THE DIVERSITY, DISTRIBUTION, AND CONSERVATION OF A POLYMORPHIC FROG (*OOPHAGA PUMILIO*) IN WESTERN PANAMA

By

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ABSTRACT

THE DIVERSITY, DISTRIBUTION, AND CONSERVATION OF A POLYMORPHIC FROG (*OOPHAGA PUMILIO*) IN WESTERN PANAMA

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The global crisis in amphibian conservation has created a need for new methods to assess population sizes and trends. I examine potential methods for assessing population trends of a small Central American poison dart frog, *Oophaga pumilio* (Anura: Dendrobatidae), in the Bocas Del Toro region of Panama where the frog is highly polymorphic and little is known about the ecology of individual populations. I pursued four lines of inquiry: 1) quantifying the changes in available habitat using satellite imagery, 2) measuring population densities for nine populations, 3) analyzing the potential for call surveys for population assessment, and 4) conducting experiments to identify factors limiting the populations. Analysis of satellite imagery for Normalized Difference Vegetation Index (NDVI) between 1986 and 1999 showed increases in habitat types associated with human development and losses in the amount of mature forest in all areas examined. Through the use of transects, I was able to assess population densities as well as relationship to edges for each of nine populations. Populations varied six-fold in density and in distribution related to forest edge. To explore the use of call surveys for population assessment, I conducted combined call and visual surveys. I found no relationship between call density and population density. Finally, I manipulated potential limiting factors such as food availability and rearing sites, identifying rearing sites as a limiting resource for these frogs. The results of this work provide important ecological information about this species such that managers may use these results to conserve at-risk populations of *Oophaga pumilio* through its range.

This work is dedicated to my parents, Wade and Kim, who have inspired me to care passionately about all that I do, as well as all of those who work tirelessly to protect amphibians.

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in Panama. The 2009 field season was very intensive, and my field assistants, Isaac Standish, Keith Erickson, and Craig Erickson were excellent assistants. They helped me in a project that by all accounts was ambitious. Without their enthusiasm and willingness, I would not have been able to do half of the collection that I did. Between field seasons, my quadrats needed monthly care, and without the help of Christian Harris at STRI, I would not have been able to make the conclusions that I did make for that portion of the study. He is a truly enthusiastic individual about any project that he is involved with, and I greatly appreciate his help in mine. In 2010, my field assistant, Tim Muhich, provided great assistance in completing the project. His enthusiasm and interest in tropical ecology was refreshing and perfect for the project. I thank all of you immensely for aiding me in completing this project.

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GENERAL INTRODUCTION

Conservation of amphibians is of great interest for a variety of reasons. Currently, approximately one third (32.5%) of amphibian species are threatened with extinction and 43.2% of amphibian species are experiencing population declines (Stuart et al. 2004). Of the 6,807 species of amphibians in the world, only 28 (0.5%) are reported to be increasing and 27.2% are stable, but many are considered “data deficient” and are likely experiencing similar declines as other species (Stuart et al. 2004). Reasons for such declines are many, including disease (Pounds et al. 2006), habitat destruction, and overexploitation. These problems are further exacerbated by decreased ability to detect amphibians due to the elusive nature of the group.

Currently, amphibians are facing an epidemic in the form of a fungus, *Batrachochytrium dendrobatidis* (*Bd*). This fungal pathogen was only recently discovered in 1999 and has been implicated in the decline of 93 amphibian species around the world with at least 43 in Central America (Lips et al. 2006). This highly virulent disease, when it invades a region, tends to result in extirpation of 50% of the amphibian species and 80% of the individuals in the remaining species (Lips et al. 2006). *Bd* is found worldwide with amphibian mortalities being directly linked to population declines in Central America and Australia (Berger et al. 1998). This disease is possibly the leading cause of all amphibian declines with many of those happening in Central America. Through the 1980s, *Bd* spread through Costa Rica and has entered Panama in the 1990s. Unfortunately the spread continues eastward toward South America (Lips et al. 2006, Woodhams et al. 2008). To make matters worse, many prolific, and often invasive frog species (e.g., *Agalychnis callidryas, Bufo marinus,* and *Leptodactylus pentadactylus*) are effective carriers of the disease (Lips et al. 2006).

Fortunately, not all species are affected by *Bd*, but many of those species are subjected to the impacts of habitat destruction and fragmentation. Fragmentation is particularly damaging because habitat fragments may not be large enough to support a population of amphibians or it may allow opportunistic edge species to invade and compete with vulnerable interior species. Home ranges of large, charismatic species like jaguar and tapir are well known (Foerster and Vaughan 2002, Maffei et al. 2004), but the extent of habitat use remains largely unknown for many amphibian species. In temperate amphibians, the onset of spring triggers migrations in many species to go to breeding ponds. Habitat destruction and conversion can negatively affect the ability of these animals to reach breeding ponds (Rittenhouse and Semlitsch 2006). Tropical amphibians may have similar migrations between dry and wet seasons, which make habitat fragmentation and destruction particularly problematic. To further complicate matters, tropical amphibian species with small ranges and small clutch sizes are most at risk of extinction (Cooper et al. 2008).

Many species of amphibians are often subject to intense pressure from human collection. Reasons for these pressures vary from sustenance as seen in the Goliath Frog (*Conraua goliath*) to intrigue in the pet trade as seen in the Kaiser's Newt (*Neurergus kaiseri*). In each instance, impact on populations can be minimal, but on some species, especially those with small ranges, collection pressure can cause declines, extirpations, and extinctions. Species that are bold and charismatic pose particular problems due to collection pressure because these are often the same species in demand in the pet trade. The Panamanian Golden Frog (*Atelopus zeteki*) is such a species. This species of toad is under great threat due to *Bd* and has recently been removed in large numbers for conservation programs to avoid extinction of the species. Many conservationists are concerned about potential illegal smuggling of these species because of its bold and bright coloration, so sites where the toads were known to been were kept very secret. Unfortunately, not all species of amphibians live in the inaccessible reaches that *A. zeteki* live in.

The conservation of the Strawberry Poison Dart Frog (*Oophaga pumilio*) is important as this species may act as an indicator species for other species. This species is common throughout the Caribbean lowlands from Nicaragua to Panama and often is one of the most abundant vertebrates in those systems (J. Lawrence, personal observation). Perhaps the most well known feature of this species is that in the Bocas del Toro region of Panama, the species goes through a massive radiation of colors, from reds and oranges to blues and blacks, and everything in between. Historically, most of the research of *O. pumilio* has focused on this radiation (e.g., Summers et al. 1999, Siddiqi et al. 2004, Hagemann and Prohl 2007, Maan and Cummings 2008, Wang and Shaffer 2008). Recent literature focuses on the evolution of color as this radiation happened within the last 10,000 years when a land bridge occurred between the mainland and the islands (Fairbanks 1989). However, little research has been done on the conservation or the ecology of this species.

The focus of this research was to understand the conservation status of populations of *O. pumilio* in the Bocas del Toro region of Panama. The objectives of my research were to:

1. determine the approximate population size of nine different populations of *O. pumilio* and the extent of suitable habitat available to them.
2. determine if the use of call surveys is a viable method for assessing population size and trends for *O. pumilio*, and
3. assess whether presence or absence of leaf litter, as well as addition of artificial rearing sites, affected population size of *O. pumilio* populations on Isla Colon over the course of one year.

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CHAPTER 1 : NATURAL HISTORY OF *OOPHAGA PUMILIO* IN THE BOCAS DEL TORO ARCHIPELAGO

Abstract

The Strawberry Poison Dart Frog (*Oophaga pumilio*) is a unique species found throughout the Caribbean lowlands of western Panama. This species is particularly noted for a geologically rapid radiation in color-pattern and size polymorphism in the Bocas del Toro archipelago of Panama. Consequently, this species has been of great interest in understanding the evolution of aposematism in anurans. The Bocas del Toro archipelago is a cluster of approximately 10 large islands that support *O. pumilio* populations. These islands have formed in the last 8,900 years as the result of sea level change. Currently, these islands are a combination of lowland rainforest, agricultural plantations, and pastureland. Agricultural plantations are typically cacao (*Theobroma cacao*), banana (*Musa* spp.), plantain (*Musa* spp.), and teak (*Tectona grandis*) with other crops such as mango (*Mangifera indica*) and papaya (*Carica papaya*) being planted locally. Temperatures in the region average 28°C during the day when these frogs are active with daily humidity averaging between 75.9% and 88.3%. The variability in habitat as well as the relatively small distances between islands makes this region ideal for studying the evolution of polymorphism in *O. pumilio.* Additionally, *O. pumilio* populations in this area exhibit high variability in population density and exposure to pet-trade harvest; thus it will be important to develop regional conservation strategies to maintain the genetic diversity in this species.

Natural History of *Oophaga pumilio*

Oophaga pumilio is a small frog in the family Dendrobatidae found throughout the Caribbean lowland rainforests from Nicaragua to Panama (Lötters et al. 2007). This species is semi-arboreal and individuals may climb up to 40m (Lawrence 2011) into the trees to access water sources found in bromeliads (Bromeliaceae) for larvae deposition. These frogs have evolved to use rearing sites containing very small amounts of water, presumably to avoid cannibalism (Summers 1999) or competition, and to avoid potential predators (Galindo-Leal et al. 2003). This species uses a diversity of sites for larval deposition, including *Heliconia* and bromeliad axils, tree holes, and even artificial structures, as shown in captive studies.

The genus *Oophaga* includes nine species in the monophyletic *Oophaga histrionicus* complex that are known as obligate egg feeders (Lötters et al. 2007). These frogs have evolved a complex system of parental care. As in most dendrobatids, newly-hatched tadpoles are transported to a water source by a parent (typically the female in *O. pumilio*). In a trait unique for the genus *Oophaga*, the females return periodically to lay infertile, nutritive eggs for the tadpoles to eat . Without this supplemental nutrition, the tadpoles would not survive. This degree of parental care is very unusual in amphibians. Consequently, sexual selection in this complex would predictably be important in the evolution of species and populations (Summers et al. 1999, Maan and Cummings 2008).

Oophaga pumilio is notable for the diverse polymorphic seen in the Bocas del Toro archipelago (Figure 1.1). Through most of its range, this species has a red body with blue or black legs (Figure 1.2). Within the last 8,900 years in Bocas del Toro, Panama (Wang and Shaffer 2008), this species in has diverged and evolved into 20 or more different, distinct populations (Wang and Shaffer 2008; Figure 1.2). Given that these islands separated from the main archipelago as recently as the last 1,000 years, these populations have diversified rapidly. The most obvious differences are the vast array of colors, from reds and oranges to blues and blacks. This divergence, however, is not limited to color. Populations exhibit size differences, with some populations being as small as 15mm in snout-vent length to as large as 24mm. These populations even exhibit behavioral differences, with some being bold and others being skittish. Observers can readily approach and, in some instances, touch frogs in bold populations whereas in skittish populations the frogs may seek cover when observers are several meters away. This radiation, consequently, has been of great interest for those studying the evolution of aposematic coloration and toxicity (Summers et al. 1999, Hagman and Forsman 2003, Wang and Shaffer 2008, Maan and Cummings 2009).

Noteworthy Field Observations of Oophaga pumilio *Behavior*

In Bocas del Toro, *O. pumilio* are most often associated with vertical substrate, and are frequently encountered on or near tree bases. Populations show a high degree of variability in this trait, however. Some populations are largely terrestrial and rarely climb into the canopy (e.g., Popa – North) while others are highly associated with vertical substrate and can be found in the canopy (e.g., Isla Colon). Frogs occur throughout the leaf litter and vegetation, but often prefer traversing on fallen branches or roots when crossing ground free of downed leaf litter. Male *O. pumilio* will tend to select calling perches that allow them to be conspicuous to conspecifics (Prohl and Hodl 1999).

Differences in behavior were also observed between populations. As noted above, some populations were very bold, and the frogs would tolerate close approach (within centimeters) or in some instances, actual contact before retreating from the observer’s presence. Other populations were skittish and individual frogs would retreat when observers were several meters away. Reasons for the differences between flight initiation distances in the respective populations is unclear. It is presumably an anti-predator response as seen in other species, but possibly influenced by habitat structure differences, including those caused by human-mediated environmental disturbances (Relyea 2003). Variables influencing escape distances might also include the increase or decrease in conspicuousness caused by increased or decreased movement, respectively. Despite current hypotheses that aposematic species tend to evolve slow movement patterns to aid in predator recognition of their unpalatable nature (Sherratt et al. 2004), short, fast hops followed by remaining still as seen in skittish populations (e.g., Solarte, Almirante, and Aguacate; Figure 1.2E, 1.2J, and 1.2M, respectively), could aid in increasing conspicuousness of these frogs. This movement pattern could attract a predators’ attention and aid the predator in recognizing the frog as unpalatable rather than simply relying on color alone. This could be especially important for more cryptically colored frogs, such as in the Aguacate population (Figure 1.2M), which, when immobile, are effectively cryptic in natural habitat. Further research will be necessary to discern the reasons for these behavioral differences among populations.

Arboreal Habitat Usage

Throughout its range, *O. pumilio* typically uses the forest floor for foraging as well as defending territories. After eggs hatch in the leaf litter, the female will transport the larvae to arboreal water sources such as bromeliads (Savage 2002). Previous research has shown that *O. pumilio* will travel up to 12m into a tree to deposit tadpoles (Young 1979).

In June 2010 on Isla Colon, Bocas del Toro, Panama, I ascended into the canopy of a *Ficus* spp. to 30m and observed an *O. pumilio* on a branch next to the climbing line. This frog examined a small bromeliad before continuing to climb up the branch higher into the canopy. In August 2009, I ascended a different tree to 40m and heard the advertisement call of a male in the tree next to the one I was climbing, at approximately the same level. Further, on both excursions, males could be heard through the entire ascent although height of males could not be determined. Other individuals have been seen as high as 46m into the canopy (J. Maher, ITEC, personal communication). As advertisement calls vary from aggressive calls in this species (Bunnell 1973), frogs heard calling in the canopy presumably were calling to establish a territory and attract mates.

Utilization of canopy resources may be more important to *O. pumilio* than previously thought. Virtually all previous research on this species has taken place at ground level; thus future studies on populations and ecology of *O. pumilio* should include a canopy component to better understand the habitat use of this species.

Bocas del Toro Natural History

The Panamanian isthmus is a unique convergence zone of North American and South American flora and fauna. This isthmus was formed and connected the two continents approximately 2.8 million years ago by the convergence of the Pacific Cocos and Nazca plates, the Caribbean plate, and the Panama microplate (Coates et al. 2005). The subduction of the Cocos plate led to the uplift of the Panamanian isthmus. The Bocas del Toro archipelago first began rising from the depths approximately 12 million years ago from volcanic activity in the region which stopped approximately 8 million years ago (Coates et al. 2003); the effects of this volcanic activity did not manifest themselves until approximately 1.6 million years ago when sea levels began to change (Collins et al. 1995). Fall of the coastal sea levels led to the development of the archipelago. The archipelago, as it is currently, developed within the last 10,000 years.

Approximately 8,900 years ago, Escudo de Veraguas first separated from the rest of the mainland. The earliest separation of the main archipelago from the mainland happened approximately 6,300 years ago when Isla Colon separated from the Soropta peninsula on the mainland. Approximately 1,100 years later, Isla Colon separated from the Bastimentos and Solarte. Bastimentos and Solarte, still together at this point, separated from the mainland 4,700 years ago, and Cayo de Agua separated about 3,400 years ago. The rest of the archipelago including San Cristobal, Isla Popa and the Bastimentos-Solarte separated within the past 1,000 years (Figure 1.3; Anderson and Handley 2002). Given that the area was likely uniform lowland forest for the last 2 million years (Anderson and Handley 2002), extant frog species or their immediate ancestors were likely dispersed throughout the region when sea levels started to rise and separate the islands from one another.

The Bocas del Toro archipelago is now an island chain that consists of dozens of islands, 11 of which support populations of *O. pumilio* (formerly *Dendrobates pumilio*, see Grant et al. 2006). Many islands are mangrove, and as such are not suitable habitat for amphibians. The archipelago and surrounding mainland areas are a mixture of plantation, pasture, secondary forest, and primary forest. People have historically occupied this area in Panama for hundreds of years, since Christopher Columbus first arrived in 1502. The islands are sparsely colonized by people, and the majority of the population can be found in Bocas Town on Isla Colon (with a population of approximately 3,500 people) and Bastimentos Town on Bastimentos. Most of the other islands are inhabited by people, but spread out in indigenous communities or family groups. This spread of people means that the archipelago is relatively heavily used. There is only one national park in the archipelago: Bastimentos National Marine Park, which encompasses the eastern half of the island as well as the surrounding coral reefs. Also in the archipelago is Escudo de Veraguas wildlife refuge at the eastern edge of the province. Unfortunately, this refuge, while home to several endemic species including a sloth and hummingbird, receives minimal protection and is seasonally used by anglers for camping on fishing trips. The rest of the Bocas del Toro archipelago does not receive any legal protection, although in some areas, such as the northern part of Isla Popa, local human communities take action to protect the forest (J. Lawrence, personal observation).

Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipterix* spp. Understory growth varies from forest to forest with some areas being largely clear with few palms as understory plants (e.g., *Chamaedorea* spp. and *Geonoma* spp.) and sub-canopy plants (e.g., *Astrocaryum alatum*) and other areas consisting of thick undergrowth of largely herbaceous plants (e.g., *Dieffenbachia* spp., *Costus* spp., and *Heliconia* spp.). Composition of the forest largely depends on the age of the forest and amount of disturbance it receives. Soil through this region is iron rich, nutrient poor clay with a thin topsoil layer as is typical for tropical regions. Pastureland is largely used for cattle ranching and results in grassy vegetation sometimes with interspersed large trees. Plantations in this area are variable, but include cacao (*Theobroma cacao*), banana (*Musa* spp.), plantain (*Musa* spp.), and teak (*Tectona grandis*) with other crops such as mango (*Mangifera indica*) and papaya (*Carica papaya*) being planted locally. Banana and cacao are the largest crops of this region with mainland areas such as Almirante and Changuinola supporting plantations for commercial growers such as Chiquita™. Small scale, organic, active or abandoned plantations of banana and cacao can actually provide benefit to *O. pumilio* with many of the denser populations occurring in these areas (e.g., Shepherd's Island and Loma Partida). Active management, especially when using pesticides and fungicides, as is typical for commercial operations, ends up reducing suitable habitat for the frogs (J. Lawrence, personal observation).

Seasons in this region, like most lowland neotropical rainforest systems, are mild as compared to temperate seasonality. The first wet season typically occurs from May through August with the other starting in late October and often extending into March (Figure 1.4; STRI 2010). For amphibians, however, this variable availability of water can strongly dictate behavioral and breeding patterns (Gottsberger and Gruber 2004). From 2006 to 2009, Isla Colon received an average of 3,312 mm of precipitation with the majority of it occurring during the wet season (Figure 1.5; STRI 2010). Seasonal temperatures range from 23.2°C to 28.6°C with daily temperatures ranging on average from 28°C during the day to 24.9°C at night. Average daily relative humidity ranges from 75.9% to 88.3%. Temperature tends to peak between 14:00h and 16:00h (which corresponds with average low daily humidity) while humidity peaks in early morning between 02:00h and 04:00h. The lowest temperatures in the day occur between 04:00h and 06:00h (Figure 1.6). Frogs were most active early in the morning until approximately 14:00h when calling and sightings would gradually diminish. Additionally, if forest humidity dropped below 75% (Figure 1.7A) or temperatures exceeded 29°C (Figure 1.7B), the number of males calling and visual encounters would decrease drastically.

APPENDIX 1

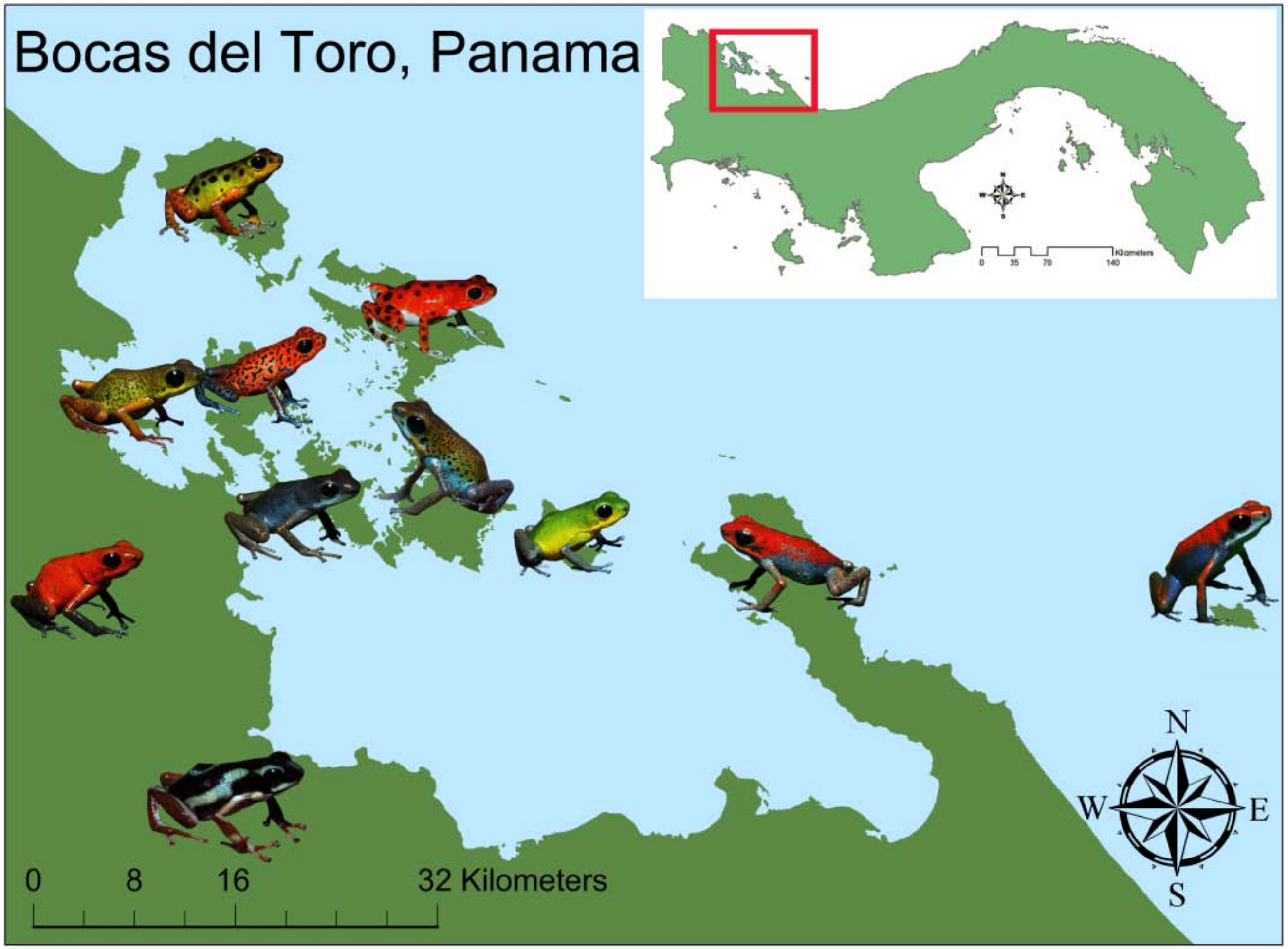

Figure 1.1: Spread of some of the different morphs of *O. pumilio* through the Bocas del Toro archipelago. Populations show high variability on islands and surrounding mainland areas in color, snout-vent length, and behavior. Given the relatively short amount of time these populations have had in isolation (Anderson and Handley 2002), this species has diverged very quickly. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

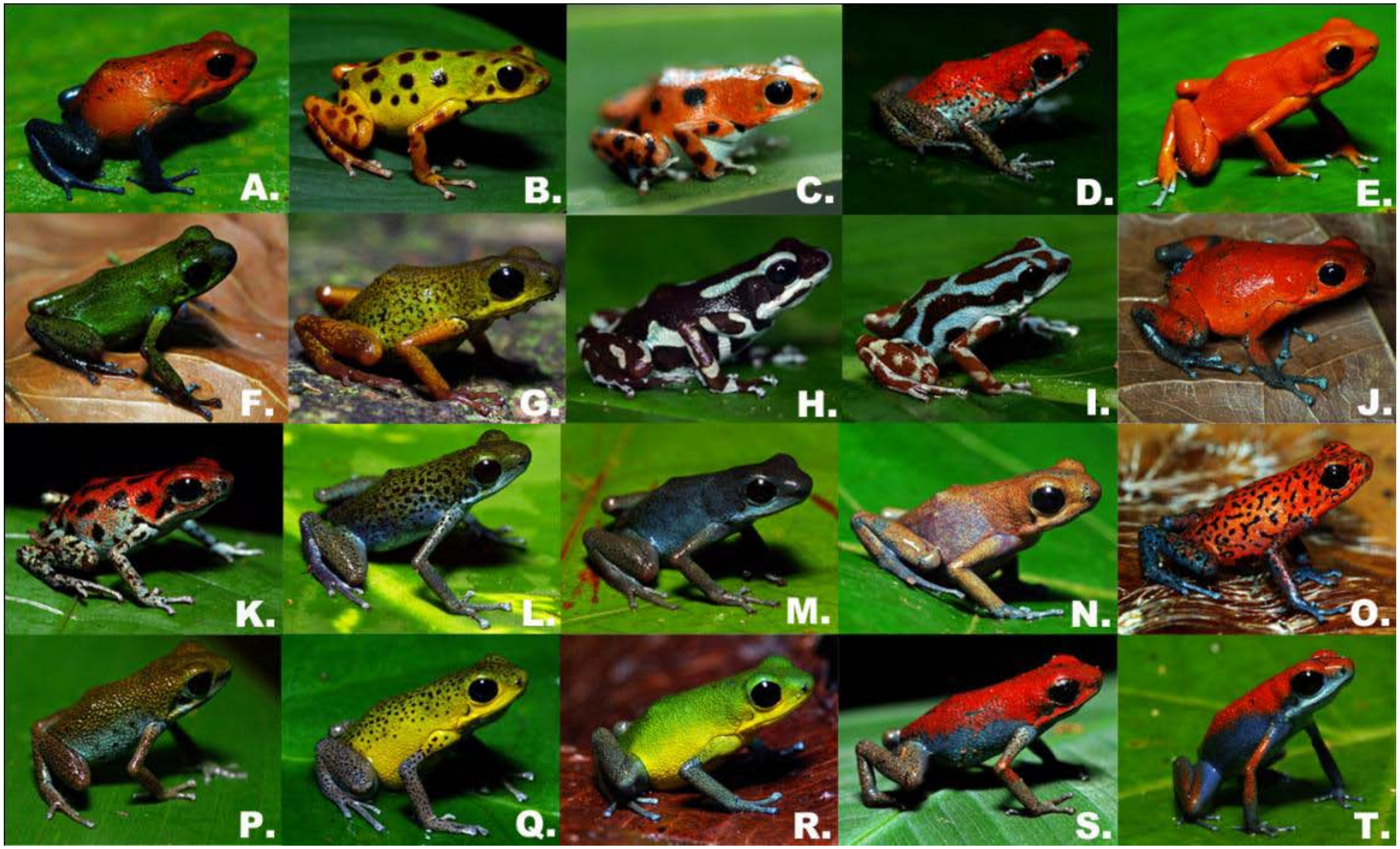

Figure 1.2: A variety of morphs from throughout the *Oophaga pumilio* range. A. Mainland Costa Rica, B. Isla Colon, C. Western Bastimentos, D. Bastimentos – Salt Creek, E. Solarte, F. Pelican Key, G. Shepherd's Island, H. Robalo River, I. Uyama River, J. Almirante, K. "Nicki," L. Loma Partida, M. Aguacate Peninsula – Cerro Brujo, N. Aguacate Peninsula – Dolphin Bay, O. San Cristobal, P. Isla Popa – North, Q. Isla Popa – South, R. Cayo de Agua, S. Valiente Peninsula, and T. Escudo de Veraguas. Most populations through the range look similar to the Costa Rican mainland population with a red body and blue/black legs.

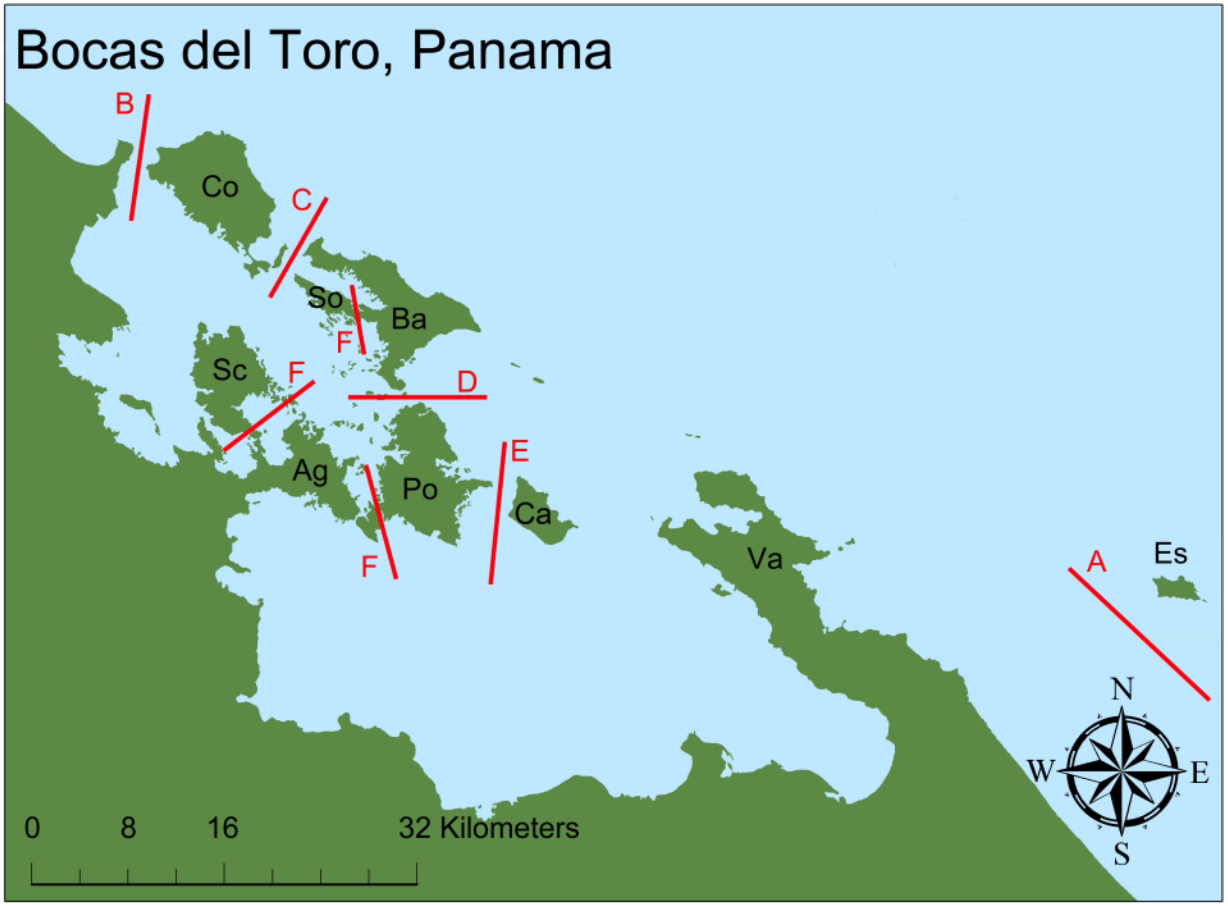

Figure 1.3: Development of the Bocas del Toro archipelago over the last 10,000 years. A. 8,900 years BP Escudo de Veraguas (Es) separates from the mainland Valiente peninsula (Va), B. Isla Colon (Co) separates from the Soropta Peninsula 6,300 years BP creating a peninsula, C. 5,200 years BP, Isla Colon separates from the rest of the peninsula, D. Bastimentos (Ba) and Solarte (So), still connected, separate from the peninsula 4,700 years BP, E. 3,400 years BP Cayo de Agua (Ca) separates from the Aguacate (Ag), F. within the last 1,000 years, Solarte separates from Bastimentos and San Cristobal (Sc),and Popa (Po) both separate from the Aguacate peninsula. Adapted from Anderson and Handley (2002).

