

Investigation of Forest Canopies as Possible Safe Havens from Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*): Hope in the Midst of a Global Amphibian Extinction Crisis

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1. Introduction

Amphibian Chytrid Fungus and the Amphibian Extinction Crisis

Amphibian populations are declining worldwide at an alarming rate, a phenomenon now considered to be an “amphibian extinction crisis” (Stuart et al. 2004; Wake & Vredenberg, 2008). Habitat loss and degradation, environmental contaminants, and emerging infectious diseases (Collins & Storfer 2003; Lips et al. 2005; Rohr et al. 2008) have been paving the road towards extinction for decades. More recently, the amphibian chytrid fungus *Batrachochytrium dendrobatidis* has been identified as a rapidly emerging amphibian pathogen, capable of acting as both the proximate and ultimate causes for amphibian extinction (Skerratt et al. 2007; Wake & Vredenberg 2008). As the etiological agent of amphibian chytridiomycosis, *B. dendrobatidis* infects the keratinized epithelial cells of amphibians and is responsible for a variety of pathological expressions, ranging from asymptomatic individuals and sporadic deaths in some amphibian species to mass mortalities and extinctions in others. In some instances, the reason for drastic interspecific variation in susceptibility can be explained by the production of antimicrobial skin peptides or the presence of anti-*B. dendrobatidis* symbiotic bacteria (Rollins-Smith & Conlon 2005; Woodhams et al. 2007 a & b), but many species appear to be highly susceptible. Despite over a decade of extensive study, it was not until recently that we understood the physiological cause for amphibian mortality – electrolyte imbalance and cardiac arrest (Voyles et al. 2009). The scope of amphibian biodiversity at risk of decline is one of alarming severity and global impact, as *B. dendrobatidis* can now be found on every continent where amphibians exist and continues to spread regionally.

Amphibian Chytrid Fungus in Mesoamerica

The plight of amphibians in Mesoamerica began to attract attention and gain momentum following the alarming amphibian declines documented in Costa Rica and Panama nearly a decade ago (Lips 1998, 1999; Lips et al. 2003). Since then, many enigmatic amphibian declines worldwide have demonstrated a strong association with the presence of *B. dendrobatidis*.

Although Costa Rica and Panama receive significant international attention and resources to understand and combat this issue, the amphibians of Honduras are also in decline and warrant similar investment (Wilson & McCranie 2004). Before our first survey for *B. dendrobatidis* in Honduras in 2007, only one report served to confirm its presence within the country, based on archived museum specimens previously collected in 2003 (Puschendorf et al. 2006). After conducting baseline herpetological surveys in Cusuco National Park, Honduras (CNP) in 2006, we were alarmed to learn that many of the park's endangered and critically endangered species were said to be experiencing enigmatic declines, according to the IUCN Red List of Threatened Species (2007). This concern prompted the establishment of our *B. dendrobatidis* investigation in Honduras, which continues to this day. In brief, we have identified CNP as the first known site in Honduras where endangered and critically endangered amphibian species are infected with *B. dendrobatidis* (Kolby et al. 2009). To date, we have now found 12 species to be infected, some of which appear to be experiencing significant declines and face a high risk of extinction (Kolby & Padgett-Flohr 2009). Most researchers consider *B. dendrobatidis* to be confined to terrestrial water sources and therefore focus on the plight of amphibian species which possess aquatic life stages in rivers and lakes. In 2008, we were alarmed to discover *B. dendrobatidis* infecting arboreal amphibians in CNP. These results prompted us to conduct the current investigation in order to better understand the threat which *B. dendrobatidis* poses to arboreal amphibian species which do not enter terrestrial water systems.

2. Current (2009) Investigation

Purpose and Brief Summary

From June-August 2009, we set out to perform the first risk analysis for the threat of *B. dendrobatidis* exposure to arboreal amphibians and the possibility for an arboreal epidemic to occur. In order to accomplish this, we conducted a multidisciplinary investigation combining amphibian radiotracking, data logging of water condition within bromeliads, and surveys for *B. dendrobatidis* infection. Since we had previously identified infection in arboreal and semiarboreal amphibians in 2008, it was important to investigate whether arboreal tank bromeliads could serve as safe havens from *B. dendrobatidis* for species which exhibit life history patterns independent of the presence of terrestrial water bodies, where *B. dendrobatidis* is

most likely to occur and accumulate. For optimal growth in culture, *B. dendrobatidis* requires aquatic environments with a pH 6 to 7 and below 32 C (Johnson & Speare 2003, 2005; Johnson et al. 2003; Piotrowski et al. 2004). Since studies have shown that the water collected by bromeliads and other phytotelmata can become quite acidic (Benzing et al. 1972; Kitching 2001), these arboreal aquatic habitats could potentially retard activity or even kill *B.*

dendrobatidis, thereby protecting amphibians which depend upon arboreal water sources. In addition, arboreal bromeliads are widely dispersed throughout CNP and can be found in regions devoid of terrestrial water bodies, allowing bromeliad-dwelling amphibians to occupy regions far from known hotspots of *B. dendrobatidis* occurrence. Therefore, it may be possible for arboreal and semiarboreal amphibian species to be environmentally, physically, and behaviorally protected from the effects of a *B. dendrobatidis* epidemic while species with terrestrial aquatic larvae are concurrently experience decline. Therefore, our risk analysis consisted of three main components: 1) *B. dendrobatidis* infection prevalence surveys of susceptible amphibian species, 2) amphibian radiotracking surveys, and 3) bromeliad habitat and water condition monitoring.

