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## Natural History and Conservation of Bolitoglossine Salamanders in Central Panama

Leslie Caren Brinkman

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**NATURAL HISTORY AND CONSERVATION OF BOLITOGLOSSINE  
SALAMANDERS IN CENTRAL PANAMA**

A Masters Thesis

Presented to

The Graduate College of  
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree  
Master of Science, Biology

By

Leslie Caren Brinkman

May 2016

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# NATURAL HISTORY AND CONSERVATION OF BOLITOGLOSSINE SALAMANDERS IN CENTRAL PANAMA

Biology

Missouri State University, May 2016

Master of Science

Leslie Caren Brinkman

## ABSTRACT

I conducted a mark-recapture study to assess the natural history of a Neotropical salamander community in three discrete 4–6 week sampling periods at Chorro Las Yayas, a protected natural area of about 8000 m<sup>2</sup> in central Panama, during an extended drought. I captured 87 *Bolitoglossa biseriata*, 17 *Oedipina complex*, and 15 *O. parvipes*. *Bolitoglossa biseriata*, had a mean mass of  $0.8 \pm 0.4$  g with a mean snout-vent length (SVL) of  $31.45 \pm 5.30$  mm. *Bolitoglossa biseriata* was active throughout the night, and its detection rate varied with season, air temperature, and amount of precipitation in the previous 24 h. A principal components analysis indicated that *B. biseriata* utilized all of the available ground-level habitats within the study site. A chytrid fungus, *Batrachochytrium dendrobatidis*, was detected on *B. biseriata* and *O. parvipes* but I did not observe any mortality resulting from infection. *Oedipina* at the site were detected mainly on wet nights. *Oedipina complex* and *B. biseriata* detections decreased significantly after the first field season as the drought continued. Drought conditions are predicted to become more common as a result of climate change; my data suggest that this could lead to a decrease in surface activity of some tropical plethodontid species. Conservationists need to be aware of the effects of drought on surface activity of these species and understand that it likely will make monitoring and detection of population changes more difficult.

**KEYWORDS:** *Bolitoglossa biseriata*, caudate, chytrid, diet, ecology, habitat, *Oedipina complex*, *Oedipina parvipes*, plethodontidae

This abstract is approved as to form and content

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Dr. Brian Greene  
Chairperson, Advisory Committee  
Missouri State University

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May 2016

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## ACKNOWLEDGEMENTS

I would like to first thank my advisor, Dr. Greene, for giving me this opportunity to develop further as a student and researcher. His support of my decision to pursue a project in another country and on a cryptic species has changed my life in a very positive way. I would like to thank the other members of my committee, Dr. Ray and Dr. Mathis, for their enthusiasm and valuable feedback.

I would like to thank my parents for encouraging my love for nature and animals from a young age. They have always been my best supporters as I pursue my passions.

I would like to thank Missouri State University, La MICA Biological Station, Autoridad Nacional del Ambiente, and Chorro Las Yayas. A special thanks to Julie Ray, Macedonio Pérez, Pastor Arcia, Mike Itgen, Monika Kastle, Ryan Frantzen, Ross Whetstone, Dana Green, Valerie Jones, Donny McKnight, Jackson Lage, Isaac Mortimer-Lotke, Josh Proal, Nicole Eller, Meredith Swartwout, Blake Klocke, Will Shoemaker, Andreas Hertz, Jordan Verga, Eric Green, Brisselle Magaña Valencia, and Dale Jackan for their contributions to the research aspect of my project in Panama.

I have sincere gratitude to Drs. Jamie Voyles, David Wake, Sean Rovito, and Sean Maher for their assistance analyzing samples and data.

Finally, I would like to thank my friends and fellow graduate students. I feel very blessed to have such a wonderful support system.

## TABLE OF CONTENTS

|                                     |    |
|-------------------------------------|----|
| Introduction.....                   | 1  |
| Amphibian Decline .....             | 1  |
| Bolitoglossini .....                | 4  |
| Conservation .....                  | 5  |
| Objectives .....                    | 7  |
| Methods.....                        | 8  |
| Study Area .....                    | 8  |
| Study Species .....                 | 10 |
| Field Work .....                    | 13 |
| Processing .....                    | 18 |
| Analyses .....                      | 22 |
| Results .....                       | 24 |
| <i>Bolitoglossa biseriata</i> ..... | 27 |
| <i>Oedipina complex</i> .....       | 35 |
| <i>Oedipina parvipes</i> .....      | 36 |
| Discussion .....                    | 38 |
| References.....                     | 46 |

## LIST OF TABLES

|   |    |
|---|----|
| Table 1. Structural Habitat Characteristics. ....   | 19 |
| Table 2. Effort and Salamander Detection Rates..... | 25 |
| Table 3. Model Set Analyzing Detectability.....     | 27 |
| Table 4. Body Size Statistics .....                 | 29 |
| Table 5. Genomic Equivalents of <i>Bd</i> .....     | 30 |
| Table 6. Loadings of Habitat Variables .....        | 34 |



## LIST OF FIGURES

|   |    |
|---|----|
| Figure 1. El Copé in Coclé Province, Panama.....                            | 8  |
| Figure 2. The Chorro Las Yayas Trail.....                                   | 9  |
| Figure 3. Chorro Las Yayas with Salamander Detection Locations.....         | 11 |
| Figure 4. A Phenotype Comparison.....                                       | 14 |
| Figure 5. The Bihourly Salamander Encounter Rates .....                     | 16 |
| Figure 6. The Tree Species Used Most by <i>Bolitoglossa biseriata</i> ..... | 20 |
| Figure 7. Boxplot of the Vertical Distance .....                            | 31 |
| Figure 8. Habitat Component Scores .....                                    | 32 |
| Figure 9. Distance Moved between Captures .....                             | 35 |
| Figure 10. Microhabitats .....  | 37 |

# INTRODUCTION

## Amphibian Decline

In 1989, scientists at the first World Congress of Herpetology began to express concern about the global decline of amphibian populations. Subsequently, a Global Amphibian Assessment revealed that amphibians were declining at a faster rate than other vertebrate groups, with the number of critically imperiled amphibian species almost doubling between 1980 and 2004 (Stuart et al., 2004). Currently, 1,961 of the 6,424 evaluated species of amphibians are considered to be threatened with extinction (IUCN, 2015). Amphibian decline is a worldwide phenomenon involving an estimated area of over 840,000 km<sup>2</sup> by 2005 (Lips et al., 2005a) that has continued to expand (Olson et al., 2013; Batista et al., 2014; Rebollar et al., 2014). Amphibian declines are thought to result from a complex interaction of habitat destruction, climate change, introduced species conflicts, unsustainable harvest for the pet trade, and infectious diseases (Collins and Strofer, 2003).

Latin America encompasses a high proportion of the world's amphibian fauna, including an estimated 5–10% that has not yet been described (Young et al., 2001). Neotropical species are among the most affected by declines, especially those concentrated in protected montane areas (Lips et al., 2005b). The pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), is associated with the decline of at least 43 species of amphibians in Central and South America (Lips et al., 2006). In Central America, the earliest specimen with *Bd* was collected in 1986 (Puschendorf et al., 2006). The first amphibian population crash documented in Central America occurred in the

Monteverde Cloud Forest Preserve in 1987 (Pounds et al., 1997). In some of the remote highlands, rapid (4–6 months) die-offs have resulted in half the amphibian diversity being lost and most of the remaining species persisting at 20% of their original abundance (Lips et al., 2006). The full scope of the decline of Latin American amphibians is unknown because of inadequate sampling in many regions, resulting in deficient knowledge about the distribution, ecology and natural history of most species (Daszak et al., 2005). In addition, the current understanding of Latin American amphibian populations may be taxonomically biased toward conspicuous anurans because of the relative difficulty sampling secretive, cryptic species such as salamanders and caecilians (Young et al., 2001; Rovito et al., 2009).

The decline of over 200 amphibian species worldwide has been attributed to chytridiomycosis, a disease caused by *Bd* (Fisher et al., 2009). *Bd* has been detected on 449 species of anurans, 106 of which are listed as threatened by the IUCN Red List (International Union for the Conservation of Nature, 2015), and 60 caudates, of which 12 are classified as threatened (Olson et al., 2013). *Bd* is an aquatic fungus with two life stages. The motile zoospore stage can persist on amphibian skin for up to 220 days (Lips et al., 2006) where they encyst on keratinized tissue and develop into a reproductive sporangia stage (Weinstein, 2009). High concentrations of *Bd* result in chytridiomycosis, characterized by hyperplasia (increased epidermal cells), hyperkeratosis (Lips et al., 2006), and atypical behavior such as lifting of the trunk and affected limbs (Crane and Mathis, 2011). It has been suggested that chytridiomycosis ultimately kills amphibians by disrupting cutaneous transport mechanisms, resulting in a loss of electrolytes (Voyles et al., 2007). *Bd* thrives in cool, moist conditions found throughout most of the montane

regions in the tropics. Species that rely on permanent bodies of water are at the greatest risk of encountering *Bd* zoospores, whereas those with terrestrial reproductive modes are less impacted (Lips et al., 2006).

The majority of Neotropical salamander species occur in cloud forests, that are roughly restricted to 800-2700 m above sea level (asl). Tropical montane cloud forests (TMCFs) account for between 2.5 and 14 percent of all tropical forests and are recognized as one of the most threatened ecosystems due to their restricted elevational range and high susceptibility to deforestation and climate change (Toledo-Aceves et al., 2011).

A wide diversity of Neotropical salamander species have radiated into specialized niches at different elevations, habitats, and microhabitats. Neotropical salamanders occupy four microhabitats: underground (fossorial), within the leaf litter and other surface objects (ground-dwelling), within and under bark (log-dwelling), and within leaf axils (arboreal; Wake, 1987). The high humidity and dense epiphytic coverage characteristic of cloud forests facilitates the use of arboreal habitats by salamanders. At elevations above the cloud forest there are fewer epiphytes and most salamander species that occur there are terrestrial specialists (Wake, 1987). Climate change in the Neotropics has apparently resulted in selective declines among cloud forest salamanders such that terrestrial microhabitat specialists in mid- to high elevation forests largely have disappeared while microhabitat generalists that rely, at least in part, on bromeliads, remain (Rovito et al., 2009).

Many bromeliad species have leaves in spiral arrangement that form phytotelma or tanks (Foster, 2001) and are considered “keystone plant species” because they are

occupied by many animal species (Richardson, 1999). Bromeliad tanks provide salamanders a protected microhabitat structure providing shelter from predators, egg deposition sites, and abundant food (Wake, 1987). Bromeliads in many premontane cloud forests exist outside of protected areas and are vulnerable to destruction for timber or mineral resources. Deforestation may affect intact forests by causing desiccation of bromeliads through increased elevation of the cloud base (Rovito et al., 2009).

Neotropical forests are predicted to become drier and warmer with climate change, causing shifts in the geographic ranges and niches of moisture-dependent species (Foster, 2001). Bromeliads are predicted to be especially vulnerable to climate change because of their dependence on cloud water deposition and their loss may have cascading effects on ecosystems, changing their physical structure, the accumulation of water and nutrients, energy processes, and the biodiversity they sustain (Benzing, 1998). If the recent decline of salamander species in the mid- to high-elevation cloud forest is a result of climate change, then the surviving salamander species soon may suffer a similar fate (Rovito et al., 2009).