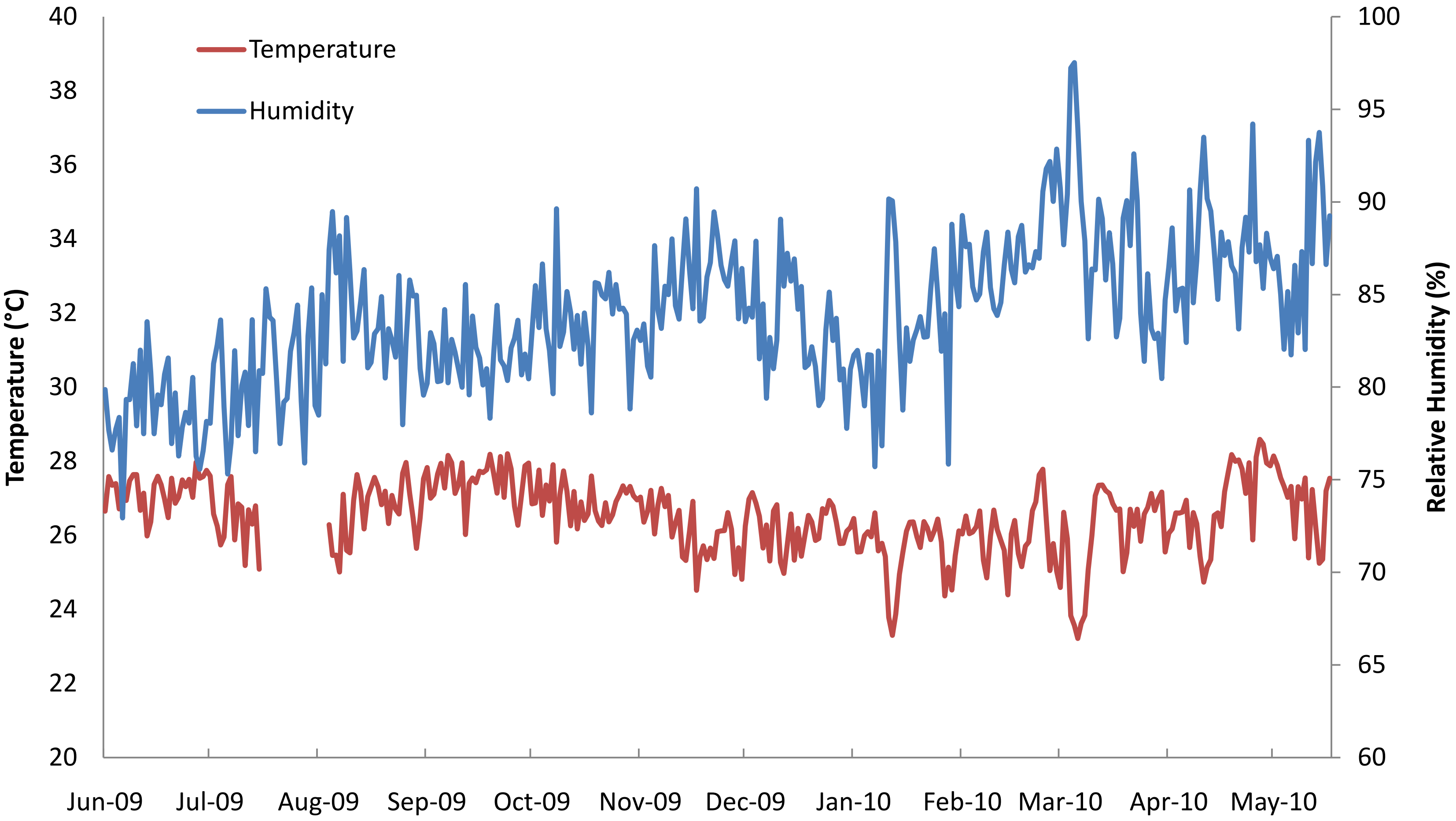

Figure 1.4: Daily temperature and relative humidity variation from the Smithsonian Tropical Research Institute's Bocas del Toro weather station from June, 1 2009 to May 18, 2010 which were all of the available data. The temperature sensor failed from July 16, 2009 through August 3, 2009, so those data were omitted (STRI 2010).

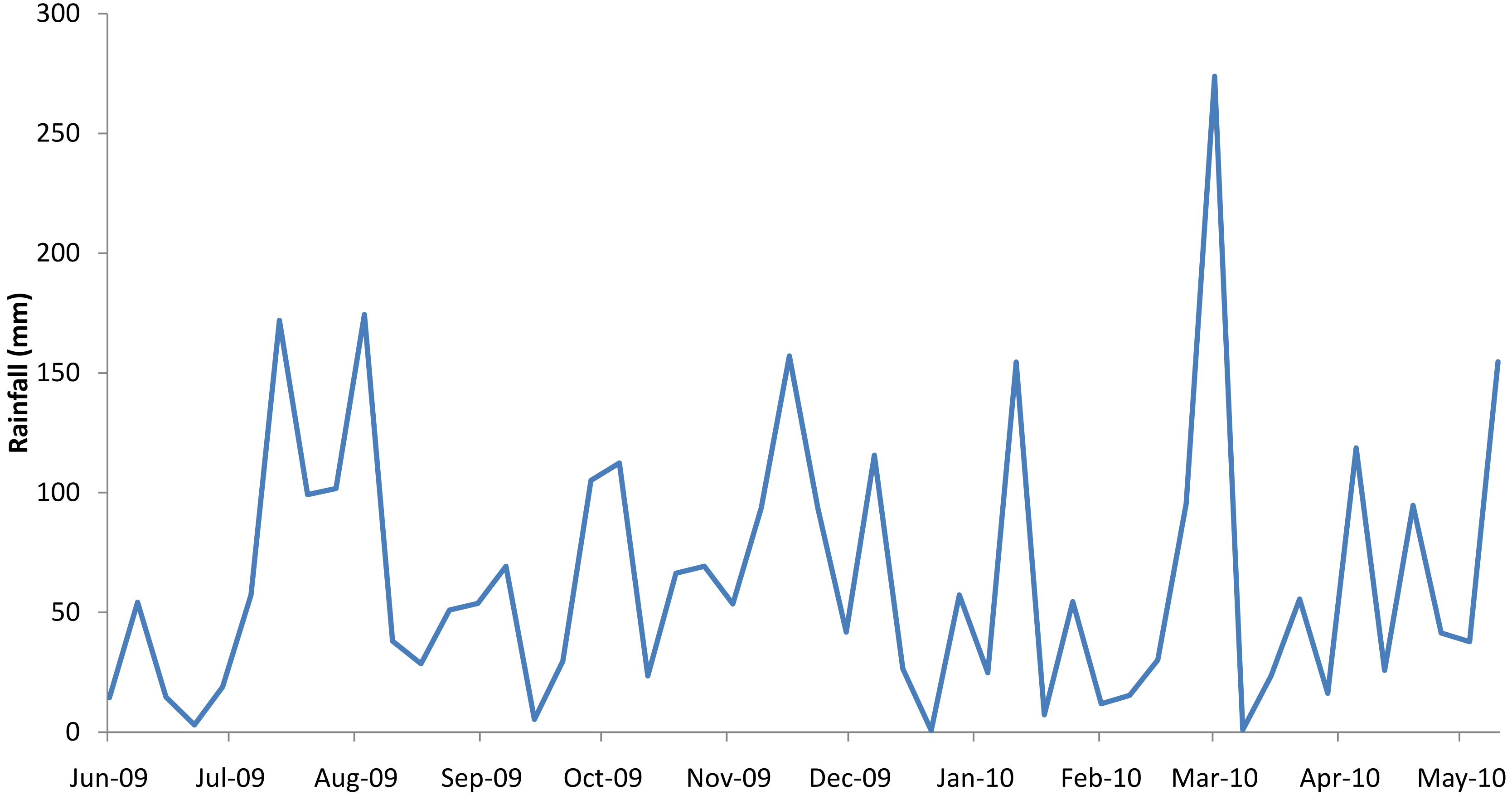

Figure 1.5: Weekly rainfall accumulations from the Smithsonian Tropical Research Institute's Bocas del Toro weather station from June 1, 2009 to May 18, 2010 which were all of the available data. Peaks in rainfall occurred in July-August 2009, November-December 2009, January 2010, and March 2010 (STRI 2010).

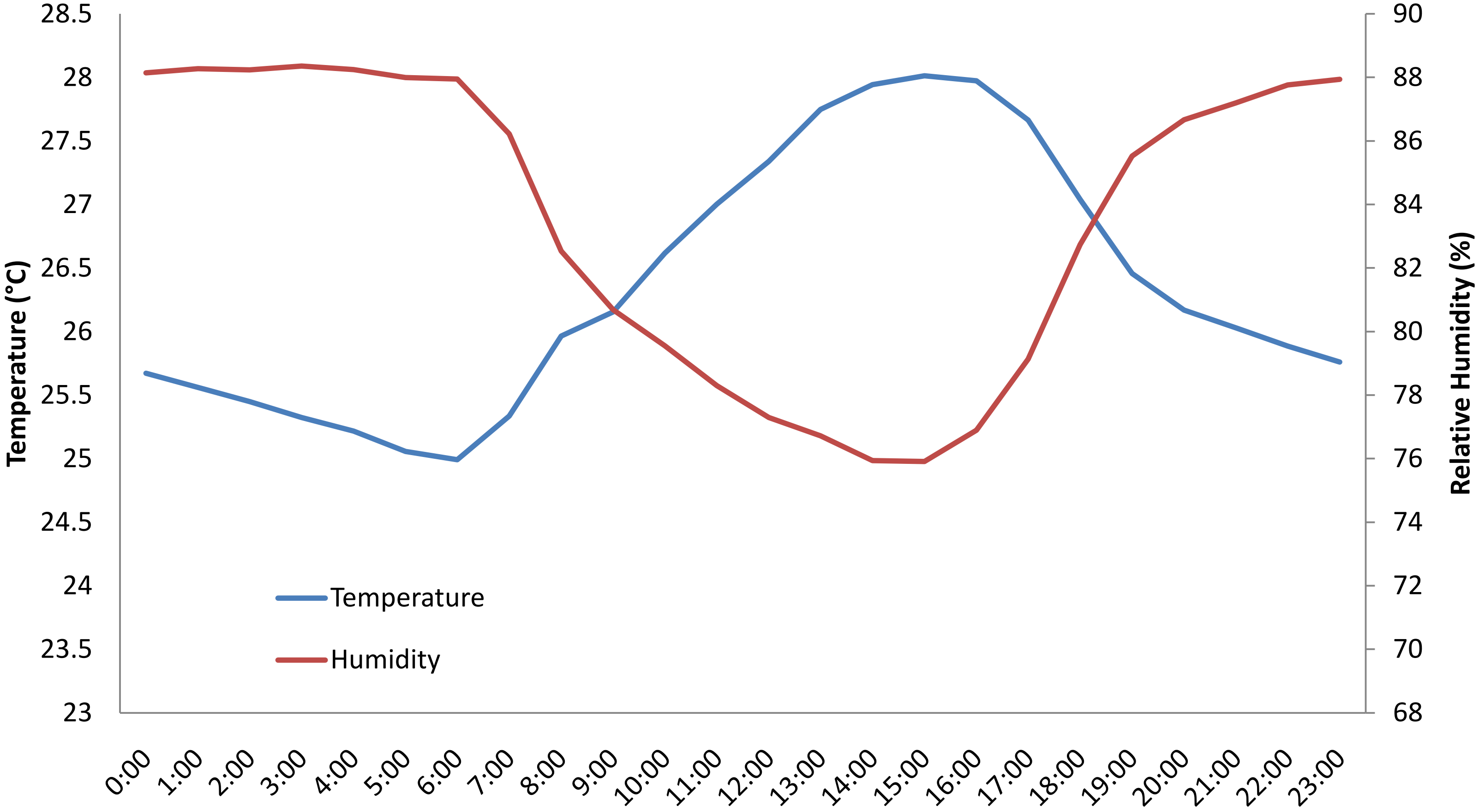

Figure 1.6: Average daily variation of temperature and relative humidity over the study period (STRI 2010). Temperature and humidity show inverse relationships, and frog breeding patterns are dictated by humidity variability during the day. Most activity in frog calling appears to occur early in the day when humidity is highest and tapers off as humidity drops.

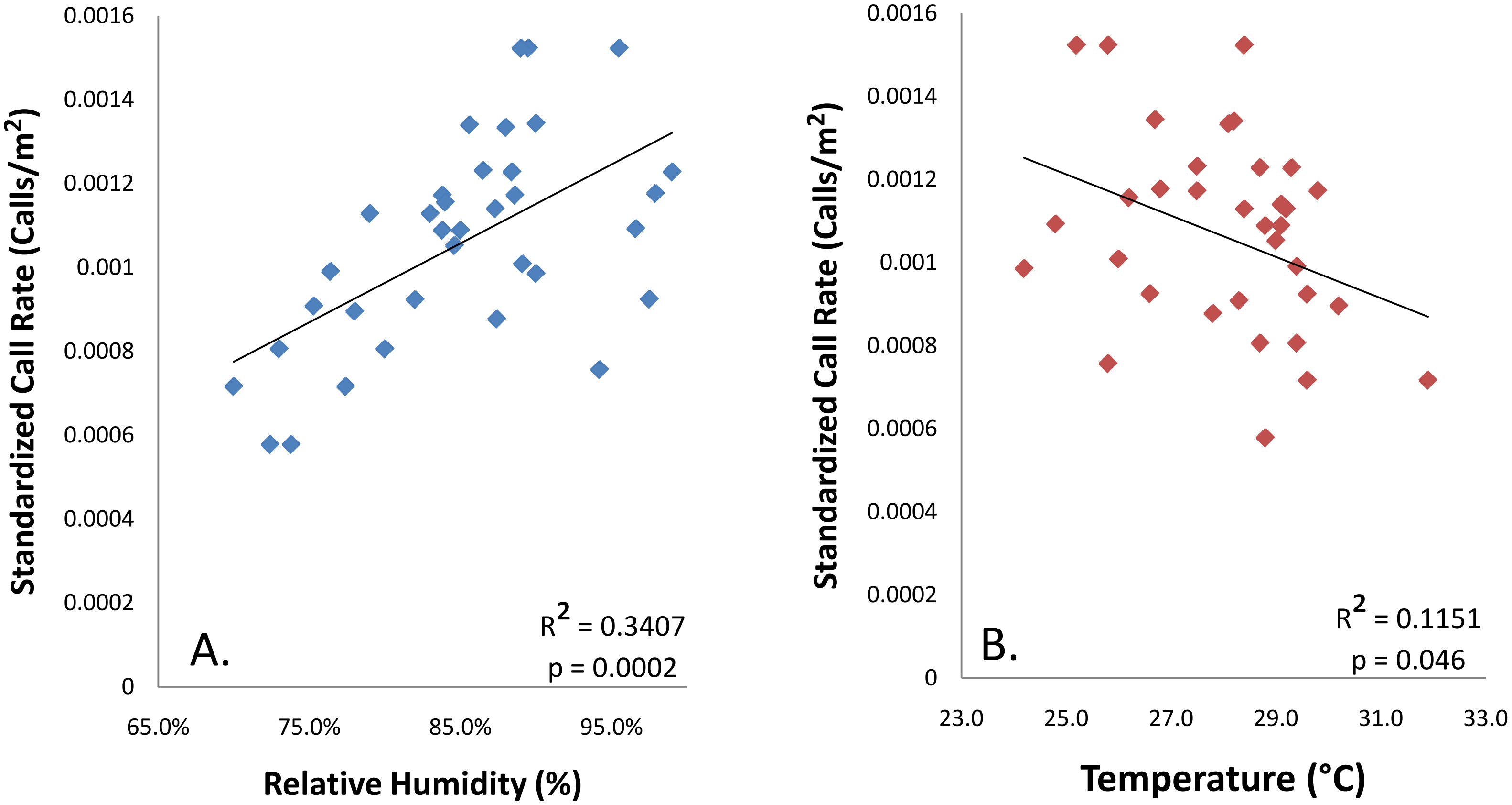

Figure 1.7: Effects of both relative humidity (A.) and temperature (B.) on the standardized call rate (call rate in relation to SVL of respective population male averages) for *Oophaga pumilio* populations. A simple linear regression was conducted for both and humidity was found to be a very accurate predictor for calling density (p=0.0002) while temperature was found to be a moderate predictor (p=0.046).

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CHAPTER 2 : POPULATION ESTIMATES AND SPATIAL DISTRIBUTIONS OF *OOPHAGA PUMILIO* IN A FOREST EDGE LANDSCAPE

Abstract

Conservation of amphibian species has been of great interest to resource managers in recent years. Success or failure of these conservation efforts often depends on the ecological knowledge about the species of interest. Among the most important characteristics to understand are the availability of habitat and how species of interest utilize the habitat. *Oophaga pumilio* is a small species of poison dart frog found throughout the Caribbean lowland rainforests of Nicaragua, Costa Rica, and Panama. This species can be found throughout closed canopy rainforest in these areas. The Bocas del Toro archipelago in Panama has historically had a sustained amount of deforestation from agriculture and tourism, but this has not yet been quantified. Using a normalized difference vegetation index (NDVI), I assessed the available habitat to *O. pumilio* for nine different frog populations using 1986 and 1999 LANDSAT images. I classified five different habitat types: colonized, pasture, plantation, mature forest, and other. Colonized, pasture, and plantation are habitat types associated with human development, and all showed increases over the study period. Mature forest, however, decreased on average 9.5% over the 13 years for the nine areas. In addition to habitat analysis, I estimated densities and reactions to forest edge of frogs for each of these nine populations using transect methods. Densities varied from 239 frogs/ha to 1351 frog/ha. Most populations did not show negative reactions to forest edge, but three (Isla Colon, Pastores, and Loma Partida) did show a negative reaction to forest edge with more frogs found in interior forest as opposed to near forest edge. Populations of *O. pumilio* show high variability in available habitat, population density, and

reaction to forest edge. Consequently, conservation efforts of this species should happen at the population-level as opposed to species-level.

