

ENVIRONMENTAL QUALITY EFFECTS AND THE ECOLOGICAL CONTEXT OF
A RAINFOREST CANOPY BROMELIAD FAUNA

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ENVIRONMENTAL QUALITY EFFECTS AND THE ECOLOGICAL CONTEXT OF
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ABSTRACT

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SUPERVISING PROFESSOR: MICHAEL R.J. FORSTNER

Canopy strata of tropical forests are one of the remaining unexplored biotic frontiers. New access techniques enabling us to reach the canopy have facilitated an increased investigation on the ecology of forest canopies and their function in tropical ecosystems. As the interface between the terrestrial environment and atmosphere, the canopy and its inhabitants are integral to ecosystem function and maintenance. Epiphytes, particularly tank bromeliads, provide microhabitat for a high diversity of fauna and flora in tropical forest canopies and are considered a “keystone resource”.

Anthropogenic perturbations are rapidly altering the landscape of the Ecuadorian Amazon and along with it the species diversity, forest dynamics, and ecosystem functions. *Aechmea zebrina* is a large tank bromeliad typically found in the high canopy

of the upper western Amazon Basin in Columbia and Ecuador. As part of this study I have identified a diverse amphibian community inhabiting these phytotelmata, yet their ecological role in forest canopies remains primarily unknown. I investigated the symbiotic relationship of *A. zebrina* bromeliads and their inhabitants, ecological factors driving this relationship, microclimate moderation by *A. zebrina* supporting faunal diversity, vertical distribution of the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, and the influence of anthropogenic forest disturbance on the anuran inhabitants of *A. zebrina*.

My research has resulted in a successful protocol for sampling the fauna of high canopy bromeliads. It has contributed to the identification and description of new bromeliad-inhabiting anuran species, along with accounts of reproductive ecology and behavior. Ecological characteristics of *A. zebrina* bromeliads were quantified and effects of microclimate moderation identified as playing a significant role in *A. zebrina* providing a viable microhabitat in the harsh canopy environment. The first occurrence of *B. dendrobatidis* in lowland Amazonian rainforests and canopy-inhabiting anurans was discovered across a vertical gradient from the forest floor to the upper canopy. Lastly, low-level anthropogenic forest disturbance along an isolated oil road was found to negatively impact the abundance, occurrence, and diversity of anuran inhabitants in *A. zebrina*. These data provide science-based support to promote social responsibility and conservation efforts throughout Amazonia.

CHAPTER I

ECOLOGY AND MICROCLIMATE OF A HIGH CANOPY TANK BROMELIAD, *AECHMEA ZEBRINA*, IN AMAZONIAN ECUADOR¹

Introduction

The rich and diverse community of epiphytic plants within neotropical rainforest canopies include specialized rainwater storage species, or phytotelmata. These phytotelmata, defined as plants or parts of plants which hold rainwater (e.g. bromeliads, fruits, inflorescences, palm fronds and tree holes) actually help moderate microclimate variables and support a rich fauna of organisms within the high canopy. In some moist tropical locations the availability of this habitat for aquatic organisms is up to 50,000 liters per hectare, literally a “wetland in the sky” (Kitching 2000; McCracken & Forstner 2006). In particular, tank bromeliads are capable of holding relatively large amounts of water and play a principal role as a “keystone resource” and microhabitat for invertebrates, vertebrates, and other plants (Nadkarni 1994).

Bromeliads of the genus *Aechmea* are distributed from central Mexico and the West Indies to northern Argentina with approximately 225 described species, occurring from sea level up to 2000 m (Smith & Downs 1979; Manzanares 2002). *Aechmea* are

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typically epiphytic and range in size from several centimeters to meters in diameter (Benzing 2000). Their roots are primarily mechanical, providing support and attachment to the host plant with little absorptive function, while also facilitating clonal reproduction (Ceusters 2008). The leaf architecture of most *Aechmea* create phytotelms (tanks) that hold water between the leaf axils or a central tank formed by the leaf rosettes. Members of the *Aechmea* genus sequester atmospheric CO₂ for photosynthesis via the Crassulacean acid metabolism pathway, a unique water conservation method typical of arid region plants. Nutrient and water absorption is performed by trichomes on the leaves and has allowed for the adaptive evolution of epiphytism in many bromeliad species. *Aechmea* is a dominant bromeliad species group in the harsh canopy environment of the northwestern Amazon Basin.

Aechmea zebrina is a large epiphytic tank bromeliad native to the Amazon regions of Ecuador and southeastern Colombia (Figure 1.1) (Smith 1953; Garden 2012). *Aechmea zebrina* occur in the upper canopy and overstory trees at vertical heights up to ~50 m and range in number of individuals from 1 to >150 on a single phorophyte tree (McCracken & Forstner 2006). The foliage is characterized by distinct silvery-white banding on green leaves (sometimes underside of leaves are varying shades of red when higher in the canopy and exposed to more sun) and the inflorescences are red and yellow. *Aechmea zebrina* has been reported to be collected by eastern lowland indigenous Kichwa people for medicinal and ornamental purposes, and is now propagated in the western lowlands of Ecuador (Benzing 2000). It has been exported and propagated by international bromeliad collectors but is not widely available, most likely due to difficulties meeting its environmental requirements under commercial cultivation.

Basic ecology, natural history, and microclimate data on *A. zebrina* in its natural habitat are deficient in the literature. While conducting a project on canopy bromeliad-inhabiting anurans in the northeastern Ecuadorian Amazon we recorded our observations for these basic data. Herein, we provide summary data on *A. zebrina* physical characteristics of height, diameter, number of mature leaves, water volume, water pH, wet mass, and height above forest floor. We also report the number of *A. zebrina*, height, and elevation above sea level for each tree. We provide a list of phorophyte (host) tree species, historic and new geographic locations, flowering phenology, and describe general structural form observations due to exposure and height in tree for *A. zebrina*. We report and analyze microclimate variables of air temperature, water temperature, and humidity from within an *A. zebrina* leaf axil and outside of the plant. We describe the herpetofauna utilizing *A. zebrina* for daytime refugia and permanent habitat. Finally, our study documents other vertebrates and invertebrates using *A. zebrina* as a resource. Taken as a whole this information supports the unique contributions to diversity enabled by this epiphyte species, but also document the environmental conditions for the species in the wild for potential application to maintenance or propagation under cultivation.

Materials and Methods

The reported observations were collected in the northeastern region of Amazonian Ecuador during fieldwork in 2004 (May-August), 2006 (May-August), and 2008 (March-December); but also include some observations from field seasons in 2002, 2003, and 2010 (i.e. flowering phenology) (Figure 1.1). *Aechmea zebrina* collections were conducted in the Yasuní Biosphere Reserve and its respective buffer zone at the Tiputini

Biodiversity Station (TBS) (0°38'14"S, 76°08'60"W), founded and operated by the Universidad San Francisco de Quito, and the Yasuni Research Station (YRS) (0°40'27"S, 76°23'51"W), founded and operated by the Pontificia Universidad Católica del Ecuador. Additionally, surveys for *A. zebrina* were conducted along 24 km of road, primarily constructed for the oil industry, in the vicinity of Dayuma (Auca oil road), Tena, and Limoncocha. Each of these locations are representative of varying stages of deforestation and fragmentation due to oil operations and colonization, from undisturbed primary forest at TBS to historical and current heavy disturbance near Dayuma. The study area is part of the Napo Moist Forests terrestrial ecoregion, and has been recognized as one of the most biologically diverse terrestrial systems on the planet (Olson & Dinerstein 2002; Bass et al. 2010).

Aechmea zebrina were sampled following methods described by McCracken & Forstner (2008). Identification of *A. zebrina* followed (Luther 1998). Only trees with >15 *A. zebrina* were selected as potential trees to be sampled so as not to decimate the bromeliad community. The tree canopy was accessed using single-rope technique (SRT), and bromeliads were collected haphazardly at estimated even vertical intervals between one another (Perry 1978) (Figure 1.2). Tree height and bromeliad elevation were recorded using a rangefinder (Nikon, Tokyo, Japan). Tree coordinates and elevation were recorded using a Differential Global Positioning System (Magellan, Santa Clara, California). We removed bromeliads by cutting the base-support rhizome, sealed the entire plant within a 55 gal. plastic bag, and lowered it to the ground in a tarp. The number of *A. zebrina* inhabiting the tree was counted and a herbarium specimen was collected from the tree to confirm identification. We transported bromeliads back to camp and processed them in a

screened tent to prevent escape of animals. Bromeliad water was poured through a 1-mm sieve to separate arthropods, leaf litter, and detritus. We measured water volume with a graduated cylinder and pH with a 3-point calibrated pH probe (Oakton, Vernon Hills, Illinois). We measured the height and diameter of bromeliads to nearest centimeter and counted the number of mature leaves. Wet mass without water was recorded using a spring scale (Pesola AG, Baar, Switzerland). We carefully dismantled each bromeliad leaf-by-leaf to collect all herpetofauna and invertebrates.

Microclimate data were collected hourly from an *A. zebrina* bromeliad in a high canopy tree, *Brosimum guianense*, at TBS. Only daytime temperature data from 0700-1800 hours were analyzed, as this time is representative of the most dramatic climatic changes in the canopy. Thermochron iButton data loggers were used to record bromeliad water temperature (resolution of 0.5°C) and Hygrochron iButton data loggers to record air temperature (resolution of 0.5°C) and relative humidity (resolution of 0.01%RH) (Maxim, Sunnyvale, California). The iButton data loggers were operated within manufacturer safe operating temperature and humidity ranges with a reported accuracy of $\pm 0.5^{\circ}\text{C}$ and $<1.0\%\text{RH}$ after software correction handled automatically using the 1-Wire Viewer Software (Maxim, Sunnyvale, California). A Thermochron data logger was placed in an outer leaf axil holding water at time of installation approximately 8 cm below the water line. Hygrochron data loggers were installed with a string tied around the leaf in the same leaf axil as the Thermochron approximately 10 cm above estimated maximum water level, and another to the supporting tree branch of the bromeliad approximately 0.5 m from the bromeliad. An analysis of variance (ANOVA) with post-hoc Tukey's HSD test was performed on the daily temperature data as a whole and on the

hourly data points. Paired t-tests were performed on the daily humidity data as a whole and on the hourly data points. We used the R 2.14.1 statistical programming environment for all analyses (R Development Core Team, 2011).

General observations of *A. zebrina* ecology and natural history were recorded during the study periods, and reflect opportunistic and qualitative data collection. Most of these observations were made during survey and sampling procedures, but also during visits to two canopy towers at TBS in *Ceiba pentandra* trees with *A. zebrina* communities and along a suspended canopy walkway between several trees with *A. zebrina*. Comments on flowering phenology and bromeliad structure and shape are the result of cumulative field journal notes on *A. zebrina* in sampled trees.

Results

A total of 240 *A. zebrina* were collected from 48 phorophyte trees during three field seasons (2004, $n=40$; 2006, $n=40$; 2008, $n=160$). Measurements of physical characters for *A. zebrina* and sampled tree data are provided in Table 1.1. We have some reservation about the accuracy of our diameter measurements for *A. zebrina* as they were taken after the individual had been placed in a plastic bag and transported back to camp. We also recorded water loss from 11.25% of sampled *A. zebrina* during removal or due to holes in transport bags for which we did not correct measurements. Analyses of these variables for effects of forest disturbance level using the 160 bromeliads sampled in 2008 found no significant differences (S.F.M. & M.R.J.F., unpublished data). Therefore, data from all sampling periods were summarized together.

Microclimate data were collected from December 9, 2008 to March 3, 2009 for a total of 85 days. The phorophyte tree, *B. guianense*, used to record microclimate data from an *A. zebrina* bromeliad was 42.5 m tall with a diameter at breast height of 130 cm. We counted 143 mature *A. zebrina* on the tree and the bromeliad used for data logger installation was 38.5 m above the forest floor and approximately 60 cm tall. The mean daytime external ambient air temperature recorded adjacent to the bromeliad was 26.8 °C with a range of 20.5-37.0 °C. Mean daytime internal ambient air temperature recorded in the bromeliad leaf axil was 26.5 °C with a range of 21.0-35.5 °C. Mean daytime water temperature inside the bromeliad leaf axil was 24.8 °C with a range of 21.0-29.0 °C. ANOVA results were highly significant ($p < 0.0001$) for mean daytime temperatures with Tukey HSD results between all three temperatures significant (external vs. internal, $p = 0.0188$; external vs. water, $p < 0.0001$; internal vs. water, $p < 0.0001$). Significance of hourly temperature comparisons was variable (see Figure 1.3), with the exception of external air temperature vs. internal water temperature. External air temperature was significantly greater than the internal water temperature during all daytime hours except 1800 hr. The mean daytime external relative humidity was 78.74% with a range of 39.31-99.51%. The mean daytime internal relative humidity was 84.70% with a range of 45.03-99.50%. Paired t-test results were highly significant ($p < 0.0001$) for mean daytime relative humidity. Internal relative humidity was significantly greater ($p < 0.0001$) than external relative humidity during all daytime hours (Figure 1.4).

Aechmea zebrina were found on 22 species of phorophyte trees ($n=33$) representative of 20 genera in 13 families (Table 1.2). The geographic locations for *A. zebrina* from herbarium records and this study are shown in Figure 1.1. *Aechmea zebrina*

has been observed flowering during every month of the year, with increased blooming starting in November and peaking in the driest months of January and February. During canopy sampling of *A. zebrina* we have observed that individuals located lower in the canopy under denser cover had less erect leaves creating more open rosettes and tended to have less white banding with darker green leaves. *Aechmea zebrina* located higher in the canopy and with greater solar radiation exposure, due to canopy architecture and tree leaf phenology, tended to have more erect leaves with much tighter or compact rosettes accompanied by more pronounced white banding and reddening of the leaves.

A total of 10 metamorphosed anuran species, 2 lizard species, 1 gecko species, and 1 snake species have been collected or observed in *A. zebrina* (Table 1.3). We observed 6 species of birds and 3 species of primates visiting *A. zebrina* for various purposes (Table 1.4). A butterfly (Family Nymphalidae) and orchid bee (Tribe Euglossini) have both been seen visiting the flowers of *A. zebrina*, and preliminary sorting of invertebrates collected from within two *A. zebrina* have identified approximately 43 morphospecies.

Discussion

Aechmea zebrina persist at the top of the harsh equatorial canopy in the northwestern Amazon. While it is relatively abundant where it occurs in undisturbed primary forest the other *Aechmea* (i.e. *A. romeroi*) are more common, and particularly in disturbed forest areas where *A. zebrina* becomes more scarce with increased open canopy area ((S.F.M. & M.R.J.F., unpublished data). Its large size and ability to hold a high

volume of water are the primary characters making it an important resource provider and microclimate moderator. The relationship between phorophyte trees and epiphytic bromeliads is typically considered to be commensal (Benzing 2000). However, when bromeliads grow to the size of *A. zebrina* with up to or over 150 individuals on a tree the stress due to mass or shading must be considered and the relationship may then be trending towards parasitism. We calculated a mean mass of 3.87 kg ($n=20$) for *A. zebrina* that were weighed after pouring off their water content, using the mean mass and adding the calculated mass for our recorded mean water volume (1.34 kg) we derive a mean total mass of 5.21 kg per bromeliad. Therefore, a tree with our mean of 66 *A. zebrina* individuals would have a minimum of 343.86 kg additional mass that it must support. When we consider a tree with 150 *A. zebrina* or more we begin to approach a metric ton (~800 kg) of additional mass to support. We consider these to be conservative estimates, as we only weighed 20 bromeliads that were centered around our means and well within 1 standard deviation for height and diameter. We also had a recorded water loss from 11.25% of sampled *A. zebrina* that we did not correct for, and our weights only include the vegetative part of the bromeliad and not the rhizome support network or any dead plants and the detritus, soil, or water they may have held. Additionally, our water volume measurements do not necessarily represent the maximum water holding capacity of *A. zebrina* because of time since and amount of last rainfall event before collection. Mean water pH (4.44) was mildly acidic and within the range that has been reported to affect development of embryonic and larval anurans (Beattie & Tyler-Jones 1992). However, we observed many aquatic invertebrates and larval anurans in our collections, and bromeliads are a known breeding site for amphibians (Benzing 2000). With a mean

height from the forest floor of 32.9 m for *A. zebrina* in trees averaging 40.4 m tall it is clear from our data that *A. zebrina* prefer the upper canopy.

Our results show that *A. zebrina* do provide microclimate moderation from the external canopy environment for both temperature and humidity. During the mid-afternoon hours (1300-1500) is when the greatest amount of microclimate moderation occurs between external and internal temperatures and humidity, particularly the differences between air and water temperatures as well as humidity. These significantly lower water temperatures in bromeliad leaf axils are important as they provide an excellent refuge for anurans and other organisms from the higher air temperatures. It is the water held within these leaf axils that facilitates the maintenance of significantly higher humidity levels in the bromeliad confines. This combination of lower temperatures, higher humidity, and presence of water enables anurans to avoid desiccation, which is likely their greatest ecophysiological threat in the canopy (Boone et al. 2003). It is probable that bromeliad size, location within canopy, and phorophyte species all influence the microclimate moderating abilities of bromeliads. The bromeliad we installed data loggers in was below our mean height recorded for *A. zebrina* ($\bar{x}=74.8$ cm) at approximately 60 cm tall, and was only about 4 m from the top of the tree (42.5 m) exposing it to a great amount of solar input and wind in the upper canopy. Larger bromeliads hold a greater volume of water allowing them to maintain sufficient water levels for microclimate moderation between rainfall events. Larger *A. zebrina* have more leaves resulting in more leaf axils and possibly increasing insulation effects from the outer most leaves towards the interior. Bromeliads located closer to tree boles and lower in the canopy are also better protected against climatic factors due to increased

surrounding canopy architecture (Cardelús & Chazdon 2005). Deciduous phorophyte tree species present a unique situation to bromeliads when they drop their leaves and expose bromeliads to increased solar radiation and wind. These factors increase daytime temperatures and decrease humidity levels, most likely increasing evaporation of water from bromeliad leaf axils. Two tree species, *Parkia multijuga* and *Ceiba pentandra*, in which we sampled *A. zebrina* had anurans present in them during their deciduous season. These initial findings of microclimate moderation by *A. zebrina* warrant further investigation of different phorophyte species, and across a gradient of elevation in the canopy and distance from tree bole.

In our study *A. zebrina* utilized 22 species of trees as phorophytes, with no discernable morphological characters or taxonomic relatedness to identify as a specific host preference. One species, *Mauritia flexuosa*, is a palm characteristic of swamp areas of forest and appears to be more of an opportunistic or even accidental phorophyte for *A. zebrina* with just a few individuals usually present. As previously mentioned, *A. zebrina* occupy deciduous trees but were also found in non-deciduous trees. The ability of *A. zebrina* to persist through several months of much increased exposure to the canopy environment brings into question their physiological modification capabilities. Some of these modifications, noted during our study, included more erect leaves and tighter rosettes resulting in deeper leaf axils with less surface area. These adaptations may help to hold more water and defend against water loss during these periods of increased exposure. Future studies of relationships between phorophyte species and *A. zebrina* may better explain tree architecture and substrate influences, and coping abilities of bromeliads in the canopy environment.

During initial bromeliad surveys in 2004, *A. zebrina* supported the most diverse and abundant anuran community of the 3 bromeliad species sampled (McCracken & Forstner 2008). We have now identified 14 species of herpetofauna occupying *A. zebrina* in an area of less than 200 km², along with a number of other vertebrates and invertebrates using this plant for different resources. Having only sampled a relatively small number of *A. zebrina* ($n=240$) across a small area, we believe future research including all species of high canopy tank bromeliads will come to reveal a large number of vertebrate and invertebrate species variably dependent upon these unique canopy resources.

The results from our cumulative work on *A. zebrina* provide evidence that further supports Nadkarni's (1994) recognition of epiphytes as a "keystone resource". The ability of *Aechmea zebrina* to acquire atmospheric nutrients, host and protect organisms that contribute and decompose organic nutrients (i.e. fecal matter and leaves), and then pass these nutrients on to other canopy organisms (i.e. crown-wash leaching and water source) characterize them as microecosystems acting as both a sink and source of canopy nutrients. Individual tank bromeliads in the canopy may be thought of as island microcosms; however, when viewed as a community interconnected by the canopy architecture they represent a three-dimensional aerial wetland. *Aechmea zebrina* create and support a highly diverse wetland community in the canopy of northwest Amazonia. Their role as a "keystone resource" make them ideal candidates for *in situ* ecological microcosm studies and of particular interest for canopy conservation concerns.

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Table 1.1: Ecological variables of sampled *Aechmea zebrina*. Summary statistics of ecological data for sampled *A. zebrina* and phorophyte tree.

Variable	Mean (\bar{x})	Standard deviation	Range	Sample size (n)
Height (cm)	75	14	45-126	240
Diameter (cm)	77	22	38-148	240
Number of mature leaves	28	6	14-46	200
Water volume (mL)	1343	656	99-3645	200
Water pH	4.44	0.53	3.14-6.08	200
Mass (kg)	3.87	0.69	2.96-4.80	20
Height above forest floor (m)	32.9	5.6	18.3-44.5	200
Number of <i>A. zebrina</i> in tree	66	40	19-159	40
Tree height (m)	40.4	5.6	28.0-49.0	40
Tree elevation (m.a.s.l.)	234.4	17.1	203.7-268.0	40

Table 1.2: *Aechmea zebrina* phorophyte species. Host tree species and number of trees sampled for *A. zebrina*.

Family	Genus	species	Number of trees sampled
Annonaceae	<i>Rollinia</i>	<i>pittieri</i>	1
Annonaceae	<i>Ruizodendron</i>	<i>ovale</i>	1
Arecaceae	<i>Mauritia</i>	<i>flexuosa</i>	2
Bignoniaceae	<i>Tabebuia</i>	<i>ochracea</i>	1
Bombacaceae	<i>Ceiba</i>	<i>pentandra</i>	2
Fabaceae	<i>Cedrelinga</i>	<i>cateniformis</i>	1
Fabaceae	<i>Lonchocarpus</i>	<i>sericeus</i>	1
Fabaceae	<i>Parkia</i>	<i>multijuga</i>	8
Fabaceae	<i>Pterocarpus</i>	sp.	1
Fabaceae	<i>Swartzia</i>	<i>multijuga</i>	1
Lauraceae	<i>Yasuni</i>	sp.	1
Lauraceae	unidentified	sp.	1
Moraceae	<i>Brosimum</i>	<i>guianense</i>	1
Moraceae	<i>Brosimum</i>	<i>potabile</i>	2
Moraceae	<i>Helicostylis</i>	<i>tomentosa</i>	1
Myristicaceae	<i>Virola</i>	<i>duckei</i>	1
Myristicaceae	<i>Virola</i>	<i>flexuosa</i>	1
Myrtaceae	<i>Calypttranthes</i>	sp.	1
Rubiaceae	<i>Wittmackanthus</i>	<i>stanleyanus</i>	1
Sapotaceae	<i>Pouteria</i>	sp.	2
Tiliaceae	<i>Pentaplaris</i>	<i>huaoranica</i>	1
Urticaceae	<i>Pourouma</i>	<i>minor</i>	1
Total			33

Table 1.3: Herpetofauna of *Aechmea zebrina*. Amphibian and reptile species utilizing *A. zebrina* as diurnal refugia or permanent habitat.

Class	Family	Genus	species
Amphibia	Dendrobatidae	<i>Ranitomeya</i>	<i>duellmani</i>
Amphibia	Dendrobatidae	<i>Ranitomeya</i>	<i>ventrimaculatus</i>
Amphibia	Hylidae	<i>Osteocephalus</i>	<i>fuscifacies</i>
Amphibia	Hylidae	<i>Osteocephalus</i>	<i>planiceps</i>
Amphibia	Hylidae	<i>Osteocephalus</i>	<i>taurinus</i>
Amphibia	Hylidae	<i>Scinax</i>	<i>ruber</i>
Amphibia	Strabomantidae	<i>Pristimantis</i>	<i>acuminatus</i>
Amphibia	Strabomantidae	<i>Pristimantis</i>	<i>aureolineatus</i>
Amphibia	Strabomantidae	<i>Pristimantis</i>	<i>orphanolaimus</i>
Amphibia	Strabomantidae	<i>Pristimantis</i>	<i>waoranii</i>
Reptilia	Phyllodactylidae	<i>Thecodactylus</i>	<i>rapicauda</i>
Reptilia	Polychrotidae	<i>Anolis</i>	<i>ortonii</i>
Reptilia	Polychrotidae	<i>Anolis</i>	<i>transversalis</i>
Reptilia	Colubridae	<i>Leptodeira</i>	<i>annulata</i>

Table 1.4: Vertebrates utilizing *Aechmea zebrina*. Mammal and bird species observed using *A. zebrina* as a resource and their activities.