### **Bolitoglossini**

All Neotropical salamanders belong to the plethodontid subfamily Bolitoglossini, which includes 13 genera and 310 of the 676 extant species of salamanders (AmphibiaWeb, 2015). Neotropical salamanders all exhibit direct development and occupy arboreal and terrestrial habitats (Wake, 1987). Most published work on such species is focused on taxonomy, systematics, and distribution (e.g., Wake and Lynch, 1976; Bolaños and Wake, 2009; Townsend, et al., 2009; Rovito et al., 2012; Acevedo et

al., 2013) with fewer studies addressing reproduction and diet (e.g., Houck, 1977; Anderson and Mathis, 1999; Ortega et al., 2009; Neckel-Oliviera et al., 2011).

Drastic declines of multiple species of plethodontid salamanders have been reported from Mesoamerica (Weinstein, 2009). Half of the species in 12 bolitoglossine genera are critically endangered, endangered, or threatened (Rovito et al., 2009). The species of salamanders that occur in the highest two elevation zones have experienced the greatest decline (Rovito et al., 2009) with four sites in Costa Rica and Panama experiencing a 47% decline on average (Lips et al., 2003). *Bd* infection appears to explain at least part of this pattern as it was detected in the rapidly declining populations (Cheng et al., 2011). While the leading factor behind salamander population declines is unknown, climate change, habitat loss, and *Bd* are all possible contributors. The cryptic nature of Neotropical salamanders has hindered study of their populations, particularly for lowland species (Parra-Olea et al., 2005).

## **Conservation**

To combat amphibian decline, the Amphibian Conservation Summit designed the “*Amphibian Conservation Action Plan*,” outlining a response to the current crisis. The plan discusses the need to understand the causes of decline, continue documenting diversity, developing and implementing conservation programs, and responding to immediate crises. Long-term amphibian conservation involves protecting key biodiversity areas and freshwater resources, instituting captive management until threats subside, controlling harvest and creating individual species action plans (Gascon et al., 2007).

Salamander conservation efforts in the Neotropics currently include research to determine hotspots and identify changes in biodiversity, preservation of habitat, research on diseases, and maintenance of captive colonies with some success in captive breeding. In September 2015, international conservation groups purchased 3.7 km<sup>2</sup> of critical habitat for endangered species including the Finca Chiblac Salamander (*Bradytriton silus*) and the Long-limbed Salamander (*Nyctanolis pernix*) to create the San Isirido Reserve (Dasgupta, 2015). These efforts could be improved with the promotion of sustainable development techniques and the spread of public awareness, which may reduce pollution and provide better habitat to native salamander populations. Research is a fundamental first step to learn about the distribution and requirements of Neotropical salamanders.

A thorough understanding of the ecology and natural history of Neotropical salamanders is important for the development of management strategies to help protect their habitats and prevent continued decline (Young et al., 2001). Data are deficient on the population size, demography, and natural history of almost all Neotropical amphibian species (Lips et al., 2005a).

Diet is an important component of salamander natural history, providing insight into seasonal fluctuations of prey availability and the impact of habitat modification (Anderson, 1991). Information about diet sheds light on the niche of a species and how it is influenced by and can affect other species, which is essential in the development of conservation strategies. A relationship between prey size and head/body size in salamanders indicates whether a species is a prey-size generalist or specialist (Maglia, 1996). Arthropods, including Acari (Río-García et al., 2014), Formicidae (Anderson and

Mathis, 1999; Ortega et al., 2009, Río-García et al., 2014), Coleoptera (Río-García et al., 2014), and larval Diptera (Ortega et al., 2009), were determined to be the most important prey taxa in various Bolitoglossine salamanders. Acari and Formicidae may be the source of cutaneous alkaloids secreted by salamanders in self-defense (Brodie et al., 1991; Hantak et al., 2013).

## **Objectives**

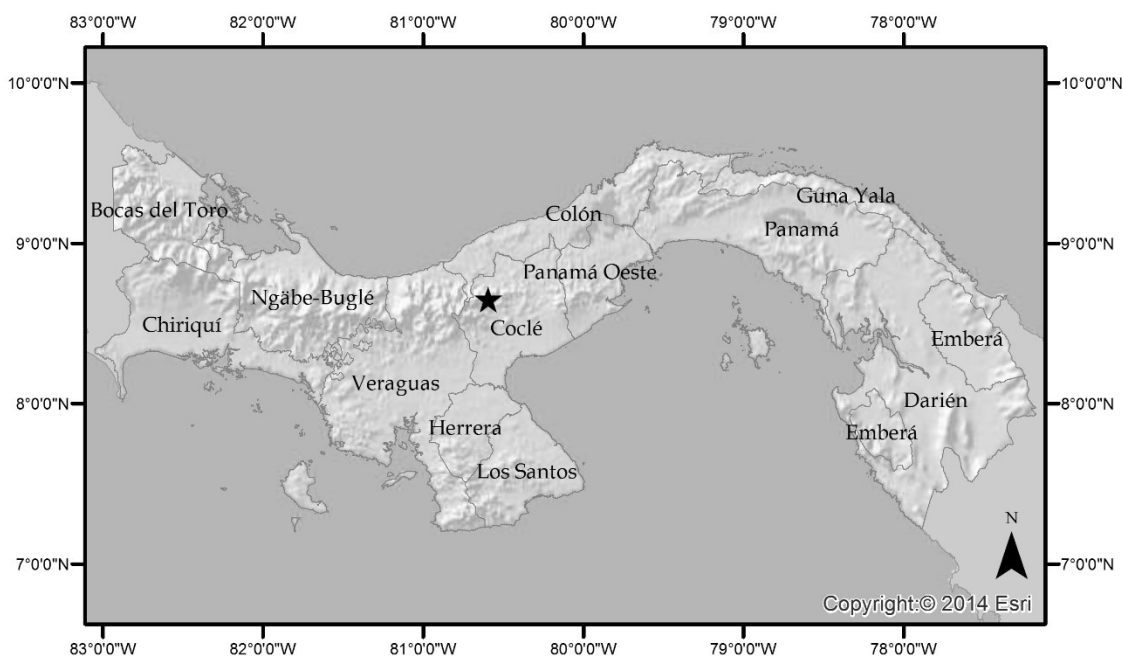
This study evaluates the natural history of three salamander species within a pre-montane cloud forest in central Panama (Fig. 1) and documents their abundance during drought conditions, which are predicted to become more common as a result of climate change. I contribute natural history information for three species of salamanders, summarizing 197 encounters collected between June 2013 and July 2014. These data provide insight into the environmental requirements of *Bolitoglossa biseriata*, *Oedipina complex*, and *O. parvipes*, and possibly their congeners, informing conservation efforts for all. It is hoped that the baseline data provided by my study may be useful in monitoring salamander populations at Chorro Las Yayas.



## METHODS

### Study Area

I conducted my study along a 327 m long concrete trail in Chorro Las Yayas in central Panama during three discrete sampling periods over two years: 01 June–29 July



**Fig. 1.** El Copé in Coclé Province, Panama, the community closest to the natural área where my study was conducted. ESRI map by Patty Ruback.

2013, 10 December 2013–06 January 2014, and 01 June–29 July 2014 (Fig. 2). The park is located at 8° 65' N, 80° 59' W. The development of the site originally was funded by El Corredor Mesoamerica Biológico del Atlántico Panameño. It is protected by the Panamanian Environmental Authority, Autoridad Nacional del Ambiente (ANAM), and is managed by the community-run group Barrigoñeros Organizados por la Conservación

del Ambiente. The temperature for June and July ranged from 20.8-28.7°C. Based on local records, the mean annual precipitation is 3,500 mm (Lips et al., 2006). The wet season runs from mid-April to mid-December, and the dry season is mid-December to mid-April with a lull in the rains in July (Ray, 2009). The study area is on a west-facing slope with a gravel (2013) or paved (2014) road passing along the east side and a river bordering it on the west. The trail heads west from a south entry point along the road and then north on the east side of the river, Río Barrigón, and east at the large waterfall towards the north exit of the park along the road (Fig. 3). The forest is dominated by large trees and small, herbaceous plants. Primates, marsupials, rodents, chiropterans, arboreal and leaf-litter snakes, frogs, a variety of birds, and a plethora of insects utilize the park (J. Ray, pers. obs.). The study area was selected because of its accessibility and regular encounters of *Bolitoglossa* along the trail (J. Ray, pers. obs.).



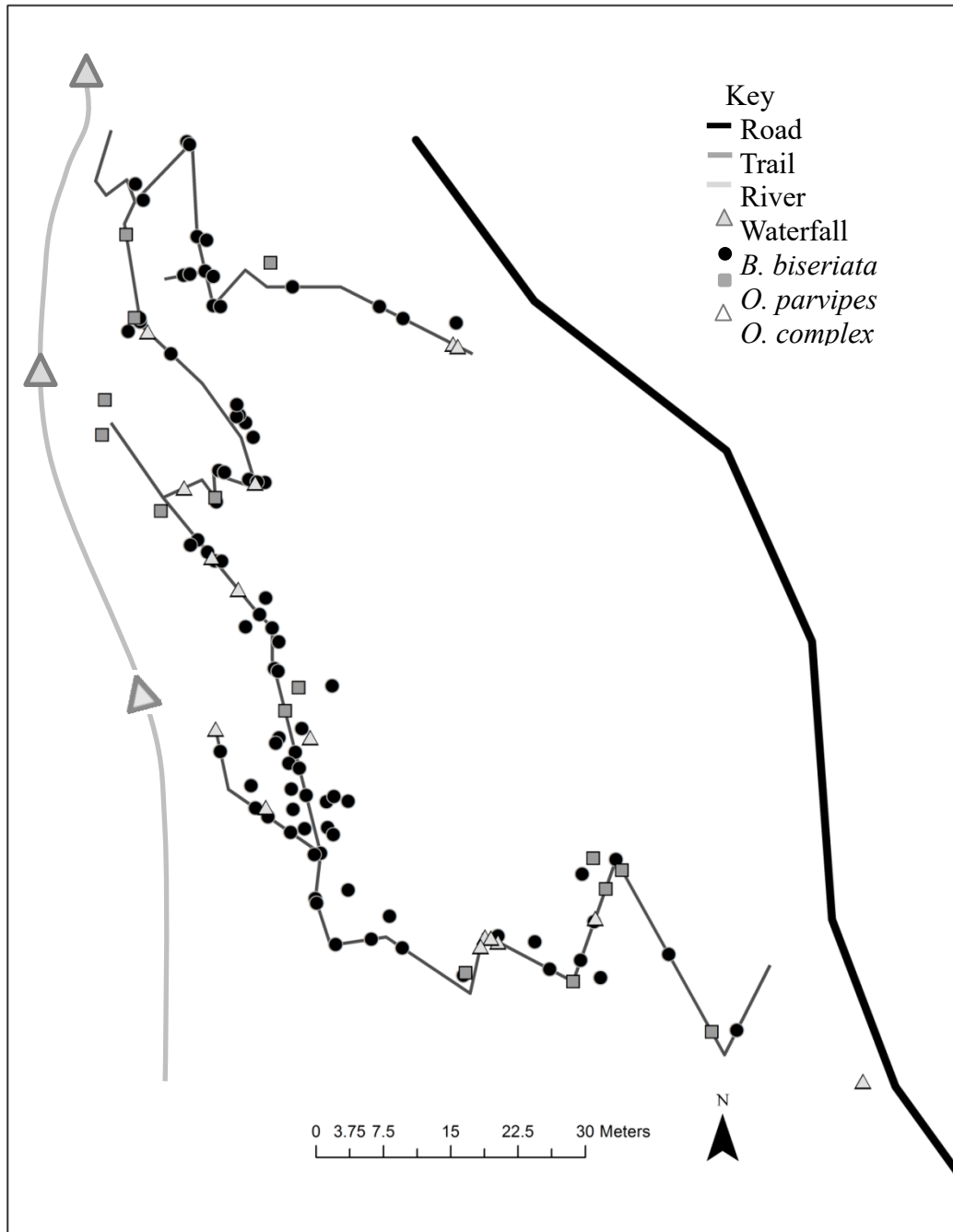
**Fig. 2.** The Chorro Las Yayas trail heading north on the western side of the study site, east of the river.