Introduction

Conservation of species depends on a number of factors including person power, money, public support, and sound ecological knowledge of the species of interest. While each of these factors can be problematic for managers for a variety of reasons, ecological knowledge of the species of interest is vitally necessary to make responsible decisions in the best interest of conserving the species into the future. Historically, this lack of knowledge can hindered conservation decisions. Further, ecological knowledge in some cases is not only necessary at the species-wide level, but is necessary where distinct and diverse populations exist (e.g., Southeast Asian Tigers, *Panthera tigris*; Lynam 2010). Population-level ecological data are often more important than that of species-level data due to the increased likelihood that these populations are extirpated for a variety of reasons (e.g., habitat destruction, disease, poaching/harvest).

While there are many causes to declines and extinctions, habitat destruction is widely considered to be the single most important factor leading to population declines and extinctions across many taxa (Harris and Pimm 2004, Venter et al. 2006, Trail 2007, Sodhi et al. 2008). In recent years, researchers have realized the impact of fragmentation of landscapes to be as damaging as outright destruction (Goosem 2007). This fragmentation can reduce the ability of animals to migrate across their range (Collingham and Huntley 2000), cause variable sensitivity towards edges (Ries and Sisk 2010), and reduce the available habitat for habitat specialists (Devictor et al. 2008). These effects of fragmentation are well known for large vertebrates that are migratory or have large home ranges (Eizirik et al. 2001, Kinnaird et al. 2003), but the effects of fragmentation on smaller species such as many reptiles and amphibians, are less well known and often overlooked. This is of particular concern due to the proclivity of tropical species to have very small ranges (Stevens 1989), especially if they occur on islands or mountains (Orme et al. 2006). Even moderate amounts of habitat destruction that may not have large impacts on larger fauna can have disastrous effects on herpetofauna.

Both amphibians and reptiles are showing declines in tropical areas (Whitfield et al. 2007). Amphibian decline in particular has garnered a great amount of interest from researchers (Lips et al. 2005, Pounds et al. 2006, Sodhi et al. 2008), possibly for the disturbing amount of decline seen in amphibians. Stuart et al. (2004) found 43.2% of amphibian species are declining with 32.5% of species considered to be globally threatened. This is far greater than the declines seen in mammals or bird species that are declining (Stuart et al. 2004), making amphibians arguably the most threatened vertebrate group in the world. Even more concerning is that 29.1% of the 6,810 amphibian species in the world are lacking any data on species trends (Stuart et al. 2004). Consequently, in recent years, we have seen a great deal of emphasis on the conservation of amphibian species. While 29.1% of amphibian species lacking data on species-level trends is concerning, many species with species-level data have phenotypically distinct populations that lack any data on population-level trends. To conserve biological diversity, conservationists must seek to conserve as much genetic diversity as possible within a species (Hanski 1998), which especially includes the conservation of diverse and distinct populations within the species.

The Strawberry Poison Dart Frog (*Oophaga pumilio*) is a small, terrestrial frog that exhibits a massive divergence of color pattern in the Bocas del Toro archipelago in Panama. This divergence of color has been of great interest to researchers for decades, primarily for the implications it has for the evolution of aposematic signals (Summers et al. 1999, Siddiqi et al. 2004, Maan and Cummings 2008). While the majority of the focus has dealt with the evolution of the species, relatively little has focused on the ecological information associated with this species. Densities of *O. pumilio* in La Selva, Costa Rica were 827 frogs/ha (Savage 2002), but no estimates for the rest of the range of the species have been done, which is of concern since species do not necessarily maintain a constant density throughout their range. This species has been collected in large numbers in recent years (Nijman and Shepherd 2010), and due to the "Least Concern" conservation status of the species (Sólis et al. 2008), management of the species or individual populations is of minimal concern. Consequently, depending on the density of individual populations and rate of habitat loss, extirpation of phenotypically unique populations is likely in the near future. To execute any effective conservation programs, ecological data such as population density, available habitat, and rate of habitat loss are necessary for management.

To assess vulnerability of frog populations in the Bocas del Toro region, I assessed the extent of available habitat to nine different *O. pumilio* populations. Additionally, to examine if negative effects of deforestation to this species, I examined relationships to edges. Fragmentation is unlikely to inhibit migration due to the territoriality of *O. pumilio*, but fragmentation could result in negative reactions to forest edges. Such effects would likely be fewer frogs found around a forest edge as opposed to forest interior, for a variety of reasons, from invasion of opportunistic edge species that may prey on frogs or compete with them to microclimate changes that are not favorable to frogs.

Study Area

I conducted this study from June 2009 to August 2009 on nine different populations in the Bocas del Toro archipelago in Panama. The Bocas del Toro archipelago is a series of islands in the Caribbean on the western side of Panama.

Seasons in the Bocas del Toro archipelago are subdued with the wet season typically occurring from May through August and another spike of rain fall in October and November (STRI 2010). Over the course of a year, Isla Colon, the largest island and where the Smithsonian Tropical Research Institute’s (STRI) weather station is located, receives an average of 3,312mm of precipitation with the majority of it occurring during the wet season (STRI 2010). Seasonal temperatures range from 23.2°C to 28.6°C with daily temperatures ranging on average from 28°C during the day to 24.9°C at night. Average daily relative humidity ranges from 75.9% to 88.3%. Temperature tends to peak between 12:00h and 13:00h while humidity peaks in early evening between 21:00h and 22:00h (J. Lawrence, unpubl. data). Frogs were most active early in the morning until approximately 14:00h when calling and sightings would gradually diminish. Additionally, if forest temperatures exceeded 29°C or humidity dropped below 75%, the number of males calling and visual encounters would decrease significantly (J. Lawrence, personal observation).

The frog populations used for this study were located on Cayo de Agua, Isla Colon, San Cristobal, Loma Partida, Pastores, Popa North, and Solarte islands with Almirante and Uyama River representing mainland areas (Table 2.1 and Table 2.2). While some areas have multiple morphs (e.g., Bastimentos island), geographic barriers are not obvious within such areas. Consequently, I could only define frog populations by the geographic barriers present.

Almirante

Almirante is a mainland area extending from the town of Almirante approximately 17km along the coast of Almirante Bay. The resident human population of the town of Almirante is approximately 8,000 with many individual settlements occurring along the coast of the bay. Almirante is now primarily a town for workers of banana plantations in surrounding areas Soil in all of the transects was nutrient poor clay with a thin topsoil layer. All transects were done on a slope that also had interspersed granite boulders throughout the area. Study areas were actively managed as a cacao plantation..

Oophaga pumilio of this population are often associated with ground substrate, but are often found on or near tree bases. Frogs are often found in leaf litter (J. Lawrence, personal observation).

Cayo de Agua

Cayo de Agua is a moderate sized island with a small resident human population which scattered throughout the island and in a couple of small communities. Forest is a mixture of primary and secondary forest with clearing for plantations becoming more and more prevalent. Soil in all of the transects was nutrient poor clay with a thin topsoil layer. Study areas were forest on the edge of a small community who maintained the forest.

Oophaga pumilio of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation).

Isla Colon

Isla Colon, the largest and most populated island in the Bocas del Toro archipelago, with a resident human population of about 3,500; the majority of which live in the main town located on southeast side of the island. In recent years, an increase of pastureland has resulted in fragmented landscapes throughout the island. Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were undisturbed forest on communal land along a path to a small community and areas were continually maintained as undisturbed forest for research of undergraduate classes by the Institute for Tropical Ecology and Conservation (ITEC).

Oophaga pumilio on this island are most often associated with vertical substrate, often being found on or near tree bases. Frogs are very rarely found in leaf litter, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation).

San Cristobal

San Cristobal is a large island with a small resident human population which scattered throughout the island and in a couple of small communities. San Cristobal is heavily fragmented and used for pastureland. Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were forest on the edge of active pasture for a small community of about a dozen people.

Oophaga pumilio of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation).

Loma Partida

Loma Partida is a small island with a small resident human population which scattered throughout the coasts of the island and in a couple of small communities. Soil in all of the transects was nutrient poor clay with a thin topsoil layer. Study areas were active cacao plantation on the edge of a small community.

Oophaga pumilio of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation). Frogs could actively be found traversing through the thick leaf litter layer.

Pastores

Pastores, the smallest island examined in this study with a very small resident human population that is scattered primarily along the coast of the island. Pastores is primarily dominated by cacao plantation with some lowland rainforest remaining. Soil in all of the transects was nutrient poor clay with a thin topsoil layer. Study areas were unused cacao plantation.

Oophaga pumilio of this population are often associated with ground substrate, but are often found on or near tree bases. Frogs are commonly found in leaf litter (J. Lawrence, personal observation). Frogs in this population, unlike others, appear to be rather gregarious with many individuals being found in the same area.

Popa North

Popa is a large island, with the northern portion having a resident human population scattered throughout the island and in a couple of small communities. Popa is constricted in the center of the island and actually has two separate morphs of *O. pumilio* and for this study, I only examined the northern population. Soil in all of the transects was nutrient poor clay with a thin topsoil layer. Study areas were forest on the edge of a small community as well as on the edge of a resort. Forests in both areas were actively maintained as intact rainforest.

Oophaga pumilio of this area are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation).

Solarte

Solarte has a small resident human population which is scattered primarily along the coast of the island. Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were continually maintained as undisturbed forest for by a private land owner (W. Stevens, personal communication).

Oophaga pumilio of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found in leaf litter, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation).

Uyama River

Uyama River is a mainland area extending from the Uyama River towards Almirante approximately 7km along the coast of Almirante Bay. This area encompasses the Uyama River as well as smaller streams that drain into Almirante Bay. The resident human population of Uyama River is scattered in individual settlements occurring along the coast of the bay. Uyama River is a mixture of primary and secondary lowland rainforest as well as teak and cacao plantations, very similar to Almirante. Soil in all of the transects was nutrient poor clay with a thin topsoil layer. All transects were done on a moderately steep slope that also had interspersed granite boulders throughout the area. Study areas were cleared of much of the undergrowth by local residents.

Oophaga pumilio of this area are often associated with ground substrate, but are often found on or near tree bases. Frogs are often found in leaf litter (J. Lawrence, personal observation).

Methods

Population Density Estimates

To estimate population density, ten 100m transect were used for each of eight population; only five transects were used for the Cayo de Agua population due to inaccessibility later in the study. Transects started at the edge of a forest and continued into the fragment 100m ending in the forest interior; a similar method is used to estimate edge effects on bird populations (Manu et al. 2007). Birds are far more mobile than small amphibians due to the ability to fly, therefore rather than perform the typical 400m-1600m transects for birds, I used 100m transects. Transects were at least 50m from one another and ran parallel to one another. Prior to beginning, preliminary data were taken on weather conditions, time, GPS location, habitat classification, and time since last rain. At the end of the transect, time and weather conditions were taken. Perpendicular distance to visible frogs was recorded. Given the conspicuousness of the frogs, the likelihood of observing most of the frogs close to the transect, and the reduced visibility further from of observing frogs the transect due to vegetation obscuring view, a half-normal method sighting function was deemed most appropriate for estimating population densities. Based on these estimates for each transect, I conducted an ANOVA ($\alpha = 0.05$) to assess whether or not mean population density was different between the nine populations observed.

As frogs were encountered, I recorded standard demographic data (i.e., snout-vent length, sex, body mass) and made note of anything unusual features on the frogs. The presence of gular patches or gular folds was used to differentiate males from females. Sex was not recorded for juveniles. Juveniles were defined as any frog that was 15% less in length than the average for the population of interest. Unusual features included missing digits, limbs, scars, or unusual colors. Such features may provide evidence as to predation pressure on a population as well as understanding population variability.

To analyze the effects of edges on each population, I grouped frogs found on transects in five meter intervals from 0m to 100m and summed each interval for all transects. I converted the number of frogs to the proportion of frogs found at each distance for each population. Using a simple linear regression for each population, I determined any relationship between distance from edge to the proportion of frogs found on the transects.

GIS Analysis

To determine the amount of available habitat in the Bocas del Toro region, LANDSAT TM 4-5 images from November 1986 and January 1999 were used. No later images were available due to damage incurred on the LANDSAT satellites. I analyzed the images using ArcMap in ArcGIS 9.2 (ESRI 1999-2006).

I used a Normalized Difference Vegetation Index (NDVI) to determine habitat types by comparing differences of near-infrared (Band 3; 0.63-0.69 μm) and visible red (Band 4; 0.76-0.90 μm) light across the landscape (Equation 2.1) to look at photosynthetic output of vegetation in the region.

Equation 2.1: $$NDVI = \frac{\lambda_{NearInfrared} - \lambda_{\mathrm{Re}\,d}}{\lambda_{NearInfrared} + \lambda_{\mathrm{Re}\,d}}$$

Vegetation types were ground-truthed from data points through Panama. Vegetation types were defined as colonized (NDVI: 0 to 0.22), pasture (NDVI: 0.23 to 0.52), plantation/secondary growth (NDVI: 0.53 to 0.76), mature forest (NDVI: 0.53 to 0.76), and other (NDVI: 0.77 to 1). Due to overlap of NDVI values in plantation and mature forest habitat types, I used Band 5 (1.55-1.75 μm), which is sensitive to water turgidity, to separate the two habitats. I took standard deviations of each cell with surrounding cells to discern the range of standard deviations in the two habitat types. Since plantations would be more homogeneous in plant growth, standard deviation values would be lower (1.05-8.13) than that of a mature forest which will show heterogeneous growth (8.14-16.72). Plantations, such as banana plantations, could not be separated from new forest growth due to similar photosynthetic values. The “other” category had high photosynthetic values, but it was unclear what these values represented, although I hypothesize that it could be a fast growing annual crop grown by local farmers due to the sporadic spread of the vegetation types.

While *O. pumilio* exhibits great capacity to capitalize on disturbed habitats such as organic cacao or banana plantations (J. Lawrence, personal observation), the available habitat estimates only used the mature forest habitat type because many plantations, especially those comprising the majority of the plantation/secondary growth value, rely heavily on pesticide and fungicide use, and therefore are not adequate habitat for amphibians.

To estimate population density of each of the nine populations, I identified each population's geographic boundary. In the case of the island populations, the whole island was defined as the geographic boundary with the exception of Isla Popa. Isla Popa has two phenotypically distinct populations (Figure 2.1) that vary both in color and in size. I separated the two populations at the constriction at the center of the island which likely acted as a barrier between the two populations when sea levels were higher in the region (Anderson and Handley 2002). The mainland populations were separated based on geographic features of the landscape that likely acted as barriers to limit gene flow between populations. These features included mountain ridges and rivers. *Oophaga pumilio* is a lowland species (Lötters et al. 2007), so rises in elevation likely act as barriers. While *O. pumilio* has the capability of crossing rivers, the ability for a 2cm frog to cross large rivers is likely minimal, at best, so these also likely act as barriers for populations. The Almirante population is likely continuous between the Changuinola River and a ridge of small mountains that extends partially onto the Aguacate peninsula (Figure 2.2). This ridge peaks at 400-500m, which is low enough for emigration of individuals from the Almirante and neighboring Uyama population, but likely acts as an effective barrier for the two phenotypically distinct populations as red frogs occur north of this ridge and mottled black frogs occur south of this ridge. The Uyama population was defined by the ridge to the Uyama River (Figure 2.2). No geographic barrier could be detected on the images, so it is unknown what barrier would be between the Uyama population and the Aguacate peninsula populations; since these are phenotypically distinct and it is known that blue Aguacate frogs occur relatively close to the mainland, I arbitrarily determined that edge of the population to be where the peninsula meets the rest of the mainland. To the southwestern side of the Almirante population and the Uyama population is Cordillera de Talamanca Mountains which acts as a barrier for the species to spread south. *Oophaga pumilio* are known to reach up to 700m in elevation (Lötters et al. 2007), so I defined 600m as the elevation barrier on the southwestern side of these populations to be conservative about estimates.

Once geographic barriers were defined, respective population density estimates were compared to the amount of mature forest available to each population to discern approximate population sizes for each population.

Results

Habitat Analysis

Area of islands and mainland areas that hosted populations of interest ranged from as small as 22.61ha (Pastores) to as large as 612.8ha (Isla Colon), while the Almirante and Uyama areas were both much larger than those of the islands (4,035.5ha and 1,892.6ha, respectively; Table 2.3). Clouds partially obscured part of Isla Colon in the 1999 image, so for consistency in changes between years, I excluded the same area covered by clouds in the 1986 image (Figure 2.3). GIS analysis exposed fairly consistent trends in all areas examined. With the exception of Cayo de Agua, all areas showed net increases of both the urban and pasture habitats. Due to size, the Almirante area dominated in all categories for increases and losses of habitats. For island populations, however, increases were as high as 2.6ha of colonized area on Solarte and 88.26ha of pastureland on Isla Colon. Cayo de Agua showed net losses in either of these habitats. All areas examined showed net increase in the plantation/secondary forest habitat, with the highest increase being 5.53ha on Isla Colon. All areas examined show a net loss of mature forest, which was deemed to be the only suitable habitat of those examined for *O. pumilio,* with the exception of Cayo de Agua, which showed a small increase of mature forest (0.32ha; Table 2.4). The "other" habitat type showed no consistent trend as it increased in some areas, decreased in others, or remained unchanged.