Common name	Class	Order	Genus	species	Activity
Golden-mantle tamarin	Mammalia	Primates	<i>Saguinus</i>	<i>tripartitus</i>	Foraging for insects and drinking
Humboldt's Common woolly monkey	Mammalia	Primates	<i>Lagothrix</i>	<i>lagothricha poeppigii</i>	Foraging and drinking
Common squirrel monkey	Mammalia	Primates	<i>Saimiri</i>	<i>sciureus</i>	Foraging
Fork-tailed Woodnymph	Aves	Apodiformes	<i>Thalurania</i>	<i>furcata</i>	Feeding from flowers
White-chinned Sapphire	Aves	Apodiformes	<i>Hylocharis</i>	<i>cyanus</i>	Feeding from flowers
White-necked Jacobin	Aves	Apodiformes	<i>Florisuga</i>	<i>mellivora</i>	Feeding from flowers
White-lored Euphonia	Aves	Passeriformes	<i>Euphonia</i>	<i>chrysopasta</i>	Foraging in leaf axils
Purple Honeycreeper	Aves	Passeriformes	<i>Cyanerpes</i>	<i>caeruleus</i>	Bathing in leaf axils
Green Honeycreeper	Aves	Passeriformes	<i>Chlorophanes</i>	<i>spiza</i>	Bathing in leaf axils

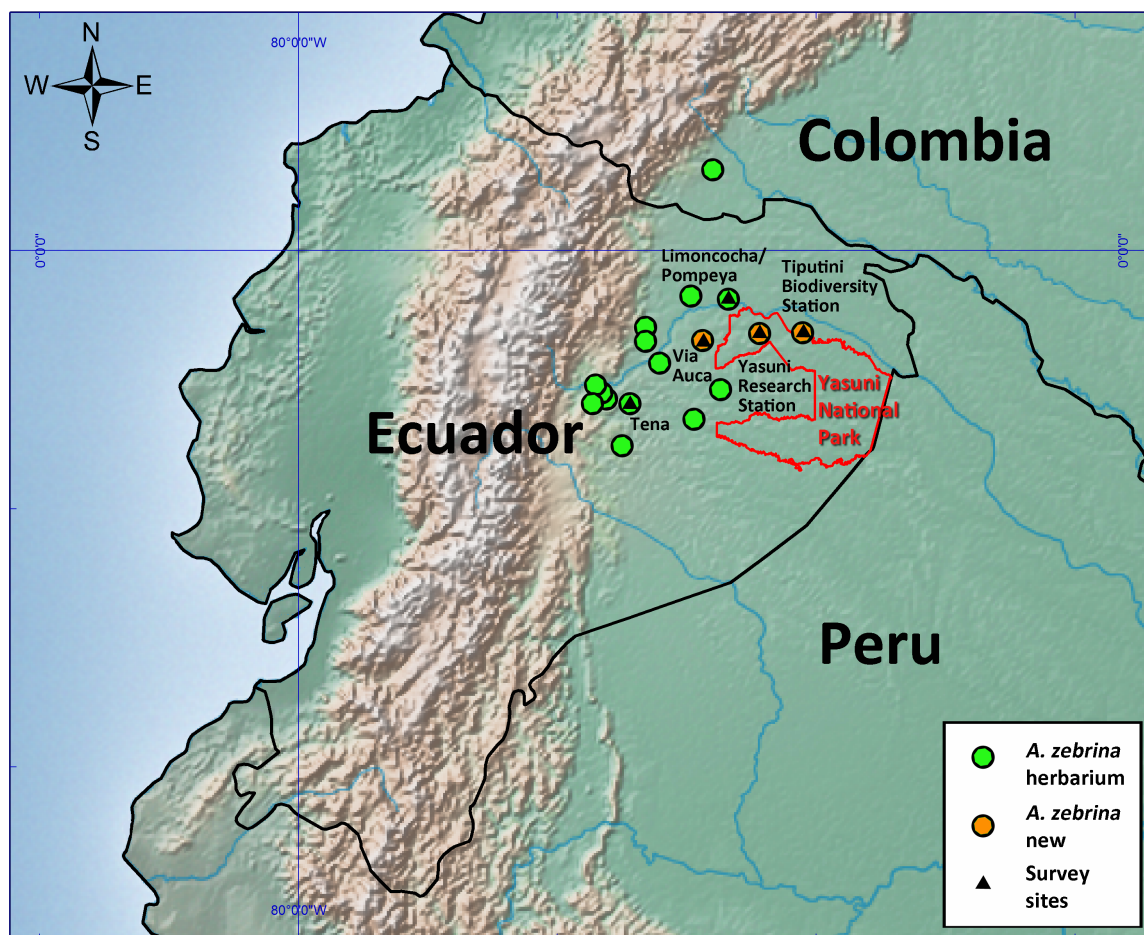


Figure 1.1: Map of *Aechmea zebrina* bromeliad observations and survey sites. Map of *Aechmea zebrina* bromeliad locations based on herbarium records and new observations during survey work. The five survey sites for this research project are included.

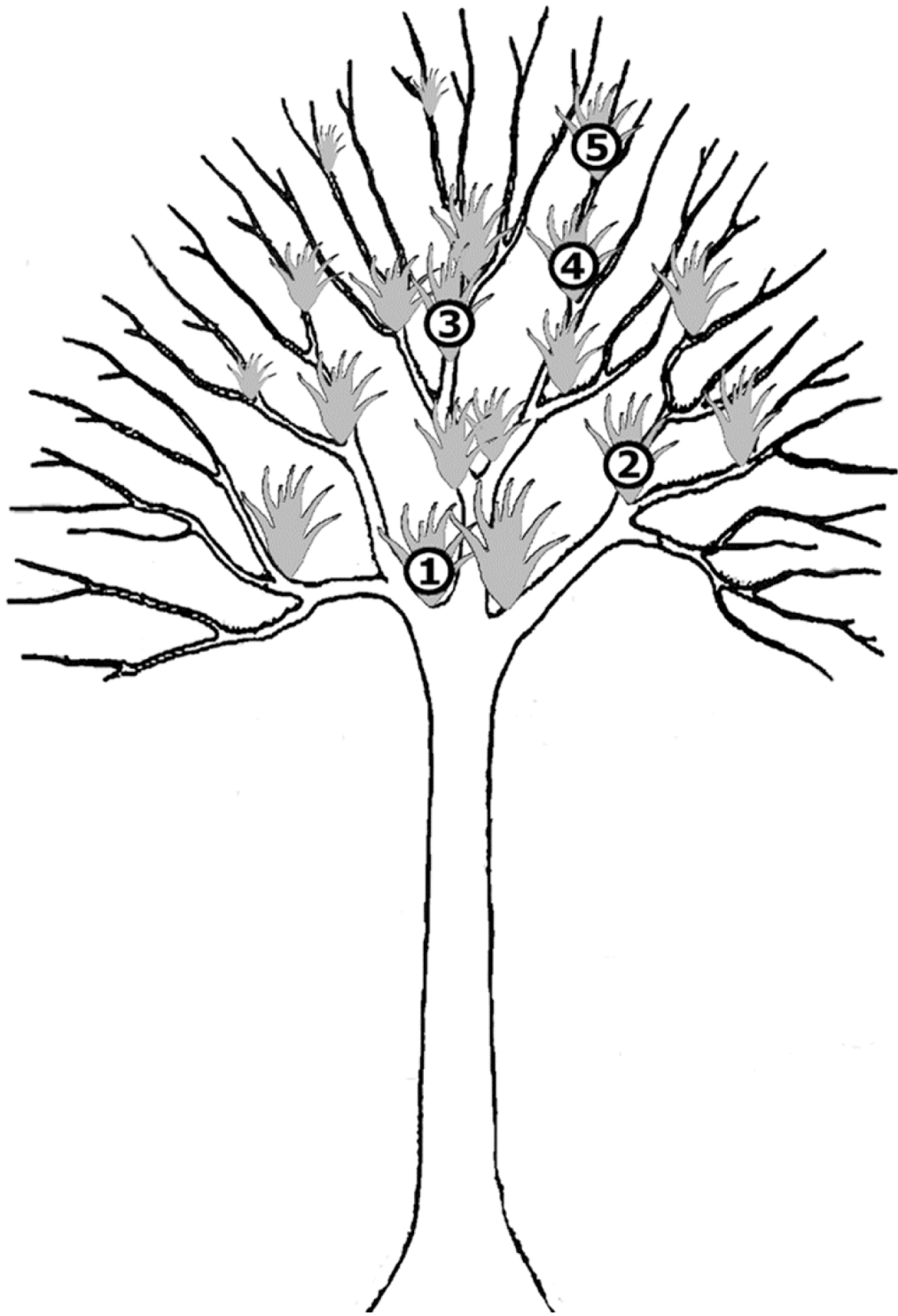


Figure 1.2: Tree schematic for bromeliad sampling. Schematic of tree with bromeliads illustrating distribution strategy for sampling units (bromeliads), numbers denote bromeliads sampled.

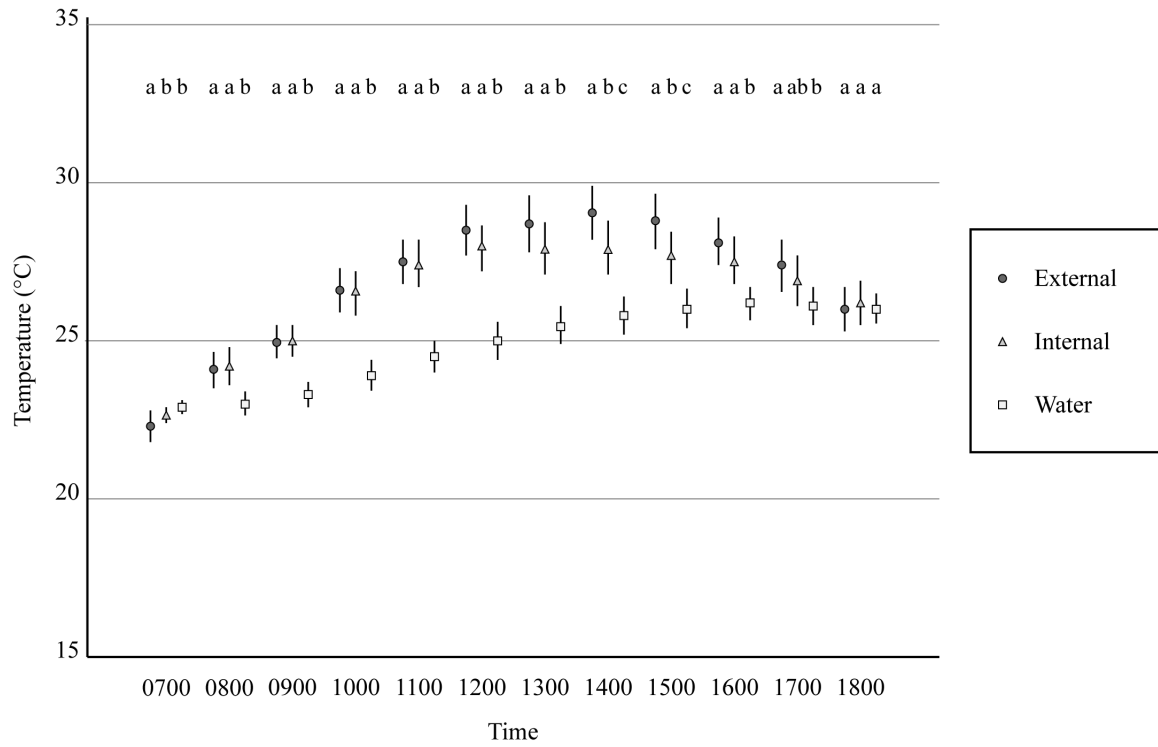


Figure 1.3: *Aechmea zebrina* microclimate temperatures. Hourly daytime mean temperatures from within and adjacent to an *A. zebrina* at 38.5 m above forest floor. External temperature recorded on support branch approximately 0.5 m from bromeliad, internal temperature recorded in bromeliad leaf axil, and water temperature in same leaf axil as temperature. Whiskers represent 95% confidence intervals and letters above code for statistical significance of hourly comparisons (ANOVA-Tukey HSD).

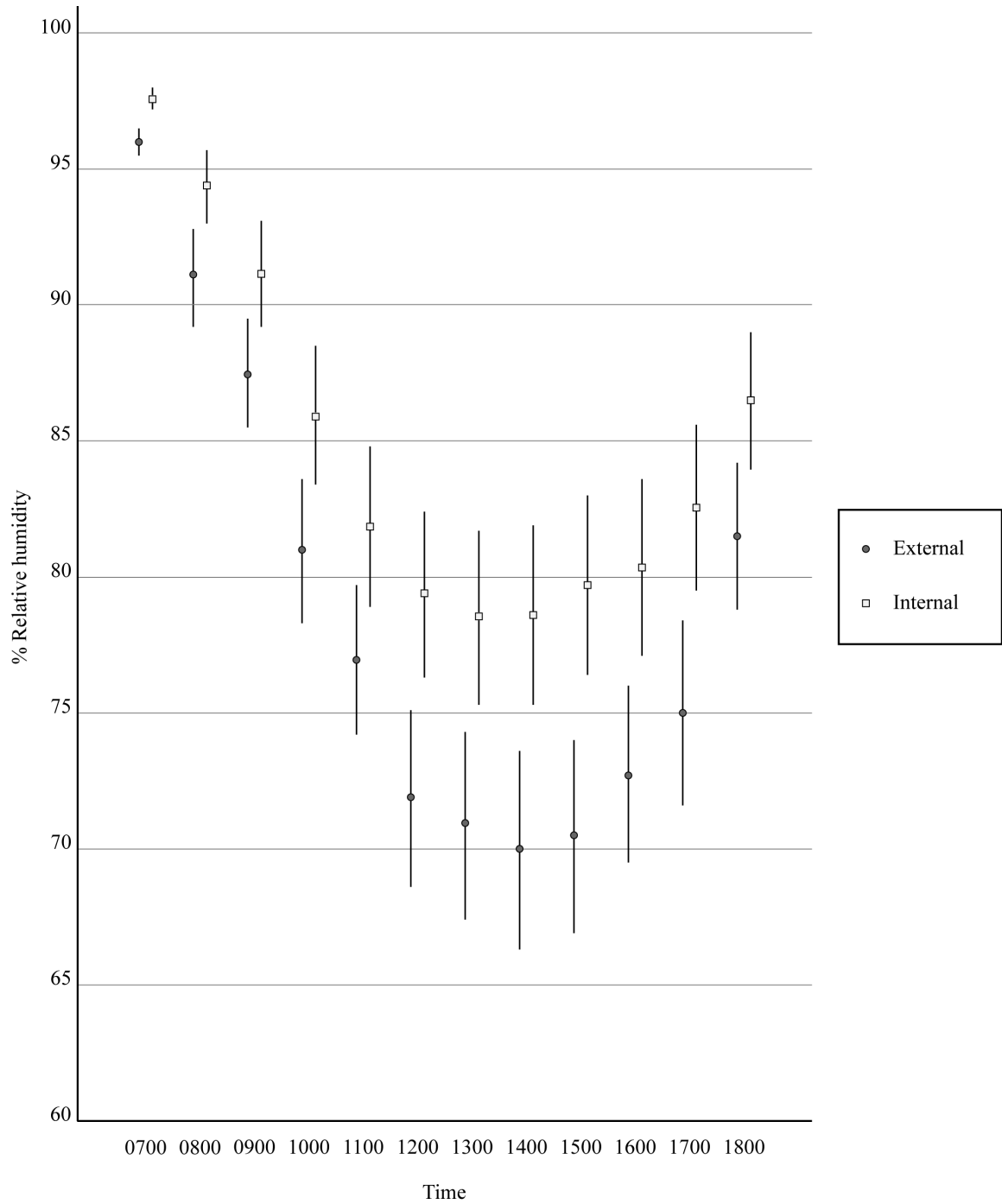


Figure 1.4: *Aechmea zebrina* microclimate humidity. Hourly daytime mean relative humidity from within and adjacent to an *A. zebrina* at 38.5 m above forest floor. External humidity recorded on support branch approximately 0.5 m from bromeliad and internal humidity recorded in a bromeliad leaf axil. Whiskers represent 95% confidence intervals.

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CHAPTER II

BROMELIAD PATCH SAMPLING TECHNIQUE FOR CANOPY HERPETOFAUNA IN NEOTROPICAL FORESTS¹

Introduction

The canopy strata of tropical forests are one of the remaining unexplored biotic frontiers. Canopy research is a relatively new discipline facilitated by recent methodological advances in canopy access techniques (Basset et al. 2003c). Forest canopies are among the most species-rich terrestrial habitats on earth, supporting approximately 40% of known extant species and estimated to hold up to 50% of the earth's biodiversity (Mitchell et al. 2002; Basset et al. 2003c). The ecological role of amphibians and reptiles in forest canopies is mostly unknown. Thus far the research focus has been on arthropods, birds, mammals, plants and ecological processes; investigations of canopy herpetofauna have only recently been documented (De Vries et al. 1997; Schiesari et al. 2003; Guayasamin et al. 2006). Kays and Allison (2001) reviewed published ecology and study methods for arboreal tropical forest vertebrates and found amphibians and reptiles to be grossly understudied compared to mammals, primarily due

¹Authors: Shawn F. McCracken and Michael R.J. Forstner. Publication: Herpetological Review, 39(2): 170-174 (2008).

to their cryptic habits and sampling difficulties. Of 752 articles on tropical forest arboreal vertebrates published between 1988 and 1998 only 4% focused on reptiles and amphibians, with the majority of those covering reptiles (Kays & Allison 2001). While many studies report arboreal occupancy by an extensive number of amphibian species, few have documented ecological characteristics besides presence/absence data based on calling males and new species descriptions (Duellman & Trueb 1986; Schiesari et al. 2003; Guayasamin et al. 2006). Most data for arboreal amphibians were obtained through collection and observation during reproduction of those species that descend from the canopy to breed in water bodies at the forest floor level (Duellman 1978a; Ron & Pramuk 1999; Duellman 2005). Standard survey techniques for amphibians, such as those at breeding sites, only encompass a small stratum (~2 m vertical height) of forest diversity (McCracken et al. 2007). Amphibians that specialize within the upper canopy remain mostly unaccounted for as a result of this limited vertical sampling bias (Guayasamin et al. 2006). More practical methods for studying canopy amphibians and reptiles is a high priority to facilitate the need for more survey and natural history work (Kays & Allison 2001).

A component of neotropical rainforest canopies that provide rich fauna microhabitats are the phytotelmata, defined as plants or parts of plants which hold rainwater (e.g. bromeliads, fruits, inflorescences, palm fronds and tree holes). In some tropical locations the availability of this habitat for aquatic organisms is up to 50,000 liters per hectare, literally a “wetland in the sky” (Kitching 2000; McCracken & Forstner 2006). In particular, epiphytic tank bromeliads are capable of holding relatively large amounts of water and play a principal role as a “keystone resource” and microhabitat for

invertebrates, vertebrates and other plants (Nadkarni 1994). Canopy bromeliad arthropod surveys have reported them as reservoirs of incredibly high biodiversity (Kitching 2000; Basset et al. 2003b). Typically, tank bromeliads occur in the upper canopy and overstory trees of lowland rainforest at vertical heights between 5-45 meters. Bromeliads normally range in number of individuals from ~5 to >150 on a single tree. Herein, we describe a technique for canopy bromeliad patch sampling of herpetofauna in lowland neotropical forests which is similar to those used in other canopy research disciplines but has not been documented for herpetofaunal investigations.

Methods

Bromeliad patch sampling was conducted during 2004 and 2006 at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito (USFQ), Orellana Province, Ecuador (00.63847°S, 076.14908°W, 217 m elev.). The vegetation type of the site has been defined as Amazonian Evergreen Lowland Forest (Palacios et al. 1999). Sampling units consisted of five bromeliads from each of 16 trees for a total of 80 bromeliads sampled. A tree was not sampled if less than 15 bromeliads of any species to be sampled were present to ensure continued persistence of the bromeliad community. Host trees were measured for diameter at 1.5 m above ground, height using a clinometer, and canopy cover using hemispherical photography with the Gap Light Analyzer (GLA) software. A leader line was positioned in the tree using a large slingshot (Sherrill™ Big Shot) which enables setting lines at 30+ meters. The canopy was accessed using single-rope technique, which should only be performed by trained and experienced individuals

(Fig. 2.2D). The lowest and highest elevation bromeliads were sampled with the remaining three sampled at estimated even intervals in between (Fig. 2.1). Before removal of each bromeliad a wide-angle photograph was taken and the following variables collected: elevation, ambient air temperature, relative humidity, barometric pressure, water temperature and pH are measured inside one of the outer leaf bracts, and a 50 mL water sample is collected by siphon. Ambient air temperature, relative humidity and barometric pressure were also collected at 1.5 m elevation. The bromeliad was removed by holding several leaves at the tips in one hand and cutting its base support stem with a pruning saw. The response of most animals is to retreat into the bromeliad bracts and therefore alleviates loss of specimens due to escape. The bromeliad was placed in a 55 gal. plastic bag with minimal disturbance, sealed, and placed in a tarp connected to a rope that is threaded through a carabiner on the climbers harness and the other end held by a ground support person. It was then gently lowered to the forest floor by the ground support person. Another photograph was taken of the site where the bromeliad was removed. After removal of the five bromeliads, a herbarium sample was collected from the tree to confirm identification and deposit in a herbarium. Bromeliads were processed at camp in a screen tent to prevent escape of animals (Fig. 2.2C). Bromeliad water was strained through a 1 mm mesh screen to separate arthropods, leaf litter, and detritus. Water volume was measured with a graduated cylinder. Bromeliads were measured, number of leaves counted, and photographed including a meter stick for scale reference (Fig. 2.2A, B). Individual leaves were removed to facilitate collection of herpetofauna, which were temporarily stored in bags for further processing. Herpetofauna species were photographed, measured and weighed. Blood or tissue samples were

collected and stored in blood storage buffer or 95% ethanol, respectively. Animals were euthanized in 10% ethyl alcohol or by ventral application of 20% benzocaine (Orajel®) and preserved using 10% formalin before being transferred to 70% ethyl alcohol for storage.

Results

In 2004 eight trees were surveyed for a total of 40 bromeliads sampled. Three species of bromeliads were sampled: 20 individuals of *Aechmea zebrina*, 17 of *Aechmea* sp., and three of an unidentified tankless bromeliad. In 2006 eight trees were surveyed for a total of 40 *A. zebrina* bromeliads sampled as part of a current study. Bromeliads were collected at elevations of 5.7–38.0 m (mean 27.0 ± 6.2 m) above ground. *Aechmea zebrina* bromeliads were 58.5–125.0 cm (mean 79.9 ± 13.9 cm, N = 40) tall and 54.0–147.5 cm (mean 89.5 ± 22.2 cm, N = 40) in diameter, *A* sp. bromeliads were 32.0–58.0 cm (mean 47.2 ± 9.7 cm, N = 17) and 54.0–94.0 cm (mean 66.8 ± 12.6 cm, N = 17) in diameter, and the unknown tankless bromeliads were 41.0–47.0 cm (mean 43.8 ± 3.1 cm, N = 3) and 33.0–43.0 cm (mean 37.3 ± 5.1 cm, N = 3) in diameter.

Thirty-four adults, 10 juveniles, 15 tadpoles, and 17 eggs of anurans representing at least four species were collected during the two survey periods. The identified adult and juvenile species included *Dendrobates (Ranitomeya) ventrimaculatus*, *Eleutherodactylus (Pristimantis) aureolineatus*, *Eleutherodactylus (Pristimantis) waoranii*, and *Osteocephalus taurinus*. Eight of the tadpole specimens were easily identified as *D. ventrimaculatus* due to their advanced stages of development. The

remaining tadpole specimens are to be identified using morphological and/or molecular techniques. One gecko, *Thecadactylus rapicauda*, was collected in an *A. zebrina* in 2006. Only one anuran was observed jumping from a bromeliad during removal and was visually identified when it landed on a nearby bromeliad before retreating into the leaf bracts.

Of the three bromeliad species, no anurans were found in the three tankless bromeliads, nine tadpoles of *D. ventrimaculatus* and three adult *E. waoranii* in five *Aechmea* sp., and the remainder in 26 *A. zebrina* (65% of *A. zebrina* sampled had anurans). All anurans were collected in bromeliads between 20.0–36.0 m (mean 28.3 ± 5.3 m) above ground.