The site is part of a premontane cloud forest at 440 m asl, and consists of secondary forest with moderate epiphytic growth. The canopy covers exceeds 75% in the majority of the park, with less cover near the river and one of the two cliff faces. The midstory is thin near the south entrance, becomes moderately dense approaching the river, and thickens considerably where the trail heads east toward the road, where it becomes thick. Prior approval for this project was obtained from the Missouri State University IRB (4 June 2013; approval 13-037) and the Autoridad Nacional del Ambiente in Panama (01 April 2013; permit SE/A 50-13).

### **Study species**

The most commonly encountered salamander species at Chorro Las Yayas is the Two-lined Climbing Salamander (*Bolitoglossa biseriata*; Fig. 4), a member of the *adspersa* species group native to Panama and Colombia (Parra-Olea et al., 2005; Solís et al., 2010). This species is small to medium in size compared to other bolitoglossines and is recognized by two distinct lateral dark stripes on an otherwise cream colored body. Individuals have complete webbing on the feet of all four limbs, and a constriction at the base of their tail (Tanner, 1962). The species occurs in humid lowland forests below 500 m asl, within bromeliads and banana trees (Solís et al., 2004). The holotype had ants, chironomid larvae, flies, and spiders in its buccal cavity at the time of collection (Tanner, 1962).

The Ridge-headed Salamander (*Bolitoglossa colonnea*) has been photo-documented at the study site (J. Ray, pers. comm.). *Bolitoglossa colonnea* is found in the



**Fig. 3.** Chorro Las Yayas map with salamander detection locations. *Bolitoglossa biseriata* is designated with a circle, *Oedipina parvipes* with a triangle, and *O. complex* with a square. The dark line on the east side represents a gravel road and the gray line on the west represents the river, Río Barrigón.

humid lowlands and promontane slopes of Costa Rica and Panama. The species is mainly arboreal, spending most of its time in bromeliads; however, it also can be found on the ground (Solís et al., 2004). *B. colonea* is characterized by the fleshy ridge between its eyes, its relatively short tail and trunk, stout limbs, and near complete webbing on its hands and feet (Savage, 2002). It is a sexually dimorphic species with females attaining larger sizes than males, and both sexes appear to be reproductive year-round (Bruce, 1997).

Two species of *Oedipina*, the Gamboa Worm Salamander (*O. complex*) and the Columbian Worm Salamander (*O. parvipes*) also occur at Chorro Las Yayas (Fig. 4). *Oedipina complex* and *O. parvipes* belong to the *Oedipina parvipes* group within the subgenus *Oedopinola*. *Oedipina complex* ranges from western Panamá to northern Ecuador, while *Oedipina parvipes* ranges from Costa Rica through Colombia. Members of the *Oedipina parvipes* group are characterized by long bodies with 17 (rarely 18) costal grooves. They often have white face masks and white spotting over much of their body. *Oedipina* occur in lowlands to lower montane regions and are fossorial or semi-fossorial (McCranie et al, 2008).

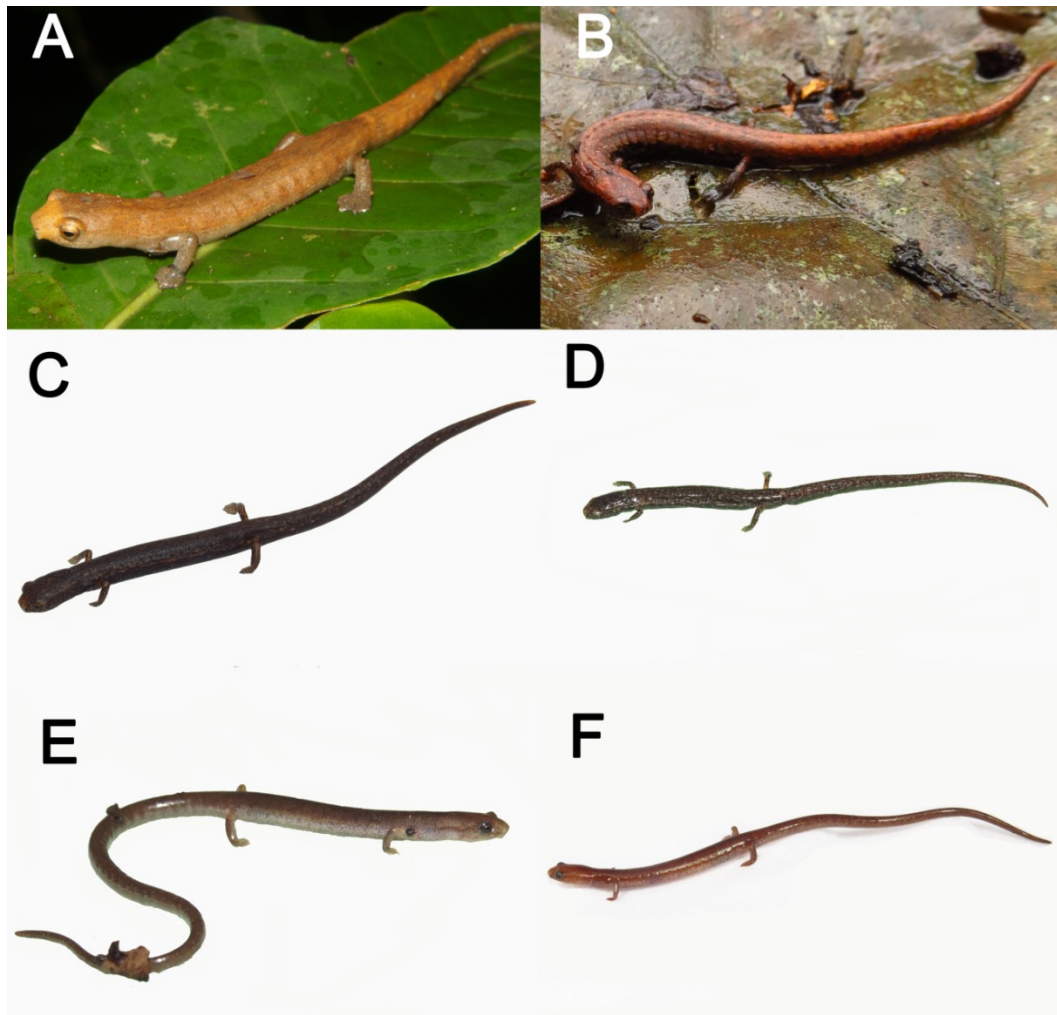
Although the IUCN has classified all three species as “Least Concern” (IUCN, 2015) their ecologies are poorly described and they would appear to be vulnerable to factors threatening other Neotropical salamanders as environmental conditions may change in the future. For example, although *Bolitoglossa* populations are not known to have been decimated by chytrid, five dead salamanders tested positive for *Bd* in El Copé/Parque Nacional G.D. Omar Torrijos H. (Lips et al., 2006).

## Field Work

I collected salamanders by hand during nocturnal visual encounter surveys (Dodd, 2010), treating the trail as a complete transect, to generate population estimates and assess activity patterns of salamanders. Immediately before each survey I recorded the date, time, temperature, humidity, and daily precipitation. Each survey was conducted using two to seven observers (which included me and field assistants) divided up to survey each side of the trail at a time. Up to three surveys of the transect were conducted each night. During the wet season of 2013, I conducted surveys with the start time randomly selected between 1800 and 0400 hr. Because I did not observe an effect of time on the number of captures in 2013, I conducted surveys either at or around dusk or dawn in subsequent seasons (Fig. 5).

We walked transects scanning the leaf litter, rails, natural cover (logs, rocks, moss), and vegetation for salamanders with a flashlight, focusing on the closest 2m to the trail (Waldron and Humphries, 2005). For each individual, I recorded the time, microhabitat, vertical distance, and angular orientation of the salamander. If a salamander was located on a leaf, then I would record whether they were facing the leaf's base or tip. Each individual was identified to species and their activity at the time of detection was recorded (Lips et al., 2001). A salamander's activity was categorized as sitting, walking, or hiding (over 50% concealed). I also documented whether a mental gland (males) or eggs (females) were present to signify if an individual was an adult in reproductive condition. I recorded the GPS coordinates of the individual for later habitat assessment.

ventilated



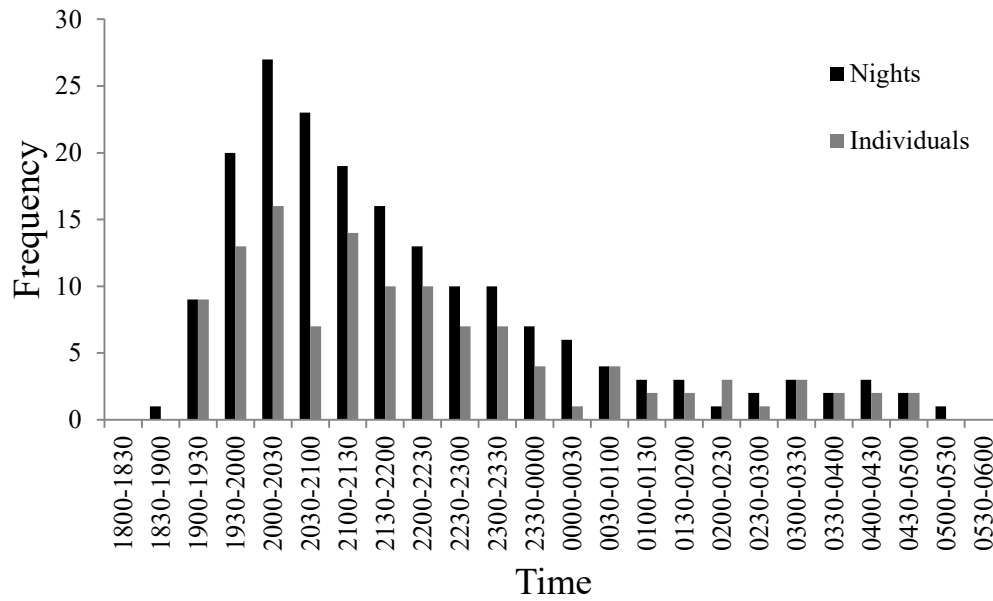
**Fig. 4.** A phenotypic comparison of the salamanders found during this study (A) *Bolitoglossa biseriata*, (B) red morph of *Oedipina complex*, (C) black morph of *Oedipina complex*, (D) black/brown morph of *Oedipina complex*, (E) square snout morph of *Oedipina parvipes*, and (F) rounded snout morph of *Oedipina parvipes*.