Across the region, the urban, pasture, and plantation habitat types exhibited yearly increases from 1986 levels. Over the 13 year span, a 0.99% per year increase was observed in the colonized habitat type across the region, but the greatest increase was observed on Uyama which showed an increase of 2.92% per year from 1986 levels. Pastureland increased, on average, 3.45% across the region, with the largest increase seen on Isla Colon at 7.61% per year, followed closely by San Cristobal at 6.5% per year, the two largest islands examined (Table 2.4). The plantation habitat type showed the largest increase of those examine at an average of 14.84% per year, but this is largely bolstered by a large increase of 60.97% per year on Cayo de Agua, but large increases were also seen on Solarte (28.7%) and Isla Colon (22.11%). Mature forest was the only habitat type that showed a decrease per year from 1986 levels, on average 0.73% loss per year with the greatest loss seen on San Cristobal of 1.56% per year followed closely by Solarte at 1.42% loss per year. As stated above, Cayo de Agua was the only area that showed a net increase of mature forest at 0.02% per year from 1986 levels. The "other" category remained unchanged (Cayo de Agua and Popa North) or showed a loss, on average 5.04% with the largest losses seen in 7.69% in Isla Colon and Solarte.

Population Density Estimates

Populations were highly variable in the density of frogs with densities, on average, ranging from as low as 2.39 ± 0.72 frogs/100m^2 in the Uyama population to as high as 13.52 ± 3.14 frogs/100m^2 in the Pastores population (Table 2.5). I did not detect any frogs in one transect in the Uyama and one in the Popa North population which were among the least dense populations. As would be expected with these large differences in populations, an ANOVA confirmed that there was a difference in population densities ($F_{8,\ 74} = 4.714$, $p = 0.0001$). I ran a *post-hoc* Tukey's HSD test to examine relationships between populations. Pastores was found to be different from Almirante ($p = 0.002$), Isla Colon ($p = 0.0001$), Popa North ($p = 0.002$), and Uyama ($p = 0.0001$, Table 2.5).

Edge Effects

Populations showed variable reactions to proximity to edge. Interestingly, both Almirante and Cayo de Agua showed a negative relationship to increased distance from edge, although neither of these relationships varied from zero ($p = 0.242$ and $p = 0.542$, respectively; Figure 2.4). All other populations showed positive relationships to increased distance from forest edges with Isla Colon, Loma Partida, and Pastores varying from zero ($p = 0.016$, $p = 0.014$, and $p = 0.006$, respectively; Figure 2.4).

Discussion

Results from this study represent the first estimates of individual population densities of *O. pumilio* through the Bocas del Toro archipelago. Densities of frogs varied greatly between populations from as 239 ± 72 frogs/ha in Uyama to 1352 ± 314 frogs/ha in the Pastores population. Numbers on average, however, were lower than the Costa Rican estimates of 827 frogs/ha (Savage 2002), that causes concern and demonstrates the need for conservation of individual populations. Pastores is an interesting population because it had the highest density of frogs of the populations examine, but it also had the smallest area (22.59ha) of those examined (with only 16.08ha of available habitat; Table 2.3). The island, for unknown reasons, has the capacity to support a large number of frogs, but due to the small size of the island, I would deem this population of particular concern for extirpation. Pelican Key, a neighboring smaller island next to Pastores, likely has similar density of frogs as Pastores (J. Lawrence, personal observation), but is far smaller than Pastores (only 2.87ha with 1.53ha of available habitat). A large amount of the forest has been cut and that which remains is on the steep slopes on the island. Unfortunately, at the current rate of deforestation of this island (J. Lawrence, personal observation), despite the high density of frogs, this morph is likely to be extirpated in the near future. While Pastores does not seem to be in threat of as rapid deforestation, this population could easily be lost if the rate of habitat loss is not closely monitored.

Interestingly, populations showed variable reactions to forest edges. Most populations did not appear to show any reaction to edges, which would be expected as this species could be considered a habitat generalist as evident from the broad range of habitat types and levels of disturbance it will tolerate. Isla Colon, Loma Partida, and Pastores all showed, however, some reaction to edges, with the Pastores population showing the greatest reactions to distance from edges (p = 0.006). This negative relationship to forest edge is of particular concern for these populations as Pastores and Loma Partida are the two smallest islands examined (22.6ha and 38.1ha respectively), and also are among the smallest islands in the archipelago where frogs exist. A modest amount of deforestation of mature forest in these areas can result in much larger negative effects than in other populations. Isla Colon is the largest island (612.8ha) in the archipelago as well as the most populated. Between 1986 and 1999, Isla Colon lost 7.33ha per year of mature forest while gaining 6.79ha per year of pastureland some of the highest rates of gain and loss seen in the region (Table 2.4). This population also exhibited a moderate level of population density at 6.81 frogs per hectare. There appears to be no apparent reason as to why these three populations would exhibit reactions to edges, and the other populations would not, but coupled with the threat of deforestation on small islands or on islands with high human populations, the conservation of these populations is important.

Mature forest habitat loss was seen throughout the region with the exception of Cayo de Agua. In general, the urban, pastureland, and plantation habitat types showed increases across the region (with Cayo de Agua showing decreases in urban and pastureland), which is what would be expected as the region increases in human population. Unfortunately, the increase comes at the expense of mature forest habitat which is the only suitable habitat of those examined for *O. pumilio* populations, as well as other rainforest species. With the increasing popularity of the Bocas del Toro archipelago as a tourist destination, these trends likely have continued beyond 1999, although satellite images were not available for a more recent analysis of trends. If the rate of increase seen from the 1986-1999 period continues, half of the mature forests in the region can be expected to be lost by 2050, with some of the areas, such as Isla Colon, likely being deforested at a much greater rate.

Conservation of *O. pumilio* is likely of low priority for a variety of reasons including the "Least Concern" conservation status by the IUCN (Sólis et al. 2008). Among these reasons is the fact that individual populations have not had official subspecies designations, and have been referred to as morphs or populations. Subspecies recognition can often allow for management of distinct populations as seen in Tigers (*Panthera tigris*; Lynam 2010), Copper-Bellied Water Snakes (*Nerodia erythrogaster*; Bufalino and Scott 2008), and Galapagos Tortoises (*Geochelone nigra*; Caccone et al. 1999). Distinct, but unrecognized populations are far more difficult for management agencies to justify for conservation. Island *O. pumilio* populations are excellent candidates for subspecies designation due to their geographic isolation from each other, as has been seen in other species (e.g., Galapagos Tortoises). Mainland populations, equally, should have subspecies designations to allow for more effective conservation programs. These mainland populations, admittedly, can yield problems for subspecies designations due to intergrade zones between populations. The Aguacate peninsula, for example, appears to be an intergrade zone ranging from blue at the northern extent and gradually changing to color forms similar to that of the Almirante population, which occurs at the southern extent of the peninsula. Intergrades, however, do not traditionally inhibit subspecies designations (e.g., Painted Turtles, *Chrysemys picta*; Conant and Collins 1998). For effective management of threatened *O. pumilio* populations, official subspecies designations are warranted for distinct populations.

APPENDIX 2

Table 2.1: Physical conditions of the nine different areas for this study. All descriptions are for areas where transects were done. Canopy species refers to notable canopy species in the areas, and understory species refers to notable understory species in the area. Neither the canopy species nor the understory species are all inclusive, but rather, represent dominant types of plant species in the area.

	Mainland or Island	*Area (Ha)*	*Forest Type*	*Canopy Species*	*Understory Species*
Almirante	Mainland	4035	Primary and Secondary Forest, Cacao and Teak plantations	*Ficus* spp., *Dipteryx oleifera*	*Heliconia* spp.
Cayo de Agua	Island	146.1	Primary and Secondary Forest	*Ficus* spp., *Dipteryx oleifera*	*Dieffenbachia* spp., *Calathea* spp., *Costus* spp.
Isla Colon	Island	612.8	Primary and Secondary Forest	*Ficus* spp., *Dipteryx oleifera*	*Astrocaryum alatum*, *Dieffenbachia* spp., *Calathea* spp., *Costus* spp., *Bromelia pinguin*
San Cristobal	Island	375.6	Primary and Secondary Forest	*Ficus* spp., *Dipteryx oleifera*	*Dieffenbachia* spp., *Calathea* spp., *Costus* spp.
Loma Partida	Island	38.1	Primary and Secondary Forest, Cacao Plantation	*Ficus* spp., *Dipteryx oleifera*	*Dieffenbachia* spp., *Calathea* spp., *Costus* spp.
Pastores	Island	22.6	Primary Forest and Cacao Plantation	*Ficus* spp., *Dipteryx oleifera*	*Heliconia* spp.
Popa North	Island	154.2	Primary and Secondary Forest	*Ficus* spp., *Dipteryx oleifera*	*Dieffenbachia* spp., *Calathea* spp., *Costus* spp.
Solarte	Island	79.3	Primary Forest	*Ficus* spp., *Dipteryx oleifera*	*Dieffenbachia* spp., *Calathea* spp., *Costus* spp., *Heliconia* spp.
Uyama	Mainland	1892	Primary and Secondary Forest, Cacao and Teak plantations	*Ficus* spp., *Dipteryx oleifera*	*Astrocaryum alatum*, *Heliconia* spp.

Table 2.2: Weather conditions for transects over the course of the study period. Mean temperature and mean relative humidity are both means of the transects done in each area. Often, weather conditions were highly variable during transects, so multiple weather conditions are listed for respective locations.

	Mean Temperature (°C)	*Mean Relative Humidity*	*Weather Conditions*
Almirante	29.0	78.5%	Cloudy
Cayo de Agua	28.2	99.0%	Cloudy or Raining
Isla Colon	26.6	93.2%	Partly Cloudy or Cloudy
San Cristobal	30.0	76.7%	Sunny or Cloudy
Loma Partida	28.2	81.7%	Sunny or Cloudy
Pastores	31.4	72.7%	Sunny
Popa North	28.8	89.4%	Sunny or Cloudy
Solarte	27.8	92.0%	Cloudy
Uyama	29.2	82.0%	Partly Sunny, Cloudy, or Raining

Figure 2.1: Two distinct populations occur on the North (A.) and South (B.) ends of Isla Popa. Populations not only vary in color pattern, but they also vary in size with the northern population averaging only 15.5± 0.14 mm and the southern population averaging 18.5 ± 0.13mm.

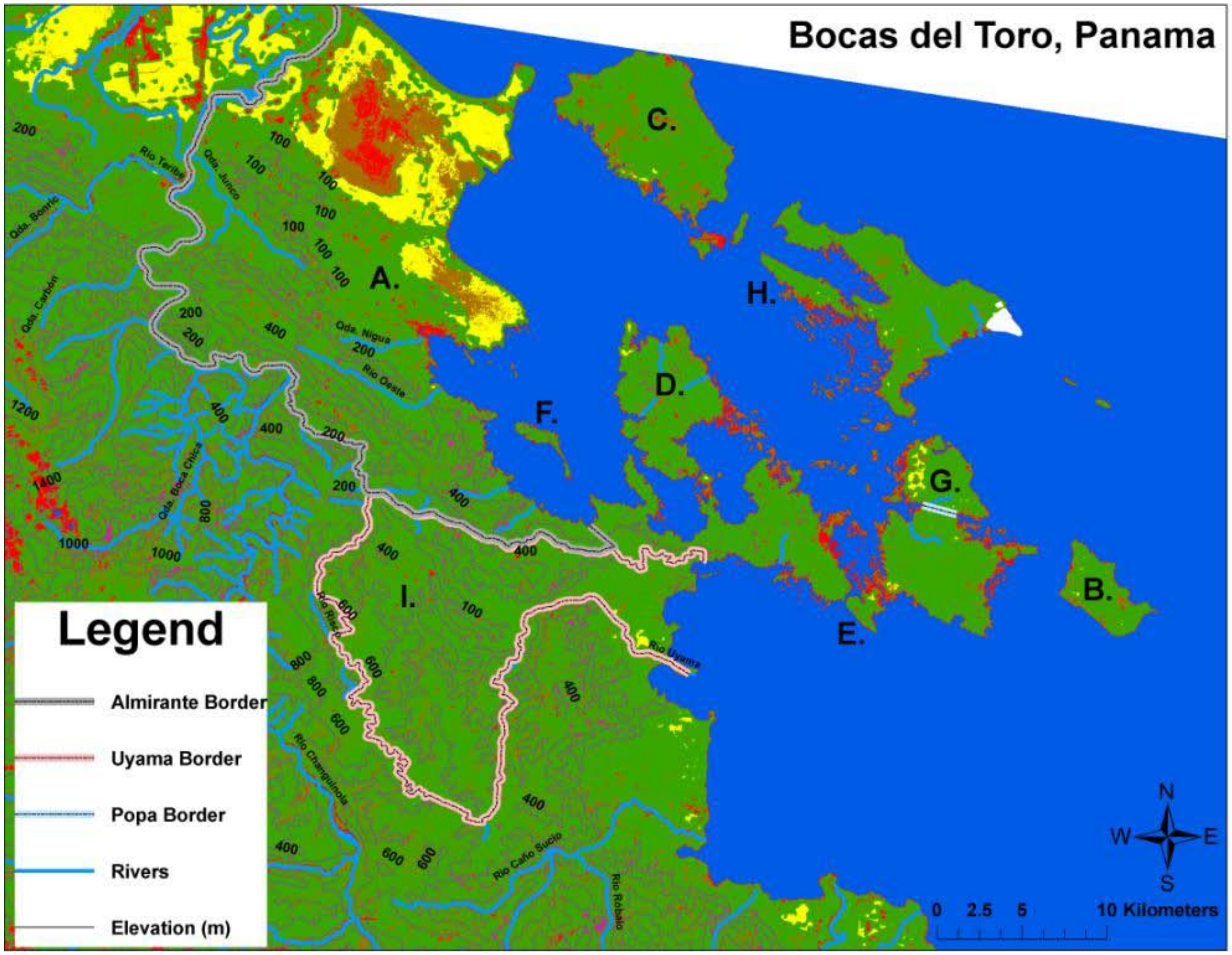

Figure 2.2: The nine different populations examined in the study. On mainland populations, areas were defined by borders to rivers and elevations above 600m which can act as effective barriers for migration between populations. The small ridge running to the Aguacate peninsula was defined as the barrier between the Almirante population and the Uyama River population. Popa North was separated at the constriction in the island which likely acted as a barrier when sea levels were higher. A. Almirante; B. Cayo de Agua; C. Isla Colon; D. San Cristobal; E. Loma Partida; F Pastores; G. Popa North; H. Solarte; I. Uyama River.