Summary of Results

***B. dendrobatidis* infection prevalence surveys**

Over the course of this investigation, 309 amphibians of 14 species were swabbed for the detection of *B. dendrobatidis* infection. Of these amphibians, 106 amphibians tested positive for infection and 203 produced negative results (Table 1). Three additional amphibians produced inconsistent positive results possibly due to weak infection, but these samples were excluded from analysis to prevent the introduction of bias into our results. Of the 14 species tested in 2009, the following six species produced positive results for infection: *Plectrohyla dasypus*, *Plectrohyla exquisita*, *Ptychohyla hypomykter*, *Duellmanohyla soralia*, *Bromeliohyla bromeliacea*, and *Lithobates maculata* (Annex 1). Although an additional eight species were tested, these six infected species composed 91% of all individuals sampled. The seven positive records of infection for *B. bromeliacea* solidify the species' susceptibility to infection. Although one of four animals sampled in 2008 produced a weakly positive result for infection, we were hesitant to prematurely declare significant results based on a single sample. We have now discovered seven additional infected *B. bromeliacea*, the significance of which lies in the fact that this species is a "bromeliad frog" which exhibits a completely arboreal life cycle,

independent of terrestrial water bodies. Six of these infected individuals were tadpoles completely confined to their bromeliad's water tank. In all instances, the bromeliads containing infected tadpoles were located at least 400m away from any significant terrestrial water body where *B. dendrobatidis* is likely to be encountered. As will be discussed later in this report, the behavioral patterns observed in two species known to carry infection revealed that neither species is likely to disperse *B. dendrobatidis* far from stream habitats. Both *Plectrohyla dasypus* and *Plectrohyla exquisita* remained relatively close to streams at all times during our study, venturing only 20m and 33m away from the streams' edge, respectively. Therefore, the confirmation of infection in *B. bromeliacea* larvae serves as a sobering reminder that there is still much to be understood about the dispersal mechanisms and persistence of this aquatic pathogen.

The highest prevalence of infection detected in 2009 was observed in two critically endangered endemic species; *P. dasypus* (48.9%) and *P. exquisita* (45.6%). Collectively, the total prevalence of *B. dendrobatidis* infection observed in CNP in 2009 was 34.3%, compared to 30.2% in 2008 and 44.4% in 2007 (Table 1). To date, 12 amphibian species have now been found infected with *B. dendrobatidis* within this cloud forest fragment, threatening 40% of CNP's amphibian diversity. Furthermore, eight of these infected species are listed either as endangered or critically endangered by the IUCN Red List of Threatened Species. Of the 12 infected species, four species (*P. dasypus*, *D. soralia*, *P. hypomykter*, and *L. maculata*) have produced positive results during all three field seasons from 2007 to 2009. As a result, this third season of annual survey data now presents us with the first opportunity to begin monitoring for long-term changes in prevalence among susceptible species (Table 2).

Between 2007 and 2008, a noteworthy decline in prevalence of infection occurred simultaneously in all four species, on average by -27.7%. Then, between 2008 and 2009 the prevalence trend reversed and simultaneously increased among all four species, but by a much lesser magnitude, on average 4.8%. The identity of the driving force(s) behind these observed trends is still a mystery, but may have grave consequences for the persistence of susceptible species, especially since this catalyst was not species-specific. It appears that a shift in *B. dendrobatidis*-associated survival has occurred, possibly mediated by a change in *B. dendrobatidis* virulence or a shift in amphibian susceptibility, and although it is too soon to predict the long-term effects it signifies the presence of a dynamic system. This contradicts the popular conception within the field of *B. dendrobatidis* research; that this pathogen is most

devastating to naive amphibian populations following initial introduction, whereas susceptible species are expected to rapidly slide towards extinction after which infection reaches a state of endemism and equilibrium in the species which persist. Although this makes good sense, the prevalence trends beginning to emerge from our long-term research suggests that sudden dramatic changes in survival can still occur even after a decade of persistence. Through the examination of museum archived material collected from CNP in 1996 we have proven that *B. dendrobatidis* has been present at our study site for at least 14 years (Kolby & Padgett-Flohr 2009), and yet our current work suggests that this system has not reached equilibrium, nor implies that it ever will. Due to the paucity of other long-term *B. dendrobatidis* field studies to draw reference from, it is too soon to assess whether our observations are representative of the majority or minority of other threatened amphibian populations infected with *B. dendrobatidis*.

Amphibian Behavior

Over the course of this study, 22 adult frogs were radiotracked and a total 262 individual locations were recorded. Of these 22 frogs, 10 were *Plectrohyla dasypus* and 12 were *Plectrohyla exquisita*. In order to follow these amphibians through the challenging terrain, each was fitted with a miniature radiotransmitter attached to a “waistbelt” consisting of a thin loop of Tygon silicone tubing, fitted just anterior to the hind legs. This technique was a modification of that demonstrated by Rathbun and Murphey (1996) in order to reduce the weight and friction of the belt. A small bead of quick-setting plumber’s epoxy was molded around the radio transmitter and extended to form a small loop, through which the Tygon belt was threaded (Fig. 1). A receiver was used to relocate and document the physical location and activity of each frog approximately every 12 hours, at 10am and 10pm (Fig. 2). Each location was marked with a red ribbon containing time and date information and after the final location was marked for that individual, all ribbons were meticulously measured and mapped to record the path followed by each amphibian. Frogs were tracked at three different river systems within the protected core zone of the park; Cantiles, El Cortecito, and El Danto (Fig. 3); and were followed for up to 10 days each.