Discussion

Visual encounter surveys, focal point observations, and inspection of individual bromeliads along a 100 m-long canopy walkway and two ~40 m high observation towers built around emergent trees at TBS–USFQ revealed 13 species of anurans; these surveys were conducted 3–4 times a year from 1998 to 2001 during the morning, afternoon, and night for 4–5 days duration (Cisneros-Heredia 2003; D. F. Cisneros-Heredia, pers. comm.). During one week in May 2002 canopy searches targeted at calling anurans were conducted using tree-climbing spurs at the Yasuni Scientific Research Station–Universidad Católica del Ecuador and resulted in the discovery of six anuran species occupying canopy habitat (S. Ron, pers. comm.). Canopy bromeliad patch sampling revealed a minimum of four species and the additional species *E. waoranii* (McCracken

et al. 2007). Results from our surveys contributed significantly to the new species description for *E. aureolineatus* and a manuscript on the reproductive ecology and behavior; they are wholly responsible for the new species description of *E. waoranii* (Guayasamin et al. 2006; McCracken & Forstner 2006; McCracken et al. 2007). Three other species found during these canopy surveys are newly described since 1999, demonstrating the value of such research techniques (Guayasamin et al. 2006). The potential for the discovery of additional new species and collection of detailed ecological data at other sites is evident in the fact that our surveys and the previous canopy surveys represent a limited sampling effort at two sites within close geographic proximity (~28 km) and similar habitat structure.

The technique provides a labor intensive but successful method for surveying the otherwise inaccessible microhabitats of the upper forest canopy strata herpetofauna. While our bromeliad patch sampling technique recovered less than a third of the number of species collected during the canopy walkway/tower surveys it represents a much less intensive sampling effort. Our sampling focused on the specific microhabitat provided by bromeliads, of which we only investigated three species. Our results indicate that the largest tank bromeliad in our surveys, *A. zebrina*, had the greatest occurrence rate with 65% of those sampled having anurans present. The use of canopy bromeliad patch sampling is also supported by the limited availability of canopy walkways and towers for research in Amazonia, and the financially prohibitive construction costs of such infrastructure for most research projects. Canopy bromeliad patch sampling can be employed anywhere the forest is accessible and facilitates the collection of independent replicate sampling units with associated biotic and abiotic factors for the analysis of

ecological correlates of species diversity and abundance in a robust sampling design. Our current study targets a species specific (*A. zebrina*) tank bromeliad microhabitat, but the technique may be applied to other species and microhabitats (e.g. tree holes/cavities) within the canopy. The technique may also be used to survey other forest canopies and their specific microhabitats.

Fauna of forest canopy habitats are at risk due to high rates of deforestation and habitat fragmentation, which are primary reasons for the rapid decline in amphibian populations worldwide with nearly one-third of all amphibians being threatened and at least 43% declining in population size (IUCN et al. 2006). The rapid exploitation of natural resources is having a profound effect on the rainforests and its inhabitants of the Ecuadorian Amazon. Yet, little is known about the effects of canopy biota loss. Epiphytes are considered hypersensitive to climatic conditions, requiring the very conditions they promote for existence (Benzing 1998; Hietz 1998; Benzing 2000). This hypersensitivity makes them particularly susceptible to forest microclimate changes as a result of anthropogenic disturbance, making epiphytes suitable as a bioindicator of diversity and forest ecosystem functions (Benzing 1998; Hietz 1998; Brighigna et al. 2002). Loss of epiphyte diversity will degrade all biodiversity within inclusive ecosystems by causing shifts in faunal resource availability, nutrient budgets and cycling, system energetics, and hydrology (Benzing 1998). Amphibians may be considered a vertebrate counterpart to epiphytes as bioindicator species and their utilization of epiphytic tank bromeliad habitat provides the researcher with a unique system for monitoring anthropogenic disturbance in forest canopies. Bromeliad patch sampling surveys are essential to documentation of the

faunal diversity in neotropical forest canopies and promoting the conservation of these important “wetlands in the sky” (McCracken & Forstner 2006).

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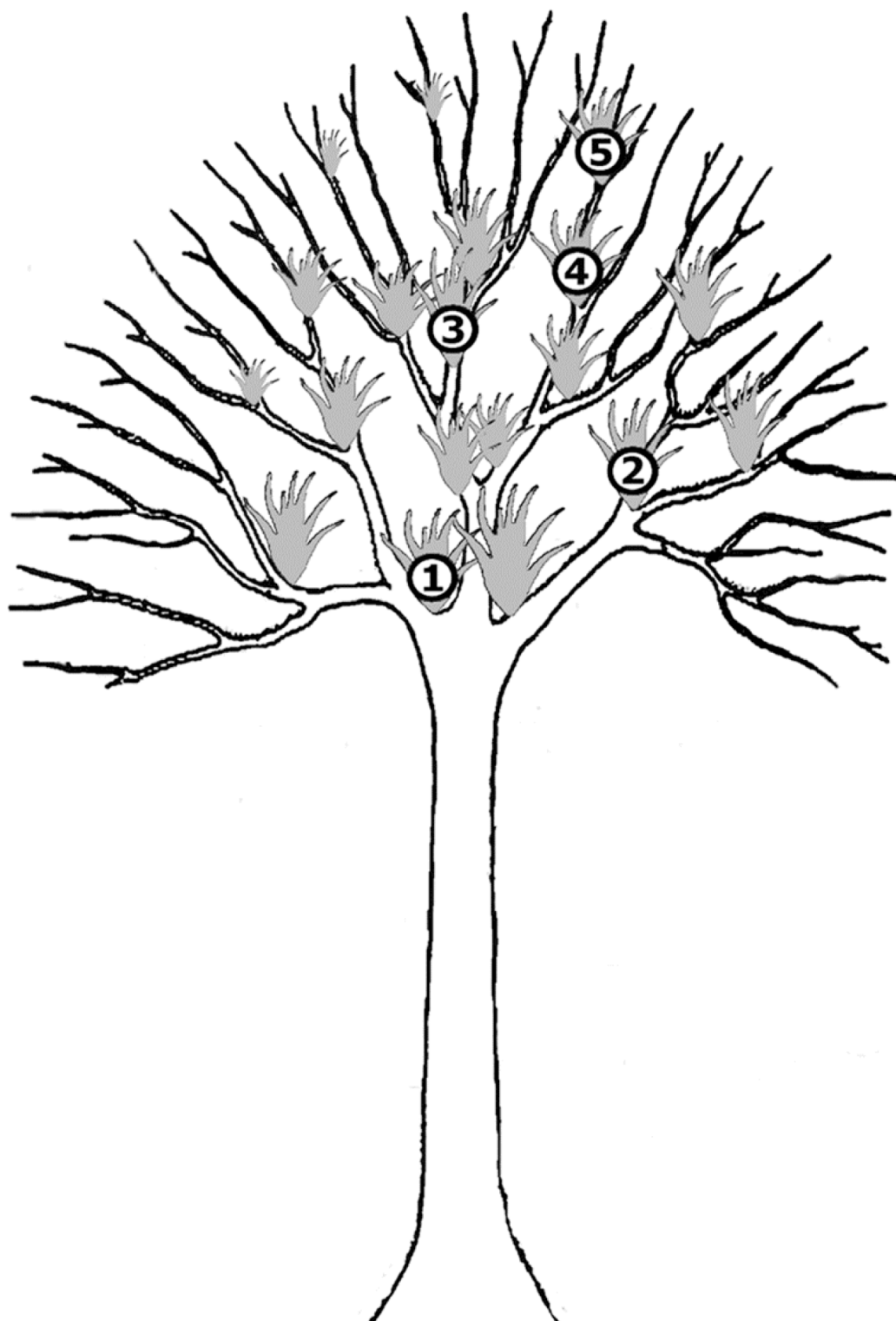


FIG. 2.1: Tree schematic for bromeliad sampling. Schematic of tree with bromeliads illustrating distribution strategy for sampling units (bromeliads), numbers denote bromeliads sampled.



Fig. 2.2: Photo details of bromeliad patch sampling methodology. A) Side view photo of *A. zebrina* bromeliad with meter stick in background. Bar = 20 cm. B) Top view of *A. zebrina* bromeliad with meter stick below. Bar = 20 cm. C) Senior author in screened tent with sampled bromeliad to prevent escape of herpetofauna. D) Senior author ascending into canopy to access bromeliads for sampling using single-rope technique (SRT) to climb.

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CHAPTER III

A NEW SPECIES OF THE *ELEUTHERODACTYLUS LACRIMOSUS* ASSEMBLAGE
(ANURA: BRACHYCEPHALIDAE) FROM THE LOWLAND RAINFOREST
CANOPY OF YASUNI NATIONAL PARK—AMAZONIAN ECUADOR¹

Abstract

A new species of *Eleutherodactylus* (Anura: Brachycephalidae) from the lowland rainforest canopy in northeastern Amazonian Ecuador is described. It is placed in the *Eleutherodactylus lacrimosus* assemblage of the greater *unistrigatus* group. It is most similar to the sympatric *E. lacrimosus*, but differs by the lack of a papilla at the tip of the snout, tubercles on upper eyelids, tubercles on dorsum, dorsal markings, and larger body size, and the presence of lateral fringes on the fingers and a tarsal fold. The new species inhabits tank bromeliads in the upper strata of the rainforest canopy at heights of 23.5–38.0 m. The effects of implementation of canopy surveys on biological diversity are briefly discussed.

¹Authors: Shawn F. McCracken, Michael R.J. Forstner, and James R. Dixon. Publication: Phyllomedusa—Journal of Neotropical Herpetology, 6(1): 21-33 (2007).

Introduction

The anuran genus *Eleutherodactylus* is one of the largest vertebrate genera in the world with 492 species currently described (Frost 2007). Their distribution ranges from the southern United States through Mesoamerica and the West Indies to southern South America (Zug *et al.* 2001). The recent publication of the Amphibian tree of life by Frost *et al.* (2006) has partitioned the former genus “*Eleutherodactylus*” (sensu lato) into five genera and placed them in the family Brachycephalidae along with an additional 11 genera, while acknowledging the probable continued nonmonophyletic state of “*Eleutherodactylus*” (sensu stricto). We acknowledge and accept *Eleutherodactylus* as nonmonophyletic and treat it here in the sensu stricto sense, but refrain from using quotation marks throughout this manuscript for ease of readability.

Ecuador is ranked number three in the world for amphibian diversity with more than 447 known species, following Brazil and Colombia, respectively (Young *et al.* 2004). In Ecuador, at least 132 species are members of the genus *Eleutherodactylus*, with 21 residing in the eastern lowlands of the Amazon basin (IUCN *et al.* 2004). Until recently, only minimal investigation of *Eleutherodactylus* in lowland eastern Ecuador has been conducted following Duellman’s exhaustive work at Santa Cecilia in the sixties and seventies (Duellman 1978b; Lynch & Duellman 1980). Furthermore, examination of the canopy anuran community has been poorly represented in the study of the Amazonian amphibian fauna (Guayasamin *et al.* 2006).

The *Eleutherodactylus lacrimosus* assemblage currently consists of 15 recognized species, and is considered a phenetic subgroup of the large *E. unistrigatus* species group

applied by Lynch and Duellman (1997) (Guayasamin *et al.* 2006, Heyer and Hardy 1991). The 15 species currently considered part of the *E. lacrimosus* assemblage are: *E. apiculatus*, *E. aureolineatus*, *E. boulengeri*, *E. brevifrons*, *E. bromeliaceus*, *E. dorsopictus*, *E. eremitus*, *E. lacrimosus*, *E. mendax*, *E. olivaceus*, *E. petersorum*, *E. prolixodiscus*, *E. schultei*, *E. tayrona* and *E. zimmermanae* (Guayasamin *et al.* 2006). The *lacrimosus* assemblage is loosely delineated by the morphological characters of a small body size and broad, flat, pointed head; and more inclusively, a niche-specific association with bromeliads, with the exception of *E. apiculatus* (Lynch and Ruíz-Carranza 1985, Heyer and Hardy 1991, Guayasamin *et al.* 2006).

The species description herein resulted from bromeliad patch sampling conducted at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito in the eastern lowlands of Amazonian Ecuador.

Materials and Methods

Morphological terminology, diagnosis, and description follow Lynch and Duellman (1997); the format follows Guayasamin *et al.* (2006). Diagnosis, description, and variation were conducted from the observation of both preserved specimens and a series of high-resolution digital color macro photographs of each specimen consisting of dorsal, ventral, and lateral views in life. Photographs were taken with a Pentax istD digital SLR camera, outfitted with a 50 mm Pentax macro lens, Pentax AF-140c ring flash, and a Pentax AF-360FGZ external flash. Specimens were euthanized and muscle tissue was removed from the left thigh of all specimens for DNA tissue sample and stored

in 95% ethanol at - 80 °C, deposited in the Frozen Tissue Collection of Dr. Michael R. J. Forstner (MJF) at Texas State University, San Marcos, Texas; then specimens were preserved in 10% formalin and stored in 70% ethanol. All specimens were deposited at the Texas Cooperative Wildlife Collection (TCWC) at Texas A&M University, College Station, Texas. Specimens and field notes were collected by S.F. McCracken between June and August 2004.

Morphological measurements follow Guayasamin (2004) and Fuentes and Barrio-Amorós (2004), taken with a Mitutoyo CD-S6”C digital dial caliper using an Olympus SZH Stereozoom microscope on preserved specimens to the nearest 0.1mm. Measurements taken include: (1) snout–vent length (SVL); (2) tibia length; (3) foot length; (4) head length; (5) head width; (6) upper–eyelid width; (7); interorbital distance; (8) eye diameter; (9) eye-to-nostril distance; (10) snout–eye distance; (11) tympanum diameter; (12) eye-to-tympanum distance; (13) internarial distance; (14) radioulna length; (15) hand length; (16) Finger I length; (17) Finger II length; (18) Finger III disc width; (19) femur length; (20) Toe IV disc width. Sexual maturity was determined by observation for the presence or absence of vocal slits in males, and examination for the presence of convoluted oviducts in females.

All specimens were collected at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito via bromeliad patch sampling using single-rope climbing technique to access canopy bromeliads. Two trees in each of four quadrat plots were surveyed; five bromeliads ($n = 40$) were removed from each tree at varying vertical heights and placed in large plastic bags to be lowered to the forest floor. Bromeliads were searched by removal of individual leaves to allow collection of all anurans within a

screened enclosure to prevent escape. Vertical height of bromeliads was obtained by attaching a 100 m reel tape measure to the tree climber's harness and positioning the reel at the base of the tree, height was recorded by a climbing assistant on the ground when the climber was level with the bromeliad being collected. Environmental data (Table 3.2) were recorded for collection sites and reported herein for occurrences of *E. aureolineatus*, and include: temperature (°C) at 0 m, 1 m and bromeliad collection site; barometric pressure (hPa) at 1 m and bromeliad collection site with a Brunton Sherpa; humidity (% relative humidity) at 1 m with a sling psychrometer; pH of water in bromeliad with a Oakton (pHTestr 30) meter; volume (L) of water in bromeliad with graduated cylinder; and diameter at breast height (DBH) of tree to the nearest 1.0 mm (Table 3.2). Spatial distribution of sampled trees is represented by altitude (m.a.s.l.) and the shortest distance (m) to the Tiputini River using the measurement tool in ArcInfo 9.1 (Figure 3.3). Identifications of plant species were done using Ribeiro *et al.* (1999) and Kreft and Köster (2004).

Species Description

Eleutherodactylus waoranii sp. nov.

Figures 3.1 (A, B, C, D, E, F), 3.2 (A, B, C, D)

Holotype - TCWC 90728, adult male collected near the border of Yasuni National Park along the Tiputini River, Provincia de Orellana, Ecuador; approximately 1 km Northwest of the Tiputini Biodiversity Station (TBS)—Universidad San Francisco de

Quito, 0° 38' 18.49'' S, 76° 08' 56.69'' W, 217 m a.s.l., collected on 15 August 2004 by Shawn F. McCracken.

Paratopotypes - TCWC 90729 and 90730, adult females, collected 15 August 2004 by Shawn F. McCracken.

Paratypes - TCWC 90731, adult female, collected 31 July 2004; TCWC 90732, adult male, collected 08 August 2004 by Shawn F. McCracken at the type locality.

Diagnosis - A member of the *Eleutherodactylus lacrimosus* assemblage contained within the greater *E. unistrigatus* group (Lynch and Duellman 1980, Lynch and Ruíz-Carranza 1985, Lynch and Duellman 1997) having (1) skin on dorsum finely shagreen, that on venter areolate; discoidal fold low; dorsolateral folds absent; (2) tympanic membrane weakly differentiated in males and slightly evident in females; tympanic annulus evident, with supratympanic fold obscuring upper and posterodorsal edges; horizontal diameter of tympanum 39–49% of eye diameter; (3) snout subacuminate in dorsal view (truncated by protruding nostrils), truncate in profile; (4) upper eyelid lacking tubercles, 50–74% of interorbital distance; cranial crests absent; (5) dentigerous process of the vomer triangular, each bearing 3–6 teeth; (6) males with vocal slits and median subgular vocal sac; white, nonspinous nuptial pads present; and white testes (mesorchium); (7) first finger shorter than the second; Fingers III–IV bearing expanded and rounded discs about twice as wide as digits; (8) fingers with narrow lateral fringes; (9) ulnar tubercles low, antebrachial largest; (10) tubercles on heel and outer edge of tarsus absent; inner tarsal fold present; (11) inner metatarsal tubercle oval, 2–3x as long as round outer metatarsal tubercle; supernumerary plantar tubercles small and low, at the

base of Toes III and IV; (12) toes with narrow lateral fringes; webbing absent; fifth toe much longer than third; (13) in life, dorsum of males bright golden-brown, that of females golden-brown with greenish tint to dark brown; venter of males translucent white, that of females white to creamy white; (14) adults small, SVL in males 19.7–21.2 mm ($\bar{x} = 20.4 \pm 1.12$, $n = 2$), in females 27.5–31.1 mm ($\bar{x} = 29.7 \pm 1.97$, $n = 3$).

Comparison with similar species - *Eleutherodactylus waoranii* is most similar to *E. lacrimosus*, both of which occur in the Yasuni region. *Eleutherodactylus waoranii* is differentiated from *E. lacrimosus* by the lack of a papilla at tip of snout (variably reported in literature for *E. lacrimosus*), tubercles on upper eyelids, tubercles on dorsum (few tubercles in *E. lacrimosus*), and dark brown markings on dorsum and hind limbs (Lynch and Schwartz 1971, Lynch and Duellman 1980, Rivero and Serna 1987, Heyer and Hardy 1991, Rodríguez and Duellman 1994, Guayasamin *et al.* 2006). It also differs by the presence of narrow lateral fringes on fingers, series of tubercles on the ulnar, inner tarsal fold, and marginally larger body size than *E. lacrimosus* (Lynch and Schwartz 1971, Lynch and Duellman 1980, Rivero and Serna 1987, Heyer and Hardy 1991, Rodríguez and Duellman 1994, Guayasamin *et al.* 2006). Lynch and Duellman (1980) reported smaller sizes for lowland (< 800 m) populations of *E. lacrimosus* compared to those on Amazonian slopes. For lowland populations they recorded a SVL for males 16.1–20.0 mm ($\bar{x} = 18.5 \pm 0.5$, $n = 18$) and for females 20.6–24.4 mm ($\bar{x} = 22.5 \pm 0.5$, $n = 18$); in *E. waoranii*, we recorded a SVL for males 19.7–21.2 mm ($\bar{x} = 20.4 \pm 1.1$, $n = 2$), and for females 27.5–31.1 mm ($\bar{x} = 29.7 \pm 2.0$, $n = 3$). *Eleutherodactylus waoranii* differs from the sympatric species *Eleutherodactylus acuminatus* by lacking a green dorsum, pointed snout, and a dark stripe from the tip of the snout extending through the eye to

above the arm or along the flank. *Eleutherodactylus waoranii* has a prominent tympanum whereas the tympanum is concealed in *E. acuminatus* (Duellman 1978, Rodríguez and Duellman 1994). *Eleutherodactylus waoranii* differs from the syntopic *E. aureolineatus* by lacking a papilla on tip of the snout and a creamy-yellow interorbital stripe extending above the eyes and dorsolaterally to the sacrum (Guayasamin *et al.* 2006). *E. waoranii* differs from all other members of the *E. lacrimosus* assemblage by its unmarked, bright to dark golden-brown dorsal coloration and absence of any tuberculation of the upper eyelid (Guayasamin *et al.* 2006).

Description of Holotype - Adult male (TCWC 90728), 21.24 mm. Head slightly wider than body, wider than long; head width 41.4% SVL; snout subacuminate in dorsal view (truncated by protruding nostrils), truncate in lateral view, comparatively short (snout–eye distance 16.0% SVL); eye–nostril distance 96.3% diameter of eyes; nostrils protuberant, weakly directed dorsolaterally; canthus rostralis angular, straight to slightly sinuous; loreal region weakly concave, sloping abruptly to lips; upper lips not flared; lacking upper eyelid tubercle; interorbital space flat, wider than upper eyelid (upper eyelid width 63.2% interorbital distance); cranial crests absent; upper eyelid lacking tubercles; temporal region slightly angled; supratympanic fold weakly defined, obscuring upper and posterodorsal edge of annulus, extending posteroventrally to just above insertion of arm; tympanic membrane weakly differentiated from surrounding skin; tympanic annulus, round, uppermost portion slightly obscured by supratympanic fold, tympanum diameter 38.7% of eye diameter, separated from eye by distance 70.0% of tympanum diameter; postrectal tubercles posteroventral to tympanic annulus, compressed and fused, forming short ridge extending ventrolaterally from tympanum; choanae round,

small, not concealed by palatal shelf of maxillary arch, separated by distance approximately twice the width of single choanae; vomerine odontophores triangular, postereomedian to choanae, separated medially by distance less than width of odontophore, with four vomerine teeth right and three left in transverse row at base of triangle (bilateral variation); tongue as wide as long, posterior border not notched, posterior 25% not adherent to floor of mouth; vocal slits elongate, posterolateral to tongue; vocal sac single, subgular.

Skin on the dorsum, head, flanks and upper surface of limbs smooth to finely shagreen; dorsolateral folds absent; skin on throat smooth; skin on venter areolate; ventral surfaces of limbs smooth, except skin of thigh below and ventrolateral to vent areolate; discoidal fold well anterior to groin; cloacal opening directed posteriorly above dorsal plane of thighs, short transverse fold or flap dorsally; cloacal sheath and enlarged tubercles absent.

Forearm slender; radio-ulna length 24.0% SVL; three low ulnar tubercles in row extending to just distal of elbow, unevenly spaced, low, antebrachial largest; hand length longer than radio-ulna length (hand length 24.6% SVL); thenar tubercle oval, twice as long as wide; palmar tubercle large, incompletely bifid, about 2x larger than thenar; four distinct supernumerary palmar tubercles present on each hand, low, round; subarticular tubercles elevated, rounded; rest of palmar surface areolate; narrow lateral fringes present on fingers; webbing absent; finger length $I < II < IV < III$, first finger slightly shorter than second; discs on fingers broad, round on Fingers I and II, expanded and rounded on Fingers III and IV, on Finger I slightly wider than width of digit, on Finger II less than 1.5x width of digit, those on Fingers III and IV at least 2x width of digit (about equal to

tympanic annulus in life); broad ventral pads with complete circumferential grooves on all discs; white nonspinous nuptial pad evident on medial surface at base of Finger I.

Hind limbs slender; tibia length 50.6% SVL; foot length 136.1% of tibia length; heels overlap considerably when flexed hind limbs held perpendicular to sagittal plane; heel tubercle absent; outer edge of tarsus lacking tubercles; inner surface of tarsus bearing row of low tubercles with a short ridge between tubercles forming an inner tarsal fold, extend from proximal inner metatarsal tubercle to just distal of heel; inner metatarsal tubercle oval, about 2x as long as wide; outer metatarsal tubercle round, subconical, about one-fourth size of inner metatarsal tubercle; supernumerary plantar tubercles small and low at bases of Toes III–IV; subarticular tubercles subconical; rest of plantar surface areolate; toes bearing narrow lateral fringes coalescing at base of toes; webbing absent; toe length $I < II < III < V < IV$; tip of Toe V reaching just beyond distal border of distal subarticular tubercle of Toe IV; tip of Toe III reaching distal border of medial subarticular tubercle of Toe IV; disc on Toe I round, slightly broader than digit proximal to disc; discs on Toes II and III broad and rounded increasingly larger; disc on Toe IV large, significantly broadened, slightly smaller than discs on Fingers III and IV; disc on Toe V slightly smaller, broadened.

