes which take up cations and may have similar effects in the water tank. In bogs, other sources of acidity have been documented including CO_2 build up, assimilatory cation intake, oxidation of reduced N and S, production of organic acids during decomposi-tion and acid deposition (Urban, 1987). A combination of these factors determines the acidity in bogs independent of microorganismal activity, and similar processes may also occur in bromeliads.

Along the same lines, the differences in pH in our study that are associated with the plant weight might instead be attributed to the inclusion of different bromeliad species (with different maximum weights, respectively). If different bromeliad species naturally produce certain levels of water acidity, it would be difficult to interpret from our data; the larger bromeliads are *Catopsis* and the smaller bromeliads are *Tillandsia*. *Tillandsia* tend to occur on the lower parts of the trees, whereas *Catopsis* were found at all heights.

The composition and quantity of organic material captured by the tank will further determine the acidity of bromeliad water. For example, snail shells can neutralize the acidity of bromeliad tank water as documented with the fascinating behavior of the freshwater crab *Metopaulias depressus* Rathbun in Jamaica (Diesel, 1989). Females of this species are known to manipulate the condition of the bromeliad where offspring was deposited by collecting snail shells to make the water more basic.

The largest bromeliads were found in the midsection of the trees (Figure 5). We are not quite sure why this is, but surmise it is a zone of optimal growing conditions and tolerable disturbance. Water is the most relevant abiotic constraint for growth of epiphytes (Zotz and Hietz, 2001), and compared with the lower understory, the midsection is exposed to more rainwater and greater light exposure. The midsection is also more likely to collect a greater volume of falling organic material and nutrients than bromeliads in the canopy. Although some beneficial growing factors (sunlight and rain capture) would intuitively peak for the bromeliads in the canopy, a higher

Table 3. The average, extreme (min and max) values and standard deviation of temperature (T) and acidity (pH) as measured in the three different datasets, averaged for all bromeliads in Base Camp (BC), Cantiles (CA) and Cortecito (CO). The first column presents the average values and the second the standard error.

Temperature and acidity	Tree						Cluster		Time	
	BC		CA		CO		CA	CO	CA	BC
T max	19.3	0.4	20.5	1.8	20.7	0.4	20.8	0.8	17.2	0.6
T min	14.8	0.2	16.2	2	17.4	0.3	14.5	0.1	13.5	0.2
T average	16.8	0.1	18.3	1.9	19	0.2	16.9	0.2	14.8	0.2
T standard deviation	1.088	0.137	0.999	0.07	0.878	0.096	1.219	0.209	0.875	0.049
pH max	5.6	0.3	6.7	0.7	5.5	0.7	6.6	0.8	5.8	0.3
pH min	3.9	0.2	4.6	0.5	4.4	0.4	4.1	0.3	4.3	0.3
pH average	4.3	0.1	5.5	0.5	4.8	0.5	4.9	0.3	5.3	0.3
T standard deviation	0.2	0.019	0.415	0.12	0.21	0.071	0.508	0.22	0.245	0.052

Numbers of bromeliads included in each average are shown in Table 1.

Table 4. Spearman Rank correlations for environmental variables and height of the bromeliad on the tree and the weight of the bromeliad. Kruskall-Wallis test for differences in low, mid and high sites of bromeliad tree attachment.

Temperaturand acidity	Height		Height Category		Weight	
	S (N = 20)	*p-value*	H (2, N = 20)	*p-value*	S (N = 20)	*p-value*
T max	0.304	0.193	2.027	0.363	-0.096	0.686
T min	0.122	0.609	3.075	0.215	0.374	0.104
T average	0.105	0.661	2.462	0.292	0.265	0.259
T standard deviation	-0.144	0.546	4.361	0.113	-0.696	**0.001**
pH max	-0.004	0.987	1.199	0.549	0.394	0.086
pH min	0.219	0.354	0.605	0.739	0.263	0.262

disturbance regime likely offsets these benefits. These bromeliads become extremely prone to physical damage from the forces of strong wind and desiccation from overexposure. The relatively small volume of water collected by bromeliads attached lowest on the trees was also illustrated by the pH data collected simultaneously at different heights (Figures 1 to 4). Following a precipitation event, bromeliads closest to the forest floor expressed only minor changes in pH, due to the smaller volume of water captured, whereas the bromeliads in the canopy collected much more water and experience drastic sudden changes in water chemistry, characterized by a rise in pH. Still, variation may occur within bromeliads at roughly the same level when differently exposed to the weather conditions (Figure 1B). Overall, water temperature variations decrease after recent precipitation events, as the rise in water levels provide a heightened buffer to air temperature changes.

In our data we observed strong diel variation in pH and water temperature. As the water in the bromeliads evaporated the temperature

Table 5. Results of models in RDA. Environmental variables are used as “species” data, the continuous variable attachment height of the bromeliads on the tree as explaining environmental variable, and the weight of the bromeliad plants as covariable.

Temperaturand acidity	Trace	*F-value*	*p-value*
T max	0.000	0.000	0.990
T min	0.011	0.222	0.666
T average	0.009	0.176	0.700
T standard deviation	0.012	0.469	0.518
pH max	0.044	1.163	0.296
pH min	0.001	0.010	0.916
pH average	0.020	0.556	0.460
T standard deviation	0.082	2.287	0.178

fluctuations became more extreme. In addition, the pH of the water in the plants became increasingly acidic; this is particularly visible in the extended "time" data series (Figure 4). After precipitation topped up the water in the bromeliads, the pH quickly spiked from acidic to basic, likely due to a significant dilution of the concentrate.

This work represents the first long-term illustration of the dynamic and unpredictable environments created by phytotelmata. Despite the information we provide, this study only scratches the surface into a largely overlooked realm of ecological exploration. Additional studies are needed to better understand these habitats and determine unique characteristics of similar habitats in different regions.

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