In addition, habitat was assessed at 30 randomly-selected points along the transect, ten each field season. The first time a salamander was captured, I retained it in a plastic container with leaves collected near the detection site. Salamanders were processed the next day and then returned to their capture locations.

If a salamander was recaptured within a week of its release it was immediately returned without any new measurements collected. If it had been over a week since we had seen and measured the recaptured salamander we would measure its current SVL and mass, and record the straight line distance from its last capture.

Near the transect, I provided artificial habitat, including cover objects on the forest floor (Hesed, 2012) and PVC refugia attached to trees (Boughton et al., 2000; Zacharow et al., 2003; Johnson et al., 2008) to assess usage of human-made refuges by salamanders. I placed twelve 0.5 m<sup>2</sup> corrugated sheets of tin cover objects along the trail in randomly selected locations (based on rail number) on flat areas of ground after displacing leaf litter from the selected area two weeks before surveys began (Hesed, 2012). I traveled up to 15 m from the trail in order to find a flat area in which I could place a cover board. Cover boards were weighed down with logs and rocks and covered with leaf litter to reduce interference from larger animals. I placed fifteen 1.27 cm diameter PVC pipes on separate trees that were at least 18 cm in diameter at DBH in randomly selected locations. Each 60cm long PVC refuge was positioned with its bottom end at 2.0 m, 2.5 m, 3.0 m, 3.5 m or 4.0 m above the ground. PVC refuges were suspended vertically from a nail driven into a tree trunk near the top end of the pipe. The PVC refugia were placed up to 5 m off the trail. I capped seven of the PVC refuges at the bottom and drilled two drainage holes 15 cm from the bottom; the other eight refuges had open bottoms.





**Fig. 5.** The bihourly salamander encounter rates of *Bolitoglossa biseriata* and effort for the 2013 wet season.

I checked cover objects and PVC refugia once or twice a week between 0800 and 1200hr and removed debris from each PVC refuge (Boughton et al., 2000). Salamanders were removed from PVC refuges using a sponge attached to a 60 cm dowel to lift them upward until they could be captured.

I also conduct two types of leaf litter surveys. One involved making 1 m<sup>2</sup> plots in areas with little slope (Fauth et al., 1989), and the other involved sorting through the leaf litter 1 m out on both sides of the transect. I surveyed seven 1m<sup>2</sup> leaf litter plots in the wet season of 2013, and 10 during the wet season of 2014. In the dry season, I surveyed all the leaf litter within 1 m of the trail, from the northern entrance to the first river entrance (57.5 m). I randomly selected locations for leaf litter plots by selecting a random rail in the hand rail and the side of the trail when starting at the south entrance. Plots were made close to the trail due to the difficulty of accessing points further away and placed in flat

areas for convenience. I measured the leaf litter depth at each plot corner as well as in the center. I cleared away leaves within a half meter buffer zone around each plot to improve detection of salamanders attempting to leave a plot (Fauth et al., 1989). The leaf litter surveys were conducted between 0700 and 1800 h with two people working in opposite directions. Leaf litter was moved with caution and all vegetation within 0.5 m of the ground was checked (Fauth et al., 1989). Each leaf litter plot was surveyed once per season to limit the amount of disturbance (Dodd, 2010), and only two were revisited in separate seasons. Captured salamanders were placed into an inflated plastic bag with moist leaf litter until the survey was complete (Lips et al., 2001). For each plot, I measured habitat variables including % ground cover, canopy cover, soil moisture, and temperature (Dodd, 2010). Once the survey was complete, salamanders were processed in the manner described below then released and the leaf litter returned.

Within a 1 m<sup>2</sup> plot centered over the point of capture of a particular salamander, I recorded information about the ground and understory vegetation (under 1 m high) including: percent of each ground cover type, the number of woody and herbaceous stems, and the number of woody and herbaceous species (Table 1). I recorded the distance from the salamander's capture location to the closest rock, log, and tree. I also recorded the following midstory (1–6 m. high) structural variables in a 10 m<sup>2</sup> plot surrounding each capture location: epiphyte load, canopy height, diameter of trees at breast height (1.37 m), and stem count (Table 1). I used the same 10 m<sup>2</sup> plot to evaluate overstory (over 6 m high) structure with the following variables: percent canopy cover, vine epiphyte load, number of trees with vines, moss epiphyte load, number of trees with moss, fern epiphyte load, number of trees with ferns, bromeliad epiphyte load, number of

trees with bromeliads, the total number of trees and an estimate of canopy height (Table 1; Fogarty and Vilella, 2003). I measured habitat variables at 30 random locations along the trail to characterize the habitat structure available along my sampling transect at Chorro Las Yayas. I used a random number generator to select the rails at which available habitat would be evaluated, the side of the trail to make the center of the plot, and whether it would be on the trail, 0.5–1 m away, or 2 m away. At each rail I would choose a microhabitat that appeared to be suitable to a salamander within the area specified (e.g. the rail at 0 m or a leaf of the tree *B. biseriata* was detected most commonly and within 1.5 m of the ground if further from the trail, Fig. 6).

## Processing

The day after capture, salamanders were removed from their temporary holding containers for processing. Zero to two salamanders were selected haphazardly to be swabbed for *Bd*. Then, each salamander was weighed ( $\pm 0.1$  g), anesthetized for stomach flushing and VIE injection, and measured for snout-vent length (SVL) and tail length (TL) ( $\pm 1$  mm). Salamanders were anesthetized with a drop of Orajel<sup>®</sup> (benzocaine) placed on the top of their head, being careful not to get any on their eyes (Cecala et al., 2007). I classified individuals smaller than the smallest reproductive adult as juvenile, and individuals that were much tinier than the average juvenile as neonate. While I recorded measurements, an assistant sifted through the leaf litter in the temporary holding container to remove any fecal matter to store it in a microcentrifuge tube with isopropyl alcohol and label the tube with the date and identification number of the salamander.

**Table 1.** Structural habitat characteristics measured in association with salamander captures and random points. Ground cover variables were assessed in a 1 m<sup>2</sup> plot centered over the capture location. Midstory and overstory variables were assessed in a 10 m<sup>2</sup>-plot centered over the capture location. A densiometer was held over the capture location to assess percent canopy cover.

| Habitat stratum | Variable | Measurement  |
|-----------------|----------|--|
| Ground cover    | % GC     | percent of each ground cover category              |
|                 | N WST    | number of understory woody plant stems             |
|                 | N WSP    | number of understory woody plant species           |
|                 | N HST    | number of understory herbaceous plant stems        |
|                 | N HSP    | number of understory herbaceous plant species      |
| Midstory        | N EPI    | number of epiphytes                                |
|                 | N TWE    | number of trees with epiphytes                     |
|                 | CANOPY   | mean height (m) of midstory trees                  |
|                 | CBH      | mean circumference (cm) of midstory trees          |
|                 | N ST     | number of midstory trees                           |
| Overstory       | PCC      | percent canopy cover using a spherical densiometer |
|                 | VINES    | number of vines growing on overstory trees         |
|                 | MOSS     | number of moss mats growing on overstory trees     |
|                 | FERNS    | number of ferns growing on overstory trees         |
|                 | BROM     | number of bromeliads growing on overstory trees    |
|                 | T VINES  | number of overstory trees with vines               |
|                 | T MOSS   | number of overstory trees with moss mats           |
|                 | T FERNS  | number of overstory trees with ferns               |
|                 | T BROM   | number of overstory trees with bromeliads          |
|                 | N T      | number of overstory trees                          |
|                 | CANOPY   | mean height (m) of overstory trees                 |

After measurements were completed I sprayed the entire body of the salamander with bottled water from the syringe, using my gloved fingers to help rub off any remaining anesthetic. Once the salamander was rinsed thoroughly I returned it to its holding container.



**Fig. 6.** The tree species used most by *Bolitoglossa biseriata* at time of detection.

To better understand the predator role of Bolitoglossids in the local ecosystem, I collected diet samples from each salamander by non-lethal stomach flushing. I placed each salamander on a moist paper towel after it was anesthetized with Orajel® applied to the top of the head (Cecala et al., 2007). Once salamanders were fully anesthetized I collected stomach contents by lavage using bottled water delivered through a blunt needle attached to a 10-cc syringe (Cecala et al., 2007). The mouth was held open with a sewing needle and water was introduced gently with enough force to eject stomach contents. I watched the fluid exiting the mouth and collecting any displaced items that emerged, and continued flushing the stomach until only water was being ejected (Legler and Sullivan, 1979). I collected prey items with tweezers and preserved them in a 2.0 ml vial of

isopropyl alcohol. I returned salamanders to their point of capture after they had recovered from anesthesia.

In the lab, I inspected diet samples under a dissecting microscope to count prey items and classify them to order (Mahan and Johnson, 2007). I dried items of the same order from each salamander sample on a plastic weigh boat in a drying oven, and recorded their dry masses to the nearest 0.001 g.

I used a capture-mark-recapture method to estimate population densities and evaluate distance traveled between captures. Each salamander was marked uniquely with Visually Implanted Fluorescent Elastomer (VIE) following a standard procedure (Northwest Marine Technology, Inc., 2008). I used VIE, a bio-compatible material that is frequently used to mark fishes, amphibians, crustaceans, and other animals because of its high retention rate and minimal impact on an animal. I injected VIE sub-cutaneously on the ventral side of the salamander near their legs using a syringe needle. VIE was injected near the base of at least two of their legs, and the order of blanks and color represented a unique ID (e.g. BXOX, representing an individual with blue VIE near its anterior right leg, orange near its posterior right leg, and no VIE on near its left legs). I used fluorescent VIE to improve its detection at night under an ultra violet flashlight (Northwest Marine Technology Inc., 2008).

Each season, I swabbed a subsample of individuals after the first capture for PCR testing to assess the prevalence of *Bd* in the salamanders at Chorros Las Yayas. To prevent cross-contamination I wore a clean pair of gloves for each salamander sampled (Brem et al., 2007). I swabbed the ventral surface of each foot and the vent five times with a cotton-tipped applicator. After swabbing, the applicator tip was broken off into a

vial containing one ml of 95% ethanol and the vial was labeled with the individual identification number. All materials that could be contaminated were discarded into a trash bag (Brem et al., 2007). Taqman real-time PCR assays were used to detect the infection status of 12 *Bolitoglossa biseriata*, two *O. complex*, and two *O. parvipes* (Boyle et al., 2004) and were performed by the Dr. Jamie Voyles' lab at New Mexico Institute of Mining and Technology.