Coloration of holotype in life - Dorsum uniformly bright golden-brown, dark outline of optic lobes clearly visible through skin; upper eyelids a slightly darker golden-green; flanks pale golden-yellow with minute brown specks; faint pale salmon color ventrolaterally and across chest, most prominent at insertion of forearm. Venter translucent white; outline of heart, liver and intestines visible; throat pale yellow; limbs uniformly bright golden-brown, with the exception of a transverse narrow brown bar on

the upper surface of the wrist and the upper surface of the heel being pale golden-yellow. The iris is bright reddish-copper with a median, horizontal reddish-brown streak. (Figure 3.1).

Color of holotype in ethanol - Dorsum light tan; canthus and nostrils brown; flanks white; venter translucent white; throat and ventral surfaces of limbs white.

Measurements of holotype and paratypes – Table 3.1.

Eleutherodactylus acuminatus – ECUADOR: **Orellana**: Tuputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, SFM 0039, 0040, 0051, 0126, 0409, 0410, and 0687. PERU: **Loreto**: Moropon, TCWC 41521.

Eleutherodactylus aureolineatus – ECUADOR: **Orellana**: Tuputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, TCWC 90334–90342 (paratypes).

Eleutherodactylus lacrimosus – ECUADOR: **Napo**: Isla Anaconda, KU 202406; Rio Alpayacu, KU 180294. ECUADOR: **Orellana**: Tuputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, SFM 0371. ECUADOR: **Sucumbios**: Lago Agrio, KU 126210; Limoncocha, KU 104623 and 106967; Santa Cecilia, KU 148895, 148897, 148905–148908, 152385, and 153022. PERU: **Loreto**: Explornapo, KU 22042

Variation - The ridge formed by the compressed and fused postrictal tubercles are variable; in one male (TCWC 90732) and one female (TCWC 90730) there is a break in the line of the ridge, forming what appears as two elongate tubercles. The number of teeth on the vomerine odontophores is variable; bilateral variation is evident in two other specimens, one female (TCWC 90731) has 3 and 5 teeth, one male (TCWC 90732) has 4

and 6 teeth, while two females (TCWC 90729, 90730) do not show bilateral variation, with 4 or 5 teeth on each odontophore. Tongue length longer than wide in TCWC 90729, 90730, and 90732; small notch in posterior border of TCWC 90730 and 90732. Ulnar tubercles marginally evident in TCWC 90729. In life, males have a bright golden-brown dorsum by night and pale golden-brown dorsum by day. Females have a darker coloration, one female (TCWC 90731) was golden-brown with a greenish tint by night and dark golden-brown by day, whereas the other two females (TCWC 90729, 90730) were dark golden-brown by night and a darker reddish-brown by day. Flanks pale golden-yellow with minute brown specks and faint pale salmon color ventrolaterally, pale salmon color absent in TCWC 90732; venter translucent white to creamy white, throat pale yellow in males and white in females. Posterior surface of thighs is variably lighter than dorsum of individuals. Iris lacking median, horizontal reddish-brown streak in TCWC 90729, 90730, and 90732. In preservative, the dorsum is creamy brown (TCWC 90732) or reddish brown (TCWC 90729, 90730); soles and palms pale tan (TCWC 90731, 90732) or pale brown (TCWC 90729, 90730).

Distribution and natural history - *Eleutherodactylus waoranii* is known only from the type locality in the upper Amazon Basin of eastern Ecuador at the Tiputini Biodiversity Station–Universidad San Francisco de Quito. The region is classified as lowland evergreen broadleaf rain forest, with an annual mean temperature of 25°C, annual mean relative humidity of 88% and annual rainfall between 2425–3145 mm (Iremonger *et al.* 1997, Balslev *et al.* 1987; Blandin 1976, Duellman 1978). TBS is on the north shore of the Río Tiputini at an elevation of 217 m, with low rolling hills up to an elevation of 310 m. The habitat consists of terra firme forest (non-flooded forest), varzea

(flooded forest), seasonally flooded forest, palm swamp, and igapó forest (black-water tributary associated forest) (Duellman 1978, Jaramillo and de Vries 2002). On 15 August 2004, the holotype and two adult females (TCWC 90729, 90730) were collected by day in a tank bromeliad (*Aechmea zebrina*), at 29.5 m height, on the trunk of a tree in terra firme forest approximately 2.5 km west-northwest of the TBS camp. On 31 July 2004, an adult female (TCWC 90731) was collected by day in a tank bromeliad (*Aechmea zebrina*), at 38.0 m height, on the trunk of a tree (*Dimorphandra sp.*) in terra firme forest approximately 1.0 km northwest of the TBS camp. On 08 August 2004, an adult male (TCWC 90732) was collected by day in a tank bromeliad (*Aechmea zebrina*), at 23.5 m on a branch of a tree in terra firme forest approximately 1.25 km northwest of the TBS camp. All individuals were found in the water-filled outer bracts of bromeliads. Vertical heights and environmental data collected for bromeliads are listed in Table 3.2. All trees and bromeliad sites were above the surrounding forest canopy, *Eleutherodactylus waoranii* is unmistakably an inhabitant of the sub- to upper canopy strata.

Eleutherodactylus waoranii lives syntopically with *E. aureolineatus*, both have been observed in the same bromeliad during bromeliad patch sampling conducted at TBS. *Eleutherodactylus waoranii* and *E. lacrimosus* are considered to exist sympatrically at TBS, no *E. lacrimosus* have been collected during the sampling effort of 80 canopy bromeliads to date.

Etymology - Named in recognition of the Waorani, an ancient indigenous tribe who have inhabited the type locality for centuries. They are notoriously excellent tree climbers and continue to persist, along with the local biota, despite widespread anthropogenic pressures in the region.

Remarks - Only one *E. lacrimosus* (SFM0371) was found at TBS on May 24, 2004 on the upper surface of a palm leaf at 1.2 m during nocturnal terrestrial quadrat surveys. Representative specimens collected after the writing of this manuscript during the June–August 2006 field season to be deposited at the Museo Ecuatoriano de Ciencias Naturales in Quito, Ecuador, after examination by the senior author.

Discussion

Eleutherodactylus waoranii is considered a member of the *E. lacrimosus* assemblage, a group which has experienced considerable historic taxonomic confusion potentially as a result of limited confirmed specimens and difficulties associated with definitive morphological characters among vouchers. The placement is based upon its niche-specific association with bromeliads, and morphological characters of a small body size, and broad, flat head, with the fifth toe much longer than third (Lynch and Ruiz-Carranza 1985, Heyer and Hardy 1991, Guayasamin *et al.* 2006).

Eleutherodactylus waoranii is recognized as a new species, differentiated from *Eleutherodactylus lacrimosus* based upon comparison to cumulative descriptions within the literature, preserved specimens, and high-resolution digital macro photographs in life of an adult male (specimen/photo voucher, SFM 0371), found at the type locality, matching the descriptions of *E. lacrimosus*. Lynch and Schwartz (1971) redescribed and figured *E. lacrimosus* in lieu of absence of the original holotype collected and described by Jiménez de la Espada (1875). Heyer and Hardy (1991) designated the specimen KU 110782, examined and figured by Lynch and Schwartz (1971), as the neotype for *E.*

lacrimosus in agreement with Lynch and Schwartz's (1971) description, "...with the minor exceptions that the upper eyelids, although flattened in preservative, appear to be moderately tuberculate and moderately developed antebrachial tubercles are present". *E. lacrimosus* (SFM 0371) from the type locality of *E. waoranii* conforms to these descriptions, with the exception of possessing a low inner tarsal fold most evident in the macro photographs. The most obvious morphological differences between the species are the lack of a papilla at the tip of the snout (variably reported in literature for *E. lacrimosus*), tubercles on upper eyelids, tubercles on dorsum (few tubercles in *E. lacrimosus*), and dark brown markings on dorsum and hind limbs, larger size, and the presence of lateral fringes on the fingers and a tarsal fold in *E. waoranii* (Lynch and Schwartz 1971, Lynch and Duellman 1980, Rivero and Serna 1987, Heyer and Hardy 1991, Rodríguez and Duellman 1994, Guayasamin *et al.* 2006).

The inclusion of sampling methods directed at little known habitats are necessary to ensure the accuracy of biodiversity estimates for a given study site. Terrestrial amphibian surveys are the historical method of sampling in tropical rainforest, only encompassing a small stratum (~ 2 m vertical height) of rainforest diversity. Investigations into the amphibian fauna of the canopy has historically only been represented by a few surveys of felled trees conducted during forest clearing operations, limiting collection of substantial data on habitat strata occupancy and utilization (Duellman 1978). Yet, where the upper strata have been examined the results are very productive. Annual canopy surveys were conducted from 1998 to 2002 along a 100 m-long system of canopy bridges at TBS, and sporadic canopy surveys were conducted during one week in May 2002 at the Yasuni Scientific Research Station–Universidad

Católica del Ecuador by students from the respective universities (Guayasamin *et al.* 2006). These surveys have resulted in the collection of 15 species; see Guayasamin *et al.* (2006) for a detailed report on the results of these surveys. The current study has also shown glimpses of additional diversity in these habitats. We found 17 specimens from three species of metamorphosed anurans and 11 individuals of unidentified larval amphibians. Two of those species are now described as new taxa in the genus *Eleutherodactylus*. Of the total 15 species revealed by canopy searches, the majority have not been collected during typical terrestrial surveys. It is hypothesized that the reported diversity, community structure, and abundance of amphibians within the Ecuadorian Amazon rainforest system is biased towards the terrestrial strata (Guayasamin *et al.* 2006). Given the rapid rate of deforestation throughout Amazonia canopy surveys are crucial for the documentation of their vertebrate taxa to facilitate the conservation of this important “wetlands in the sky” (McCracken and Forstner 2006). In future sampling efforts, the incorporation of sampling techniques that include the vertical forest strata are necessary to increase the accuracy of the herpetofaunal diversity within a given area of study.

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Table 3.1: Adult *Eleutherodactylus waoranii* measurements. Measurements (in mm) of adult *Eleutherodactylus waoranii* (H = Holotype).

	TCWC 90728 (H)	TCWC 90732	TCWC 90729	TCWC 90730	TCWC 90731
Sex	Male	Male	Female	Female	Female
SVL	21.2	19.7	30.7	31.1	27.5
Tibia length	10.7	11.0	15.8	16.3	15.2
Foot length	14.6	13.4	21.3	22.7	20.1
Head length	7.0	7.2	11.7	12.0	11.2
Head width	8.8	8.6	14.4	14.1	12.2
Upper-eyelid width	1.8	1.9	2.9	2.9	2.0
Interorbital distance	2.9	2.6	3.9	4.0	4.1
Eye diameter	2.7	2.5	3.8	4.2	3.2
Eye-to-nostril distance	2.6	2.7	3.7	4.3	3.0
Snout-to-eye distance	3.4	3.5	4.8	5.0	4.2
Tympanum diameter	1.0	1.1	1.8	1.7	1.6
Eye-to-tympanum distance	0.7	0.6	1.5	1.5	1.3
Internarial distance	1.6	1.5	2.1	2.1	1.9
Radioulna length	5.1	5.2	7.4	7.5	6.7
Hand length	5.2	5.6	8.6	8.6	7.0
Finger-I length	3.5	3.6	5.8	5.6	4.8
Finger-II length	3.6	3.7	6.0	5.9	5.1
Finger-III disc Width	0.9	0.8	1.6	1.6	0.9
Femur length	9.5	10.0	13.4	13.2	12.5
Toe-IV disc width	0.8	0.8	1.3	1.6	1.0

Table 3.2: Bromeliad environmental data. Vertical heights and environmental data collected for bromeliads with occurrences of *Eleutherodactylus waoranii*.

Environmental parameters	Bromeliads		
	PMT5B1	PMT6B2	ADT8B2
Vertical height (m)	38.0	23.5	29.5
Water volume (L)	1.240	1.195	1.830
Temp.(°C) @ 0m	24.5	23.8	26.5
Temp.(°C) @ 1m	25.0	24.0	24.5
Temp.(°C) @ bromeliad	24.0	23.0	25.0
hPa @ 1m	1015	1017	1018
hPa @ bromeliad	1015	1014	1018
% relative humidity @ 1m	92.0	95.0	99.0
Water pH	5.45	4.88	4.19
DBH (cm) of tree	289.0	47.0	56.5

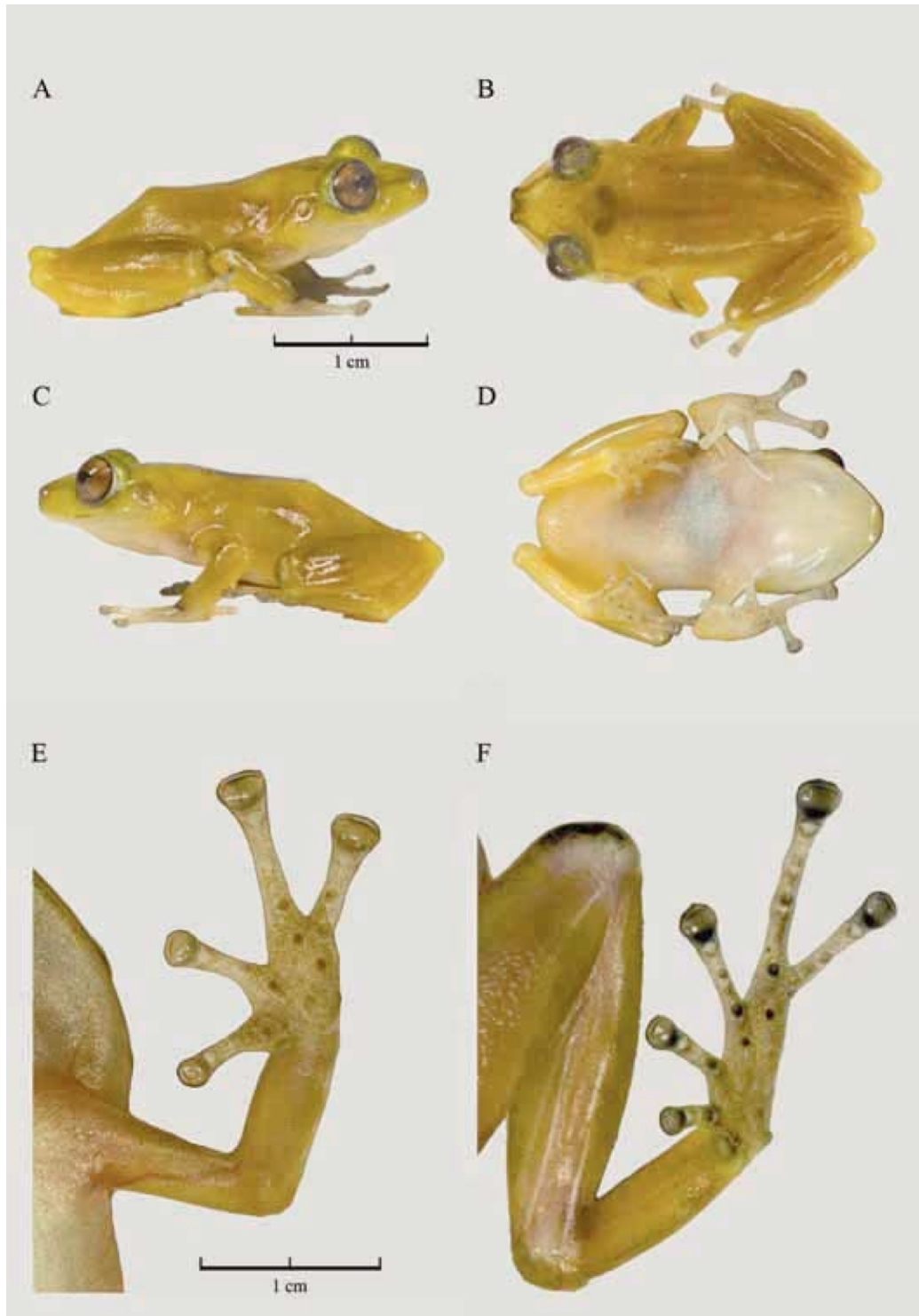


Figure 3.1: *Eleutherodactylus waoranii* specimen plates. *Eleutherodactylus waoranii* (TCWC 90728, holotype, SVL = 21.2 mm, male) in lateral (A, C), dorsal (B), and ventral (D) views; and plantar view of left hand (E) and left foot (F) of *E. waoranii* (TCWC 90731, paratype, SVL = 27.5 mm, female). Photograph by Bejat A. McCracken.

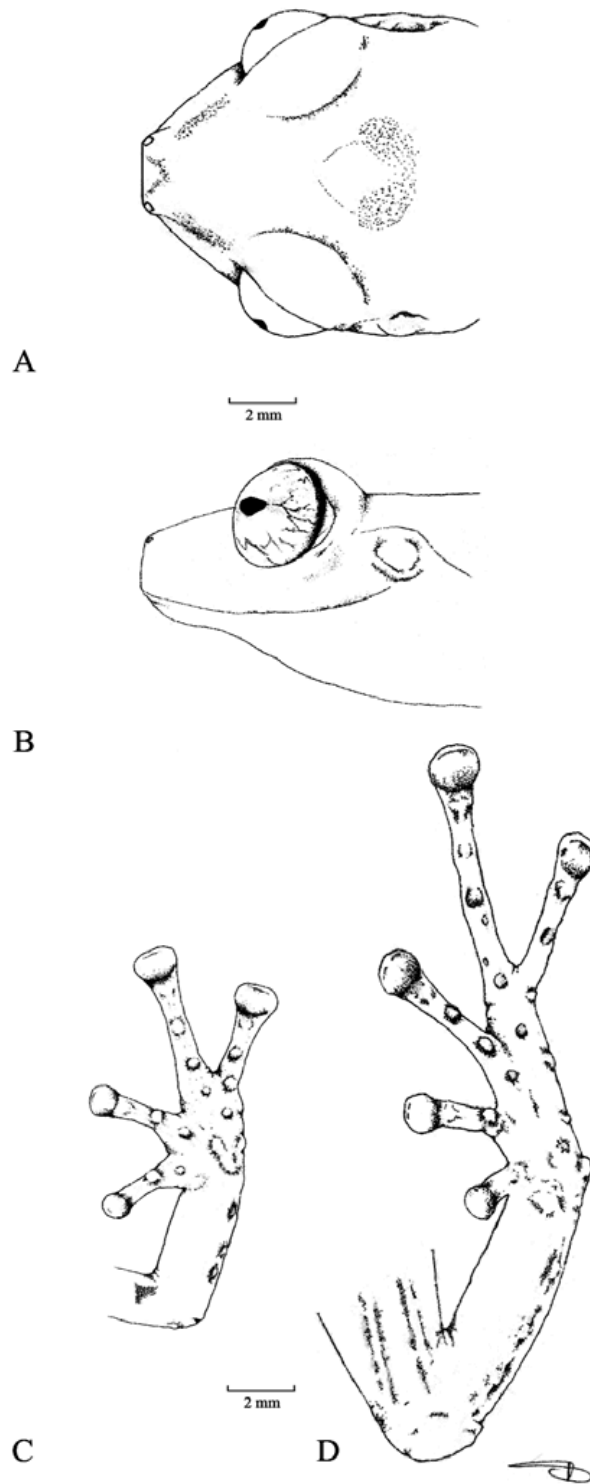


Figure 3.2: *Eleutherodactylus waoranii* specimen drawings. Dorsal (A) and lateral (B) views of head, and ventral views of hand (C) and foot (D) for the holotype of *Eleutherodactylus waoranii* (TCWC 90728), holotype, SVL = 21.2 mm, male). Drawings by Tana Ryan.

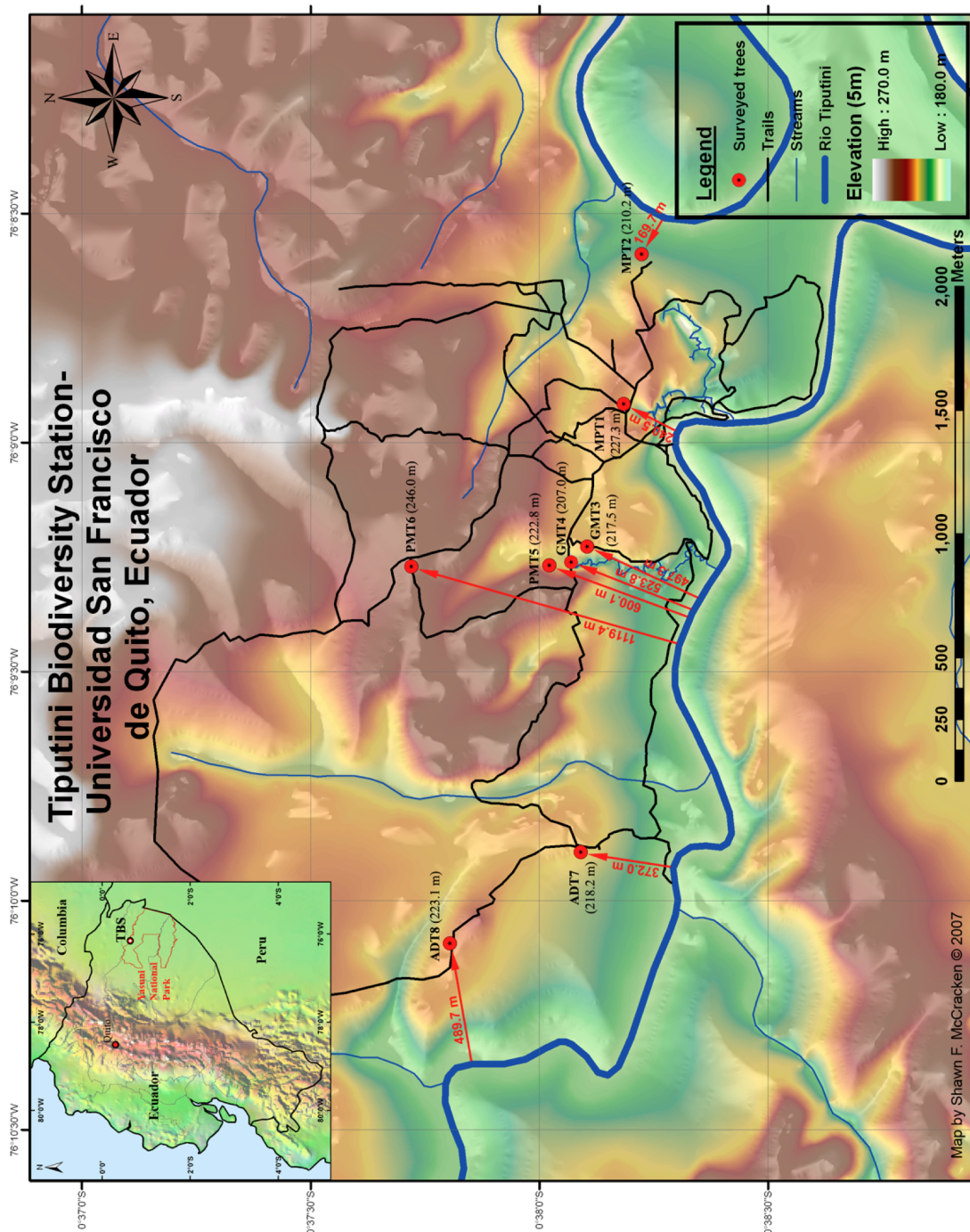


Figure 3.3: Map of bromeliad patch sampling trees. Map of trees surveyed by bromeliad patch sampling at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito, Ecuador. Tree distance shortest to the Tiputini River in red and elevation in black. Inset map shows location of TBS and outline of Yasuni National Park in red.

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CHAPTER IV

REPRODUCTIVE ECOLOGY AND BEHAVIOR OF *ELEUTHERODACTYLUS* *AUREOLINEATUS* (ANURA: BRACHYCEPHALIDAE) IN THE CANOPY OF THE UPPER AMAZON BASIN¹

Abstract

Exploration and investigation of the canopy strata anuran fauna of the Upper Amazon Basin has led to the recent discoveries of new species of *Eleutherodactylus* utilizing the microhabitat within bromeliads. Detailed information on the ecology and natural history of these communities is scarce due in part to the difficulty of accessing their habitat. New sampling methods for rainforest canopies have allowed for the collection and observation of the herpetofauna utilizing this habitat. Sexual size dimorphism is examined for the newly described *Eleutherodactylus aureolineatus*, and confirms placement in the *unistrigatus* species group and *lacrimosus* assemblage. The microhabitat, vocalization characteristics, reproductive behavior and egg deposition site are also described. The data and observations herein contribute to the overall

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understanding of the characters and relationships which define the *lacrimosus* assemblage.