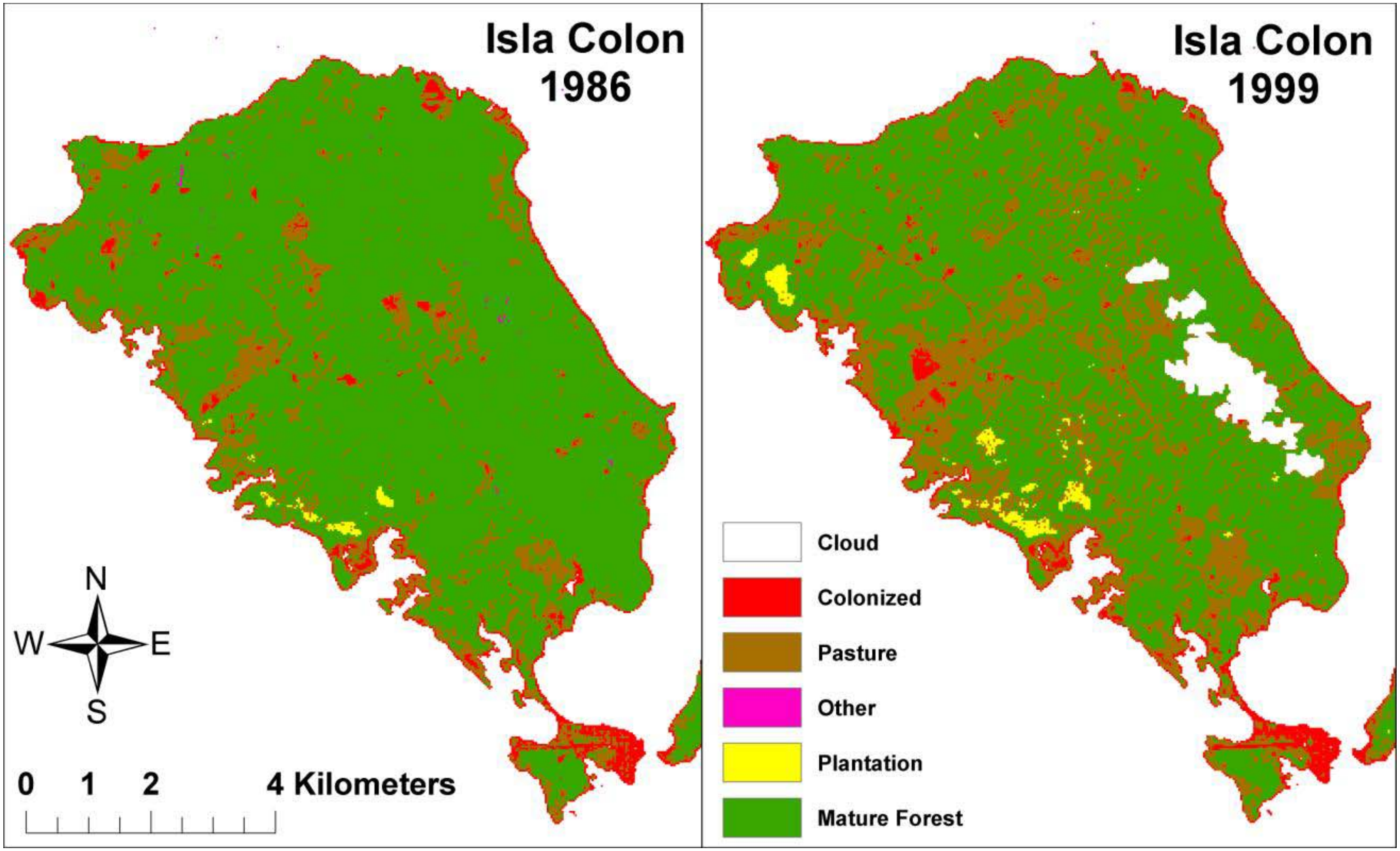

Figure 2.3: Comparison of Isla Colon in 1986 and 1999 to show the amount of cloud cover in 1999. This portion of cloud covered 22.4 hectares of area on the island in 1999. Consequently, the same area in 1986 was omitted from analysis in order to keep consistency between habitat changes between years.

Table 2.3: The amount of each habitat type on the nine areas examined in hectares in 1999. The “other” category displayed very high photosynthetic values in NDVI, and it is hypothesized that this category possibly represents a very fast growing annual crop grown by locals as it does not show a consistent pattern as seen in plantation crops such as banana. The change category represents the net gain/loss of habitat from 1986.

	Colonized		*Pasture*		*Plantation*		*Mature Forest*		*Other*		*Total*
	Area	Change	Area	Change	Area	Change	Area	Change	Area	Change	*Area*
Almirante	197.2	+41.3	867.25	+221.4	599.9	+112.8	2362.1	-336.99	8.94	-50.43	4035
Cayo de Agua	5.42	-0.75	10.68	-4.35	5.46	+4.85	124.52	+0.32	0.02	+0	146.1
Isla Colon	30.25	+1.14	177.49	+88.26	7.46	+5.54	375.13	-95.27	0	-0.44	612.8
San Cristobal	21.66	+1.76	62.42	+28.58	1.84	+0.08	286.59	-24.87	0.37	-4.82	372.9
Loma Partida	7.04	+0.04	7.26	+0.95	1.33	+0.62	22.2	-1.39	0.18	-0.17	38.01
Pastores	3.05	+0.14	3.37	+0.78	0	+0	16.08	-0.27	0.11	-0.59	22.6
Popa North	10.94	+1.71	28.04	+8.76	14.83	+4.69	102.17	-15.42	0	+0	155.9
Solarte	12.15	+2.6	20.33	+6.58	1.11	+0.87	41.98	-9.5	0	-0.01	75.6
Uyama	21.52	+5.92	250.91	+109.1	11.75	+6.44	1603.1	-101.75	5.25	-19.31	1892

Table 2.4: The net change in habitat type per year from 1986 to 1999. The hectare per year category represents the net gain/loss of habitat type each year over the 13 year period. The percent per year category represents the percent gain/loss of year habitat type from the 1986 levels. The "other" category displayed very high photosynthetic values in NDVI, and it is hypothesized that this category possibly represents a very fast growing annual crop grown by locals as it does not show a consistent pattern as seen in plantation crops such as banana.

	Colonized		*Pasture*		*Plantation*		*Mature Forest*		*Other*	
	Ha/yr	%/yr	Ha/yr	%/yr	Ha/yr	%/yr	Ha/yr	%/yr	Ha/yr	%/yr
Almirante	+3.18	+2.04%	+17.03	+2.64%	+8.68	+1.78%	-25.92	-0.96%	-3.88	-6.53%
Cayo de Agua	-0.06	-0.93%	-0.33	-2.22%	+0.37	+60.97%	+0.02	+0.02%	+0	+0%
Isla Colon	+0.09	+0.3%	+6.79	+7.61%	+0.43	+22.11%	-7.33	-1.56%	-0.03	-7.69%
San Cristobal	+0.14	+0.68%	+2.2	+6.5%	+0.01	+0.36%	-1.91	-0.61%	-0.37	-7.16%
Loma Partida	+0.005	+0.04%	+0.07	+1.15%	+0.05	+6.72%	-0.11	-0.45%	-0.01	-3.75%
Pastores	+0.01	+0.38%	+0.06	+2.33%	+0	+0%	-0.02	-0.13%	-0.05	-6.51%
Popa North	+0.13	+1.43%	+0.67	+3.49%	+0.36	+3.57%	-1.19	-1.01%	+0	+0%
Solarte	+0.2	+2.1%	+0.51	+3.68%	+0.07	+28.7%	-0.73	-1.42%	-0.001	-7.69%
Uyama	+0.46	+2.92%	+8.39	+5.91%	+0.5	+9.34%	-7.83	-0.46%	-1.49	-6.05%

Table 2.5: Density (Frogs/100m^2) estimates and associated standard errors for each population examined. I ran ten transects for each population with the exception of Cayo de Agua, which I ran five. In one transect in both Popa North and Uyama, only one frog was found on the transect, so an estimate for each transect could not be made, so those transects were omitted from analysis. I used half-normal distributions for estimates.

Almirante		*Cayo de Agua*		*Isla Colon*		*San Cristobal*		*Loma Partida*		*Pastores*		*Popa North*		*Solarte*		*Uyama*	
Den.	SE	Den.	SE	Den.	SE	Den.	SE	Den.	SE	Den.	SE	Den.	SE	Den.	SE	Den.	SE
4.27	1.33	5.74	1.39	2.46	0.4	6.81	1.13	6.61	1.1	13.52	3.14	4.09	1.39	7.08	1.66	2.39	0.72

Table 2.6: Results of a Tukey's Honest Significant Different test between population densities for nine different populations of *Oophaga pumilio* after an ANOVA ($F_{8,\ 74} = 4.714$, $p = 0.0001$). I ran ten transects for each population with the exception of Cayo de Agua, which I ran five. In one transect in both Popa North and Uyama, only one frog was found on the transect, so an estimate for each transect could not be made, so those transects were omitted from analysis.

	Cayo de Agua	*Isla Colon*	*San Cristobal*	*Loma Partida*	*Pastores*	*Popa North*	*Solarte*	*Uyama*
Almirante	0.9997	0.9954	0.9605	0.9761	**0.0019**	1	0.9308	0.9951
Cayo de Agua		0.9466	0.9999	0.9999	0.1007	0.9995	0.9998	0.9461
Isla Colon			0.5467	0.6106	**0.0001**	0.9981	0.4650	1
San Cristobal				1	0.0673	0.9507	1	0.5628
Loma Partida					0.0526	0.9689	0.9999	0.6249
Pastores						**0.0021**	0.0913	**0.0001**
Popa North							0.9177	0.9979
Solarte								0.4828

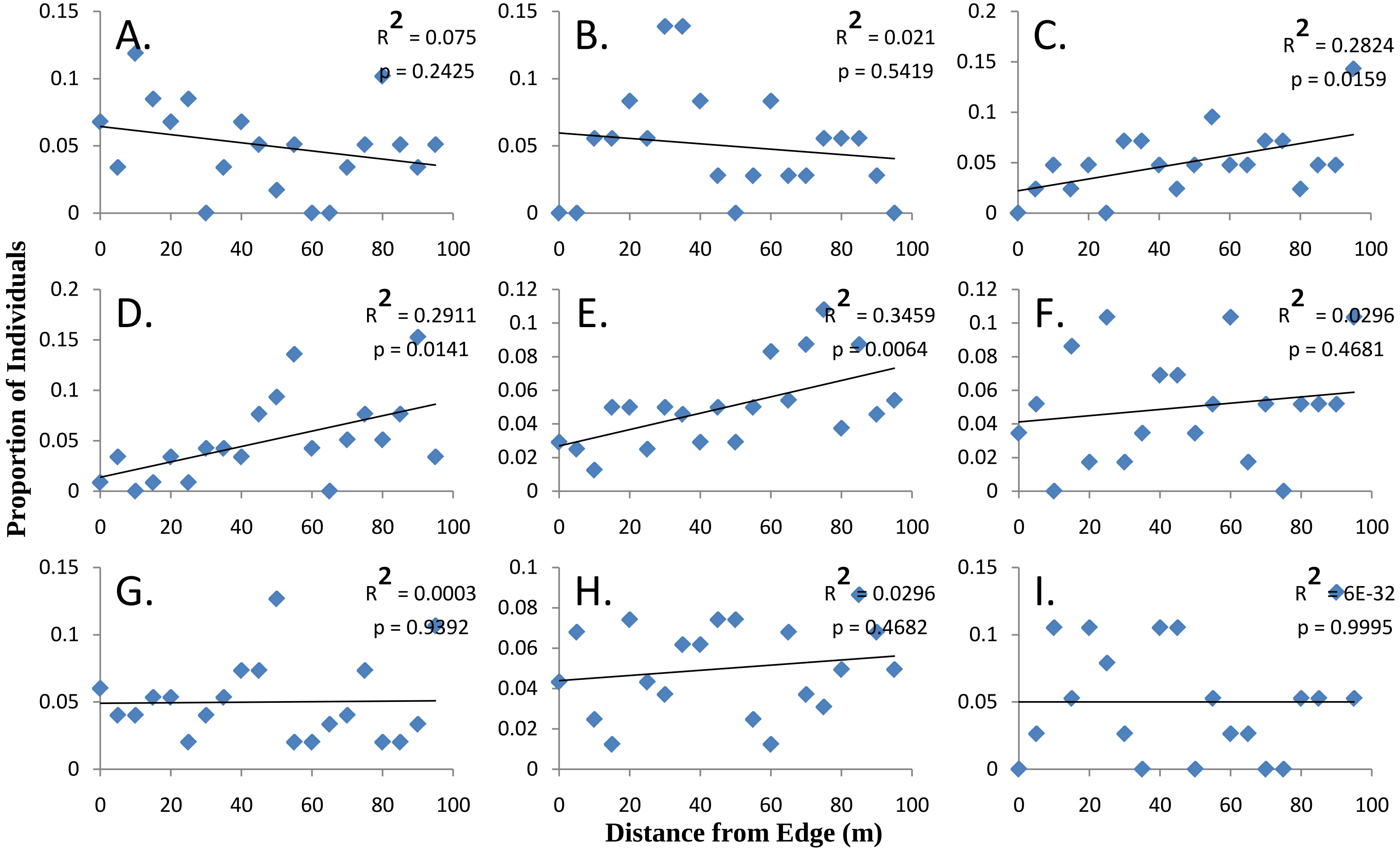

Figure 2.4: Simple linear regressions examining relationships to edges of the nine populations examined. Frogs were grouped by 5m intervals and then the proportion of individuals in each population was compared to distance along the transect. Populations examined include A. Almirante, B. Cayo de Agua, C. Isla Colon, D. Loma Partida, E. Pastores, F. Popa North, G. San Cristobal, H. Solarte, and I. Uyama.

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CHAPTER 3 : CALL DENSITY AND ACOUSTIC NICHE PARTITIONING IN *OOPHAGA PUMILIO*

Abstract

Call surveys offer a valuable method to monitor anuran population trends due to the temporal breeding habits these animals have, as well as, by and large, their close associations with water. Many temperate locations have adopted citizen science programs to monitor local anuran populations using call surveys and road transects. These surveys, however, are not commonly done in the tropics, possibly for a variety of reasons. I approached the use of call surveys for estimating population density of a small terrestrial poison dart frog, *Oophaga pumilio*, in seven different populations in Bocas del Toro, Panama. By conducting three minute call surveys, and searching for all individual frogs in a 10m radius of the survey point, I was able to directly compare the number of calling males to the number of frogs in a given area. Call density did not show a relationship to population density ($p = 0.153$), but humidity was found to be an accurate predictor of number of males calling ($p = 0.002$). Despite there being differences in population densities (ANOVA, $p = 0.001$), the lack of a relationship between the number of calls and population density possibly suggests acoustic niche partitioning between males in this species to avoid call overlap. This study also highlights the need species-specific analysis for the accuracy of call surveys to estimate population trends as some species, such as this one, do not show a linear relationship between number of males calling and population density.

Introduction

Because most amphibians have limited, concentrated locations to raise larvae, they will often congregate around these locations during the breeding season. These aggregations of amphibians, especially anurans, provide managers the opportunity to sample species that may otherwise be difficult to sample during nonbreeding times (e.g., hylid treefrogs). Since managers can observe population trends, demographics, and reproductive output all at once, such breeding gatherings are important for managing threatened species. While no accurate method has been developed to analyze population trends quantitatively using call surveys, methods exist to qualitatively assess population trends (Mossman et al. 1998, Genet and Sargent 2003).

Call surveys are commonly done around breeding sites to estimate population size. Many states and Canadian provinces have active citizen science based programs to qualitatively assess population trends (e.g., Lepage et al. 1997, Mossman et al. 1998, Genet and Sargent 2003). These programs typically will rank the amount of calls along a scale to estimate the number of males calling over the course of the breeding season (Mossman et al. 1998). This qualitative method is primarily used due to oversaturation of calling males and the inability to distinguish individuals in a chorus (Genet and Sargent 2003). This is a particularly useful technique in temperate areas as most amphibians depend on the seasonally available water sources in order to breed. There is a general assumption that a linear relationship exists between population trends and calling intensity, but few studies actually examine this relationship (Mossman et al. 1998).

Call surveys do not, however, appear to be commonly used with tropical anurans. This could be in part due to the minimal amount of seasonality as compared to temperate regions (Janzen 1967). Despite this reduced amount of seasonality, many anuran species show punctuated breeding seasons based on amount of rainfall over the course of a year (Gottsberger and Gruber 2004). Minimal use of call surveys could also be due to the amount of acoustic interference from non-target species especially insects (Paez et al. 1993). Given the number of species, especially threatened species (Myers et al. 2000), found in tropical regions, a method such as call surveys that allow for detection and qualitative analysis of populations may provide a useful tool when it comes to tropical amphibians. Universal use, however, may not be ideal with species that do not have punctuated breeding seasons as well as species that do not have centralized breeding locations.

Oophaga pumilio is a species of poison dart frog that occurs in the Caribbean lowland rainforests from Nicaragua to Panama. Unlike many anurans, this species utilizes small volumes of water often found in arboreal epiphytes (e.g., Bromeliacae) for larval deposition (Lötters et al. 2007). Given that these epiphytes are broadly distributed through a lowland rainforest landscape (Fischer and Araujo 1995), *O. pumilio* is not constrained to centralized breeding locations in a forest patch. This species, like most dendrobatids, has a prolonged breeding season with no noticeable spike in reproductive effort in relation to environmental conditions (Gottsberger and Gruber 2004). As a result, opportunistic call surveying around a water source is not an option for species such as *O. pumilio*.

Oophaga pumilio are expected to be uniformly distributed in suitable habitat. Accordingly, I hypothesize that this relatively uniform distribution of calling males through a landscape (McVey et al. 1981) may allow call surveying in a quantitative method. This study reports the results of surveying seven different populations of *O. pumilio* using a quantitative method to assess if calling intensity correlates with population density.

Study Areas

I conducted this study during May and June 2010 on seven different populations in the Bocas del Toro archipelago in Panama. The Bocas del Toro archipelago is a series of islands in the Caribbean on the western side of Panama.