In summary, the movement and habitat use of *P. exquisita* and *P. dasypus*, two critically endangered species endemic to CNP, was found to differ significantly. In total, 262 individual locations were recorded, averaging 12 locations for each frog. In general, *P. exquisita* moved

more frequently and greater distances between observations than did *P. dasypus*, and was often found perched in an exposed position, whereas *P. dasypus* remained sheltered an additional 25% of the time. On average, *P. exquisita* remained four times farther away from the stream's edge (in horizontal distance) than did *P. dasypus*. Frogs of both species usually spent several days in the same general area before moving to new locations. On average, *P. dasypus* moved 2.9 m horizontally every 12 hours, versus 5.2 m for *P. exquisita* and both species occasionally moved much greater distances within that temporal span; up to 26.2 m and 60 m, respectively. In addition, although habitat occupancy of the two species overlapped geographically, it appeared to be vertically stratified. All *P. dasypus* in this study remained within 10 m of the ground, on average within 2 m of the ground, while *P. exquisita* was often perched twice as high in the trees.

On multiple observations, some frogs of both species displayed a strong preference for a specific location and returned to inhabit the exact same diurnal retreat sites occupied on previous days. These sites were always in sheltered locations, including specific tree cavities and moss-covered tree trunks. Not all individuals exhibited strong associations with certain features or locations; some frogs traveled in unidirectional patterns and never visited the same location twice (Fig. 4). Arboreal perch sites selected by *P. exquisita* were surprisingly thin for such massive frogs, branches within reach to measure averaged 5.4 cm in circumference (Fig. 5). Although branches nearly twice this circumference appear more suitable to comfortably cradle the belly of these frogs, it seems likely that this behavior may provide an effective anti-predatory measure. Any approach by a sizeable reptilian or mammalian predator would likely collapse the branch before reaching close proximity and allow *P. exquisita* to escape immediate danger.

To the best of our knowledge, no other amphibian research projects have integrated *B. dendrobatidis* infection surveys into amphibian radiotracking studies. By doing so, the conservation value of the data produced can be significantly greater than that of either study conducted alone. For instance, the goal of our radiotracking was to document behavior characteristic of *P. dasypus* and *P. exquisita*, but since some amphibians become lethargic and unresponsive when infected with *B. dendrobatidis*, it is possible that radiotracked animals could exhibit subdued behavior atypical of the species. Consequentially, this would negatively affect important conservation decisions to be made on the basis of behavior; such as the determination of critical habitat boundaries and the captive requirements for maintaining assurance populations. Had we not concurrently collected *B. dendrobatidis* infection data to integrate with our

behavioral monitoring results, it would have been impossible to determine whether the variation observed between highly active and sedentary individuals (as seen in Fig. 4) was caused by a loss of fitness due to infection or rather the reflection of individual preference and variation. By taking this into consideration throughout our investigation, we were able to determine that the behavior we documented was valid and that *B. dendrobatidis* did not appear to exert a measureable effect on the behavior of radiotracked adults which tested positive for infection: two *P. dasypus* and one *P. exquisita*. These three individuals were alert and healthy and appeared to manifest the infection asymptotically.

Our survey was too short-term to determine whether these three infected individuals possessed resistance to chytridiomycosis, or whether we had simply sampled these amphibians shortly after the infection occurred. As such, this is an area which warrants continued investigation: *B. dendrobatidis* infection status during longer-term tracking sessions. Over the course of weeks rather than days, might an infected adult succumb to or overcome *B. dendrobatidis* infection? Additionally, are certain behavioral patterns correlated with such a change in infection status? Answers to these questions would help conduct rapid assessments of the fate of infected frogs and help explain the dynamics of *B. dendrobatidis* infection within certain populations. All 22 frogs tracked during this study were directly exposed to stream habitats saturated with this pathogen, but collectively only 13.6% developed infection versus 63.4% of co-occurring larvae and subadults. There are few instances where amphibians have displayed the ability to naturally overcome infection in the wild, but one such way is through behavioral modification such as increased basking to elevate skin temperature (Berger et al. 2003; Woodhams et al. 2003). In the current study, adult *P. dasypus* were often located in sheltered diurnal retreat sites close to the ground or stream as compared with the arboreal basking *P. exquisita*, yet in this instance, the contrasting behavior appeared to have no effect on either species' risk of developing infections. In total, adult infection prevalence surveys for both species produced very similar results; 13% for *P. exquisita* (23 adults sampled) and 13.8% for *P. dasypus* (29 adults sampled).