Introduction

Eleutherodactylus aureolineatus was recently described by Guayasamin *et al.* (2006) from Provincia de Sucumbios and Provincia de Orellana of northeastern Ecuador, and Departamento de Loreto of northeastern Peru at elevations below 350 m.a.s.l. *Eleutherodactylus aureolineatus* is primarily an inhabitant of large tank bromeliads in the upper strata of the rainforest canopy (> 20 m vertical height) (Guayasamin *et al.* 2006). It is a member of a phenetic subgroup called the *Eleutherodactylus lacrimosus* assemblage of the greater *Eleutherodactylus unistrigatus* species group applied by Lynch and Duellman (1980, 1997) (Heyer and Hardy 1991; Guayasamin *et al.* 2006). The assignment was loosely based on morphological characters and preference of bromeliad habitat. Herein we specifically examine those characters most relevant to the assignment of this new species to the *unistrigatus* group and *lacrimosus* assemblage. We examined adult type specimens of *E. aureolineatus* for sexual size dimorphism (SSD) in snout-to-vent length (SVL), head width, tibia length and tympanum diameter (TD). The *lacrimosus* assemblage as a whole is not well known. We were able to closely evaluate the habitat, advertisement call, reproductive behavior and egg deposition for this new species. We intend to provide both additional data on this new species and contribute to the knowledge of the *lacrimosus* assemblage.

Methods and Materials

Raw morphometric data were used for the adult type specimens ($n = 16$) in Guayasamin *et al.* (2006) (see Table 4.1). Data for type specimens (TCWC 90334-90342) collected by the author were taken during bromeliad patch sampling surveys at the Tiputini Biodiversity Station (TBS)–Universidad San Francisco de Quito ($0^{\circ}38'18.52''$ S, $76^{\circ}08'56.66''$ W) in the eastern lowlands of Amazonian Ecuador between June and August 2004. Single-rope climbing technique (SRT) was used to access canopy bromeliads in two trees from each of four terrestrial quadrat strip plots. Five bromeliads ($n = 40$) were removed from each tree and placed in large plastic bags to be lowered to the forest floor and transported to camp. Individual leaves were removed to allow collection of all anurans within a screened enclosure to prevent escape. Following Guayasamin (2004) and Fuentes and Barrio-Amorós (2004), morphological measurements were taken from preserved specimens to the nearest 0.1mm. Because of a small sample size ($n = 16$) the Mann-Whitney U Test was used for the analyses. Analyses for SSD were performed on snout-to-vent length (SVL), head width, tibia length and tympanum diameter using the the raw morphometric data for adult specimens of *E. aureolineatus* with a significance level of $\alpha \leq 0.05$. Statistical analyses were performed with SPSS version 14.0.

Spatial distribution of sampled trees is represented by altitude (m.a.s.l.) and the distance (m) due north of the Tiputini River using the measurement tool in ArcInfo 9.1 (Figure 4.1). Vertical height of bromeliads was obtained by attaching a 100m reel tape measure to the tree climber's harness and positioning the reel at the base of the tree, height was recorded by a climbing assistant on the ground when the climber was level

with the bromeliad being collected. Environmental data were recorded for collection sites and reported herein for occurrences of *E. aureolineatus*, and include: temperature (°C) at 0 m, 1 m and bromeliad collection site; barometric pressure (hPa) at 1 m and bromeliad collection site with a Brunton Sherpa; humidity (% relative humidity) at 1.5m with a sling psychrometer; pH of water in bromeliad with a Oakton (pHTestr 30) meter; volume (L) of water in bromeliad with graduated cylinder; and diameter at breast height (DBH) of tree to the nearest 0.1 cm.

One individual (TCWC 90335) was collected on 31 July 2004 and recorded on 8 August 2004. When the bromeliad occupied by this individual was sealed in the plastic transport bag this male began calling. Upon opening the bag a total of 4 individuals were observed within bracts; two adult males (TCWC 90335 and 90338), a gravid adult female (TCWC 90337) and a juvenile female (TCWC 90336). To facilitate call recording and observation of reproductive behavior the bromeliad was placed in a low (1.5 m above ground) tree crotch and sealed in a mosquito net attached to surrounding trees to prevent escape. Daily sweep netting provided prey which was then placed within the mosquito net. Call data was recorded using a Marantz PMD201 portable tape recorder with an Azden SGM-2X shotgun microphone on THAT's 60 minute metal cassette tapes at 0.5 m from calling individual. Call digitizing and analysis was performed using Raven version 1.3 (Cornell Laboratory of Ornithology, 2006). A sampling rate of 44.1 kHz was used to digitize signals at a size of 16 bits. Figure of spectrogram calculated using fast Fourier transform (FFT) with a window size= 512 samples, window overlap= 50% and window type= Hann. Call parameters terminology follows Duellman and Trueb (1994).

Eggs were measured immediately following deposition and before preservation using dial calipers to the nearest .01 mm. Eight were preserved in 95% ETOH and nine were preserved in 10% formalin.

Results

Sexual Size Dimorphism (Table 4.1). – SVL in males was 19.7–28.8 mm ($\bar{x} = 23.6 \pm 2.7$, $n = 9$) and 26.3–30.5 mm ($\bar{x} = 27.5 \pm 1.6$, $n = 7$) in females. Tibia length in males was 11.5–15.4 mm ($\bar{x} = 12.8 \pm 1.5$, $n = 9$) and 13.5–15.4 mm ($\bar{x} = 14.5 \pm 0.7$, $n = 7$) in females. Head width in males was 8.7–12.0 mm ($\bar{x} = 9.5 \pm 1.1$, $n = 9$) and 10.2–12.2 mm ($\bar{x} = 11.1 \pm 0.7$, $n = 7$) in females. Females were found to be significantly larger for SVL (Mann-Whitney U, $Z = -2.701$, $P = 0.007$), head width (Mann-Whitney U, $Z = -2.489$, $P = 0.013$) and tibia length (Mann-Whitney U, $Z = -1.960$, $P = 0.050$). Tympanum diameter in males was 1.0–1.5 mm ($\bar{x} = 1.2 \pm 0.2$, $n = 9$) and 1.2–1.5 mm ($\bar{x} = 1.3 \pm 0.1$, $n = 7$) in females. No difference between males and females was found for tympanum diameter (Mann-Whitney U, $Z = -1.439$, $P = 0.150$).

Habitat. – *Eleutherodactylus aureolineatus* occurred in four of the 40 sampled bromeliads. These four bromeliads were found in just two of the sampled trees, two in each tree. Both trees are located in primary terra firme forest following a ridgetop (Figure 4.1). The first tree (PMT5) was identified as *Dimorphandra* sp.1 of the subfamily Caesalpinioideae in the family Leguminosae, according to Ribeiro *et al.* (1999). The second (PMT6) could be positively identified only to the family Leguminosae (Ribeiro *et al.* 1999). All four large tank bromeliads (PMT5B1, PMT5B4, PMT6B1 and

PMT6B2) were identified as *Aechmea zebrina* (Kreft and Köster 2004). Vertical heights and environmental data collected for bromeliads are reported in Table 4.2.

Vocalization (Figure 4.2). – One adult male (TCWC 90335) called nightly, 31 July 2004 through 14 August 2004, from the collected bromeliad under the mosquito netting. Calling began around 18:00-19:00 hr and continued until approximately 01:00 hr. The other adult male (TCWC 90338) was never observed calling during this time. On 8 August 2004, the advertisement call of TCWC 90335 was recorded from 21:00-21:30 hr at an air temperature of 22.5°C and 97% humidity. 325 calls belonging to 15 call groups with duration of 1771.35 seconds (S) were analyzed. The advertisement call consists of a single note chirp repeated in call groups of 7-52 ($\bar{x} = 21 \pm 11$, $n = 15$) calls, with an interval between call groups of 19.94-242.60 ($\bar{x} = 79.98 \pm 65.30$, $n = 14$) S. Call rate 16.7-40.4 ($\bar{x} = 29.7 \pm 5.8$, $n = 15$) per minute. Dominant frequency (=fundamental) 1690.9-4254.8 ($\bar{x} = 3078.4 \pm 164.6$, $n = 325$) Hertz (Hz); notes variable with no frequency modulation to a slight upward modulation. A single harmonic structure present at a frequency of 7616.6-10797.7 ($\bar{x} = 9000.9 \pm 380.5$, $n = 325$) Hz. Note duration was 15.1-42.0 ($\bar{x} = 25.9 \pm 4.6$, $n = 325$) milliseconds; with an interval between notes of 0.88-8.85 ($\bar{x} = 2.03 \pm 0.90$, $n = 325$) S. Amplitude modulation present in each note with beginning most intense and ending least intense; most notes weakly divided in two to three incomplete pulses with some notes single pulsed.

Reproductive behavior and egg deposition. – On 13 August 2004 at 19:30 hr the adult female (TCWC 90337) and adult male (TCWC90335) were found amplexant in the axillary position on the outer leaf edges of the collected bromeliad under the mosquito netting. At 23:45 hr they were no longer in amplexus. At 02:00 hr on 14 August 2004,

the pair was once again observed in amplexus. At 05:45 hr on 14 August 2004, the pair was no longer in amplexus and 17 white eggs were found clumped at the outer base of the bromeliad on the leaf litter and detritus buildup. Egg diameter was 3.2 to 3.7mm ($\bar{x} = 3.5 \pm 0.04$, $n = 17$).

Discussion

Sexual size dimorphism (SSD) has long been a characteristic observed in anurans, yet its biological significance is poorly understood for most situations (Hayek and Heyer 2005). SSD in *Eleutherodactylus* is prominent throughout the genus, but few or no museum specimens exist for some sexes within a species (Lynch and Duellman 1997). The variables SVL, tibia length and head width, were used in this analysis of SSD because of greater confidence in the accuracy of measurement (Hayek and Heyer 2005). Historically, morphometric data other than SVL has been transformed or converted to ratios of the measures; Hayek and Heyer (2005) provide significant tests to the contrary and show that these transformations often greatly reduce the observed effect size. Females were found to be significantly larger than males for the targeted morphometrics, further supporting the existence of SSD in the genus *Eleutherodactylus*. Morphometric data for anurans has also been a commonly used method for classifying species into groups, subgroups and assemblages (Heyer and Hardy 1991; Lynch and Duellman 1997; Guayasamin *et al.* 2006). Morphological characters of a small body size and broad, flat, pointy head; fifth toe much longer than third; and more inclusively, a niche-specific association with bromeliads define the *lacrimosus* assemblage (Lynch and Ruíz-Carranza 1985; Heyer and Hardy 1991; Guayasamin *et al.* 2006). Members of the *unistrigatus* species group are also characterized as not expressing SSD in tympanum diameter

(Lynch and Duellman 1997). SSD for tympanum size was not significant in the analysis, reinforcing placement of *E. aureolineatus* in the *unistrigatus* species group.

Frogs of the genus *Eleutherodactylus* are primarily inhabitants of terrestrial and low (< 4 m) arboreal microhabitats; a few species have been recorded in bromeliads up to 13 m (Lynch and Duellman 1997). *Eleutherodactylus aureolineatus* occupies bromeliads in the middle to upper strata of the rainforest canopy (19-45 m), with few individuals having been found on low vegetation (~ 1.5 m) at night (Guayasamin *et al.* 2006). Ecological data for individuals ($n = 14$) collected from bromeliads show all trees were located in terra firme forest, although bromeliads were sampled in only one tree (GMT4) located in a seasonally flooded stream plain during the authors sampling (Guayasamin *et al.* 2006). *Eleutherodactylus aureolineatus* has been found occupying the same bromeliad as *Osteocephalus planiceps* and *Dendrobates ventrimaculatus* (Guayasamin *et al.* 2006).

Advertisement calls for members of the *lacrimosus* assemblage have been noted for several species with only one having been recorded, *Eleutherodactylus zimmermanae* (Heyer and Hardy 1991). Heyer and Hardy (1991) proposed that recordings would help in establishing the ranges of species within the assemblage. In Guayasamin *et al.* (2006), *E. aureolineatus* was observed calling from a Ceiba tree over a leaf at night ca. 45 m; we observed individuals calling from the outer edges of *Aechmea zebrina* leaves at night. *Eleutherodactylus aureolineatus* was observed calling on the evenings of cloudy or rainy days from May-August 2004. Eleutherodactylines typically deposit direct development eggs in terrestrial sites amongst leaf litter (Duellman 1978; Duellman and Trueb 1994). Egg deposition sites have only been reported for two species within the *lacrimosus*

assemblage: *E. tayrona* eggs on bromeliad leaves in a single layer and *E. zimmermanae* on land (Lynch and Ruíz-Carranza 1985; IUCN 2004). Despite the dearth of information on egg deposition, our observation of eggs deposited at the base of a bromeliad was still surprising. This new deposition site within the *lacrimosus* assemblage may be linked to environmental factors of its arboreal habitat.

The lack of ecological and natural history data for the *lacrimosus* species assemblage has made character delineation difficult for those investigating the relationships. The investigation described herein is beneficial in that it provides data and methods enabling researchers to identify parameters for determining range and distributional patterns of the species based on landscape patterns. The continued implementation of canopy surveys and bromeliad patch sampling efforts is necessary to gather the data needed to further determine the relationships among the species within the assemblage.

Acknowledgments

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Herbertson and Robert Winters for field work assistance. Mark Mulligan – King's College London for continued support. Floyd W. Weckerly for review and comments on the manuscript.

Table 4.1: Adult *Eleutherodactylus aureolineatus* measurements. Morphological measurements for types, paratopotypes and paratypes of *Eleutherodactylus aureolineatus* (Guayasamin *et al.* 2006).

Specimen	Sex	SVL	Shank	Head	Tympanum
			length	width	diameter
TCWC 90337	female	28.6	14.9	12.2	1.5
TCWC 90340	female	26.3	14.2	11.2	1.4
TCWC 90342	female	26.4	15.4	11.0	1.4
QCAZ 19534	female	27.8	14.8	11.2	1.2
KU 220426	female	26.6	13.5	10.4	1.2
DFCH-USFQ 736	female	30.5	14.9	11.8	1.5
CRG 931	female	26.4	13.8	10.2	1.2
TCWC 90335	male	21.3	12.1	8.7	1.3
TCWC 90338	male	26.1	15.4	10.6	1.3
TCWC 90339	male	28.8	15.3	12.0	1.5
QCAZ 20712	male	24.3	12.9	9.3	1.1
KU 106967	male	19.7	11.5	8.7	1.1
KU 148902	male	22.5	11.5	8.7	1.1
KU 148906	male	22.7	11.9	8.8	1.0
DFCH-USFQ 734	male	24.2	12.2	9.5	1.3
DFCH-USFQ 443	male	22.7	12.7	9.0	1.3

Table 4.2: Bromeliad environmental data. Vertical heights and environmental data collected for bromeliads with occurrences of *E. aureolineatus*.

Environmental parameters	Bromeliads			
	PMT5B1	PMT5B4	PMT6B1	PMT6B2
Vertical height	38.0	35.5	23.5	23.5
Water volume (L)	1.240	1.654	1.050	1.195
Temp.(°C) @ 0m	24.5	24.5	23.8	23.8
Temp.(°C) @ 1m	25.0	25.0	24.0	24.0
Temp.(°C) @ bromeliad	24.0	24.0	23.0	23.0
hPa @ 1m	1015	1014	1017	1017
hPa @ bromeliad	1015	1015	1014	1014
% relative humidity @ 1m	92.00	92.00	95.00	95.00
Water pH	5.45	4.97	5.30	4.88
DBH (cm) of tree	289.0	289.0	47.0	47.0

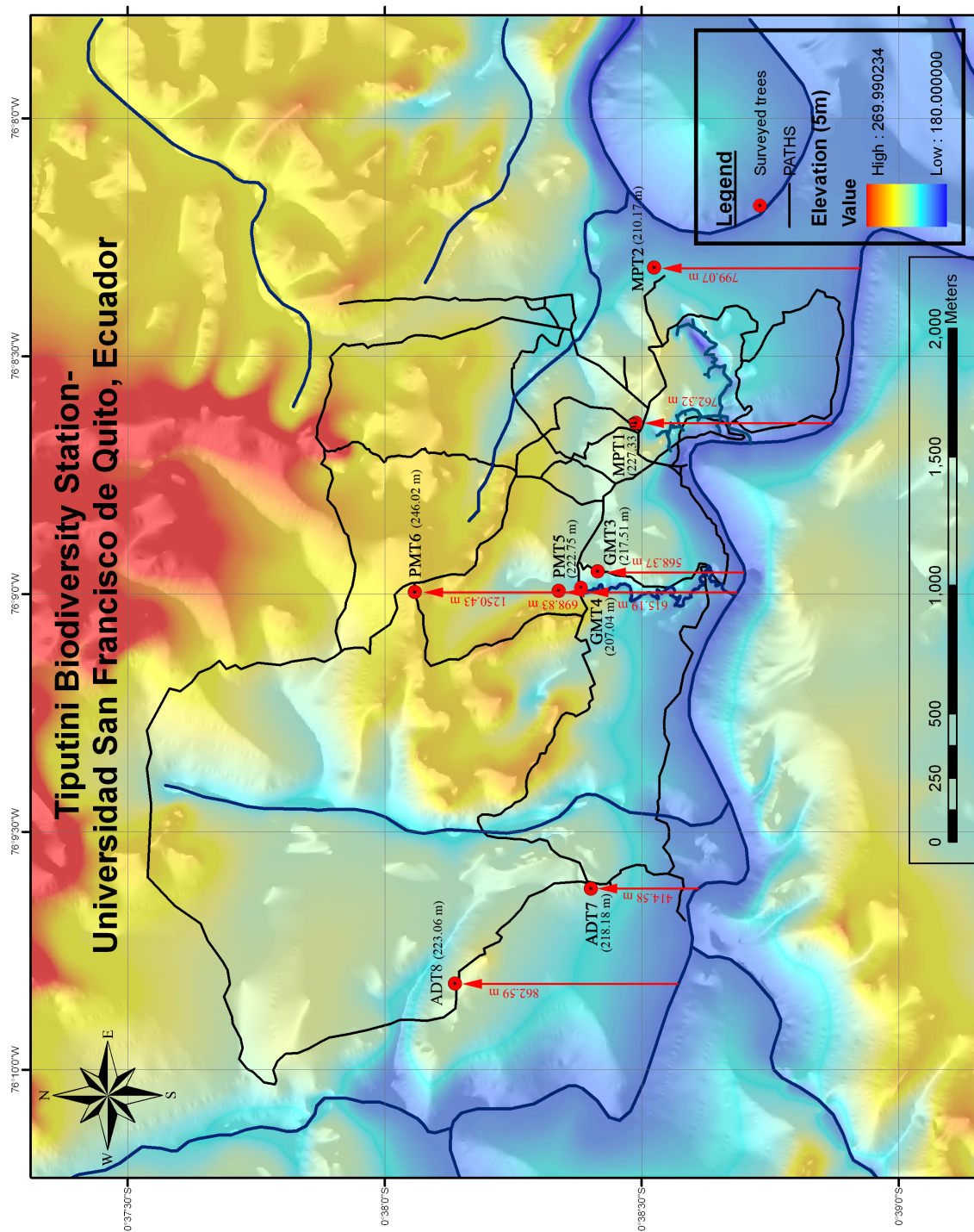


Figure 4.1: Map of bromeliad tree sampling. Map of trees sampled for canopy anurans within bromeliads at the Tiputini Biodiversity Station–Universidad San Francisco de Quito, Ecuador. Tree distances due north of river in red and elevation in black.

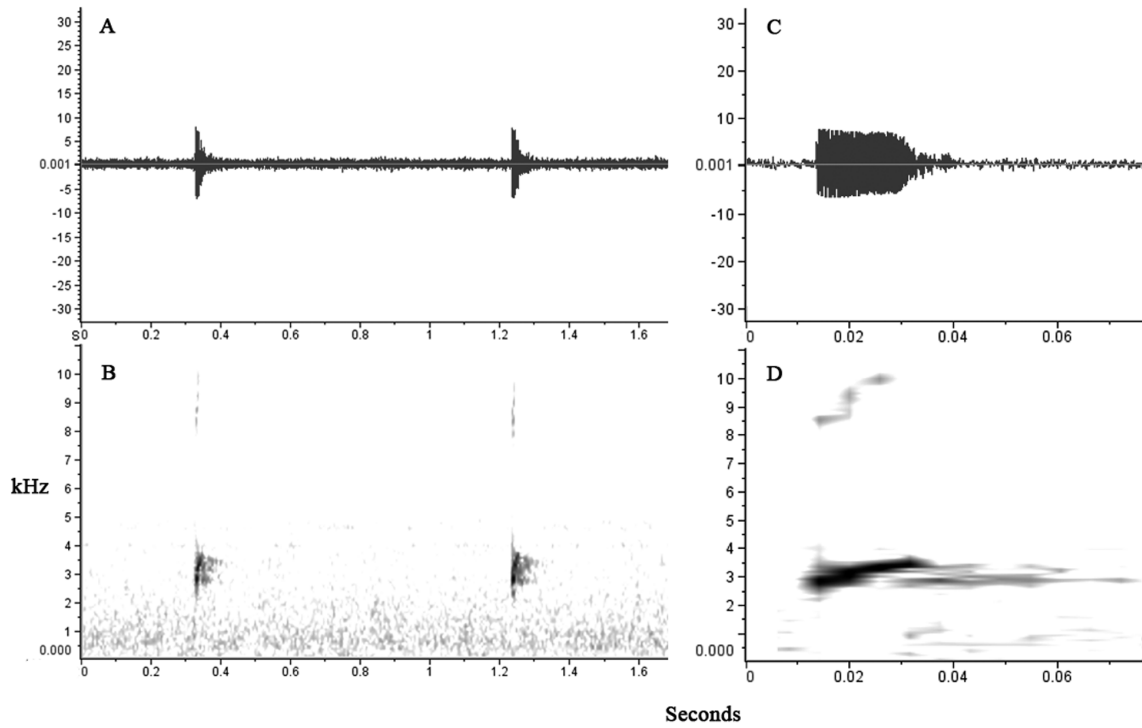


Figure 4.2: Audio analyses visualization for *Eleutherodactylus aureolineatus*. Waveform (A) and spectrogram (B) of two consecutive calls of *Eleutherodactylus aureolineatus*. Waveform (C) and spectrogram (D) of a single call, showing greater detail of signal. Recorded in the field at 22.5°C air temperature.

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CHAPTER V

DETECTION OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN AMPHIBIANS FROM THE FOREST FLOOR TO THE UPPER CANOPY OF AN ECUADORIAN AMAZON LOWLAND RAINFOREST¹

Introduction

During the last two decades significant declines and extinctions of amphibians have been observed worldwide (Blaustein & Wake 1995; Houlahan et al. 2000; Stuart et al. 2004). The Neotropics are among the most impacted areas, with sudden losses of anuran species noticed during the 1980s in Costa Rica, Ecuador and Venezuela (Pounds & Crump 1994; Pounds et al. 1997; Young et al. 2001; Ron et al. 2003). Population declines and losses have been more significant at high elevation (above 1000 m) with one study showing all species of *Atelopus* with sufficient data (n=28) declining and 75% disappearing (La Marca et al. 2005). A serious contributor to amphibian declines worldwide is the fungus *Batrachochytrium dendrobatidis* (*Bd*), which was present in the Neotropics before declines were noticed in the 1980's (Berger et al. 1999; Speare & Berger 2000; Bosch et al. 2001; Bradley et al. 2002; Lips et al. 2005). *Bd* is now

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implicated as one of the causes of the extinction of several species of anurans from these regions (Ron et al. 2003; La Marca et al. 2005; Merino-Viteri et al. 2005). While *Bd* has been detected in amphibians from many high elevation sites in the Neotropics (Ron & Merino 2000; Young et al. 2001; Lips et al. 2003), low elevation sites in the Neotropics have received comparatively little attention and thus reports on the detection of *Bd* at these sites are less common (Puschendorf et al. 2006; Oliveira de Queiroz Carnaval et al. 2006).

The aim of this study was therefore to examine the occurrence of *Bd* in amphibian species of a Neotropical lowland rainforest, i.e., at sites in the Upper Amazon Basin of eastern Ecuador at low elevation (about 200 m). This study included analyses of amphibians occupying ecological niches above the forest floor and shrub layers, specifically the mid- to upper canopy, because the upper strata of Amazonian rainforests plays an integral role in ecosystem function and data are missing for the interaction of *Bd* with amphibians in this important microhabitat.

Methods

Samples were collected from sites surrounding Tiputini Biodiversity Station – Universidad San Francisco de Quito (-0.6384694, -76.1490806, 217 m elevation), at the border of Yasuni National Park in the eastern lowlands of Ecuador (Figure 5.1) during May to August of 2004 and 2006. The vegetation of the region has been defined as Amazonian Evergreen Lowland Forest, where annual rainfall averages 2425-3145 mm, temperature averages 25°C (range is 15°-38°C), and average humidity is 88% (Blandin

Landívar 1976; Duellman 1978a; Balslev et al. 1987). This area is part of the Napo refugium, a subset of the Amazonian system, and it is likely to represent some of the oldest undisturbed forests and greatest biodiversity in the system (Prance 1982; Bass et al. 2004). Amphibians from the forest floor and shrub layer were collected near the banks of the Rio Tiputini at elevations between 190-250 m during visual encounter surveys. Amphibians occupying higher strata were collected during bromeliad patch sampling following the procedure outlined by McCracken and Forstner (2008).