Seasons in the Bocas del Toro archipelago are very subdued with the wet season typically occurring from May through August and another spike in rain fall in October and November (STRI 2010). Over the course of a year, Isla Colon, the largest island and where the Smithsonian Tropical Research Institute's (STRI) weather station is located, receives an average of 3,312mm of precipitation with the majority of it occurring during the wet season (STRI 2010). Seasonal temperatures range from 23.2°C to 28.6°C with daily temperatures ranging, on average, from 28°C during the day to 24.9°C at night. Average daily relative humidity ranges from 75.9% to 88.3%. Temperature tends to peak between 12:00h and 13:00h while humidity peaks in early evening between 21:00h and 22:00h or early morning (J. Lawrence, unpubl. data). Frogs are most active early in the morning until approximately 14:00h when calling and sightings would gradually diminish. Additionally, if forest temperatures exceeded 29°C or humidity dropped below 75%, the number of males calling and visual encounters decreases substantially (J. Lawrence, personal observation).

The populations used for this study were Isla Colon, Solarte, San Cristobal, Pastores, Almirante, Popa North, and Loma Partida due to the variety of sizes, colors, and types of habitat that each population represents. By using this variety, trends can be attributed to the species rather than individual variation in populations (Table 3.1).

Isla Colon

Isla Colon, the largest and most populated island in the Bocas del Toro archipelago, is approximately 612.8 hectares in size with a resident population of about 3,500, the majority of which live in the main town located on southeast side of the island. Isla Colon is primarily dominated by a mixture of secondary and primary lowland rainforest. In recent years, an increase of pastureland has resulted in fragmented landscapes throughout the island. Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*. Understory growth varied significantly from survey to survey with some being largely clear with few palms as understory plants (e.g., *Astrocaryum alatum*) and other surveys consisting of thick undergrowth of largely herbaceous monocots (e.g., *Dieffenbachia*, *Calathea*, *Costus*, and *Bromelia pinguin*). *Oophaga pumilio* are most often associated with vertical substrate, often being found on or near tree bases. Frogs are very rarely found in leaf litter, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation). Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were undisturbed forest on communal land along a path to a small community.

Solarte

Solarte is approximately 79.3 hectares in size with a small resident population which is scattered primarily along the coast of the island. Solarte is primarily dominated by primary lowland rainforest. Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*. Understory growth remained relatively sparse through all surveys with the majority of undergrowth being herbaceous monocots (e.g., *Dieffenbachia, Calathea,* and *Costus*), small palms, and heliconia. *Oophaga pumilio* of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found in leaf litter, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, Personal Observation). Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were continually maintained as undisturbed forest for by a private land owner (W. Stevens, personal communication).

San Cristobal

San Cristobal is a large island that is approximately 375.6 hectares in size with a small resident population which scattered throughout the island and in a couple of small communities. San Cristobal is heavily fragmented and used for pastureland. Forest is a mixture of primary and secondary forest. Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*. Understory growth remained moderate through all surveys with the majority of undergrowth being herbaceous ariod plants (e.g., *Dieffenbachia, Calathea,* and *Costus*) and tree saplings. *Oophaga pumilio* of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, Personal Observation). Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were forest on the edge of active pasture for a small community of about a dozen people.

Pastores

Pastores, the smallest island examined in this study, is approximately 22.6 hectares in size with a very small resident population which is scattered primarily along the coast of the island. Pastores is primarily dominated by cacao plantation with some lowland rainforest remaining. Forest vegetation typically consists of few large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*, with cacao making the majority of the canopy cover. Understory growth remained extremely sparse through all surveys with the majority of undergrowth being small herbaceous ariod vines or occasional Heliconia stands. *Oophaga pumilio* of this population are often associated with ground substrate, but are often found on or near tree bases. Frogs are commonly found in leaf litter (J. Lawrence, personal observation). Frogs in this population, unlike others, appear to be rather gregarious with many individuals being found in the same area. Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were unused cacao plantation.

Popa North

Popa is a large island, with the northern portion being approximately 154.2 hectares in size with a small resident population which scattered throughout the island and in a couple of small communities. Popa is constricted in the center of the island and actually has two separate morphs of *O. pumilio* and for this study, I only examined the northern population. Forest is a mixture of primary and secondary forest. Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*. Understory growth remained moderate to heavy through all surveys with the majority of undergrowth being herbaceous ariod plants (e.g., *Dieffenbachia, Calathea,* and *Costus*) and tree saplings. *Oophaga pumilio* of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation). Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were forest on the edge of a resort. Forest was actively maintained as intact rainforest.

Loma Partida

Loma Partida is a small island that is approximately 38.1 hectares in size with a very small resident population which scattered throughout the coasts of the island and in a couple of small communities. Forest is a mixture of primary and secondary forest, with large portions being cacao plantation. Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*. Understory growth remained very sparse through all surveys with the majority of undergrowth being herbaceous aroid vines (e.g., *Dieffenbachia, Calathea*, and *Costus*). *Oophaga pumilio* of this population are often associated with vertical substrate, typically being found on or near tree bases. Frogs are very rarely found high in vertical substrate and more often are found between 0-1m, and often prefer traversing on fallen branches or roots across the ground (J. Lawrence, personal observation). Frogs could actively be found traversing through the thick leaf litter layer. Soil in all of the surveys was nutrient poor clay with a thin topsoil layer. Study areas were active cacao plantation on the edge of a small community.

Methods

At each study location detailed above, I established five 10m radius call survey circles. Each circle was at least 100m away from any other circle and was located within a forested area where frogs could be heard calling. Call surveys were conducted by standing at the center of each circle and two observers listened for calling males. Sites were situated in forest where undergrowth was moderately clear with very little undergrowth in the surrounding area. These sites were chosen so that calls would be unimpeded by barriers such as leaves, branches and other debris. This allowed the maximum number of individuals to be heard during the survey. Following site selection, environmental data on humidity, temperature, and weather conditions for each survey were recorded. A three minute call survey was then conducted, following with an intensive survey of the surrounding area for any resident frogs. The number of males calling that could be individually distinguished from one another was recorded by two observers sampling the same survey circle at the same time. Some individuals could be heard at a distance away from the survey area, but due to the distance, these could not be easily distinguished as individual males and tended to mix with ambient noise. Each survey was averaged between the two observers to get the average number of males calling for each survey.

Once call surveys were completed, the 10m radius circle was then intensively surveyed for resident individuals. A 10m radius was estimated to be the maximum distance that a call would travel and still be distinguishable as an individual male, thus call estimates could directly be linked to population density estimates within the same area. Starting at the outside of the circle, observers slowly walked in a spiral toward the center of the circle conducting a visual encounter survey looking for frogs within the survey area. The spiral method allowed for thorough coverage and overlap of the survey area so that all frogs could be found within the survey plot should some individuals be more skittish than others. As frogs were encountered, they were collected to avoid counting individuals multiple times. For each collected frog, snout-vent length was measure and sex recorded. Males were identified by the presence of a gular patch discoloration which indicates recent vocal sac expansion (Gardner and Graves 2005). Popa North frogs were small and had blue bellies, so vocal sac discoloration was not a reliable method for sexing those frogs. On several occasions, frogs were sexed as female based on lack of a gular patch only to have them start calling while handling them. For this reason, the Popa North population was excluded from data analysis when sex was involved.

Data were analyzed in two ways: 1) comparing differences in frog density between the seven populations, and 2) examining relationships between population density and calling male density. When comparing the differences between population densities, the data were analyzed using a single-factor ANOVA followed by pairwise Tukey's HSD test to examine pairwise differences. To determine if a relationship existed between population density and call density, I analyzed the data with a simple linear regression. All analyses were done using the statistical package R.

Results

Frog populations differed in average density of individuals based on the visual encounter survey (ANOVA, $F_{6, 24} = 3.857$, $p = 0.01$; Table 3.2). Differences were observed between Pastores and Isla Colon (Tukey's HSD, $p = 0.0029$). Populations varied eight-fold across populations. Most notably, the Isla Colon population had low density with an average of 3.6 frogs/100m^2 and the Pastores population had high density with an average of 25.9 frogs/100m^2. It is unknown as to reason that the Pastores population has such a high density, however, I did observe thick leaf litter and a large amount of invertebrate fauna, which could contribute to a large population due to an increase in the amount of food resources for the frogs.

Call densities also differed between populations. Because frog calls vary in intensity and volume in relation to body size (Wilbur et al. 1978), call densities were standardized to body size by dividing the observed call density (number of calls/m^2) by the average snout-vent length of males of each respective population.

A multiple linear regression was conducted to examine any relationship between the standardized call density and frog density, temperature, or humidity. Humidity was log10 transformed to ensure a normal distribution. Neither frog density nor temperature showed a difference in relation to standardized call density (p = 0.156; p = 0.635, respectively). A difference was observed, however, in humidity (p = 0.024; Figure 3.1). Similarly, a multiple regression was performed to examine any possible relationship between standardized call density and male density, temperature, or humidity. Despite large differences between the number of males observed between populations, the density of males did not show any relationship to calling frequency (p = 0.501), nor did they show any relationship to temperature (p = 0.783). As with population density, humidity was found to be an accurate predictor (p = 0.042) of calling frequency.

Sex ratios varied from population to population, with some populations having more males while others had more females as detected by the presence of gular patches. For this analysis, I excluded the Popa North population because sexing using gular patches was unreliable for this population. More males were found at Isla Colon (66.6% male), Solarte (59.4% male), San Cristobal (55.8% male), with Loma Partida (57.0% male) and only Pastores (44.8% male) having more females. I used a simple linear regression to determine mean proportion of apparent males compared to mean population density which yielded a p-value of 0.011 and an associated R^2 value of 0.914 (Figure 3.2).

Discussion

Despite there being an eight-fold difference between Pastores population and the Isla Colon and Almirante populations in population density, there appears to be no linear trend in calling density that follows this increase in frog density. This is of particular note due to the territorial nature of *O. pumilio*. What likely occurs with these populations is that a linear trend is observable in small populations where the number of males is limited, but these data suggest that none of these populations were at such a low point. Most interesting is that male density was different between populations, and despite this difference, there appeared to be no difference in the calling effort between populations.

Sex ratios, as detected using gular patches, varied in a linear fashion in relation to population density. This relationship provides interesting insight into possible function of these populations. Once a population reaches a particular density, the number of males calling, as evident from the presence of gular patches, reduces to make a population appear to be more dominated by females because a smaller proportion of males are calling at any given time. This suggests that as population density increases, individual males are less likely to call continually over the course of a day to avoid territorial disputes. This reduction in the amount of calling males could have implications for conservation methods if resource managers seek to manage populations for a particular proportion of males or females. Additionally, calling is a fast method for collectors to find frogs, which could result in the removal of dominant individuals within the population.

As would be expected, calling density showed a strong relationship to relative humidity, with the number of calling males increasing in a linear fashion with increasing humidity. This factor appears to the most predictive variable for the amount of frogs calling at any given time, and given this, any management that takes place based on results from call surveys needs to take environmental conditions into account.

These results do suggest intraspecific acoustic niche partitioning in these species. With the highly territorial nature of this species (Gardner and Graves 2005), it would be predicted that territorial interactions would increase as population density increases. Gular patch development occurs in males who have called recently and cause discoloration to the vocal sac. Given this evidence, a number of males identified in this study may have called before surveys started, which suggests many more males in the population are calling than the number of males heard during the course of this study. Since males compete with one another in acoustic frequency, it is beneficial for males to alternate calling so that individuals do not overlap and reduce the fitness for both parties. Other anurans exhibit this acoustic niche partitioning behavior (Duellman and Pyles 1983), and *Dendropsophus* (=*Hyla*) *microcephala* has been shown to perform fine scale adjustments to its call to fall in a window of time with the least amount of acoustic interference (Bourne and York 2001). A reduction in territorial behavior, such as calling, could be explained by a dear-enemy effect (Temeles 1994) where individuals show a reduced amount of territoriality towards familiar conspecifics as compared to strangers. While this hypothesis has merit in a number of different taxa such as Fiddler Crabs (*Uca mjoebergi*; Booksmythe et al. 2010), lizards (Lopez and Martin 2002), and other frog species (Owen and Perrill 1998, Lesbarreres and Lode 2002), no experimental evidence suggests that *O. pumilio* demonstrate this effect (Bee 2003, Gardner and Graves 2005).

The results of this study highlight the need for further research on the accuracy of using call surveys as a surrogate for tracking population densities over time. The use of citizen science can be a very powerful tool for managing wildlife populations and understanding community trends over time, as evidenced by the successful National Audubon Society Christmas bird count initiative (Butcher et al. 1990). With the current decline in amphibian populations around the world (Houlahan et al. 2000) and especially in Latin America (Lips et al. 2005), methods to monitor population trends are sorely needed. Call surveys offer a cheap and effective method to observe presence or absence of species in a given region, but the current methods only allow for presence-absence data to be collected. It is tempting for managers to use such methods to infer population trends over time, but caution should be taken with this approach. With some species, this may be a valid method for examining population trends, but for territorial species such as *O. pumilio*, this method does not appear to be useful for predicting patterns. For many species, environmental factors such as humidity, as in this study, or rainfall (Gottsberger and Gruber 2004), drive calling frequency in anuran populations, not population density.

APPENDIX 3

Table 3.1: Physical descriptions of the different study populations with the relevant information for each population. Vegetation type refers to the area studied, although vegetation does vary across the population range. Temperature and humidity ranges are over the study period.

Location	*Island or Mainland*	*Vegetation*	*Frog Coloration*	*Average Temperature*	*Average Humidity*
Isla Colon	Island	Primary forest		26.0°C	95.1%
Solarte	Island	Primary forest		28.8°C	89.3%
San Cristobal	Island	Primary and Secondary forest		28.7°C	85.4%
Pastores	Island	Cacao plantation		26.1°C	90.8%
Popa North	Island	Primary forest		28.7°C	83.7%
Loma Partida	Island	Cacao plantation		30.0°C	75.7%

Table 3.2: Pairwise results of a Tukey's HSD Test on population density of the different populations in the study after an ANOVA ($F_{6,\ 28} = 3.857$, $p = 0.01$). Due to low sample size, many populations were not different from one another, but those that were different are indicated in bold (α=0.05).

	Loma Partida	*Pastores*	*Popa North*	*San Cristobal*	*Solarte*
Isla Colon	0.2542	**0.0029**	0.4815	0.6389	0.3329
Loma Partida		0.3389	0.9976	0.9802	0.9999
Pastores			0.1624	0.0991	0.2593
Popa North				0.9998	0.9998
San Cristobal					0.9945

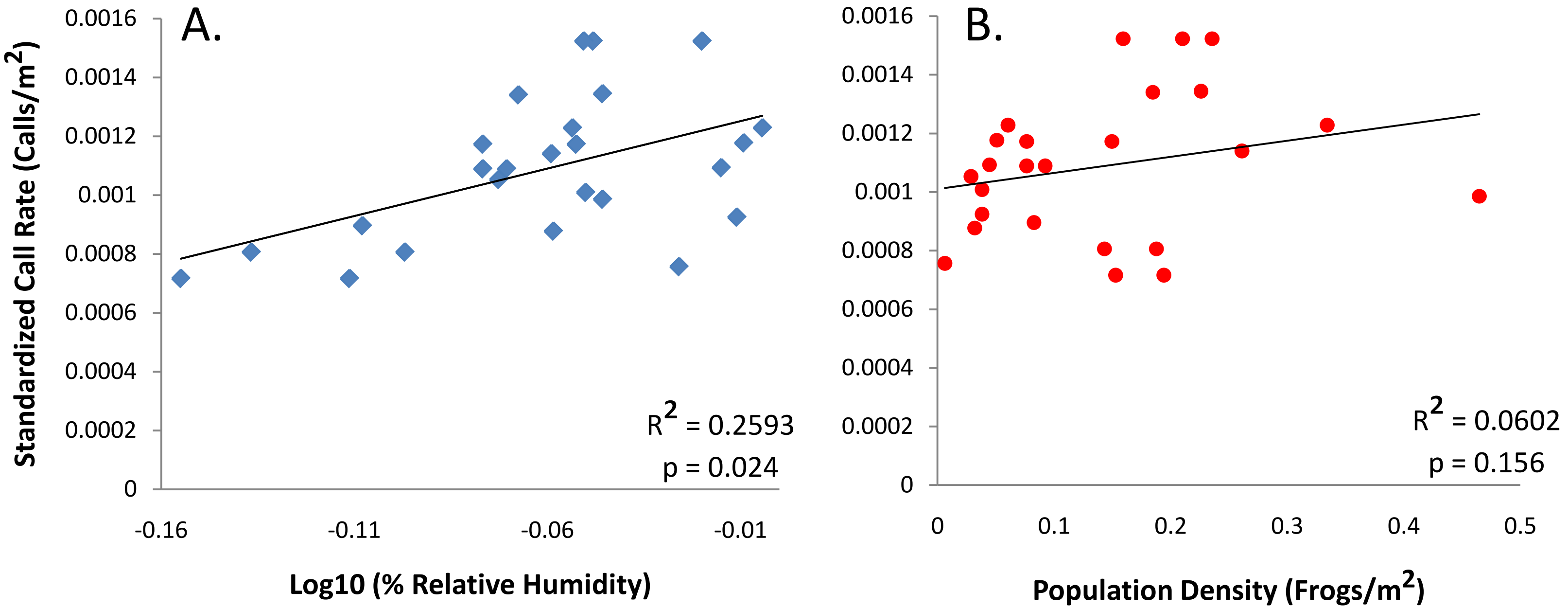

Figure 3.1: Linear regressions of A. Log10 transformed relative humidity and B. Population density in relation to a standardized call rate. Call rates were standardized by dividing the observed call density by the average male snout-vent length of the respective population in order to remove potential impacts body size has on calling intensity.