## **Analyses**

I used binary logistic regression in IBM SPSS Statistics 20 (IBM Corp., Armonk, NY; [www.ibm.com](http://www.ibm.com)) to evaluate the influence of environmental conditions on salamander activity using an information theoretic framework. I used the uncorrected Akaike information criterion (AIC) because sample sizes were large relative to the number of parameters estimated in each model. I also described detection rates for each salamander species by dividing the number of captures for each species by the number of complete transects. I used one-way ANOVAs to test for seasonal differences in precipitation and air temperature followed by Tukey's tests to identify mean differences for significant ANOVA results. Seasonal population estimates for *Bolitoglossa biseriata* were calculated using the Schnabel Method (Schnabel, 1938). Linear density was calculated for each species every season. I assessed differences in the number of captures and days between captures for males, females and juveniles with a Kruskal-Wallis test.

I assessed the usage of available habitat structure using a principal components analysis (PCA) on the correlation matrix of habitat variables. Variables that were highly inter-correlated ( $>0.9$ ) or that were not significantly correlated with any other variables

were removed from the PCA or consolidated and the analysis was performed again. A scree plot was used to select components retained for evaluation. Usage of pubescent vs. smooth leaves was compared using a Fisher's Exact Test. I used a one-way ANOVA to test for differences in vertical distance between the three age classes.

I tested for differences in mass and SVL for males and females with a Mann-Whitney U-test. I used Spearman's Rank Correlations to examine associations between growth rate (mm/day) and body size measures (SVL and mass), and between the mean meters moved per day and SVL. I used a Welch's t-test to compare the average dry mass of the total stomach contents of *Bolitoglossa biseriata* collected on plant and rail microhabitats (Whitlock and Schluter, 2009) and to compare the dry masses of adult vs. juvenile stomach contents. I used Spearman's rank correlation to assess the correlation between salamander SVL and mass to the dry mass of their total stomach content.

All statistical tests were conducted using Minitab 16 (Minitab, Inc. State College, PA; [www.minitab.com](http://www.minitab.com)), unless stated otherwise. Variables were assessed for normality and heterogeneity of variances prior to their use in ANOVA. Means are followed by standard deviation. Statistical significance was accepted at  $\alpha = 0.05$ .



## RESULTS

Of the three sampling periods, the 2014 wet season had the highest percentage of survey days without rain, 17 out of 43 (39.5%). The 2013 wet season was the wettest sampling period with 12 out of 42 (28.6%) survey days without rain. The dry season (Dec-Jan) had 6 out of 20 (30%) survey days without rain. In the dry season and 2014 wet season the early morning rain was quickly absorbed by the soil. Moisture that remained on the surface evaporated throughout the day, leaving the ground dry during most nocturnal surveys. Toward the end of the 2014 wet season, the majority of the plants near the trail were wilting.

The dry season had a significantly higher average precipitation level than both wet seasons ( $F=4.22$ ;  $df=2, 105$ ;  $p=0.017$ ), whereas the precipitation in the 2013 and 2014 wet seasons was not significantly different ( $T=0.53$ ,  $df=84$ ,  $p=0.598$ ). Rain typically fell in the afternoon during the wet season of 2013, and in the early morning during the dry season and the wet season of 2014. The air temperature was significantly higher during the first wet season ( $F=11.28$ ;  $df=2$ ;  $p<0.01$ ) than the other two field seasons, but average air temperature only varied between 23.5 and 26.6°C throughout my study.

My field assistants and I spent 195.1 person hours surveying in the 2013 wet season, followed by 79.5 person hours in the 2013 and 2014 dry season, and 189.0 person hours in the wet season of 2014. Between June 2013 and July 2014 I captured 122 salamanders of two genera and three species: *Bolitoglossa biseriata*, *Oedipina complex*, and *O. parvipes*. *B. colonnea* has been reported at the site (Ray, pers. obs.), but was not

found during this study. Salamander capture rates per complete transect surveys were higher during wet seasons (0.88 in 2013 and 1.7 in 2014) than the dry season (0.72; Table 2). Additionally, the probability of finding a salamander on a given transect was 0.658. In the wet season of 2013 I completed 64 complete transects that had 107 salamander capture events (97 *B. biseriata*, eight *O. complex*, and two *O. parvipes*). In the dry season I completed 25 full transects that had 18 salamander capture events (15 *B. biseriata*, three *O. parvipes*). In the 2014 wet season I completed 17 full transects that had 15 salamander capture events (10 *B. biseriata*, one *O. complex*, and five *O. parvipes*). The relatively low number of complete transect surveys in 2014 resulted from a high incidence of interrupted sampling events due to aggressive hymenopterans. When comparing the wet seasons of 2013 and 2014, the number of *Bolitoglossa biseriata* captured decreased by 35%, *Oedipina complex* decreased by 54.5%, while the number of captured *O. parvipes* individuals doubled.

**Table 2.** Effort (person hours) and salamander detection rates per season.

|          | Effort<br>(person<br>hours) | Species             | #<br>Individuals | Detection rate<br>(captures/person hr) |
|----------|-----------------------------|---------------------|------------------|--|
| Wet 2013 | 195.1                       | <i>B. biseriata</i> | 107              | 0.548                                  |
|          |                             | <i>O. complex</i>   | 11               | 0.056                                  |
|          |                             | <i>O. parvipes</i>  | 5                | 0.026                                  |
| Dry      | 79.5                        | <i>B. biseriata</i> | 20               | 0.252                                  |
|          |                             | <i>O. complex</i>   | 0                | 0.000                                  |
|          |                             | <i>O. parvipes</i>  | 3                | 0.038                                  |
| Wet 2014 | 189.0                       | <i>B. biseriata</i> | 34               | 0.180                                  |
|          |                             | <i>O. complex</i>   | 6                | 0.032                                  |
|          |                             | <i>O. parvipes</i>  | 8                | 0.042                                  |

Season and air temperature explained the largest amount of variation in capture rate, followed by the amount of precipitation in the 24 h preceding the survey (Table 3). The detection rate of *Bolitoglossa biseriata* in a transect survey during wet season 2013 was 0.706, it dropped to 0.393 in the dry season, and went up to 0.407 in the 2014 wet season. The probability of finding a *B. biseriata* on a complete transect was 0.734 in wet season 2013, 0.482 in the dry season, and 0.650 in the 2014 wet season. *Oedipina complex* and *O. parvipes* were encountered less frequently than *B. biseriata* across all seasons. The detection rate of *O. complex* on a complete transect in wet season 2013 was 0.17, which dropped to <0.001 in the dry season, and went up to 0.30 in wet season 2014. The probability of finding *O. complex* on a complete transect was 0.141 in wet season 2013, 0.0 in the dry season, and 0.200 in the 2014 wet season. The detection rate of *O. parvipes* in a complete transect survey during wet season 2013 was 0.08, and went up to 0.11 in the dry season, and to 0.35 in the 2014 wet season. The probability of finding *O. parvipes* on a complete transect was 0.063 in the 2013 wet season, 0.074 in the dry season, and 0.150 in the 2014 wet season.

*Bolitoglossa biseriata* was the most commonly encountered species with 87 individuals captured over three seasons. In wet season 2013 there were an estimated 76 individuals (95% confidence interval 61–106). Estimated population sizes dropped to 44 individuals in the dry season (95% CI= 13–74) and 46 individuals in wet season 2014 (95% CI= 30–98). The linear density of *B. biseriata* was 0.23 salamanders/m in wet season 2013, 0.13 in the dry season, and 0.14 in wet season 2014. *Oedipina complex* was the second most abundant with 17 individuals, followed by *O. parvipes* with 16

individuals. I could not calculate population estimates for the two *Oedipina* species due lack of recaptures.

**Table 3.** Model set analyzing survey conditions and season on detectability of *Bolitoglossa biseriata* using binary logistic regression in Minitab 16. The number of parameters in each model is shown in the np column.

| Model                     | AICc    | $\Delta$ AIC | np |
|---------------------------|---------|--------------|----|
| p(Temp+Season)            | 143.894 | 0            | 3  |
| p(Temp+Season+Precip)     | 145.208 | 1.314        | 8  |
| p(Temp+Precip)            | 149.761 | 5.867        | 6  |
| p(Temp)                   | 152.515 | 8.621        | 1  |
| p(Season+Precip)          | 158.175 | 14.281       | 7  |
| p(Previous)               | 160.253 | 16.359       | 1  |
| p(Season)                 | 160.408 | 16.514       | 2  |
| p(Season+Surveyors)       | 161.608 | 17.714       | 3  |
| p(Precip)                 | 166.535 | 22.641       | 5  |
| p(Cloud)                  | 166.709 | 22.815       | 1  |
| p(Time)                   | 167.229 | 23.335       | 1  |
| p(Precip+Obs)             | 167.684 | 23.79        | 15 |
| p(Surveyors)              | 169.793 | 25.899       | 1  |
| p(Surveyors+Previous+Obs) | 169.974 | 26.08        | 12 |
| p(Obs)                    | 170.325 | 26.431       | 10 |
| p(Surveyors+Obs)          | 170.528 | 26.634       | 11 |
| p (Weather)               | 172.113 | 28.219       | 2  |

### ***Bolitoglossa biseriata***

I captured 87 *Bolitoglossa biseriata* ranging from 20.73–43.62 mm SVL (mean= 31.45  $\pm$  5.30 mm) and masses of 0.2–1.9 g (mean= 0.8  $\pm$  0.4 g; Table 4). Three individuals were particularly small (SVL 20.73.7–22.18 mm) and may have hatched recently, seven were

juveniles, and 80 were adults. I found no difference in SVL ( $t= 1.27$ ;  $df= 32$ ;  $p> 0.05$ ) and mass ( $t= 1.67$ ,  $df= 32$ ,  $p> 0.05$ ) between males and females. Thirty-three salamanders (37.9%) had tails at various stages of regeneration at the time of their first capture and three individuals (3.4%) lost their original tail between captures. I found a correlation between SVL and mass ( $R_s= 0.886$ ,  $df= 83$ ,  $p<0.001$ ) and SVL and head width ( $R_s= 0.820$ ,  $df=83$ ,  $p<0.001$ ). I did not find a correlation between SVL ( $R= 0.08$ ,  $df= 20$ ,  $p> 0.05$ ) or mass ( $R= -0.24$ ,  $df= 31$ ,  $p> 0.05$ ) with daily growth rate. A single *B. biseriata* mortality occurred after processing and may have resulted from anesthetic being left on the salamander too long. Twelve *B. biseriata* *Bd* swabs were analyzed and one of the salamanders tested positive for *Bd* (Table 5).

I flushed prey from 37 of the 57 *Bolitoglossa biseriata* examined. Formicidae were the most abundant prey category ( $n= 261$ ) followed by Acari ( $n= 33$ ) and Coleoptera ( $n= 31$ ). Most of the biomass was Formicidae (total dry mass= 0.1016 g), followed by Coleoptera (total dry mass= 0.0085 g) in adult salamanders and Araneae (total dry mass= 0.0043 g) in juveniles. Formicidae were encountered in the most salamanders (33), followed by Coleoptera (16) and Acari (16). I found two unique items: a member of Siphonaptera and a plant seed. I did not find a correlation between salamander body mass ( $R_s= 0.14$ ,  $df= 30$ ,  $p> 0.05$ ) or SVL ( $R_s= 0.13$ ,  $df= 30$ ,  $p> 0.05$ ) with the dry mass of their prey. There was no significant difference in prey dry mass between salamander microhabitat categories at time of detection ( $t= 1.11$ ,  $df= 29$ ,  $p> 0.05$ ). Fecal samples were largely unrecognizable pieces with a few pieces of exoskeleton from Formicidae, Orthoptera, Acari, Coleoptera, Diptera, and Araneae.