Toe clips and skin from thigh muscle samples were used to test for the presence of *Bd* (Table 5.1). Samples were grouped into three categories (fossorial/forest floor, shrub/sub-canopy, and upper canopy) along the vertical axis based on historical and current collection locations for each species tested. Species placed in the fossorial/forest floor category are those which utilize subterranean habitat such as burrows and holes or typically occur amongst the forest floor leaf litter. Thirty-six individuals of 15 species in the order Anura were tested from the fossorial/forest floor category. The second category contains species which occupy the forest understory (shrub/sub-canopy). Whereas some of these amphibians may occasionally be found on the forest floor, they are most commonly found on low vegetation (< 2 m); a few animals which were tested and placed in this category have been found up to 4 m. Twenty-nine individuals of 12 species in the orders Anura and Caudata were tested from the shrub/sub-canopy category. The final category contains species found in the upper canopy occupying tank bromeliad habitat above 4 m (4-38 m). Twenty-one individuals of 4 species in the order Anura were tested from the upper canopy layer.

Water temperature was collected in three tank bromeliads of the species *Aechmea zebrina* located in the upper canopy of an emergent tree, *Parkia multijuga*, at 32, 34, and 35.5 m vertical height and within 2 m horizontal distance from the tree bole. One Thermochron iButton (model # DS1922L) temperature logger was placed in an outer leaf bract of each bromeliad a minimum of 8 cm below the water level. Data loggers were set to collect water temperature every 30 minutes (35.5 m) and every 60 minutes (32 m, 34 m) at a resolution of 0.5°C with an accuracy of $\pm 1^\circ\text{C}$ from -30°C to $+70^\circ\text{C}$. Duration of total data collection varied from 43 to 142 days from 31 March 2008 to 24 August 2008. Water pH was taken during bromeliad patch sampling according to McCracken and Forstner (2008) for five *A. zebrina* in each of 18 trees for a total of 90 bromeliads during field seasons in 2006 and 2008. An Oakton pHTestr 30 was used to collect water pH at a resolution of 0.01 pH with an accuracy of ± 0.01 pH; a three point calibration was performed before each tree sampled.

A nested PCR approach that has shown increased sensitivity of detection in samples with low template numbers and is outlined in detail in (Gaertner et al. 2008) was used for detection of *Bd*. This approach used primers ITS1f (5' CTT GGT CAT TTA GAGC GAA GTA-3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC-3') targeting conserved regions of the 28S and 18S rRNA to amplify the 5.8S rRNA gene and the flanking internal transcribed spacer (ITS) of all fungi (White et al. 1990). PCR products from this reaction were purified and then used as a template for the subsequent PCR reaction using the *Bd*-specific primer set Bd1a (5' CAG TGT GCC ATA TGT CAC G-3') and Bd2a (5' CAT GGT TCA TAT CTG TCC AG-3') (Annis et al. 2004). The product from this reaction was examined by gel electrophoresis (2% agarose in TAE buffer)

(Sambrook et al. 1989) for a fragment of approximately 300 bp (Annis et al. 2004). DNA from a sample positive for *Bd* from a previous study (Gaertner et al. in press) and sterilized distilled water were used as positive and negative controls, respectively. PCR products from samples showing the 300 bp amplicons were then sequenced using the CEQ 8800 Quickstart Kit with the addition of 5% DMSO to the reaction mix on a CEQ 8800 sequencer (Beckman Coulter, Fullerton, CA). The sequences were validated against the GenBank/EMBL databases using BLASTn (Pearson & Lipman 1988).

Results

Eighty-six amphibians of two orders (Anura N = 84, and Caudata N = 2) belonging to 7 families and 31 species were screened (Table 5.1) using nested PCR analysis (Gaertner et al. 2008). Of those tested, 17 individuals (20%) representing eight species were positive for *Bd*. *Bd* was detected from within each of the strata, with the fossorial/floor group consisting of nine positive from 36 individuals tested (25%), shrub/sub-canopy group consisting of one positive from 29 tested (3%), and the canopy group consisting of seven positive of 21 tested (33%) (Figure 5.2). Comparative sequence analysis of all 17 amplicons retrieved from PCR amplification with existing sequences of *Bd* available in GenBank/EMBL databases confirmed the detection of *Bd*. All sequences exhibited more than 99% similarity, with all sequences of the 5.8S rRNA gene being identical to the published sequence of *Bd* (AY997031), and those of seven amplicons displaying small differences in the ITS regions (Table 5.2).

Water temperatures recorded inside *A. zebrina* bromeliads at 32 m were 20.5-30.5°C (23.8 ± 1.9 ($\bar{x} \pm SD$), N = 1369), at 34 m they were 20.0-31.0°C (24.1 ± 1.9 , N =

3422), and at 35.5 m was 20.5-32.5°C (24.5 ± 2.5 , N = 2017). The mean pH recorded was 4.48 ± 0.67 (N = 90).

Discussion

The presence of *Bd* was demonstrated in amphibians of the Upper Amazon Basin of eastern Ecuador. *Bd* has been detected from several high altitude sites in the Neotropics (Ron & Merino 2000; Young et al. 2001; Lips et al. 2003), however, detections at low elevation sites are less common (Oliveira de Queiroz Carnaval et al. 2006). Our results have shown that *Bd* is present in Amazonian lowland rainforests at elevations less than 300 m. This finding is significant because even though amphibian declines are not as prevalent at low elevations (Puschendorf et al. 2006; (Oliveira de Queiroz Carnaval et al. 2006), *Bd* is present and could be contributing to declines. Amphibians may also be serving as a reservoir for *Bd* which could then move to and infect amphibians at higher elevation sites.

Another important finding of this study is the demonstration of clinical signs of chytridiomycosis and a positive detection for *Bd* by one individual (*Leptodactylus pentadactylus*) included in the study. In laboratory experiments, *Bd* grew best and was most lethal under cool (22°C) moist conditions (Woodhams et al. 2003; Piotrowski et al. 2004) suggesting that montane species should be the most likely candidates for declines. This scenario is certainly supported by studies in the area with one study failing to find even a single apparently healthy population of *Atelopus* at elevations above 1000 m (La Marca et al. 2005). Despite the apparent virulence of *Bd* at high elevation sites, there are very few accounts of population declines caused by *Bd* infection at low elevations. The

discovery of *L. pentadactylus* at this elevation showing clinical signs suggests that *Bd* may play a more important role in lowland declines than previously thought.

All previous studies on *Bd* have used amphibians caught at or near ground level. This allows for testing of only the portion of amphibians contained in the lower strata of a structurally complex system. We detected *Bd* on amphibians inhabiting all strata of the forest which demonstrates that searches not including vertical strata may be lacking important data on the system. Furthermore, we found evidence that the occurrence of *Bd* infection on amphibians along a vertical axis is non-random in this system. Infection by *Bd* was found to be significantly higher in the fossorial/floor and canopy groups, showing 25% and 33% of individuals infected, respectively, than in the shrub/sub-canopy group with only one individual (3%) infected. Each of the strata of the rainforest has unique microclimate characteristics that could potentially affect *Bd* infections. In this case the availability of water may play a role in the prevalence of infection in each of the groups. Amphibians of the fossorial/floor group have water available in the form of streams and standing pools and the canopy group has access to water captured in phytotelmata, primarily tank bromeliads. Because *Bd* is transmitted via aquatic zoospores (Longcore et al. 1999b), the absence of an abundance of standing water available to amphibians of the shrub/sub-canopy group may be reducing their exposure to the fungus. Previous studies have demonstrated that environmental conditions at the landscape level can have strong effects on host-pathogen dynamics (Woodhams et al. 2006), and future studies should include the investigation of interactions of *Bd* with environmental conditions, including water availability, on both the landscape and microhabitat level.

Environmental parameters for *Bd* have been found to have a significant influence on its pathogenicity (Andre et al. 2008; Piotrowski et al. 2004). Piotrowski et al. (2004) found isolates of *Bd* to grow and reproduce (in culture) between 4-25°C and pH 4–8. Optimal growth of *Bd* zoospores occurred at temperatures of 17-25°C and pH 6-7 (Piotrowski et al. 2004). Mortality rates in studies of *Bd*-infected frogs exposed to ambient temperatures of 17-25°C have been >50%, although several studies show increased survival and decreased infection rates with temperatures >22°C (Andre et al. 2008; Berger et al. 2004; Carey et al. 2006; Kriger and Hero 2007; Woodhams et al. 2003). Exposure to temperatures >25°C has been shown to kill *Bd* zoospores and cure infected frogs (Berger et al. 2004; Kriger and Hero 2006; Piotrowski et al. 2004; Woodhams et al. 2003). While *Bd* prefers a near neutral pH, swimming zoospores were found in cultures at pH 4 and a temperature of 23°C for 14 days (Piotrowski et al. 2004).

In our study, basic environmental water parameters collected from *A. zebrina* tank bromeliads indicate optimal temperature conditions for the persistence of *Bd* in the canopy. While pH levels were lower than previously reported as optimal conditions in fungal culture experiments, it remains unclear what effect this has *in situ*. *Pristimantis aureolineatus* and *P. waoranii* are known permanent inhabitants of *A. zebrina* bromeliads and with 44% of those individuals screened in this study testing positive it seems evident that *Bd* is present in canopy phytotelmata. However, definite proof of this assumption requires detection of *Bd* directly in water of the bromeliads. Many other species of anurans utilize bromeliad habitat for egg and tadpole deposition sites and may act as a reservoir for transferring the pathogen between sites. Several species of Dendrobatidae are of particular concern since they transfer tadpoles from terrestrial deposition sites to

bromeliads in the canopy for final development. This broad traverse of habitats may facilitate the movement of *Bd* between terrestrial and canopy water sources, additional studies are needed to track potential movement of *Bd* along the vertical axis.

Four ecological traits are commonly associated with amphibian populations in decline including: aquatic mode of life, occurrence at mid to high altitudes, low fecundity and endemic distribution (Laurance et al. 1996; Lips 1998; Williams & Hero 1998; Lips et al. 2003). The observation of clinical signs of chytridiomycosis at a low elevation site and the characteristics of microhabitat utilized by permanent canopy anuran inhabitants infected by *Bd* demonstrate that the threat of chytridiomycosis may still be significant at sites even in the absence of those traits.

Acknowledgments

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Table 5.1: Amphibian *Batrachochytrium dendrobatidis* test results. Amphibian species collected from three vegetation strata (canopy, shrub/sub-canopy, fossorial/floor) in Yasuni National Park, Ecuador, and analyzed for *Batrachochytrium dendrobatidis* (*Bd*).

Amphibian species	Number of samples analyzed	Number of <i>Bd</i> -positive samples
Canopy		
<i>Hypsiboas boans</i>	1	0
<i>Osteocephalus taurinus</i>	4	0
<i>Pristimantis aureolineatus</i>	7	4
<i>Pristimantis waoranii</i>	9	3
total	21	7
Shrub/sub-canopy		
<i>Dendropsophus parviceps</i>	2	0
<i>Hypsiboas cinerascens</i>	1	0
<i>Hypsiboas geographicus</i>	1	0
<i>Hypsiboas lanciformis</i>	2	0
<i>Osteocephalus planiceps</i>	2	0
<i>Scinax cruentommus</i>	2	0
<i>Scinax ruber</i>	1	0
<i>Pristimantis acuminatus</i>	3	0
<i>Pristimantis altamazonicus</i>	6	0
<i>Pristimantis ockendeni</i>	6	1
<i>Pristimantis peruvianus</i>	1	0
<i>Bolitoglossa equatoriana</i>	2	0
total	29	1
Fossorial/floor		
<i>Rhinella margaritifera</i>	2	0
<i>Rhinella marina</i>	2	0
<i>Engystomops petersi</i>	6	1
<i>Leptodactylus andrea</i>	1	0
<i>Leptodactylus discodactylus</i>	5	2
<i>Leptodactylus hylaedactyla</i>	1	0
<i>Leptodactylus pentadactylus</i>	5	4
<i>Leptodactylus rhodomystax</i>	1	1
<i>Leptodactylus wagneri</i>	1	0
<i>Chiasmocleis bassleri</i>	3	0
<i>Syncope antenori</i>	2	0
<i>Oreobates quixensis</i>	2	0
<i>Pristimantis lanthanites</i>	1	1
<i>Pristimantis malkini</i>	2	0
<i>Strabomantis sulcatus</i>	2	0
total	36	9

Table 5.2: *Batrachochytrium dendrobatidis* samples base pair sequence differences.
Base pair differences of sequences generated from nested PCR reactions of samples collected from Yasuni National Park, Ecuador, using existing GenBank entry AY997031 for reference positions.

Accession Number	Difference
FJ232005	1 bp insertion at base 68 (A)
FJ232006	1 bp insertion at base 68 (A) deletion of positions 80-86
FJ232007	1 bp insertion at base 68 (A)
FJ232009	SNP at position 47 (G to T)
FJ232019	deletion of positions 13-20 SNP at position 55 (T to A)
FJ232020	deletion of positions 36-39 SNP at position 51 (A to G)
FJ232021	deletion of positions 31-33

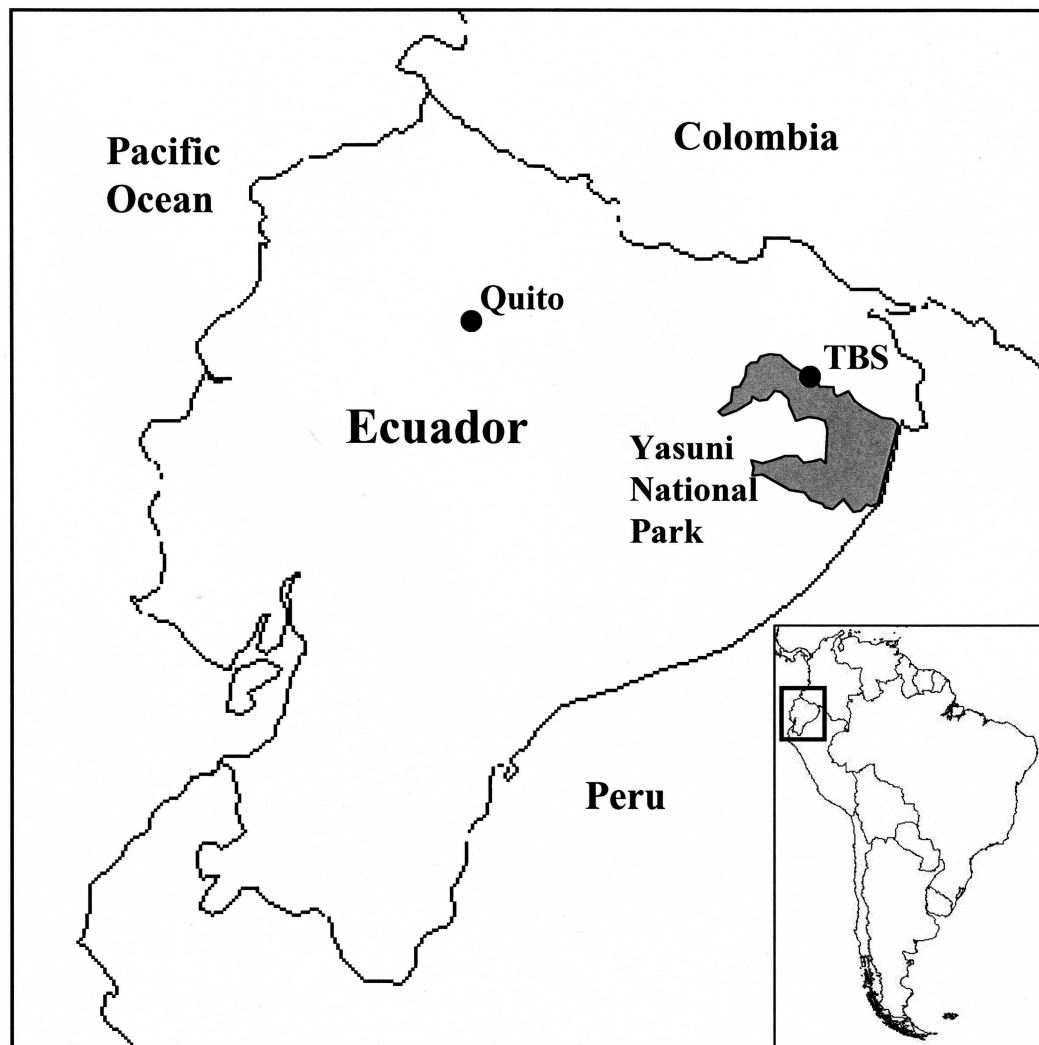


Figure 5.1: Map of *Batrachochytrium dendrobatidis* sampling site. Schematic presentation of the sampling site within the Yasuni National Park near Tiputini Biodiversity Station (TBS) – Universidad San Francisco de Quito, in the Upper Amazon Basin of eastern Ecuador used for collection of amphibians to be tested for infection by *Batrachochytrium dendrobatidis*.

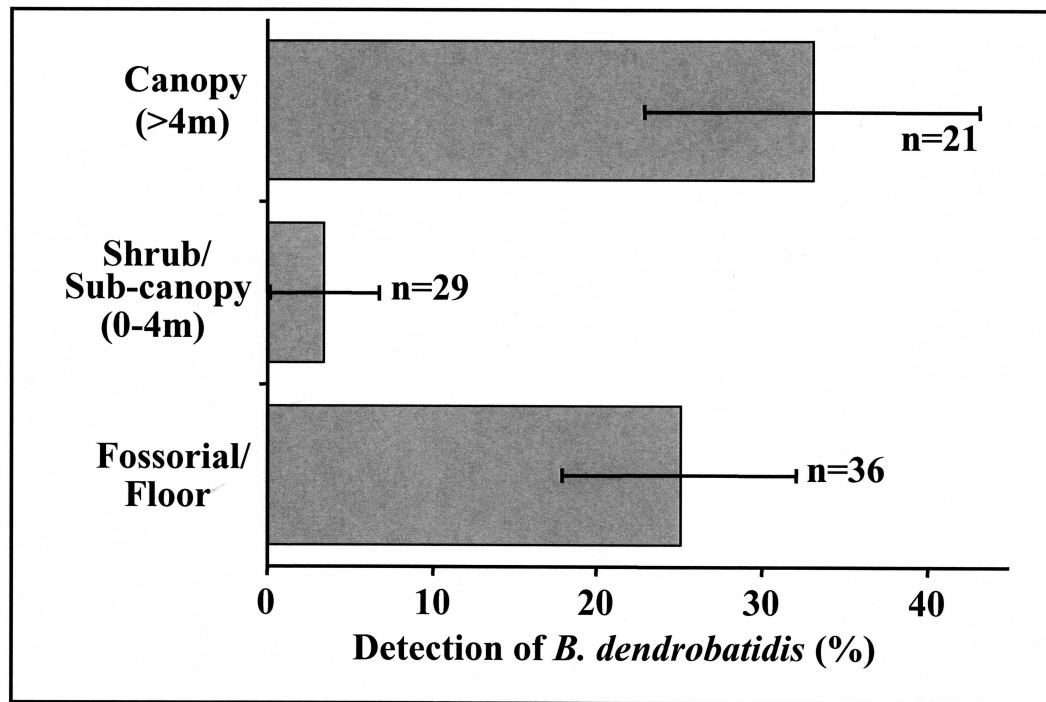


Figure 5.2: Detection of *Batrachochytrium dendrobatidis* across vertical forest strata. Detection of *Batrachochytrium dendrobatidis* ($\% \pm 95\%$ confidence intervals, calculated in Microsoft Office Excel) in samples from amphibians collected within the Yasuni National Park, Ecuador, at three different vertical strata groups (fossorial/floor; shrub/sub-canopy, 0-4 m; and canopy, > 4 m).

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CHAPTER VI

LOW-LEVEL ANTHROPOGENIC DISTURBANCE EFFECTS AND SPECIES COMPOSITION IN A WESTERN AMAZONIAN CANOPY ANURAN COMMUNITY¹

Abstract

Forest canopies are among the most species-rich terrestrial habitats on earth and one of the remaining unexplored biotic frontiers. Tank bromeliads provide microhabitat for a high diversity of fauna and flora in tropical forest canopies and are considered a keystone resource. A number of amphibians inhabit these phytotelmata, yet their ecological role and status in forest canopies remains mostly unknown. For this study, anurans were collected from an upper canopy tank bromeliad (*Aechmea zebrina*) in trees at ~20-45 m (\bar{x} = 33 m) above the forest floor. Bromeliads were sampled from trees located along ~24 km trails in undisturbed primary rainforest and the south-central portion of the Maxus oil road within the Yasuní Biosphere Reserve of Amazonian Ecuador. The Maxus road where bromeliads were sampled has experienced relatively little deforestation aside from infrastructure development for petroleum extraction operations (i.e. roads, pipelines, processing facilities and related disturbances) as

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compared to the northern roadway that is experiencing extensive colonization and associated deforestation. 95 individuals from 10 species were collected from 180 bromeliads in 32 trees. Generalized linear mixed models were used to assess the effects of disturbance and a suite of ecological factors on the occupancy and abundance of anurans collected. Full dataset analysis revealed bromeliads in undisturbed forest had a significantly greater occupancy and abundance of anurans than those along the Maxus road, while recorded ecological factors had no relationship. Reduced species set analyses demonstrated that anuran occupancy was highest in undisturbed forest; abundance showed mixed significance results although it was also greatest in undisturbed forest, and again the recorded ecological factors had no relationship. This study is the first to investigate upper canopy anurans utilizing tank bromeliad microhabitat and reveals that even the minimal footprint of petroleum extraction operations, primarily roads, in rainforest environments can have significant impacts on a unique anuran community. Based on these results, it is recommended that future petroleum development treat rainforest habitat as an offshore system where roads are not used and current access roads be protected from colonization and further deforestation.

Introduction

The upper canopy of tropical forests remain one of the few relatively unexplored biotic frontiers, yet are among the most species-rich terrestrial habitats on Earth, supporting up to 50% of known extant species (Basset et al. 2003b; Lowman & Schowalter 2012). Species richness in the canopy is supported by a diversity of

microhabitats, creating unique ecological niches. A key component of tropical rainforest canopies providing rich faunal microhabitats are phytotelmata, defined as plants or parts of plants that hold rainwater (e.g. bromeliads, inflorescences, and tree holes) (Armbruster et al. 2002). In some moist tropical locations the availability of this habitat is up to 50,000 liters per hectare, literally a “wetland in the sky” (Kitching 2000; McCracken & Forstner 2006). In particular, epiphytic canopy tank bromeliads are capable of holding a large volume of water and play a principal role as a “keystone plant resource” and microhabitat for invertebrates, vertebrates, and other plants (Terborgh 1986; Nadkarni 1994). Bromeliads have been reported as reservoirs of incredibly high biodiversity in previous bromeliad arthropod surveys (Kitching 2000; Basset et al. 2003a). Most previous studies were completed without ever actually entering the canopy. Indeed, research in the canopy is still a relatively new discipline facilitated by recent methodological advances in canopy access techniques (Basset et al. 2003c). Thus far, canopy research has largely focused on arthropods, birds, mammals, plants, and ecological processes; investigations of upper canopy herpetofauna have only recently been documented (De Vries et al. 1997; Schiesari et al. 2003; Guayasamin et al. 2006; McCracken et al. 2009).

In a recent worldwide assessment habitat loss was found to be associated with a large percentage of amphibian declines (Stuart et al. 2004). Ecuador has the highest deforestation rate (28.6% of 1990 forest area lost by 2010) and one of the worst environmental record in South America (CIA 2007; FAO 2010). In the Ecuadorian Amazon, petroleum operations have been the driving force of deforestation and pollution (Finer et al. 2008). Physical alterations of environments, such as road building for access

to oilfields, directly contribute to deforestation (Finer et al. 2009). These roads allow access and enable settlers to exploit these regions with agriculture, hunting, logging, and mining operations causing greater environmental degradation (O'Rourke & Connolly 2003; Finer et al. 2009). Agricultural colonization by small scale farmers following oil roads and pipeline paths has resulted in a nearly 2% per year deforestation rate in the Ecuadorian Amazon, greater than any other Amazon nation (Barbieri et al. 2009; Carr 2009). Rapid exploitation of natural resources in the Ecuadorian Amazon is having a profound effect on the forest and its indigenous inhabitants (Finer et al. 2009). Yet, little is known about consequences of these anthropogenic changes on canopy biota.