Although the 22 frogs tracked in this study appeared to be restricted to stream environments, it is still possible that both *P. dasypus* and *P. exquisita* occasionally wander further away from permanent water bodies than is currently known and utilize arboreal bromeliads. On two occasions we documented *P. dasypus* within arboreal bromeliads, which is significant for a

stream-dwelling species. One of these was an individual being radiotracked which selected a small bromeliad about 2.5m up a broadleaf tree as its daytime retreat. Although this location was only 4 m away from a stream, on a second occasion we discovered another *P. dasypus* within a bromeliad, nearly 600m away from any stream and 10m above the ground. This individual was not one of those which we radiotracked, but was instead encountered during an opportunistic bromeliad survey. Further work is necessary to determine how often *P. dasypus* disperses away from stream habitats and whether similar behavior is displayed by *P. exquisita*. According to James McCranie, other species of stream-breeding Plectrohylids have been found within bromeliads as much as an hours' walk away from the nearest stream (pers. comm. 2009).

Despite hundreds of hours of amphibian stream surveys we have conducted in CNP from 2006 to 2009, the paucity of tadpoles and complete lack of froglets of *P. exquisita* continues to be an unsettling enigma. In 2002, McCranie and Wilson reported that "Tadpoles of this species were taken in the Rio de Cusuco in May and August along with those of *P. dasypus* and *P. hypomykter*....Metamorphosing froglets were active at night and inactive during the day on low vegetation near the Rio de Cusuco in May, July, and August." Although we have been able to locate adults from June to August each year, tadpoles have become extremely scarce and we have yet to witness a single tadpole reach metamorphosis. Climate change and *B. dendrobatidis* are exerting increasing pressure on the amphibians of CNP and it is possible that these forces may have already resulted in a reduction in fecundity of *P. exquisita* and an increase in juvenile mortality. Although adults remain locally common in some streams, their abundance should be perceived with cautious optimism. The absence of successive seasons of juvenile recruitment may soon result in a rapid population decline as the current adult population continues to age. We hope that further behavioral studies and *B. dendrobatidis* surveys will elucidate the plight of *P. exquisita* and help to protect additional imperiled amphibian species in CNP.

In summary, many of the amphibian species found in CNP, are little studied and poorly understood. No behavioral studies have been conducted on any of these species and this is a growing concern as many Honduran species are now listed as critically endangered and reported to be declining. For many of these species, including those which served as the focus of our behavioral monitoring: *P. dasypus* and *P. exquisita*, declines are occurring within national parks and protected areas where the presence of *B. dendrobatidis* is causing harm regardless of habitat protection. Since this pathogen can only be controlled in animals maintained in captivity, our

knowledge (or lack thereof) of species-specific amphibian behavior and natural history may eventually prove to be the keystone factor in the successful long-term maintenance and propagation of species in captivity after the last of their kind has become extinct in the wild. It may be quite some time before we learn how to reintroduce susceptible species back into their *B. dendrobatidis*-infected sites of origin and although the ex situ zoo community has made great strides in amphibian captive husbandry practices, some species have not yet bred in captivity. It is important to consider the intricate behavior and environmental complexities experienced by many species in the wild and the difficulty in reproducing such conditions in a captive environment. As a result, further behavioral and environmental studies will not only strengthen the ability to successfully maintain these species in captivity long-term, but will also help us understand the poorly understood mechanisms of *B. dendrobatidis* exposure and transmission in these species.

Bromeliad Habitat and Water Monitoring

Over the course of two months, we collected a total 1,486 hours of high-resolution data points of pH and water temperature from 40 arboreal tank bromeliads. Bromeliads were sampled at six study sites across CNP (Fig. 3). In each bromeliad, water temperature and pH levels were measured with data loggers (PHTEMP101, Midgetech, Inc.) affixed with two independent probes; one for temperature (RTDS-4-3/16-6-36, Midgetech, Inc.) and another for pH (pH1, Midgetech, Inc.) (Fig6.). Data loggers were programmed to record values in continuous 30-minute intervals at a resolution of 0.1°C and 0.1 pH. Recorded data was classified into three groups for analysis: “Tree”, “Opportunistic Cluster” and “Extended Time”. The “Tree” data set was collected from three clusters of trees spanning the Park’s core zone. Each cluster was characterized by the presence of three trees located within 30 meters of one another, which each possessed bromeliads suitable for study within three general strata above the ground; those between the forest floor and 5 meters (“low”), those between 5 and 15 meters up (“mid”), and those 15 meters and above (“high”). The data loggers were affixed at height by using the double rope canopy access climbing technique (DRT), and the highest bromeliad monitored was attached to the tree 34.1 meters above the ground. The “Opportunistic Cluster” dataset is comprised of two sets of five bromeliads each which were found growing in close proximity to one another and attached within 2.5 meters of the ground. The “Extended Time” series is the

result of a monitoring session which spanned four weeks, the longest continuous data set recorded. Information on the total number of measurements, length of recording time and the number of bromeliads monitored for each data set is presented in Table 3.

The water temperature and pH values for all bromeliads were collectively analyzed and presented in Table 4. Some data sets were excluded from analysis due to the presence of erroneous values indicative of electronic malfunction. The average pH of every bromeliad remained acidic and fluctuated between 4.3 ± 0.1 and 5.5 ± 0.5 and extreme pH values recorded measured as high as 9.3 and as low as 3.3. The average water temperature of the bromeliads varied by site and collectively measured between 14.8 ± 0.2 °C and 19.0 ± 0.2 °C. The highest water temperature recorded was 31 °C, and the minimum was 13.8 °C. A visual representation of a “Tree” data set recorded at the Base Camp study site can be seen in Figure 7.