**Table 4.** Body size statistics for *Bolitoglossa biseriata*, *Oedipina complex*, and *O. parvipes*, including mean snout-vent length (SVL) and mass with standard error. Mean mass is included for individuals with more than one-third of their tail.

| Species             | Age class | Sex    | Total captured | SVL range (mm) | Mean SVL (mm $\pm$ SD) | Mean mass (g $\pm$ SD) | Gravid |
|---------------------|-----------|--------|----------------|----------------|------------------------|------------------------|--------|
| <i>B. biseriata</i> | all       |        | 87             | 20.73–43.62    | 31.45 $\pm$ 5.30       | 0.8 $\pm$ 0.4          |        |
|                     | juvenile  |        | 7              | 20.73–23.94    | 22.45 $\pm$ 1.29       | 0.3 $\pm$ 0.1          |        |
|                     | adult     | male   | 13             | 26.57–39.52    | 31.9 $\pm$ 3.43        | 0.8 $\pm$ 0.2          |        |
|                     |           | female | 23             | 24.14–43.62    | 34.68 $\pm$ 6.32       | 1.0 $\pm$ 0.5          | 5      |
|                     |           | unkn   | 44             | 24.15–36.69    | 30.85 $\pm$ 3.69       | 0.7 $\pm$ 0.2          |        |
| <i>O. complex</i>   | all       |        | 17             | 25.52–35.91    | 30.62 $\pm$ 2.72       | 0.5 $\pm$ 0.1          |        |
|                     | juvenile  |        | 3              | 25.52–27.67    | 26.83 $\pm$ 1.15       | 0.4 $\pm$ 0.1          |        |
|                     | adult     | male   | 0              |                |                        |                        |        |
|                     |           | female | 3              | 28.17–35.91    | 33.26 $\pm$ 4.41       | 0.5 $\pm$ 0.1          | 3      |
|                     |           | unkn   | 11             | 29.15–33.21    | 30.93 $\pm$ 1.07       | 0.5 $\pm$ 0.1          |        |
| <i>O. parvipes</i>  | all       |        | 15             | 21.21–39.12    | 34.35 $\pm$ 4.50       | 0.7 $\pm$ 0.2          |        |
|                     | juvenile  |        | 4              | 21.21–33.21    | 28.53 $\pm$ 5.14       | 0.5 $\pm$ 0.1          |        |
|                     | adult     | male   | 0              |                |                        |                        |        |
|                     |           | female | 1              | 37.73          |                        | 0.6                    | 1      |
|                     |           | unkn   | 10             | 34.41–39.12    | 36.34 $\pm$ 1.39       | 0.7 $\pm$ 0.1          |        |

I encountered surface active *Bolitoglossa biseriata* from 1800 to 0530 h along the entire transect (Fig. 3). The majority, 70 captures (74.5%), were sitting motionless when found, 16 (17%) were walking, and the remaining were dangling, hiding, or moving their head. All *B. biseriata* were found above ground on the hand rails or on live vegetation. Seventy-nine captures (43.4%) were horizontal in comparison to the ground, followed by 46 captures (25.3%), which were ascending diagonally, while the rest were either diagonally descending (n= 19, 10.4%), vertically ascending (n= 27, 14.8%) or vertically descending (n= 11, 6.0%). The *B. biseriata* found on plants were primarily on the upper

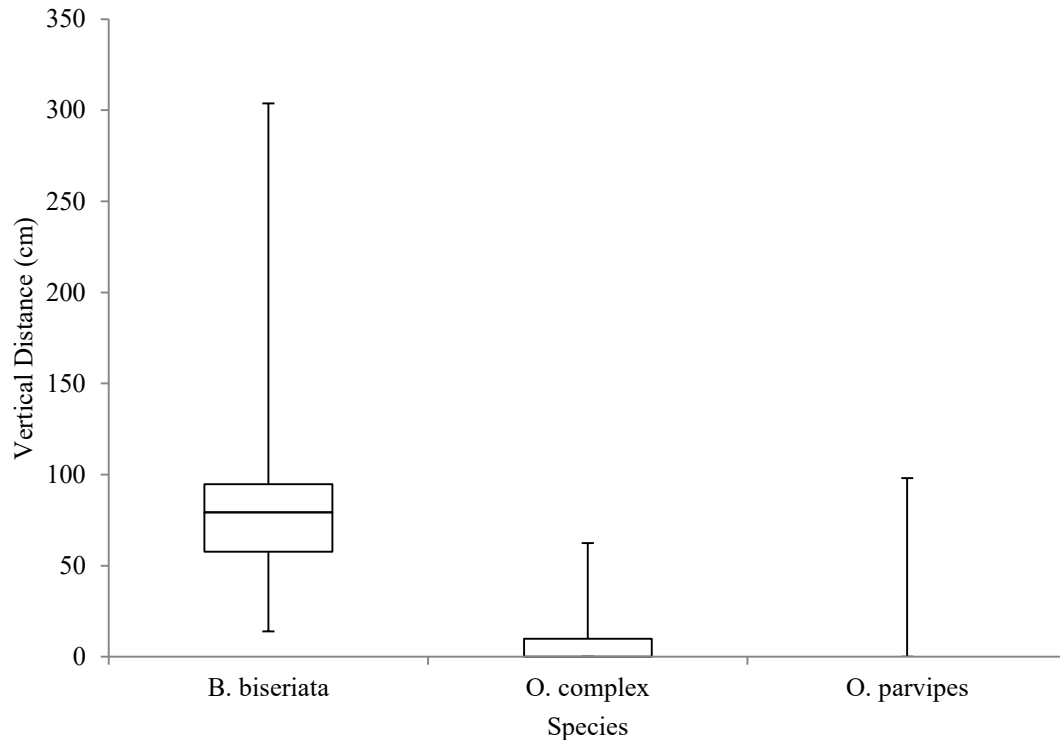
**Table 5.** Genomic equivalents of *Bd*. Average copy number represents the number of gene copies/cell of bacteria. Genome equivalents are the number of zoospore equivalents.

| Sample         | Results | PCR results<br>(average copy<br>number) | Swab Result<br>(Genome<br>Equivalents) | <i>Bd</i> Positive |
|----------------|---------|---|--|--------------------|
| <i>Bbis</i> 10 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 11 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 12 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 13 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 17 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 21 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 42 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 48 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 52 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 53 | n,n,n   | 0                                       |  | No                 |
| <i>Bbis</i> 54 | n,n,p   | 0.853523552                             | 682.8188419                            | Yes                |
| <i>Bbis</i> 55 | n,n,n   | 0                                       |  | No                 |
| <i>Ocom</i> 4  | n,n,n   | 0                                       |  | No                 |
| <i>Ocom</i> 5  | n,n,n   | 0                                       |  | No                 |
| <i>Opar</i> 2  | n,n,p   | 14.88148403                             | 11905.18723                            | Yes                |
| <i>Opar</i> 3  | n,n,n   | 0                                       |  | No                 |

surface of broad leaves (68), the underside of a broad leaf (2), or on the stem of a broad-leaved plant (7). Nine individuals were encountered on spikemosses (*Selaginella* sp.) and one was found on a compound leaf. Three individuals were encountered on vines and one was located on a blade of dead grass. Of the salamander captures detected on leaves 28 (78%) were facing toward the stem, seven (19%) were facing away from the stem and one (3%) was sitting transverse during the initial encounter. I was unable to check for salamanders in living bromeliads because of accessibility logistics and did not find any salamanders in fallen bromeliads. Salamanders were associated with plants with non-pubescent leaves ( $\chi^2 = 91.39$ ;  $df = 92$ ,  $p < 0.05$ ), with 51.8% of the captures on woody species and 82.4% facing toward the stem. The mean vertical distance for all *B. biseriata*

locations was  $79.7 \pm 39.6$  cm (range 14.0–303.8 cm; Fig. 7), and the means of vertical distance for each age class were not significantly different ( $F = 0.49$ ,  $df = 85$ ,  $p = 0.61$ ).

Highly correlated variables in the PCA included the number of midstory trees with epiphytes (removed), the number of epiphytes in the midstory, and the number of different epiphytes and trees with epiphytes in the overstory, which were reduced to the average number of trees with large epiphytes (bromeliads and ferns). The PCA of structural habitat variables revealed that 25% of the variation in habitat structure was explained by a gradient of natural vs. anthropogenic structures along the trail/forest



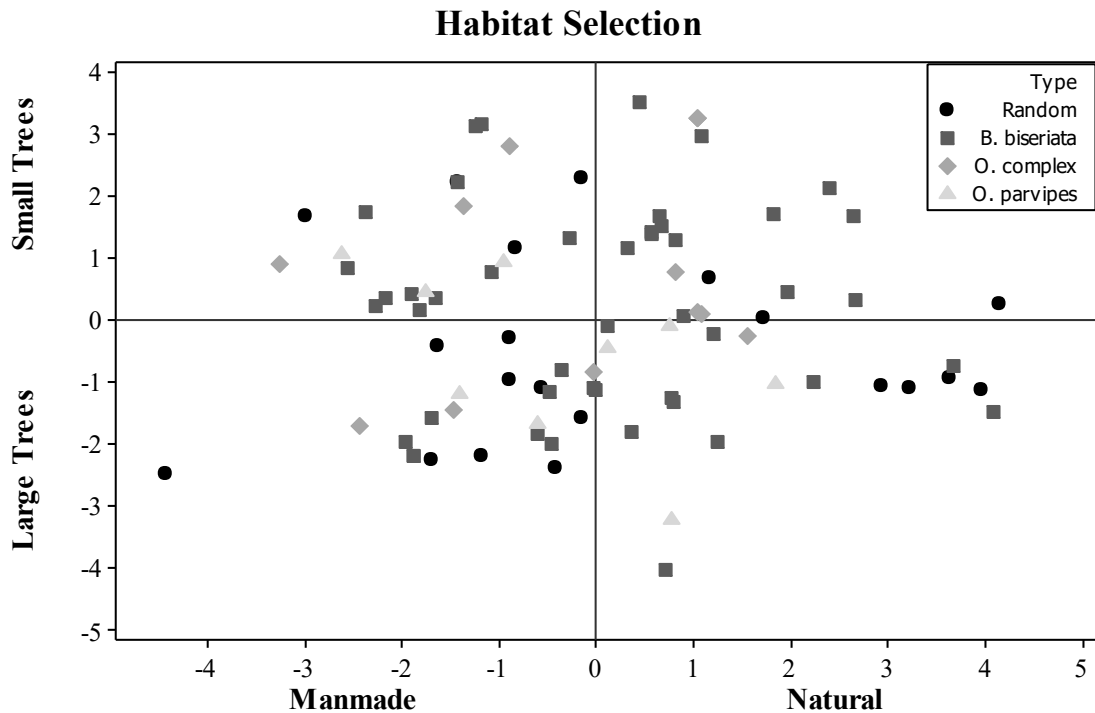
**Fig. 7.** Box plot of the vertical distance for each species at the time of detection.

interface (PC 1) and herbaceous plant density (PC 2). A plot of the component scores suggests that *Bolitoglossa biseriata* utilize most of the habitat structure defined by these



gradients (Fig. 8, Table 6). A MANOVA comparing component scores of random locations and salamander locations found no significant difference.