Habitat variables, biotic and abiotic, affecting the diversity and composition of ecological communities in tropical forest canopies are influenced by anthropogenic disturbance (Ozanne et al. 2003; Lowman & Schowalter 2012). Determining which factors are affected by anthropogenic disturbance is a fundamental goal of conservation community ecology (Krebs 2008). In tropical ecosystems, the data are confounded by a large number of undescribed species and abundance of individuals in co-occurring species assemblages (Godfray et al. 1999; Armbruster et al. 2002). The strategy of most community ecologists to avoid these complications has been to restrict their studies to a single taxonomic group at the family or guild level, thus introducing potentially inaccurate explanations of patterns and processes in complete ecological communities (Southwood 1987; Purvis & Hector 2000; Basset et al. 2004). Tank bromeliads provide a model organism for inquiries of naturally complete communities with a taxonomically rich fauna living in a structurally discrete habitat. Sampling the complete anuran community of an epiphytic canopy tank bromeliad in conjunction with measures of

habitat variables across differing anthropogenic disturbance levels provides an opportunity to identify factors influencing species assemblages in an ecologically defined natural community. Do these sky wetlands really represent mesocosms in the classic sense?

We investigated occupancy and abundance of anurans inhabiting the epiphytic canopy tank bromeliad *Aechmea zebrina*, occurring in undisturbed and disturbed lowland rainforest of the Yasuní Biosphere Reserve (Yasuní) in eastern Ecuador. In addition, a suite of habitat factors (e.g., host tree height and bromeliad water pH) were measured as potential predictors of anuran occupancy status, abundance, species richness, and as correlates of undisturbed versus disturbed forest for *A. zebrina*. We tested for differences in *A. zebrina* anuran occupancy and abundance for measured factors between forest disturbance levels. We hypothesized that *A. zebrina* sampled along an oil access road edge with few forest clearings and a minimal footprint through primary forest would reveal little to no impact on the anuran community. Overall, we sought to provide an extensive characterization of habitat parameters and anuran inhabitants associated with *A. zebrina* bromeliads, while determining the effects of oil roads on these organisms in the northwestern region of Yasuní.

Methods

Study Area and Species

The study was conducted in the northwestern portion of Yasuní in eastern Ecuador, composed of Yasuní National Park, Waorani Ethnic Reserve, and their

respective buffer and transition zones (Finer et al. 2009) (Figure 6.1). We performed additional survey work along a network of oil roads to an extent of approximately 20 km west of the Yasuní buffer zone. Yasuní covers approximately 1.7 million ha of the Napo Moist Forests terrestrial ecoregion; it is characterized by a low elevation range of approximately 190-400 m above sea level (m.a.s.l.) with numerous ridges of 25-70 m, and a dense network of tributaries to the Napo River (Olson & Dinerstein 2002; Finer et al. 2009; Bass et al. 2010). The major habitat type is *terra firme* forest (non-inundated upland forest), with interspersed *várzea* (seasonally inundated areas by white-water rivers or tributaries) and *igapó* (forest and seasonally inundated areas associated with black-water tributaries) (Prance 1979; Salovaara et al. 2004). In the vicinity of the northwestern portion of Yasuní, rainfall averages 2425-3145 mm, temperature averages 25°C (ranges 15°-38°C), and humidity averages 88% (Blandin Landívar 1976; Duellman 1978a; Balslev et al. 1987). Yasuní holds some of Ecuador's largest oil reserves, which is the country's primary export and accounts for the majority of government revenues (Finer et al. 2009; Bass et al. 2010). Oil operations are the primary driver of both direct and indirect sources of deforestation in the reserve (Wunder 2003a; Finer et al. 2009). Bass et al. (2010) reported that Yasuní holds world record species diversity for several taxa including the highest documented landscape scale herpetofauna diversity – 150 species of amphibians and 121 species of reptiles.

The Tiputini Biodiversity Station (TBS) (0°38'14"S, 76°08'60"W), founded and operated by the Universidad San Francisco de Quito, was the location of control sites in undisturbed forest (Figure 6.1). The station lies on the border of Yasuní National Park within the buffer zone and has over 30 km of well-marked and mapped trails. The

Yasuni Research Station (YRS) (0°40'27"S, 76°23'51"W), founded and operated by the Pontificia Universidad Católica del Ecuador, lies within Yasuní National Park and was used as a base for access to low-level disturbance sites along the Maxus oil road (Figure 6.1). Most forest clearing along the Maxus has been limited to the northern section of the road nearest the Napo River where immigrant Kichwa peoples have begun practicing large-scale slash and burn agriculture. The central and southern stretches of the Via Maxus, in the vicinity of YRS, are occupied by a few small clusters of indigenous Waorani Indians who historically were semi-nomadic hunter-gatherers with small subsistence farms (Franzen 2006; Finer et al. 2009). Limited forest clearing has occurred along this stretch of the road even though the Waorani are more sedentary; the majority of cleared area is occupied by oil installations and infrastructure. However, the Waorani are beginning to adopt Kichwa agricultural practices for subsistence and market sales resulting in increased deforestation along the southern stretch of road. Today these areas are still surrounded by large tracts of undisturbed forest.

A. zebrina is a large epiphytic canopy tank bromeliad, relatively common in the lowland Amazon region of eastern Ecuador and extends into the southern Amazon region of neighboring Colombia (Figure 6.1). It can grow >1 m tall and wide, and hold nearly 4 L of water between its leaves (S.F.M., unpublished data). Typically, *A. zebrina* occurs in the upper canopy of overstory trees at vertical heights of 18-45 m, ranging in number of individuals from 1 to >150 on a single tree (S.F.M., unpublished data). *A. zebrina* was chosen as the sampling unit in this study due to its relative abundance, large size, high number of individuals within a phorophyte tree community, previous confirmation of a

diverse amphibian assemblage, and to control for any differences in inter-species community assemblages and microclimate (McCracken & Forstner 2008).

Study Design and Sampling Technique

We surveyed 24 km of trail at TBS (undisturbed) and road at YRS (low disturbance) to a distance of 50 m or 100 m, respectively, into the forest on both sides for all phorophyte trees with *A. zebrina* communities. Suitable trees were required to have a minimum of 15 *A. zebrina* so as not to decimate the community as a result of sampling. Coordinates for each tree were recorded with a differential global positioning system (Ashtech, Santa Clara, California) and distance from trail or road center was measured using a rangefinder (Nikon, Melville, New York). We graded trees for overall health and crown structure based on climbing safety and ease of access. We randomly selected 16 trees for removal of 5 *A. zebrina* bromeliads, totaling 80 bromeliads at each disturbance level (a total of 160 bromeliads sampled). Tree survey's and bromeliad sampling took place between April and November of 2008, with bromeliad sampling conducted at two different time intervals for each disturbance level in order to mitigate any climatic influences. Yasuní's rainfall and temperatures are considered aseasonal with January being the driest and warmest month, during which we did not sample (Pitman et al. 2002).

A. zebrina were sampled following methods described by McCracken & Forstner (2008). We accessed the tree canopy using single-rope technique (SRT), and bromeliads were collected haphazardly at estimated even vertical intervals between one another (Perry 1978) (Figure 6.2). Tree height and bromeliad elevation were recorded using a

range-finder (Nikon Inc., Melville, New York). We removed bromeliads by cutting the base-support rhizome, sealed it in a 55 gal. plastic bag, and lowered it to the ground in a tarp. The number of *A. zebrina* inhabiting the tree was counted and a herbarium specimen was collected from the tree to confirm identification. We transported bromeliads back to camp and processed them in a screened tent to prevent escape of animals. Bromeliad water was poured through a 1-mm sieve to separate arthropods, leaf litter and detritus. We measured water volume with a graduated cylinder and pH with a 3-point calibrated pH probe (Oakton, Vernon Hills, Illinois). We measured the height of bromeliads to nearest centimeter and counted the number of mature leaves. We carefully dismantled each bromeliad leaf-by-leaf to collect all herpetofauna.

Data Analyses

Before performing analyses, we conducted graphical data exploration to check for normality, homogeneity, and collinearity of explanatory variables (Bolker et al. 2009; Zuur et al. 2010). We used generalized linear mixed models (GLMMs) for occupancy and abundance analyses of non-larval anurans inhabiting *A. zebrina* bromeliads. First, we performed occupancy and abundance analyses using the full dataset of all anuran species collected in *A. zebrina*. We then did analyses on a reduced number of species datasets based on *a priori* knowledge about their use of canopy microhabitats. The reduced datasets consisted of known permanent canopy-dwelling anurans (*Ranitomeya* spp. and *Scinax ruber* removed from full dataset) and another with only known permanent bromeliad-inhabiting anurans (*Pristimantis aureolineatus* and *P. waoranii*). For a few species natural history data is uninformative as to whether these are permanent bromeliad-inhabitants, we therefore restricted this dataset to the two species known as

Aechmea spp. inhabitants based on our previous bromeliad sampling (S.F.M, unpublished data). The use of GLMMs are ideal for ecological studies involving nonnormal data (i.e. binary or count data) with random effects, and allow models to be fit with appropriate error distributions for the response variable (Bolker et al. 2009). Incorporating random effects into the models accounts for the potential non-independence of subsampled data points in a nested design; and GLMMs fit with a Poisson distribution and individual-level random effect or negative binomial distribution without an individual-level random effect allow for overdispersion (Bolker et al. 2009). We fit GLMMs using the laplace approximation in package “glmmADMB” (ver. 0.7.2.11) in the R 2.14.1 statistical programming environment (R Development Core Team, 2011).

We used GLMMs to test for forest disturbance impact and the influence of recorded physical and environmental variables on anuran occupancy of *A. zebrina* bromeliads using binomial error distribution, with a logit link function. We included the following factors as fixed effects to test for their potential role in anuran bromeliad occupancy: forest disturbance, tree height, bromeliad elevation in tree, number of *A. zebrina* in tree, bromeliad height, number of mature bromeliad leaves, bromeliad water volume, and bromeliad water pH. We began analyses with a full model containing all fixed effects and their interaction with forest disturbance to test for differences in their effect between forest disturbance levels. We included tree as a random effect to account for potential non-independence of bromeliads sampled in the same tree (five bromeliad samples nested within 32 trees for a total of 160 bromeliads sampled). We carried out model reduction by first removing non-significant interaction terms and then main fixed effects using the Akaike Information Criterion (AIC) to determine best fit models

(Burnham & Anderson 2002). Models within $\Delta AIC \leq 2$ were considered similar to best fit models for support of the data and reported herein (Burnham & Anderson 2002).

Significance tests for parameter estimates of fixed effects were done using Wald Z-tests, which provide a more robust test when sample sizes are small as compared to likelihood ratio test (Bolker et al. 2009).

To test for forest disturbance impact on anuran abundance we used GLMMs with the same fixed effects, interaction terms, and random effect as described above for occupancy analyses. The R package “glmmADMB” provides the ability to fit Poisson and negative binomial error distributions with or without zero-inflation, both of these distribution families and their variants have been found to work best for count data (Lindén & Mäntyniemi 2011). We took advantage of this capability to fit the full model and conduct model reduction using the following error distributions with a log link function: Poisson, zero-inflated Poisson, Poisson with individual-level random effect (log-normal Poisson), type 1 negative binomial (NB1, linear mean-variance relationship), zero-inflated NB1, type 2 negative binomial (NB2, quadratic mean-variance relationship), and zero-inflated NB2. We again used the AIC to perform model reduction and rank models according to best fit of the data.

Results

We identified and mapped 56 trees (23 per 100 ha) along trails in undisturbed forest at TBS and 44 trees (8 per 100 ha) in disturbed forest along the Maxus oil road at YRS with ≥ 15 *A. zebrina* bromeliads. Trees sampled for *A. zebrina* in undisturbed and

disturbed forest locations were separated by an average of 29 km. We collected 95 metamorphosed anurans representing 10 species from 180 bromeliads in 32 trees (Table 6.1). A total of 8 species were found in undisturbed and 5 in disturbed forest, with only 3 species shared. The rarity of most species did not allow for acceptable diversity indices comparison. Anurans were present in 36 bromeliads (45%) from 15 of 16 trees sampled in undisturbed forest, while 20 bromeliads (25%) from 12 of 16 trees were occupied by anurans in disturbed forest. Interaction terms included in all models to test for differences in recorded habitat variables between disturbance levels were not significant.

Only forest disturbance had an effect on anuran occupancy in all models. In the full species dataset there were 44.4% less *A. zebrina* occupied by anurans in disturbed forest than undisturbed forest ($\beta = -0.898$, $Z = -2.62$, $p = 0.009$). The fixed factors for bromeliad elevation in tree, bromeliad water volume, and bromeliad water pH were retained in best fit models within $\Delta AIC \leq 2$, but were not significant (Table 6.1). There was a 48.5% decrease in the number of anuran occupied *A. zebrina* in disturbed forest for the reduced species dataset of canopy-dwellers ($\beta = -0.956$, $Z = -2.69$, $p = 0.007$). The fixed factors of bromeliad water volume, number of *A. zebrina* bromeliads in tree, bromeliad height, and bromeliad water pH were retained in best fit models within $\Delta AIC \leq 2$, but were not significant. In the dataset for the two permanent bromeliad inhabitants (*P. aureolineatus* and *P. waoranii*) there were 44.8% less *A. zebrina* occupied by anurans in disturbed forest than undisturbed forest ($\beta = -0.828$, $Z = -2.21$, $p = 0.027$). The number of *A. zebrina* bromeliads in tree and bromeliad water pH were the only non-significant fixed factors retained in best fit models within $\Delta AIC \leq 2$ for the permanent bromeliad inhabitants.

Similarly, only forest disturbance had an effect on anuran abundance. The best fit model for the full species dataset, as determined by AIC, was based on the log-normal Poisson distribution. However, we report the model based on the type 1 negative binomial distribution because it was within $\Delta\text{AIC} \leq 2$, more parsimonious for number of factors used, and both models produced similar results (Table 6.2). The abundance of anurans in the full species dataset was 44.3% less in disturbed forest compared to undisturbed forest ($\beta = -0.792$, $Z = -2.61$, $p = 0.009$). No other factors were retained in either best fit models for the full dataset. The best fit model for the canopy-dwellers dataset was based on the log-normal Poisson distribution with forest disturbance level as the only retained fixed factor, but 3 other models were within $\Delta\text{AIC} \leq 2$ and reported mixed significance effects for forest disturbance level (Table 6.2). There were 48% less permanent canopy-dwelling anurans in disturbed forest than undisturbed forest ($\beta = -0.723$, $Z = -2.11$, $p = 0.035$). In the dataset for permanent bromeliad-inhabiting anurans the best fit model was based on the log-normal Poisson distribution with forest disturbance level as the only retained fixed factor, but 2 other models were within $\Delta\text{AIC} \leq 2$ and reported similar results (Table 6.2). There were 44.8% less permanent bromeliad-inhabiting anurans in disturbed forest than undisturbed forest ($\beta = -0.672$, $Z = -1.77$, $p = 0.076$).

Discussion

Our results show that forest disturbance associated with oil access roads and infrastructure has a negative effect on anurans utilizing the microhabitat of *A. zebrina*

bromeliads in the upper canopy of eastern Ecuador's lowland rainforest. The limited studies on terrestrial amphibians in Neotropical lowland rainforest associated with forest clearing, fragmentation, and edge effect have generally reported negative effects on amphibian community diversity and abundance (Pearman 1997; Schlaepfer & Gavin 2001; Bell & Donnelly 2006). However, these anthropogenic disturbances do sometimes have positive or no effects when the focus is on particular species groups (e.g., Hylidae) (Pearman 1997; Urbina-Cardona et al. 2006). Typically, anthropogenic disturbance effects are correlated with habitat variable differences among disturbance levels or types; including soil moisture, temperature, fragment size, distance from clearing or edge, and leaf litter depth (Pearman 1997; Bell & Donnelly 2006; Semlitsch et al. 2007). Our study found no differences in habitat variables, both abiotic and biotic, between undisturbed and disturbed forest. Several habitat variables were retained in best-fit models within $\Delta AIC \leq 2$ for anuran occupancy and abundance, but only the number of *A. zebrina* in a sampled tree showed a mild (non-significant) negative effect in the canopy-dweller and bromeliad-inhabitant abundance datasets. While the number of *A. zebrina* per tree was the most closely related habitat variable predicting anuran abundance in the reduced datasets, it did not differ significantly between forest disturbance levels. We did not quantify size of road clearing, oil infrastructure sites (e.g. processing facilities), or Waorani clearings along the 24 km of the Maxus road we surveyed. However, it does appear that the cumulative effects of deforestation and fragmentation along roads may be having an impact on the occurrence of trees with *A. zebrina* communities.

The mean distance of 29 km separating undisturbed and disturbed forest sampling locations helped ensure that oil operations, hunting, and forest product harvesting by

Waoranii and Kichwa peoples conducted in the vicinity of the Maxus road were not potential factors of concern for bromeliads sampled in undisturbed forest. Hunting and forest product harvest, including *A. zebrina* harvest by Kichwa for medicinal and ornamental purposes, may have an impact on bromeliad-inhabiting anurans due to a reduction in canopy predators (Benzing 2000). Canopy mammals and birds forage in bromeliads and primate predation of an anuran in *A. zebrina* has been observed (S.F.M., unpublished data). Waoranii and Kichwa hunting in forest bordering the Maxus oil road is causing a decline in mammal populations, and this reduction in mammalian predators (primarily primates) may have a positive impact on anurans inhabiting the canopy (Franzen 2006; Suárez et al. 2009).

The initial sampling design for this study was to include a high-disturbance location along an older network of oil roads. A preliminary survey for trees with *A. zebrina* suitable for sampling was conducted in Summer 2006 along the central portion of the Auca oil road that was to be used for the high-level disturbance sites (Fig. 6.1). The Auca road is heavily deforested and fragmented due to uncontrolled colonization; the majority of road frontage has been cleared for crops and pasture with other colonizers moving in behind these farms, resulting in a quasi-parallel pattern of deforestation and fragmentation (Sierra 2000). Upon return for sampling in 2008, trees with *A. zebrina* no longer existed along either side of 50 km of the road, including a section that extends into Yasuní within 20 km of trees sampled along the Maxus road. Herbarium records and other road surveys confirm the presence of *A. zebrina* from throughout the surrounding region (Fig. 6.1) (S.F.M., unpublished data). A light aircraft flight was taken by S.F.M. on November 15, 2008 which crisscrossed the Auca road region to search for *A. zebrina*

in emergent trees within areas of remaining intact forest that were not accessible from road surveys. During this flight 15 trees with *A. zebrina* communities were identified, the majority being greater than a kilometer from the nearest road and the closest approximately 450 m from a road that showed no signs of clearing in the immediate area. Although somewhat anecdotal, evidence from road and aerial surveys indicate that *A. zebrina* are intolerant of deforestation but to a lesser extent forest fragmentation.

A. zebrina bromeliads in disturbed forest along the Maxus road had significantly lower occupancy by anurans than in undisturbed forest, with nearly twice as many *A. zebrina* occupied by one or more anurans in undisturbed forest. This was unexpected due to the relative intactness of the forest within our survey area along the road. There were a total of 6 oil operation facilities and 13 clearings by Waorani for home sites or crops along 24 km of surveyed road. The largest of these clearings extend a distance of approximately 100 m of road frontage with most being considerably smaller, and by our account represent a small percentage of the 480 ha surveyed for trees with *A. zebrina* along the Maxus road. The Maxus oil road is the strictest controlled road against non-indigenous settlers in existence for more than 15 years in eastern Ecuador (Finer et al. 2009). Where we conducted our sampling, most stretches of the road have primary forest right up to the right-of-way edge on either side. The fact that no recorded habitat variables differed between disturbance levels is another indicator of the quality of the forest now bordering the Maxus road.

In disturbed forest we found a significantly lower abundance of anurans in *A. zebrina* for the entire community and canopy-dwellers datasets. While differences in abundance for the permanent bromeliad-inhabitants were not significant, they did follow

a similar trend with nearly half as many anurans being observed in disturbed forest. This is evident in the full dataset that had a fractionally smaller reduction in percent abundance of anurans (44.3%) in disturbed forest as compared to that for the permanent bromeliad-inhabitants dataset (44.8%). A potential reason for the non-significance is the relatively small sample size and an excess of zeros in the dataset effecting the power of the analysis (Seavy et al. 2005; Zuur et al. 2009).

While we did not analyze species diversity, it was the combined observation of several species represented as singletons or doubletons and nearly twice the number of permanent bromeliad-inhabitants in undisturbed forest that contributed to observed differences in the two larger datasets. *Scinax ruber* was one species of particular interest found only in disturbed forest trees in close proximity to the Repsol-YPF Northern Production Facility, the largest cleared and developed site in our study area. *S. ruber* is arboreal and considered a “pest” species because of its invasiveness and propensity to displace native species (Solís et al. 2011). It’s primary habitat is disturbed or cleared areas and is not considered an upper canopy inhabitant, although Pauly et al. (2005) reported observations of *S. ruber* on a canopy tower in Panama at heights up to 25 m (Duellman 1978a). The canopy tower is in close proximity to the Panama Canal and the town of Gamboa, an area of historic and current anthropogenic disturbances and most likely reason for its presence. In our sampling for this study and previous work in undisturbed forest at TBS (130 bromeliads from 3 species and 140 quadrat surveys) we have not collected *S. ruber*, yet it has been observed around the few buildings in the camp area (S.F.M., unpublished data). Therefore, we do not consider *S. ruber* an upper canopy

bromeliad inhabitant; rather, it is an invasive species to the upper canopy near areas of moderate to heavy human disturbance where its populations have exploded.

In the absence of habitat variable correlation with anuran occupancy or abundance and no differences in these variables between forest disturbance levels, as is common in other studies, it is difficult to explain the observed negative effect on anurans inhabiting canopy bromeliads of what we consider low-impact forest disturbance. Epiphytes, including bromeliads, contribute significantly to maintaining microclimatic conditions in tropical forest canopies by reducing wind turbulence and temperature which, in turn, reduces evapotranspiration maintaining elevated relative humidity (Moffett 1994; Freiberg 2001). Epiphytes are considered hypersensitive to changes in climatic conditions, requiring the very conditions they promote for existence (Benzing 1998; Hietz 1998; Benzing 2000). This hypersensitivity makes them particularly susceptible to forest microclimate changes resulting from anthropogenic disturbance, thus epiphytes and their inhabitants are suitable bioindicators of diversity and forest integrity (Benzing 1998; Hietz 1998; Benzing 2000; Brighigna et al. 2002). While it is generally accepted that bromeliads contribute to canopy microclimate moderation, basic knowledge of the internal microclimatic factors supporting the diverse and abundant faunal communities within this ecologically distinct microhabitat is scarce (Nadkarni 1994; Benzing 1998; Stuntz et al. 2002). Canopy perturbations, whether anthropogenic or natural, can alter microclimate conditions in forest canopies causing disruptions to ecosystem functions and biodiversity, with local to global effects on climatic variables such as increased temperatures and reduced precipitation (Foley et al. 2003; Lowman & Schowalter 2012).

Disruption of these microclimatic factors may be the driver for reduced presence and abundance of anurans at our disturbed forest sites. Another explanation may be pollution from road dust or waste gas flaring at oil facilities entering the bromeliad tank water and directly poisoning anurans or disrupting the food web. Road dust from large trucks and heavy equipment traveling the Maxus road has been observed penetrating the canopy at elevations of approximately 35 m and a distance of 26 m from the road edge (S.F.M., unpublished data). Road noise and vibrations have also been found to impact some organisms including amphibians (Canaday & Rivadeneyra 2001; Andrews et al. 2008). Lastly, the possibility exists that a reduction in avian and canopy mammal predators due to human hunting and forest disturbance has had a positive effect on bromeliad-inhabiting anurans and our results might have been more significant had hunting not been a factor (Dew et al. 2003; Franzen 2006; Suárez et al. 2009).

Our study provides a unique initial look into the anuran fauna of a large upper canopy tank bromeliad and the negative effects on this community as a result of low-impact forest disturbance due to road edge and limited deforestation. We suggest several avenues of research for future studies to better unravel the impacts on canopy diversity resulting from what is considered limited forest disturbance by natural resource extraction operations. First, data collection and analyses of bromeliad microhabitat presence, abundance, and diversity associated with deforestation and fragmentation area. Second, monitoring of microclimate factors within and adjacent to upper canopy bromeliads at stratified levels of forest disturbance to determine correlates of differences. Third, bromeliad tank water quality testing for pollutants deposited with road dust, generated as part of the petroleum refining processing, and from agricultural burning. Fourth, quantify

the predation rates by canopy predators on bromeliad-inhabiting anurans to estimate effects of their decline. Further canopy research would provide us with a better understanding of how tropical forest canopy ecosystems, including their diversity and services, are disrupted by direct and indirect human activities that result in cascading effects right down to the forest floor.