Size distribution of bromeliads (by total mass) was unevenly distributed on the trees, with the largest bromeliads located near the midsection. These bromeliads were significantly larger than bromeliads closer to the ground ($p = 0.019$, $H = 2$, $N=20$) or the canopy ($p = 0.025$, $H = 2$, $N=20$). We also detected an inverse relationship between water temperature variability and bromeliad size and found that the average water condition was more acidic in smaller bromeliads. In this study, no correlation was found between average water temperature and bromeliad size. Furthermore, no correlation was observed between attachment height of the bromeliad on the tree and the average pH of the water.

In summary, we were able to detect conditions in some bromeliads (i.e. highly acidic water and elevated temperatures) which fell outside the optimal growth parameters for *B. dendrobatidis*, which has been demonstrated to be pH 6 to 7 and below 28 °C (Johnson & Speare 2003, 2005; Johnson et al. 2003; Piotrowski et al. 2004). Although average water temperatures did remain within the optimal growth limit, occasional significant spikes in pH and temperature were observed which may have a greater impact on the virulence and persistence of *B. dendrobatidis* than the averages of these parameters. Regardless of the size of the bromeliad, the acidity typically increased as the water level decreased. Increases in pH, combined with an increasing propensity for temporary desiccation may prove to be the driving force dictating possible environmental suppression of arboreal *B. dendrobatidis* epidemics.

Conclusion

As stated earlier, the overarching purpose of this investigation was to perform the first risk analysis for the threat of *B. dendrobatidis* exposure to arboreal amphibians and the possibility for an arboreal epidemic to occur. In summary, the data we have collected proves that arboreal amphibian species are indeed exposed to *B. dendrobatidis* and that this pathogen can remain virulent and infectious even outside of terrestrial water bodies. This is clearly demonstrated by the infection of *B. bromeliacea* tadpoles in arboreal bromeliads approximately 600m away from the nearest stream habitat. Exactly how *B. dendrobatidis* arrived at these bromeliads is an enigma, and the fact that it was able to remain viable and virulent during a non-aquatic dispersal pathway is extremely alarming in light of controlling future global dispersal. In some bromeliads, even though we detected environmental parameters believed to suppress the productivity of *B. dendrobatidis* in culture, amphibians were still found infected even when the pH dropped as low as 3.53. Although the detection of *B. dendrobatidis* infection infers previous and/or current virulence, the mere presence of its DNA does not allow us to discern between active and retarded *B. dendrobatidis* at the moment in time when our samples were collected. Additional studies and experiments are necessary to determine whether *B. dendrobatidis* continues to be virulent following exposure to the extreme conditions we observed. Regardless, it's disheartening to discover that *B. dendrobatidis* can disperse into and remain active within arboreal bromeliads even for some period of time, despite the stark contrast from a terrestrial aquatic habitat.

In order to address these emerging concerns, we wish to continue and expand this project. The goal of our next phase of research to be conducted in 2010 is twofold: 1) To identify the high-risk pathways of *B. dendrobatidis* dispersal by quantifying the frequency of dispersal caused by amphibians, arthropods (flying insects and aquatic crustaceans), wind/rain, and human locomotion, and 2) Evaluate the risk of extinction faced by each of CNP's 16 endangered and critically endangered amphibian species, based on amphibian ecology, the 3-dimensional distribution of *B. dendrobatidis* in CNP, and current and former *B. dendrobatidis* infection surveys and population trends.

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Table 1. *Batrachochytrium dendrobatidis* infection summary 2007-2009, Cusuco National Park, Honduras. Number of animals sampled per year, prevalence, and IUCN Red List Category Listings (Critically Endangered, CR; Endangered, EN; Near Threatened, NT; Least Concern, LC).