Of the 31 individuals of *Bolitoglossa biseriata* that were recaptured, 16 (52%) were found within 1.5 m of a previous capture of the same individual (Fig. 9). Thirty-four individuals were captured more than once and, sixteen of those who were recaptured were located more than twice (up to 7 captures). There was a 0.46 recapture rate for the entire study and 74 recapture events. The average number of meters traveled per day between captures was not correlated with SVL ( $R_s = 0.16$ ,  $df = 28$ ,  $p > 0.05$ ). The average



**Fig. 8.** Habitat component scores for each species.

distance travelled per day between capture events did not differ between 20 juveniles and nine adults ( $t = 0.57$ ,  $df = 27$ ,  $p > 0.05$ ). Salamanders were found along the entire transect. However, linear spacing of all individuals tended toward overdispersion (variance-to-mean ratio = 1.15) with captures concentrated at two locations. Thirty-seven (23.3%) captures were at the lowest elevation, around 400 m asl, and 59 (37.2%) were within 25 m of the river bordering the western side of the transect.

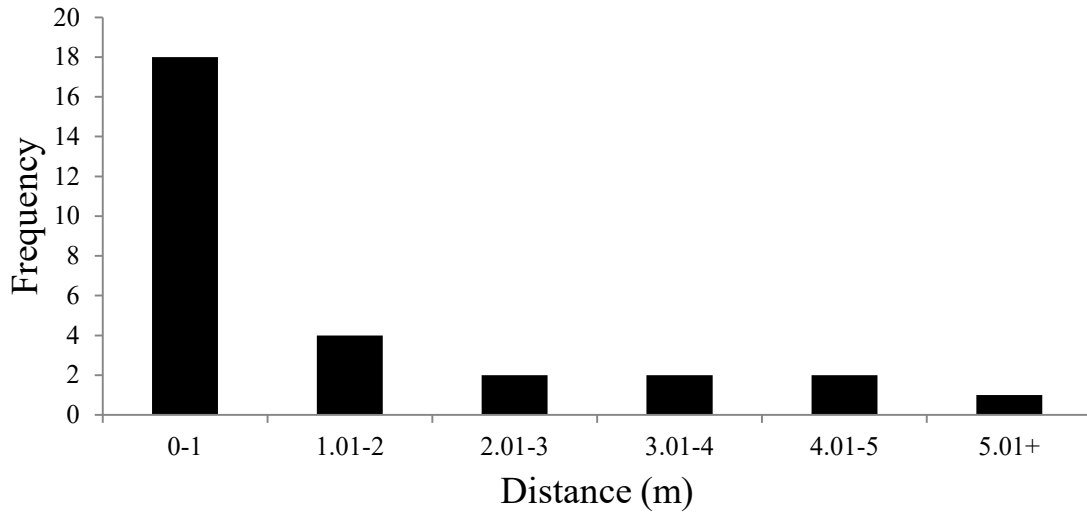
While conducting surveys I occasionally witnessed *B. biseriata* engaged in noteworthy activities. On 14 July 2013 at 2324 h I observed an encounter between two individuals. A male was observed nose-tapping and walking toward a gravid female from approximately 4 m away. The pair came into contact once on top of a post of the wooden hand railing. The female placed her front right foot on the male's flank, just behind his front leg, then moved away and walked quickly down the rail. The male nose tapped and then walked slowly up the rail, in the opposite direction of the female.

I also observed two separate individuals transitioning from apparent night time to day time locations. One individual was found at 0417 h on the top rail and moved less than a meter to a space between the post and handrail. The other individual was found at 0508 h and walked less than a meter from the leaf of an *Antherium* sp. to the base of the stem and faced upward.

Only two *B. biseriata* were not found in association with transect sampling. One individual was discovered *ca.* 150 m off the trail on a leaf of grass within 2 m of the gravel road that passes along the eastern side of the site. Another was found during the day on six different occasions within the same capped PVC refuge posted 2 m above the ground. This individual was found only in the refuge when water was present in the cap.

**Table 6.** Loading (correlations) of habitat variables on the first four principal components (I–IV) of a principal component analysis. Highly correlated variables are bold.

| Variable                         | I             | II           | III           | IV           |
|----------------------------------|---------------|--------------|---------------|--------------|
| Eigenvalue                       | 3.262         | 2.752        | 2.292         | 1.891        |
| % Variance Explained             | 0.136         | 0.115        | 0.096         | 0.079        |
| Understory (<1m)                 |               |              |               |              |
| %Rock                            | 0.123         | 0.074        | -0.053        | 0.142        |
| %Debris                          | 0.298         | 0.024        | 0.006         | 0.003        |
| %Bare                            | 0.225         | 0.04         | -0.215        | 0.213        |
| %Leaf litter                     | 0.113         | 0.209        | <b>-0.319</b> | 0.004        |
| %Herb                            | 0.207         | 0.275        | <b>0.341</b>  | 0.129        |
| %Woody                           | 0.231         | 0.191        | -0.174        | 0.055        |
| %Manmade                         | <b>-0.395</b> | 0.013        | 0.067         | 0.175        |
| Leaf litter depth                | 0.135         | 0.061        | -0.167        | 0.083        |
| #Woody species                   | <b>0.317</b>  | 0.15         | -0.256        | 0.066        |
| #Woody stems                     | <b>0.358</b>  | 0.089        | -0.26         | -0.07        |
| # Herb species                   | 0.134         | <b>0.421</b> | 0.126         | 0.095        |
| # Herb stems                     | 0.162         | <b>0.379</b> | 0.109         | 0.149        |
| Midstory (1-6m)                  |               |              |               |              |
| # Epiphytes                      | 0.211         | 0.171        | 0.299         | 0.161        |
| Canopy height                    | 0.162         | 0.101        | 0.187         | 0.052        |
| Circumference                    | 0.069         | 0.203        | 0.065         | <b>0.405</b> |
| # Trees                          | 0.147         | 0.113        | <b>0.318</b>  | 0.299        |
| Overstory (>6m)                  |               |              |               |              |
| Percent Canopy Cover             | -0.046        | 0.039        | <b>-0.362</b> | 0.266        |
| Avg # trees with large epiphytes | -0.163        | <b>0.378</b> | -0.228        | <b>0.310</b> |
| # Trees                          | -0.156        | <b>0.33</b>  | -0.231        | <b>0.391</b> |
| Canopy height                    | 0.042         | 0.214        | -0.037        | 0.049        |
| Distance to tree                 | -0.211        | 0.208        | 0.093         | <b>0.353</b> |
| Distance to rock                 | 0.024         | 0.007        | 0.136         | 0.246        |
| Distance to log                  | -0.253        | 0.071        | -0.107        | 0.008        |
| % Structure                      | 0.167         | 0.201        | -0.057        | 0.218        |



**Fig. 9.** Distances moved between captures of *B. biseriata*.

This individual was present only in the refuge when water was present in the cap.

### ***Oedipina complex***

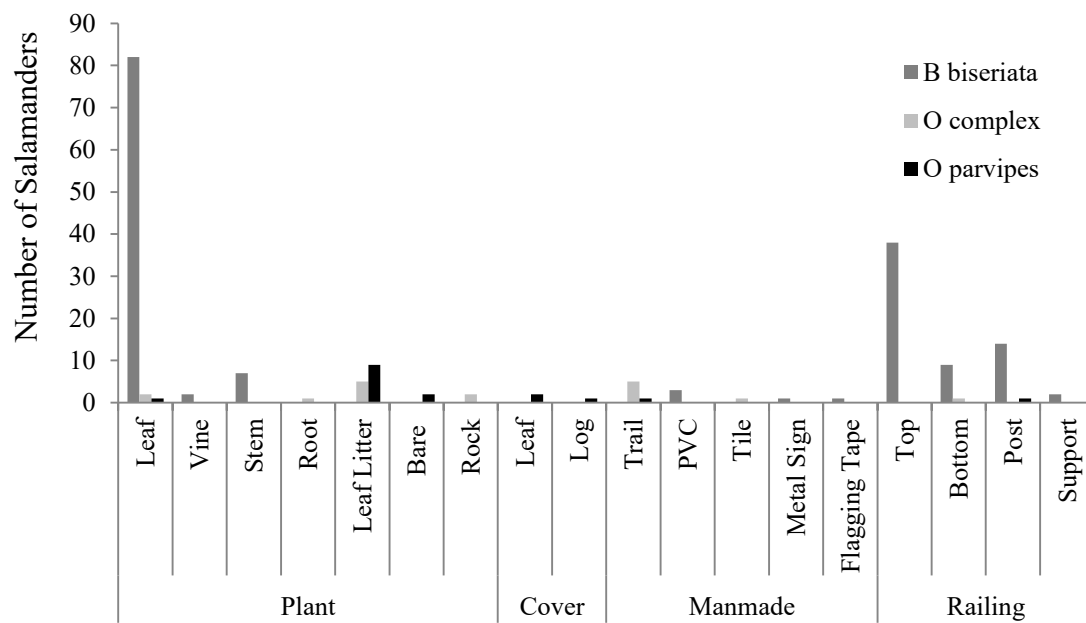
I only captured *Oedipina complex* in the wet seasons, including 11 in 2013 and six in 2014. Individuals ranged from 25.52–35.91 mm SVL (mean=  $30.62 \pm 2.72$  mm) and between 0.3–0.6 g in mass (mean=  $0.5 \pm 0.1$  g; Table 4). Three (17.6%) had a regenerated or regenerating tail. I was unable to distinguish between the sexes except when eggs were visible. Two *O. complex* *Bd* swabs were analyzed and both tested negative (Table 5).

Seventeen *Oedipina complex* were found between 1920 and 0205 h throughout the transect from 390–454 m asl. Ten (58.8%) were found moving when captured, including four of five sitting on the concrete trail, three of four captures on the leaf litter, two found on rocks, and one found on the rail. Single individuals, none of which were moving, were each located on a root, a tile near the south entrance of the trail, on the walkway railing, and on a spikemoss. The six (35.3%) salamanders found above the ground were at an overall mean vertical distance of  $8.8 \pm 16.0$  cm (Fig. 7).

### *Oedipina parvipes*

I captured one neonate, three juveniles, and 11 adult *Oedipina parvipes*. Most of these captures were in the 2014 wet season (n= 8), though I found individuals in each season (wet 2013, n= 4; dry 2013-14, n= 3). Individuals ranged from 21.21–39.12 mm SVL (mean=  $34.35 \pm 4.50$  mm), and had a mass between 0.3–0.9 g (mean=  $0.7 \pm 0.2$  g; Table 4). One individual was likely a neonate with an SVL of 21.21 mm compared to the next smallest *O. parvipes*, which had an SVL of 29.47 mm. Only one (6.7%) was missing its original tail. Two *O. parvipes* *Bd* swabs were analyzed and one tested positive (Table 5).