The detrimental effects of petroleum operations on tropical forests have been well documented in eastern Ecuador and around the world (Wunder 2003b; Finer et al. 2008). While further research is needed, our study reveals that even a minimal footprint by petroleum extraction operations, primarily roads, can have significant impacts on a unique anuran community in perhaps the most biologically diverse place on the planet (Bass et al. 2010). Based on these results, we re-emphasize the recommendations of Bass et al. (2010) to permit no new terrestrial access routes into Yasuní or its buffer zone and establish a moratorium on future exploration and extraction operations. It is recommended that any future petroleum development treat tropical forest habitat as an offshore system where land-based access is not used and current access roads be protected from colonization and further deforestation.

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Table 6.1: Model results for anuran occupancy. Best-supported models ($\Delta\text{AIC} \leq 2$) for anuran occupancy of *A. zebrina* bromeliads for all species, canopy-dwellers, and permanent bromeliad-inhabitants. Significant p-values for fixed effects in bold.

Dataset	Model	Error distribution	Fixed effects	β^d	SE	Z	P	ΔAIC	
Full ^a									
	f1bin	Binomial	Intercept	-0.201	0.225	-0.89	0.372	-	
			Forest disturbance	-0.898	0.342	-2.62	0.009		
	f2bin	Binomial	Intercept	-0.565	0.424	-1.33	0.183	1.0	
			Forest disturbance	-0.892	0.343	-2.60	0.009		
			Water volume	<0.001	<0.001	1.02	0.310		
	f3bin	Binomial	Intercept	0.491	1.028	0.48	0.630	1.7	
			Forest disturbance	-0.814	0.351	-2.32	0.020		
			<i>A. zebrina</i> elevation	-0.036	0.032	-1.12	0.260		
			Water volume	<0.001	<0.001	1.20	0.230		
	Canopy ^b								
	c2bin	Binomial	Intercept	0.060	0.364	0.16	0.869	-	
			Forest disturbance	-0.937	0.358	-2.62	0.009		
Number of <i>A. zebrina</i>			-0.007	0.005	-1.43	0.152			
c1bin	Binomial	Intercept	-0.354	0.227	-1.56	0.120	0.1		
		Forest disturbance	-0.956	0.355	-2.69	0.007			
c3bin	Binomial	Intercept	-0.365	0.484	-0.75	0.450	0.2		
		Forest disturbance	-0.917	0.360	-2.55	0.011			
		Water volume	<0.001	<0.001	1.34	0.181			
		Number of <i>A. zebrina</i>	-0.008	0.005	-1.67	0.096			

^a Dataset analyzed with all anurans collected from *A. zebrina* bromeliads.

^b Dataset analyzed using only canopy-dwelling anurans.

^c Dataset analyzed using only permanent bromeliad-inhabiting anurans.

^d Coefficient estimate.

Table 6.1: Continued.

Dataset	Model	Error distribution	Fixed effects	β^d	SE	Z	P	ΔAIC
Canopy ^b								
	c4bin	Binomial	Intercept	0.622	1.598	0.39	0.697	1.8
			Forest disturbance	-0.921	0.360	-2.56	0.011	
			Water volume	<0.001	<0.001	1.34	0.179	
			Number of <i>A. zebrina</i>	-0.008	0.005	-1.73	0.084	
			Water pH	-0.218	0.337	-0.65	0.518	
Bromeliad ^c								
	b1bin	Binomial	Intercept	-0.570	0.242	-2.35	0.019	-
			Forest disturbance	-0.828	0.374	-2.21	0.027	
	b2bin	Binomial	Intercept	-0.261	0.374	-0.70	0.049	0.9
			Forest disturbance	-0.807	0.371	-2.17	0.030	
			Number of <i>A. zebrina</i>	-0.005	0.005	-1.03	0.300	
	b3bin	Binomial	Intercept	1.237	1.622	0.76	0.446	2.0
			Forest disturbance	-0.820	0.375	-2.19	0.029	
			Number of <i>A. zebrina</i>	-0.005	0.005	-1.12	0.261	
			Water pH	-0.331	0.351	-0.94	0.345	

Table 6.2: Model results for anuran abundance. Best-supported models ($\Delta\text{AIC} \leq 2$) of anuran abundance in *A. zebrina* bromeliads for all species, canopy-dwellers, and permanent bromeliad-inhabitants. Significant p-values for fixed effects in bold.

Dataset	Model	Error distribution	Fixed effects	β^d	SE	<i>Z</i>	<i>P</i>	Δ AIC
Full ^a								
	f1lnp	Log-normal Poisson	Intercept	-0.897	0.253	-3.54	<0.001	-
			Forest disturbance	-0.801	0.329	-2.44	0.015	
	f1nb1	Type 1 negative binomial	Intercept	-0.373	0.204	-1.83	0.068	2.0
			Forest disturbance	-0.792	0.303	-2.61	0.009	
Canopy ^b								
	c1lnp	Log-normal Poisson	Intercept	-0.943	0.263	-3.58	0.000	-
			Forest disturbance	-0.723	0.342	-2.11	0.035	
	c2lnp	Log-normal Poisson	Intercept	-0.555	0.365	-1.52	0.013	0.0
			Forest disturbance	-0.699	0.335	-2.09	0.037	
Number of <i>A. zebrina</i>			-0.006	0.004	-1.40	0.163		
	c3lnp	Log-normal Poisson	Intercept	-1.44	0.760	-1.90	0.058	0.2
			Forest disturbance	-0.600	0.332	-1.81	0.071	
			Number of <i>A. zebrina</i>	-0.007	0.004	-1.61	0.108	
			<i>A. zebrina</i> leaf number	0.032	0.024	1.35	0.175	
	c4lnp	Log-normal Poisson	Intercept	-2.033	1.061	-1.92	0.055	1.6
			Forest disturbance	-0.656	0.341	-1.93	0.054	
			Number of <i>A. zebrina</i>	-0.008	0.005	-1.77	0.077	
			<i>A. zebrina</i> leaf number	0.029	0.024	1.23	0.219	
			<i>A. zebrina</i> elevation	0.024	0.303	0.80	0.422	

^a Dataset analyzed with all anurans collected from *A. zebrina* bromeliads.

^b Dataset analyzed using only canopy-dwelling anurans.

^c Dataset analyzed using only permanent bromeliad-inhabiting anurans.

^d Coefficient estimate.

Table 6.2: Continued.

Dataset	Model	Error distribution	Fixed effects	β^d	SE	Z	P	ΔAIC
Bromeliad ^c								
	b1lnp	Log-normal Poisson	Intercept	-1.178	0.300	-3.92	<0.001	-
			Forest disturbance	-0.672	0.379	-1.77	0.076	
	b2lnp	Log-normal Poisson	Intercept	-2.009	0.823	-2.44	0.015	0.8
			Forest disturbance	-0.592	0.377	-1.57	0.117	
			<i>A. zebrina</i> leaf number	0.029	0.026	1.11	0.265	
	b3lnp	Log-normal Poisson	Intercept	-1.767	0.840	-2.10	0.035	1.1
			Forest disturbance	-0.548	0.369	-1.48	0.138	
			Number of <i>A. zebrina</i>	-0.006	0.005	-1.29	0.196	
			<i>A. zebrina</i> leaf number	0.034	0.026	1.31	0.191	

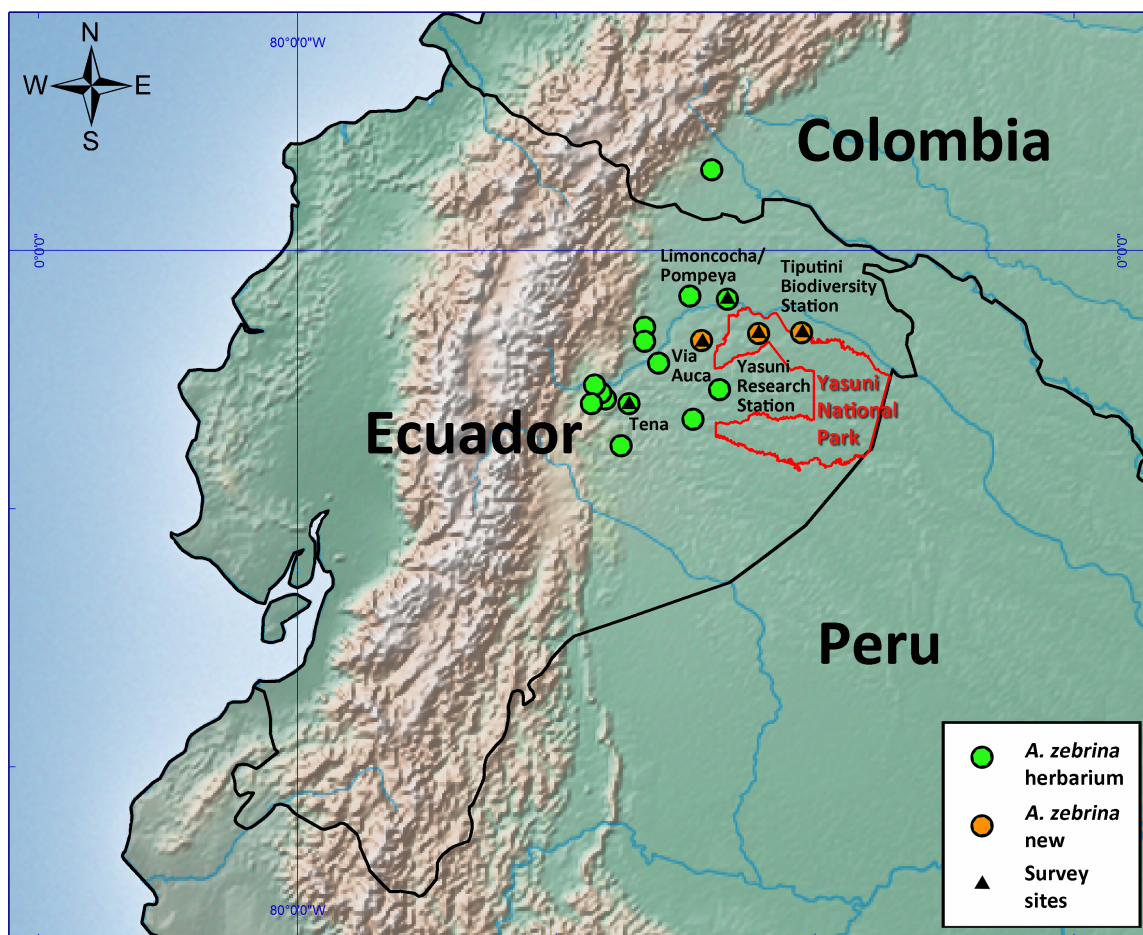


Figure 6.1: Map of *Aechmea zebrina* bromeliad observations and survey sites. Map of *Aechmea zebrina* bromeliad locations based on herbarium records and new observations during survey work. The five survey sites are labeled.

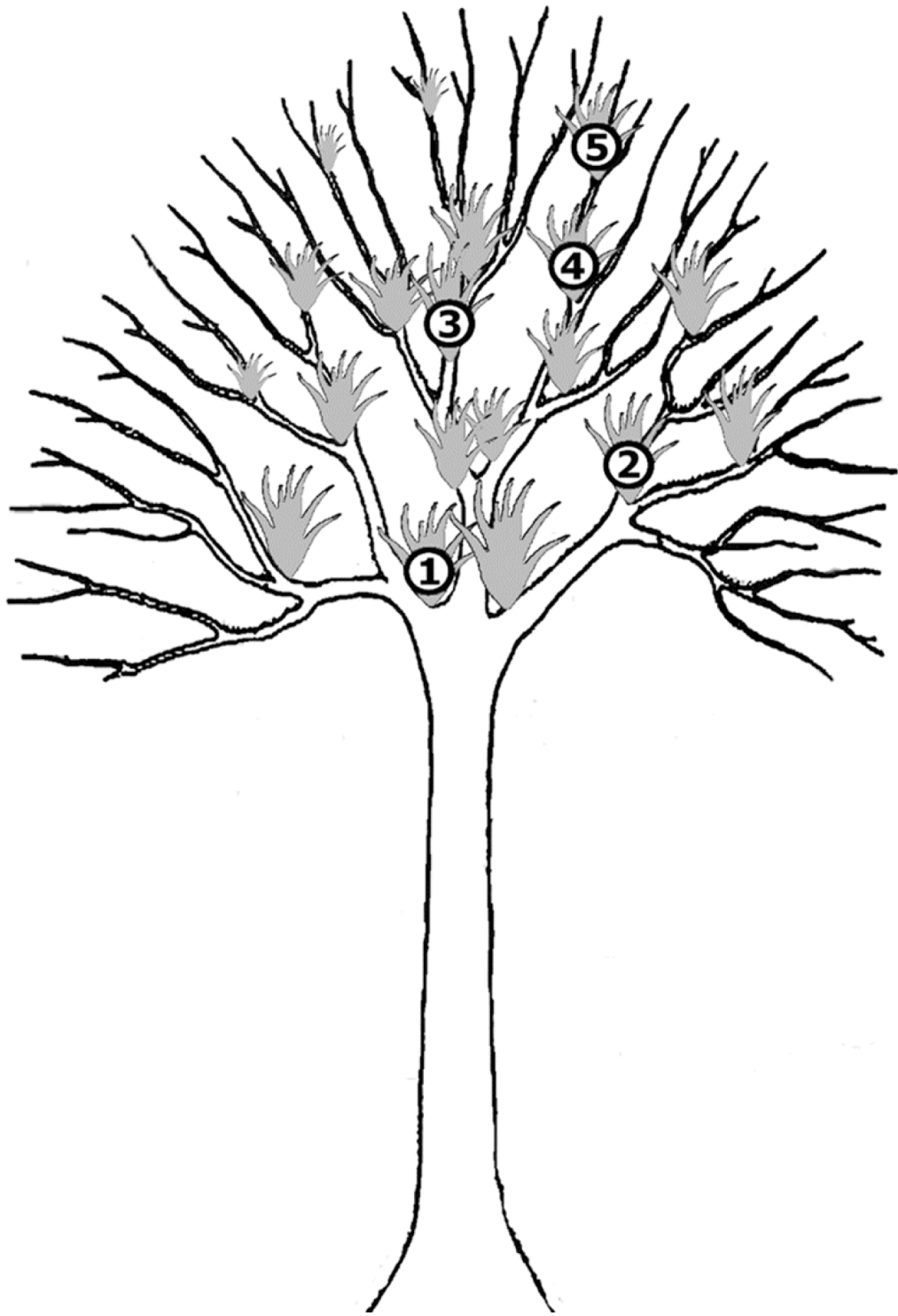


Figure 6.2: Tree schematic for bromeliad sampling. Schematic of tree with bromeliads illustrating distribution strategy for sampling units (bromeliads), numbers denote bromeliads sampled.

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CHAPTER VII

CONCLUSION

The diverse community of epiphytic plants in neotropical rainforests include the specialized rainwater storage species of Bromeliaceae, many of which live high in the canopy and are capable of holding large volumes of water. These canopy tank bromeliads provide microhabitat for a high diversity of fauna and flora and have been considered a “keystone resource”. Advancement in our knowledge of rainforest canopy inhabitants and their role in ecosystem function and maintenance has been hindered by our inability to easily access this abundant habitat. Technical advances in climbing methods derived from rock climbing and spelunking have allowed researchers to begin investigating the canopy environment and a new biological discipline has emerged from these efforts over the past 20 plus years. The development of a bromeliad patch sampling technique for herpetofauna and other taxa as part of this dissertation provides future researchers a methodology for collecting qualitative and quantitative data for canopy bromeliads and their inhabitants (McCracken & Forstner 2008). Using these methods, I sought to document the specialized rainwater collecting bromeliad community in the lowland rainforests of Amazonian Ecuador, in one of the first intensive investigations of a high

canopy tank bromeliad anuran community. I was able to evaluate the keystone bromeliad resource itself, *Aechmea zebrina*, but also provide data on the diversity and health of the anuran community. The final sampling design enables a statistical contrast between the anuran community in the bromeliads of undisturbed forests and that living in the bromeliads immediately adjacent to roadway disturbance regimes.

Aechmea zebrina is a conspicuous canopy bromeliad with its large size, contrasting silvery-white and green leaves, and brilliant red and yellow stalked inflorescence. Native to the Amazon regions of Ecuador and southwestern Colombia, *A. zebrina* was observed on a variety of phorophyte species at elevations up to 50 m above the forest floor. At a mean size of 75 cm ($n=240$) and capable of retaining up to 4 L of rainwater, and a mean of 66 individuals per tree ($n=40$), with up to 159 individuals per tree, these bromeliad communities create and support a three-dimensional wetland in the sky. *Aechmea zebrina* provides critical microhabitat and serves as a valuable resource to other canopy dwelling organisms. In this study, which occurred across a relatively small geographical area, 14 species of herpetofauna were found residing within *A. zebrina*, along with three species of primates and 6 species of birds observed utilizing it as a resource. Two of the anuran species, *Pristimantis aureolineatus* and *P. waoranii*, were new to science and described as part of the project (Guayasamin et al. 2006; McCracken et al. 2007). Additionally, the reproductive ecology and behavior of *P. aureolineatus* was reported (McCracken & Forstner 2006). These species, along with others collected as part of this project and as parts of previous research, contributed to a 2010 publication on the biodiversity and conservation significance of Yasuni National Park; with a world record

number of 150 species of amphibians found within the 6 km² of the Tiputini Biodiversity Station, USFQ (Bass et al. 2010).

The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been implicated as one of the greatest threats to amphibians worldwide and is already responsible for wide-spread deaths and possible anuran species extinction events (Pounds et al. 2006). It is known to have infected >350 species (Fisher et al. 2009). In this study, we examined for the occurrence of *Bd* in amphibians occupying ecological niches along the vertical axis from the forest floor to the high canopy in the Amazonian lowlands of eastern Ecuador (McCracken et al. 2009). Additionally, we tested for suitable conditions for the persistence of *Bd* in the aquatic environment of *A. zebrina* bromeliads. We detected *Bd* on amphibians inhabiting all strata of the forest, which demonstrates that searches not including vertical strata may be lacking important data on the system. Furthermore, we found evidence that the occurrence of *Bd* infection on amphibians along a vertical axis is non-random in this system. Infection by *Bd* was found to be significantly higher in the fossorial/floor and canopy groups, showing 25% and 33% of individuals infected, respectively, than in the shrub/sub-canopy group with only one individual (3%) infected. Because *Bd* is transmitted via aquatic zoospores, the absence of an abundance of standing water available to amphibians of the shrub/sub-canopy group may be reducing their exposure to the fungus (Longcore et al. 1999a). Environmental parameters for water temperature and pH in the leaf axils of *A. zebrina* bromeliads were found to be within the ranges acceptable for *Bd* zoospore growth and reproduction, with a mean water temperature of 23.8-24.5°C in three bromeliads at varying heights above the forest floor and a mean water pH of 4.48 from 90 bromeliads. Another important finding of this study

is the demonstration of clinical signs of chytridiomycosis and a positive detection for *Bd* by one individual (*Leptodactylus pentadactylus*) included in the study. Finally, this study was the first to report the presence of *Bd* in the Amazon lowland rainforests and, in particular, within the high canopy bromeliad-dwelling anuran community.

The final chapter of the dissertation addresses the effects of low-level anthropogenic disturbance on ecological factors and the anuran community of *A. zebrina* bromeliads. Area of disturbance and impact intensity for the treatment (disturbed) sampling sites were not quantified, but were determined based on age of oil access roads and qualitative estimates for the number of clearings along the 24 km survey area. The initial sampling design for this study was to include a high-disturbance location along an older network of oil roads where a preliminary survey (2006) had identified trees with *A. zebrina* communities. Upon return for sampling in 2008, trees with *A. zebrina* no longer existed along either side of 50 km of the road. As a consequence, only the low-level anthropogenic disturbance location would be used for sampling of a treatment location. This location, the Maxus road, primarily consisted of an oil concession access road with a total of 6 oil operation facilities, one scientific research facility, and 13 clearings by the indigenous Waorani for home sites or crops along the 24 km road survey area. Otherwise, most stretches of the road where surveying and sampling of *A. zebrina* occurred was primary forest up to the right-of-way edge on either side. A total of 10 species of anurans were found to inhabit *A. zebrina* bromeliads, with 5 species unique to undisturbed forest and only two unique to low-level disturbed forest. Occupancy of *A. zebrina* bromeliads by anurans was significantly greater in undisturbed forest at Tiputini Biodiversity Station for all species groups analyses. Anuran abundance in *A. zebrina* was significantly greater

in undisturbed forest for all species groups analyzed with the exception of the permanent bromeliad-inhabitants group consisting of just *P. aureolineatus* and *P. waoranii*.

However, they did follow a similar trend as the other species groups with nearly half as many anurans being observed in disturbed forest. A potential reason for the non-significance is the relatively small sample size and an excess of zeros in the dataset effecting the power of the analysis (Seavy et al. 2005; Zuur et al. 2009).

No habitat variables of sampled *A. zebrina* differed between undisturbed and low disturbance forests. In the absence of habitat variable correlation with anuran occupancy or abundance and no differences in these variables between forest disturbance levels, as is common in other studies, it is difficult to explain the observed negative effect on anurans inhabiting canopy bromeliads of what is consider low-impact forest disturbance.

Negative impacts on bromeliad microclimate due to road edge effect or pollution of bromeliad leaf axils water systems from road dust or airborne particles are the primary hypothesized contributors to reduced anuran occupancy, abundance, and species richness in *A. zebrina* associated with low-level forest disturbance in this study.

This research provides a unique initial look into the anuran fauna of a large upper canopy tank bromeliad and the unexpected negative effects on this community as a result of low-impact forest disturbance due to road edge and limited deforestation. While a negative effect on anuran occupancy, abundance, and species richness was observed due to low-level forest disturbance there still remains the question of causality. Additional research is necessary to identify the related environmental correlates of forest disturbance and the canopy bromeliad anuran community. However, the significantly lower anuran occupancy and abundance levels in *A. zebrina* along roadsides (nearly 50% lower)

provide evidence that even a limited amount of forest disturbance has an influence on species dynamics. If limited forest disturbance has such an effect, then the impact of increased levels of disturbance due to uncontrolled human access to forested areas via oil roads will have an exponential negative impact. The Via Auca region west of Yasuni National Park in Ecuador is a clear example of deforestation driven by the colonization of oil access roads.

Unfortunately, the termination of petroleum exploration and extraction operations is currently not a feasible option for developing nations due to heavy reliance on income from these natural resources. Most of these nations lease large tracts of land to multinational firms who develop and manage these petroleum operations. It is important these nations recognize the value of their intact forests over the long-term in the same way as they realize the value of the petroleum underground in the short-term. By requiring these companies to adhere to the same strict environmental regulations applied in developed nations and treat rainforest operations as an offshore project where roads are not built they could reap the benefits of both resources with considerably less impact over the long-term to their forested natural resource base. The research reported herein provides knowledge of canopy bromeliad ecology applicable to promoting environmental stewardship, ecosystem maintenance, and assisting conservation decision-making in Amazonia. It is science-based knowledge such as this that can empower stakeholders in forest conservation, from indigenous inhabitants to government official, with the evidence necessary to convince corporate stakeholders that the application of best-practice technology and development are in their best interest too. Foresight in environmental stewardship and pioneering technological advances in forest preservation,

as part of natural resource acquisition, is a “win-win” for all stakeholders involved and future generations.

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VITA

Shawn F. McCracken, son of John and Mary Ann McCracken, was born in Akron, Ohio and moved to Houston, Texas at age seven. Since 1997, he and his wife, Bejat, have traveled and conducted research in the Amazon Basin. In 1999, he founded the TADPOLE Organization to promote and conduct amphibian conservation in the Amazon Basin. Finally, in 2005 he graduated cum laude from Texas State University-San Marcos with a B.A. in Biology. In the fall of 2005, he entered the Ph.D. program in Aquatic Resources at Texas State under the advising of Michael R. J. Forstner. During his graduate studies he has worked as an instructional assistant for Organismal Biology, Genetics and Ornithology, research assistant under Dr. Forstner, assistant curator of the M. R. J. Forstner Frozen Tissue Collection, and Federal Houston toad monitor. In 2007, he received an NSF Graduate Research Fellowship and an NSF GK-12 Fellowship with Project Flowing Waters from 2010-2012. Shawn has been recognized numerous times by Texas State, the Biology Department, and national and international organizations for his academic and research achievements in the form of research grants, scholarships, and awards.

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