| Species | # 2007 | #2008 | #2009 | % 2007 | % 2008 | % 2009 | IUCN |
|---------------------------------------|--------|-------|-------|--------|--------|--------|------|
| <i>Bolitoglossa conanti</i> | 2 | 6 | 11 | 0% | 33% | 0% | EN |
| <i>Bolitoglossa diaphora</i> | 1 | 5 | 7 | 0% | 20% | 0% | CR |
| <i>Bolitoglossa dofleini</i> | 0 | 7 | 0 | n/a | 14.3% | n/a | NT |
| <i>Bolitoglossa dunni</i> | 0 | 4 | 5 | n/a | 0% | 0% | EN |
| <i>Bromeliohyla bromeliacea</i> | 0 | 4 | 41 | n/a | 25% | 17.1% | EN |
| <i>Bufo valliceps</i> | 1 | 9 | 0 | 0% | 11.1% | n/a | LC |
| <i>Craugastor charadra</i> | 4 | 2 | 1 | 0% | 0% | 0% | EN |
| <i>Craugastor coffeus</i> | 8 | 2 | 0 | 0% | 0% | n/a | CR |
| <i>Craugastor laevisimus</i> | 1 | 0 | 0 | 0% | n/a | n/a | EN |
| <i>Craugastor milesi</i> | 0 | 0 | 0 | n/a | 0% | n/a | CR |
| <i>Craugastor rostralis</i> | 41 | 1 | 1 | 4.9% | n/a | 0% | NT |
| <i>Cryptotriton nasalis</i> | 1 | 2 | 1 | 0% | 0% | 0% | EN |
| <i>Duellmanohyla soralia</i> | 53 | 120 | 38 | 39.6% | 17.5% | 21.1% | CR |
| <i>Hyalinobatrachium fleischmanni</i> | 2 | 0 | 0 | 0% | n/a | n/a | LC |
| <i>Isthmohyla melacaena</i> | 0 | 1 | 0 | n/a | 0% | n/a | NT |
| <i>Oedipina tomasi</i> | 1 | 0 | 1 | 0% | n/a | 0% | CR |
| <i>Lithobates maculata</i> | 38 | 27 | 22 | 63.2% | 29.6% | 40.9% | LC |
| <i>Plectrohyla dasyopus</i> | 59 | 128 | 92 | 78.0% | 47.7% | 48.9% | CR |
| <i>Plectrohyla exquisita</i> | 1 | 9 | 57 | 0% | 11.1% | 45.6% | CR |
| <i>Ptychohyla hypomykter</i> | 40 | 92 | 31 | 57.5% | 32.6% | 35.5% | CR |
| <i>Smilisca baudinii</i> | 0 | 1 | 0 | n/a | 0% | n/a | LC |
| Total | 253 | 420 | 309 | 44.4% | 30.2% | 34.3% | |

Table 2. *Batrachochytrium dendrobatidis* infection levels observed at different life stages within *Plectrohyla dasypus*, *Ptychohyla hypomykter*, *Duellmanohyla soralia* and *Lithobates maculata*, sampled in Cusuco National Park, Honduras in 2007, 2008, and 2009, respectively. All specimens less than 50% adult SVL were classified as juveniles.

| | <i>P. dasypus</i> | | | <i>P. hypomykter</i> | | | <i>D. soralia</i> | | | <i>L. maculata</i> | | |
|----------|-------------------|------|------|----------------------|------|------|-------------------|------|------|--------------------|------|------|
| | 2007 | 2008 | 2009 | 2007 | 2008 | 2009 | 2007 | 2008 | 2009 | 2007 | 2008 | 2009 |
| Larvae | 69.2 | 42.9 | 28.6 | 52 | 33.3 | 40 | 45.2 | 11.5 | 28.6 | 58.3 | 26.1 | 38.9 |
| Juvenile | 92.6 | 67.6 | 69 | 66.7 | 61.5 | 58.3 | 60 | 36.4 | 30 | 100 | n/a | 66.7 |
| Adult | 25 | 21.4 | 13.8 | 0 | 6.3 | 0 | 8.3 | 25.8 | 7.1 | 100 | 50 | 0 |
| Total | 78.0 | 47.7 | 48.9 | 57.5 | 32.6 | 35.5 | 39.6 | 17.5 | 21.1 | 63.2 | 29.6 | 40.9 |

Table 3. Information on the location, elevation of the study site, name of the data series, the total number of bromeliads monitored, the length of the recording time and the number of measurements obtained.

| Location | Elevation (m) | Data series | # | Time | Measurements |
|-------------------|--------------------------|-----------------------|----------|---------------------|---------------------|
| Base Camp (BC) | 1641 | Tree | 9 | 7 days | 338 |
| Cantilles (CA) | 1831 | Tree | 9 | 7 days 23 hours | 383 |
| El Cortecito (CO) | 1493 | Tree | 9 | 3 days 20 hours | 185 |
| Base Camp (BC) | 1623 | Opportunistic Cluster | 5 | 10 days 22.30 hours | 526 |
| Cantilles (CA) | 1979 | Opportunistic Cluster | 5 | 4 days 1 hour | 195 |
| Cantilles (CA) | 1831 | Extended Time | 3 | 28 days 4 hours | 1353 |

Table 4. Overall averages, averages of extreme (min and max) values, and standard deviation of temperature (T) and acidity (pH) as measured in the three different datasets, averaged for all bromeliads in Base Camp (BC), Cantilles (CA) or Cortecito (CO). The first column presents the average values and the second the standard error.

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



(A)



(B)

Figure 1. Adult *Plectrohyla exquisita* (A) and *Plectrohyla dasypus* (B) fitted with a Tygon waistbelt and miniature radiotransmitter



(A)



(B)

Figure 2. Radiotracking for *P. exquisita* and *P. dasypus* in Cusuco National Park (Pictured: Jonathan Kolby and Merlin Jocque) (A) and the relocation of a sleeping *P. exquisita in situ*, outlined by the red circle (B).