Fifteen *Oedipina parvipes* were found during transect surveys between 1929 and 0008 h from 401–438 m asl. An additional individual was found during a leaf litter survey at 1000 h. The majority of captures (n= 9, 56.3%) were within the leaf litter, two captures were on bare ground, and four single captures occurred on a leaf, under a log, on the trail, and climbing a railing post (Fig. 10). Only juveniles were observed above the ground. I recaptured one individual seven surveys after its first capture in leaf litter 2.1 m from its original location. The recapture rate was 0.06 for the entire length of the study. The majority of *O. parvipes* also were found moving.



**Fig. 10.** Microhabitats used at the time of detection.

## DISCUSSION

Some *Bolitoglossa* (13%) and *Oedipina* (11%) species are listed as data deficient by the International Union for the Conservation of Nature (2015). An additional 27% of *Bolitoglossa* species and 50% of *Oedipina* species have not been evaluated (Amphibiaweb, 2015). Although the species involved in this study are listed as Least Concern, both *Oedipina* are experiencing a decreasing population trend (Castro et al., 2008; Solís et al., 2008). Given the vulnerability of Neotropical salamanders to environmental disturbance, and the paucity of natural history data for these organisms, my study provides baseline data useful in guiding monitoring and management efforts. Information about the natural history of these species also may help to conserve related species that are threatened with extinction (36% of *Bolitoglossa*, 33% of *Oedipina*; Amphibiaweb, 2015).

Bailey et al. (2004) studied the capture probability of plethodontid salamanders in the Great Smoky Mountains National Park from 1999-2001 and reported that elevation and vegetation appeared to have the strongest influence on surface population size and conditional capture probability and the probability of detecting an animal given that it is present in the super-population, but not necessarily accessible (i.e. near the surface). Bailey et al.'s (2004) estimate of average conditional capture probability for 10 species was  $0.30 \pm 0.01$  and was higher for salamanders on low-elevation/disturbed/deciduous sites, and showed some evidence of varying among genera. In my study, the probability of capturing a salamander during a complete transect was 0.658 but varied greatly between seasons and among genera with *Bolitoglossa* being captured the most. Bailey et

al. (2004) reported that the average surface population did not vary between years but recapture probability was only  $0.07 \pm 0.004$  and varied little spatially, indicating that a large proportion of the population was unavailable for capture at any given time. My recapture rate for *B. biseriata* was much higher than the temperate plethodontids, while the recapture rate of *Oedipina parvipes* was similar, which suggests that the arboreal species is more available for sampling than the fossorial species at this site and in other studies.

Heatwole (1962) found that *Plethodon cinereus* vertical distribution varied seasonally with salamanders retreating deeper underground as conditions became dry and moving closer to the surface during wet conditions. Additionally, Heatwole (1962) found that microtopographical features that influence moisture content had a profound influence of salamander horizontal distribution. These findings may explain why more salamander captures in my study occurred downhill and closer to the river during dry conditions and why I only located *Oedipina* on nights following a rain event in which water was still available on the surface. *Bolitoglossa* utilized arboreal habitats much more frequently than *Oedipina*, and therefore are more individuals were available for detection and did not appear to be as reliant on standing moisture on the substrate.

One factor that may affect salamanders at my study site is climate change. Climate change, which is predicted to result in shifts in distributional ranges, and potentially ecological niches, poses a threat to most Neotropical salamanders (Foster, 2001). Climate change is predicted to decrease the annual rainfall, increase the length of the dry season, and increase variability in inter-annual rainfall (Bawa and Markham, 1995; Hulme and Viner, 1998; IPCC, 2007). As drought conditions become more



frequent, there may be a drastic change in the biota of the tropics. My study was conducted in both the wet and dry seasons of 2013 and 2014, which were exceptionally dry compared to previous years. In 2014 the rain that did fall was absorbed quickly into the soil and I saw plants wilting. Air temperature and precipitation had the largest influence on salamander detection rate, suggesting that dry conditions limit the level of surface activity by the salamanders at this site. Future biophysical modeling may shed light on the amount of time that would be available for surface activity under predicted climate change conditions. If these species are unable to adapt physiologically or behaviorally to account for reduced precipitation, less time spent foraging and searching for mates may lead to population declines (Spotila, 1972). My data suggest that the salamanders at this site are unlikely to relocate to moister environments because of their low level of movement.

Dead Bolitoglossine salamanders have been found in Panama, including *Oedipina parvipes* (Weinstein, 2009). The chytrid fungus, *Bd* has been detected in museum specimens of several species collected between 1994- 2009 in Guatemala (*B. engelhardti*, *B. flavimembris*, *B. franklini* x *B. lincolni*, *B. lincolni* and *B. occidentalis*). In Monteverde, Costa Rica, specimens of *B. robusta*, *O. poelzi*, and *O. uniformis* collected during a year of major decline (1987), had *Bd* (Cheng et al., 2011). Pasmans et al. (2004) also found *Bd* on wild caught *B. doflini*. Three individuals of *B. rufescens* that were experimentally infected with *Bd* revealed high susceptibility to *Bd* infection and suffered mortality once the average level of infection intensity was around 10,000 zoospore equivalents (Cheng et al., 2011). Woodhams et al. (2006) also found that *B. colonnea* has 0% growth inhibition of *Bd* based off of their skin peptides. I detected *Bd* on *Bolitoglossa*

*biseriata* and *O. parvipes*. More sampling will be necessary to determine how prevalent *Bd* is at Chorro Las Yayas.

*Bolitoglossa colonnea* was not found during my study but was photo-documented in Chorro Las Yayas in December of 2012, six months before my first field season. Therefore, it would seem that *B. colonnea* should have been detected during my study. The 2012 specimen was found in the dry season under very wet conditions near the largest waterfall at the northwestern portion of the trail. Bruce (1997) reported that *B. colonnea* in Costa Rica were found mostly at night on the upper surface of broad-leaved herbaceous plants and that the likelihood of detection was similar on wet and dry nights. The apparent absence of *B. colonnea* in my study may have resulted from low detectability during the drought, low abundance, or recent population declines. Future sampling will be necessary to determine if this species is still present at Chorro Las Yayas or has been extirpated recently.

Chorro Las Yayas was chosen for this salamander population study due to its high rate of salamander encounters relative to nearby sites. For example, herpetofaunal surveys have yielded only one *Bolitoglossa biseriata* and two *Oedipina* sp. at La MICA Biological Station since 2010. Unfortunately, the single trail running through the Chorro Las Yayas site provided the only sampling opportunity because the steep terrain of the site precluded safe access to much of the surrounding forest. Because the trail involved only certain portions of the study area, and provided different habitat structure than the surrounding forest, my results may not represent completely salamander populations at Chorro Las Yayas. May and Donnelly (2009) reported higher frog abundance on trails than off trails in a low-land rainforest in southeastern Peru. Because I was not able to

sample away from the trail, I am not certain if the salamander densities that I estimated are representative of the study area or are only indicative of trail habitat.

Another limitation to this study is the restricted timeframe in which sampling was conducted. My study was conducted only in June, July, and a part of December and January, which limits my understanding of how the population fluctuates throughout the year and may have decreased my chance of observing seasonally variable behaviors. The limited duration and coincidental timing of my study with an El Niño event also resulted in all data being collected during relative drought conditions. Thus, my June and July data were not collected under typical wet season conditions and likely misrepresent expected salamander encounter rates for this season.

Although my study potentially was biased by convenience sampling, the relatively robust sample sizes I obtained have provided the only quantitative data for the salamander species captured during my study. The abundance data collected are particularly useful in providing a baseline for comparisons if my surveys are replicated in future monitoring efforts.

I only located *Bolitoglossa biseriata* above the ground, primarily in small herbaceous plants and saplings. Thus, *B. biseriata* appears to be a primarily arboreal species that occurs relatively close to the ground. However, I was only able to sample within about 2 m of the ground and am uncertain to what extent this species may have used microhabitats at greater heights. Bruce (1997) reported that *B. colonnea* were also primarily arboreal but were found only on herbaceous plants. My study site was unusual in comparison to other forests because of the presence of a pedestrian walkway with a wooden handrail containing cracks and holes that were used frequently by salamanders.

This handrail was utilized by many invertebrates, which may have provided foraging opportunities for the salamanders. Presumably *B. biseriata* also forage in living vegetation. The majority of *B. biseriata* detected on a leaf at the time of their first encounter were facing toward the stem, which may be a useful foraging strategy for capturing prey traveling onto the leaf from the stem.

I used tin cover objects and arboreal PVC refugia to test if artificial structures would be practical for surveying Neotropical salamanders. I did not observe salamanders using the cover boards but limited use of a PVC pipe by one *Bolitoglossa biseriata* was observed during the second wet season of my short-term study. The PVC often contained water when the surrounding habitat was dry and may have contributed to use of this artificial microhabitat by the salamander. Further sampling effort is needed to determine if PVC refugia may serve as a sampling method for arboreal salamanders. Studies of tree frogs using PVC refugia also had success with t-shaped retreats and found that success varied among species and age classes (Boughton et al., 2000). Additionally, Zacharow et al. (2003) reported that body size of frogs was correlated with pipe diameter. Further examination using varying pipe lengths and shapes would be of interest to evaluate the use of artificial structures as a sampling method for tropical salamanders.

It appeared that the salamanders at my site face a high level of predation with almost 40% of *B. biseriata* exhibiting tail regeneration, though no threats were identified during this study. The prevalence of salamanders with regenerating tails was similar to that documented in a study of *Plethodon cinereus*, with 45% of territorial animals with regenerated or broken tails (Mathis, 1991). I did not observe directly any salamander depredation events during my study. However, snake species known to consume

*Bolitoglossa* (Brodie, 1977; Ducey et al., 1993) have been documented at the study site (J. Ray and L. Brinkman, pers. obs.). Many species of salamanders respond to snakes by directing the attention of the snake toward their tail in an effort to protect their head. Additionally, salamanders that lack or produce minimal toxic skin secretions are more likely to autotomize their tails (Ducey et al., 1993). These behaviors may lend insight as to why so many individuals had broken tails.

The *Bolitoglossa biseriata* I observed typically remained stationary while individuals of *Oedipina* were active, which may have represented a sampling artifact because of the difficulty of observing *Oedipina* that are not moving. *Oedipina* are presumed fossorial or semi-fossorial (Dunn, 1924) and typically are captured within surface cover, including logs, moss, or within sand and gravel near streams (Hilton, 1946). On multiple occasions I encountered individuals during transect surveys climbing (on rails, plants, or stairs) but most were located on or in the leaf litter or crossing the trail. The majority of *Oedipina* that I captured were encountered during wet conditions, which is also typical of temperate fossorial plethodontids such as *Plethodon cinereus* that maximize surface activity during rainfall events (Taub, 1961).

The availability of data on basic natural history, including habitat usage, reproduction, dispersal abilities and diet is essential for the development of management plans and captive breeding programs. My study has provided population estimates and natural history data for three species of Neotropical salamanders during drought conditions. Such baseline data are valuable for monitoring the population trends at Chorro Las Yayas if comparative surveys are conducted in the future. The data I collected on the demographics, habitat use, activity periods, and time of reproduction will

be valuable if *ex situ* conservation measures become necessary in the future. If the species I studied become species of conservation concern, the information about their habitat requirements will be valuable for highlighting important areas within their respective ranges that should be protected. Early detection of any potential threats will improve greatly the chance to conserve this community, and potentially, other communities as well.

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