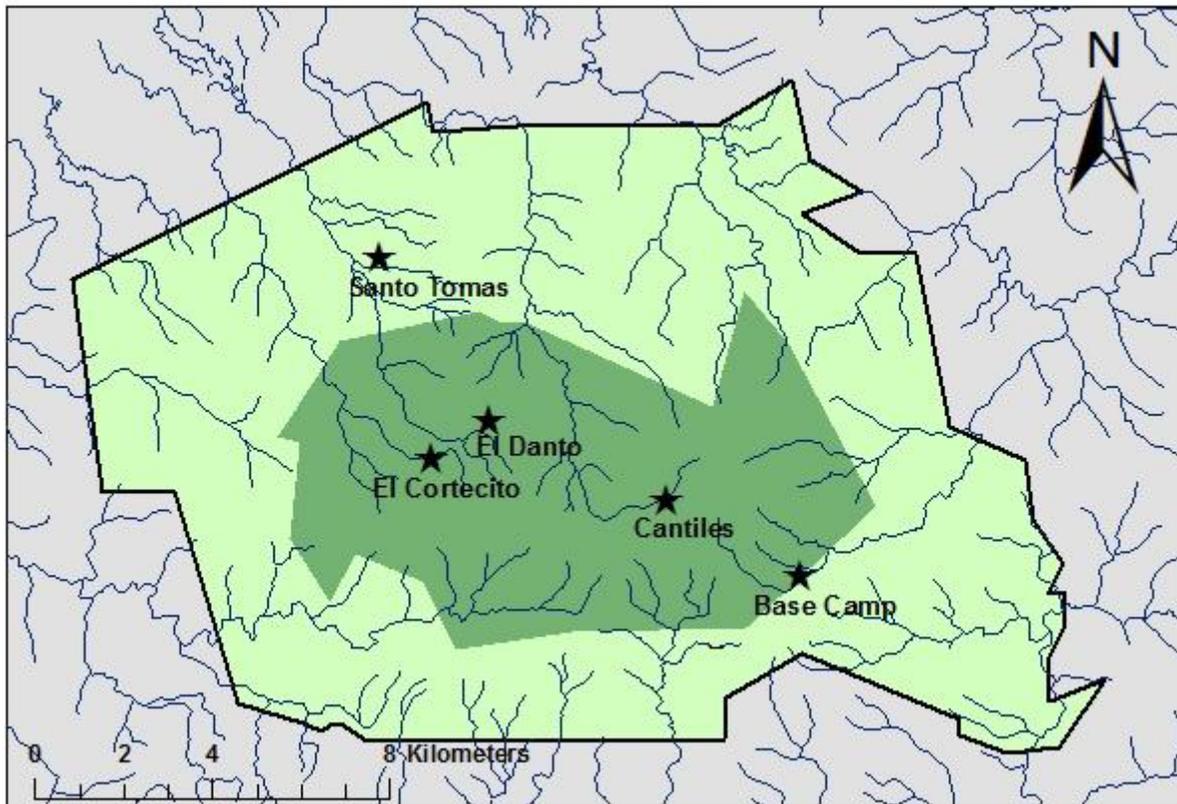
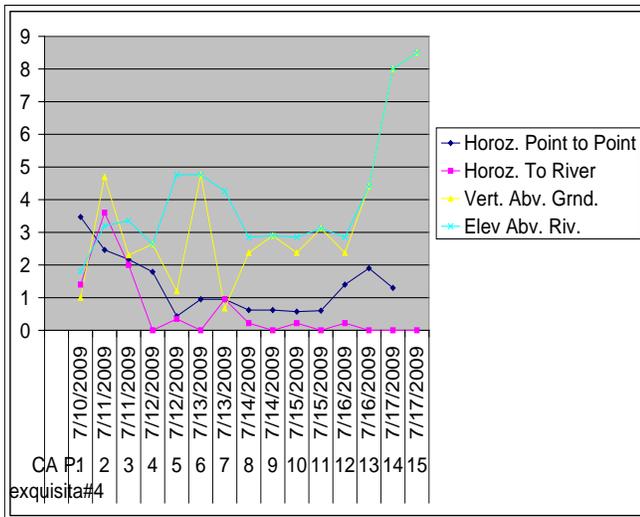
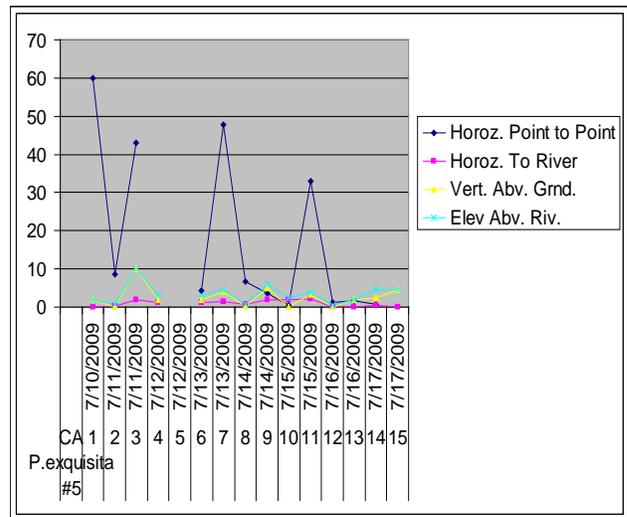


Figure 3. Sites of major sampling effort in Cusuco National Park, Honduras in 2009. Base Camp situated at 15.50°N , -88.2°W , WGS84. Black outline: Cusuco National Park; light green: park buffer zone; dark green: park core zone; blue lines: streams. Stars: regions of concentrated survey and sampling efforts; *Batrachochytrium dendrobatidis* has been detected in each region.



(A)



(B)

Figure 4. Two adult *Plectrohyla exquisita* radiotracked for seven days, from 10 July 2009-17 July 2009, at Cantiles River in Cusuco National Park. The x-axis represents location number and date and the y-axis is distance in meters. Dark blue line: horizontal distance between subsequent locations; pink line: horizontal distance from the river's edge; yellow line: vertical height above the ground; light blue line: approximate elevation above the river. An adult female which exhibited site fidelity and often revisited the same diurnal retreat site on a tree stump (A), and an adult male which made four significant nightly movements each in excess of 30m, while following a unidirectional path downstream (B).



Figure 5. Exceptionally thin perch substrates frequently selected by *P. exquisita*.



Figure 6. PHTEMP101 Data Logger *in situ* with pH and temperature probes extending into bromeliad tank (*Tillandsia guatemalensis*). The unit is sealed within a plastic bag to protect the electrical components from precipitation and humidity.

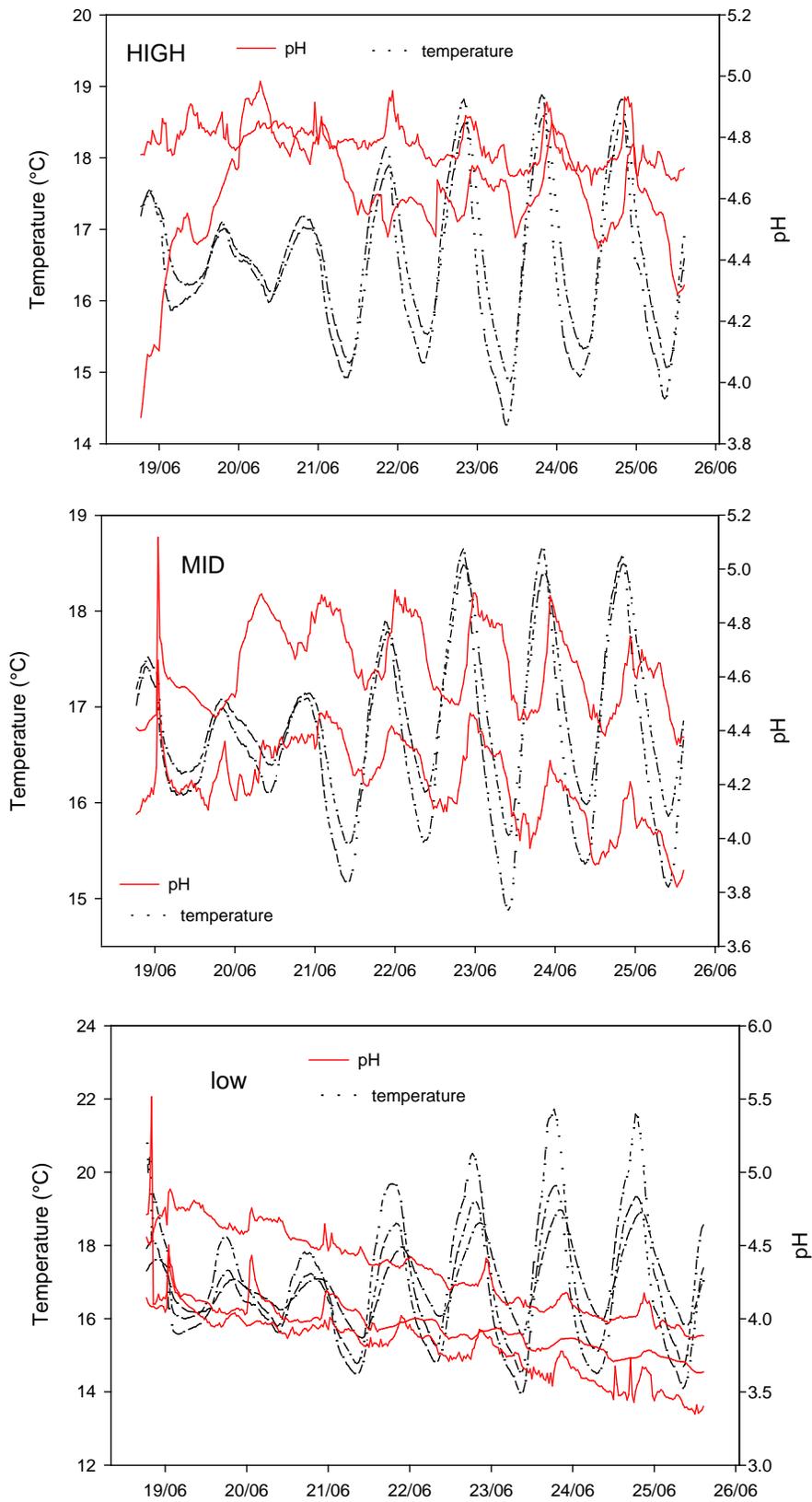


Figure 7. Water temperature and pH values recorded from a series of “Tree” bromeliads at BC.

Annex 1

Chytrid-infected amphibian species detected in CNP in 2009

Plectrohyla dasypus

Critically Endangered; highly range restricted and endemic to CNP



Plectrohyla exquisita

Critically Endangered; highly range restricted and endemic to CNP



Duellmanohyla soralia

Critically Endangered, highly fragmented & restricted distribution



Bromeliohyla bromeliacea

Critically Endangered, highly fragmented & restricted distribution



Ptychohyla hypomykter

Critically Endangered; wide distribution but highly fragmented populations



Lithobates maculata

Least Concern; wide distribution