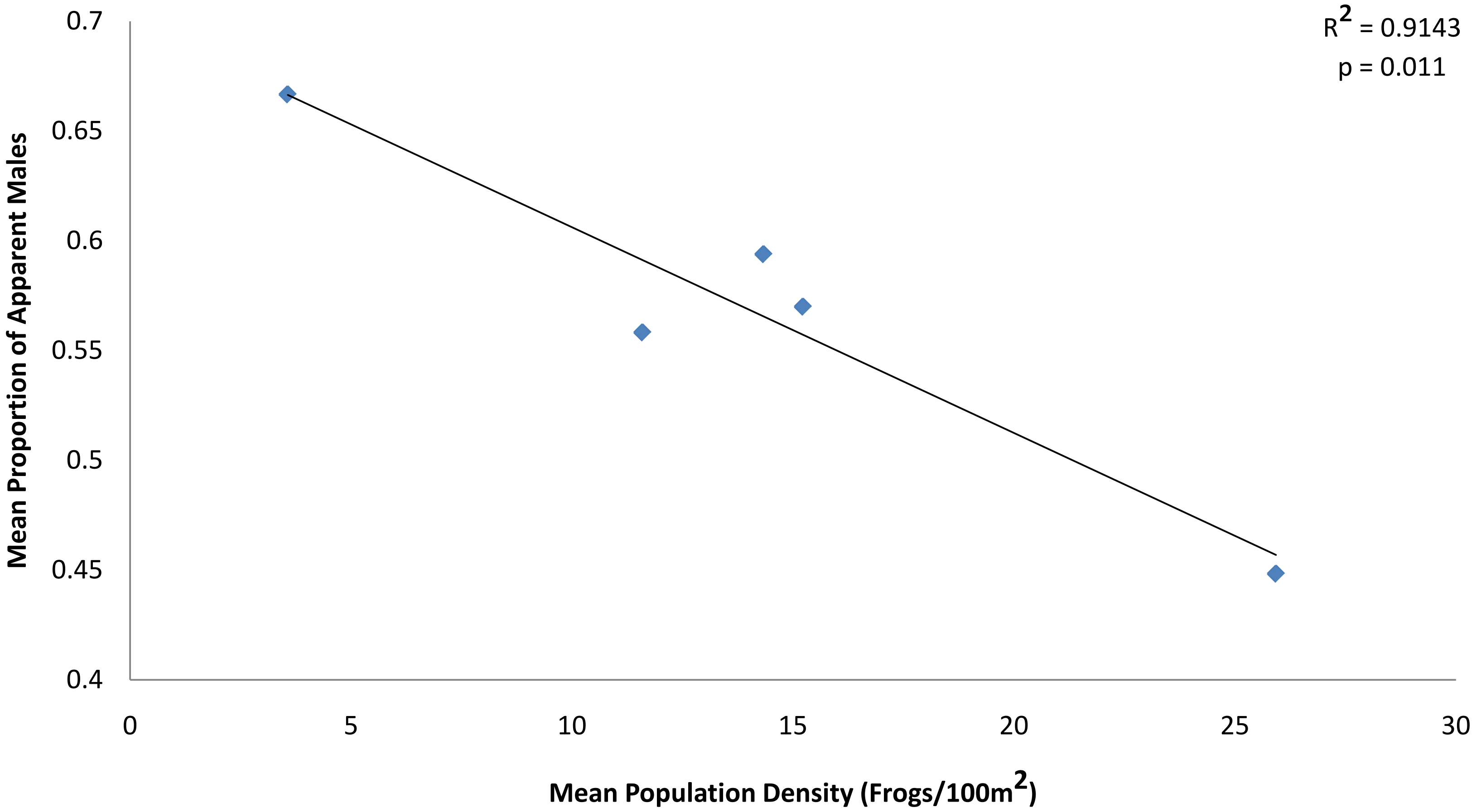

Figure 3.2: Relationship of the mean proportion of males and mean population density for Isla Colon, San Cristobal, Loma Partida, Popa North, and Pastores. A simple linear regression yielded a p-value of 0.011 with an associated R^2 value of 0.9143 showing a negative relationship between male density and population density, possibly suggesting acoustic niche partitioning in *Oophaga pumilio*.

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CHAPTER 4 : DEMOGRAPHIC CHANGES IN A POPULATION OF THE STRAWBERRY POISON DART FROG (*OOPHAGA PUMILIO*) AFTER THE ADDITION OF ARTIFICIAL REARING SITES

Abstract

It has long been understood that the distribution of species is influenced by the distribution and availability of resources. Resource availability not only determines species assemblages, but can also influence reproductive behavior, including male and female distribution. The Strawberry Poison Dart Frog, *Oophaga pumilio*, is a small, territorial species of frog that displays a notable and varied divergence in color and pattern in the Bocas del Toro region of Panama. Given the inherent vulnerabilities of these localized and insular populations, determining effective conservation methods in this region will be particularly critical in preserving genetic diversity in this species. Augmenting or manipulating limiting resources may provide an effective method of enhancing population stability or growth. To test this hypothesis, I established and observed six quadrats (with three replicates) over a year's time, and manipulated rearing sites, and habitat in the form of addition or removal of leaf litter. Artificial rearing sites were large diameter drinking straws folded in half to mimic bromeliads. Quadrats were monitored in June 2009, July 2009, August 2009, May 2010, and June 2010. Population density was not affected by addition or removal of leaf litter, but addition of rearing sites was found to have an effect; on average, the addition of rearing sites approximately doubled the population. Thus, increasing the availability of rearing sites may offer an effective conservation strategy for populations threatened with extirpation, whether from habitat loss, exploitation, or other factors. It is possible that the use of large diameter drinking straws may offer resource managers a cheap and effective method for increasing populations of small dendrobatid frogs, at least where the availability of breeding sites is limiting.

Introduction

The development of conservation strategies for amphibians has been of great interest to resource managers in recent years, as a recognized global amphibian decline has unfolded due to a variety of factors including habitat loss (Lips et al. 2005), disease (Lips et al. 2006), climate change (D'Amen and Bombi 2009), and direct exploitation (Garner et al. 2009). Given the issues associated with the amphibian decline, conservationists are actively exploring a variety of methods best to conserve target species into the future. Such methods can include captive breeding programs, both in situ and ex situ (e.g., *Atelopus zeteki*; Amphibian Ark 2011)), habitat protection and restoration (e.g., *Rana aurora draytonii*), and legal restrictions on exploitation (e.g., *Neurergus kaiseri*). There is considerable debate among conservation biologists, however, as to the most effective conservation methods (Balmford et al. 1996, Blaustein and Kiesecker 2002). While there is merit to each argument, any particular conservation method will almost certainly not prove universally effective for all amphibian species, or even for closely related species within a particular taxon.

Amphibians, as compared to other terrestrial vertebrates, provide resource managers with unique challenges due to their biphasic reproductive strategies (and associated habitats necessary for these) and secretive lifestyles. Amphibians have some of the most diverse reproductive habits of any vertebrate group, with 39 distinct reproductive strategies known to occur within the taxon (Haddad and Prado 2005). Amphibian reproductive strategies range from the production of large numbers of eggs with aquatic larvae fending for themselves (e.g., *Rana catesbeiana*), to laying singular eggs with a great deal of parental investment in larval care (e.g., *Oophaga pumilio*). Complicating conservation initiatives, reproductive strategies and population trends remain unknown for many species; population data is lacking for roughly 29% of all amphibian species (Stuart et al. 2004). Since it is estimated that about 43.2% of amphibian species are declining due to a variety of stressors, the conservation of amphibians remains a complex but critical problem (Blaustein and Kiesecker 2002).

Conservation methods that seek to maximize reproductive success of threatened amphibian species may prove to be a viable option for quickly increasing populations. For species subjected to collection pressure (e.g., *Dendrobates auratus*; Nijman and Shepherd 2010), such methods, combined with knowledge of survivorship and habitat use, could allow for a regulated, sustainable harvest of certain populations. In recent years, habitat improvement and restoration, such as pond creation, has been pursued by a number of conservation groups (Denton et al. 1997). Anthropogenic ponds may have notable conservation value to pond-breeding amphibian communities (Brand and Snodgrass 2010), but unfortunately, due to the diverse reproductive habits of the taxon (Haddad and Prado 2005), such methods will not be of benefit to all amphibian species. As in mammals, and reptiles, some amphibians have developed highly specialized modes of parental care, including nest guarding (e.g., *Plethodon* species), larval transport and rearing (e.g., *Gastrotheca* species), and oophagy (e.g, *Oophaga* species; Haddad and Prado 2005). In these amphibian species, as in some birds (Newton 1994), availability of offspring rearing sites may be an obvious limiting factor influencing amphibian population size in a given habitat (Donnelly 1989). Thus, increasing the numbers of rearing sites could be a prime target for conservation initiatives hoping to increase population sizes for such species.

Oophaga pumilio is a small species of dendrobatid found throughout the Caribbean lowlands from Nicaragua to Panama. This semi-arboreal species will climb trees up to 40m to access water sources found in bromeliads for larval deposition (Lawrence 2011). These frogs have evolved the ability to use rearing sites containing small amounts of water, presumably to avoid competition with other dendrobatids and to avoid potential predators. This species will use a wide variety of sites for tadpole deposition, including *Heliconia* and bromeliad axils, tree holes, or even artificial structures, as shown in studies on captive animals. Using a method that capitalizes on this limiting resource will aid researchers in detecting, studying, and conserving small amphibians that depend on availability of rearing sites.

In Bocas del Toro, Panama, *O. pumilio* are threatened with a variety of perturbations including deforestation (see Chapter 2) and collection (Nijman and Shepherd 2010). In recent years, fragmentation and habitat loss has been on the rise through the region (see Chapter 2), causing concern for the long term persistence of unique populations of this species through the archipelago. Additionally, thousands of frogs are being collected each year for the pet trade (Nijman and Shepherd 2010), and those numbers only include those that are legally collected. While the species has the potential to be a renewable resource for local collectors and exporters, an increased understanding of the species’ ecology and conservation needs will be required to formulate and implement a management plan in the region.

By manipulating some of the potential limiting resources (e.g., rearing sites, food availability, substrate) for an *O. pumilio* population, it may be possible to better understand their relative importance in determining population size and dynamics. Therefore, I investigated availability of rearing sites was a primary limiting factor in *O. pumilio* populations in Bocas del Toro, Panama. If rearing sites are limiting to the population and frogs will use artificial rearing sites, then the addition of artificial rearing sites to a habitat patch should lead to population changes in both size and in demographic composition. The addition or removal of food resources (or a presumed surrogate such as leaf litter) should have little impact on the population.

This work has potential importance as a conservation tool for threatened populations in the genus *Oophaga* as well as for facultative egg feeders such as those in the genus *Ranitomeya* and other arboreal breeding amphibians.

Study Area

I conducted the following study on Isla Colon in the Bocas del Toro archipelago of Panama. Isla Colon, the largest island in the Bocas del Toro archipelago, is approximately 61km^2 in size with a resident human population of about 3,500, the majority of whom live in the main town located on southeast side of the island. Isla Colon is primarily dominated by a mixture of secondary and primary lowland rainforest. In recent years, an increase of pastureland has resulted in forest fragmentation throughout the island. Forest vegetation typically consists of large emergent trees such as *Ficus* spp. and *Dipteryx oleifera*. Understory growth varied from forest to forest with some quadrats being largely clear with a few palms as understory plants (e.g., *Astrocaryum alatum*) and other quadrats consisting of thick undergrowth of largely herbaceous ariod plants (e.g., *Dieffenbachia* spp.). All areas examined also included a large number of small (<10cm) trees. Soil was nutrient poor clay with a thin topsoil layer. Study areas were continually maintained as undisturbed forest, for use in research by undergraduate classes, by the Institute for Tropical Ecology and Conservation (ITEC).

Seasons in this region are mild with a wet season typically occurring from May through August and then another one starting in late October and often extending into March (STRI 2010). For local amphibians, this seasonal availability of water can strongly dictate behavioral patterns (Donnelly 1998). From 2006 to 2009, Isla Colon received an average of 3,312mm of precipitation with the majority of it occurring during the wet season (STRI 2010). Seasonal temperatures range from 23.2°C to 28.6°C with daily temperatures ranging on average from 28°C during the day to 24.9°C at night. Average daily relative humidity ranges from 75.9% to 88.3%. Temperature tends to peak between 14:00h and 16:00h (which corresponds with average low daily humidity) while humidity peaks in early morning between 02:00h and 04:00h. Lowest temperatures in the day occur between 04:00h and 06:00h. Frogs were most active early in the morning until approximately 14:00h, when calling and sightings would gradually diminish. Additionally, if forest temperatures exceeded 29°C or humidity dropped below 75%, the number of males calling and visual encounters decreases (J. Lawrence, unpubl. data).

Methods

I established three replicates of six 10m x 10m quadrats on Isla Colon in June 2009 in three different areas on the island for a total of 18 quadrats. Quadrats were chosen at random in both primary and secondary forest. Within each set of six quadrats, each received a different treatment: (1) Leaf Litter Added (LLA), (2) Leaf Litter Removed (LLR), (3) Rearing Sites Added (RSA), (4) Rearing Sites Added and Leaf Litter Added (RSA + LLA), (5) Rearing Sites Added and Leaf Litter Removed (RSA + LLR), and (6) a Control (Figure 4.1). Replicates were at least 2km apart so that migration from one replicate to another by frogs was very unlikely. Individual quadrats within a replicate were 100m apart which would limit migration of frogs from one quadrat to another. *Oophaga pumilio* are highly territorial and are not known to travel great distances to new habitat types (McVey et al. 1981), so 100m was deemed sufficient for minimizing the possibility of migration between sites.

Once quadrats were established and before any manipulation occurred, quadrats were sampled for frogs to determine a baseline for the population size of each area. After the initial sampling in June 2009, quadrats were resampled in July 2009, August 2009, May 2010, and June 2010. Quadrats were sampled by two people walking side by side back and forth through the quadrat to look for any frogs within the quadrat so that the quadrat was examined twice to reduce likelihood of missing any individuals. Once frogs were found, they were caught, weighed, and photographed for digital measurement and mark-recapture analysis.

Individual frogs were identified by the unique spotting pattern on the dorsum of each frog. This negated some of the potential difficulties (i.e., infection) normally associated with traditional marking methods, particularly toe-clipping (Ott and Scott 1999). All frogs were photographed using a Pentax K10D and a Sigma 105mm EX Macro lens with a ring flash for uniform lighting. Spot patterns remain constant through time with only minor changes occurring to the pattern over the course of a year (Figure 4.2). While slight changes in pattern could be observed, the frog's overall pattern would remain constant and, as a result, proved to be a reliable method for mark-recapture analysis in these frogs over the course of this study.

Artificial Rearing Sites

In this study, plastic drinking straws were used as artificial rearing sites (ARS) for the *O. pumilio*. Straws were 190mm in length with an opening diameter of ~13mm. To mimic bromeliads and facilitate water collection, I cut the ends of the straws to form a lip at a 30° angle to form a point at each end. Straws were moderately translucent with alternating white stripes and colored stripes. A number of different colored straws (red, orange, yellow, green, blue) were used, to study the preference and use of particular colors.

Two-hundred fifty straws were added to nine quadrats evenly distributed throughout the quadrat, with colors evenly spread throughout the study plots. Straws were folded in half to form a water collection point (thus mimicking a small bromeliad) and attached to vines, trees, and other woody stemmed plants from 0.5m to approximately 2.5m off of the ground. Zip-ties were used to hold straws in the V position and secure them to the smaller plants. For larger trees, I used fence staples to secure straws (Figure 4.3). In each case, care was taken not to crush the straw body and avoid breaking of straws. After straws were secured in the test quadrats, they were left to fill with water as it rained. The remaining nine quadrats were controls.

Once quadrats were sampled for frogs, the rearing site quadrats were examined for use of artificial rearing structures by *O. pumilio*. While sampling for frogs, if any were found in relation to ARS (e.g., in or on an ARS), the individual was noted. Straws were examined for tadpole deposition and the position and color of each straw containing a larva was recorded.

Leaf Litter

Previous research indicates that the numbers of invertebrates increase as the amount of leaf litter increases (Facelli 1994), therefore, leaf litter resources were manipulated as a surrogate to examine potential effects of food availability; food resources would include ants (Saporito et al. 2004), mites (Saporito et al. 2007), and millipedes (Saporito et al. 2003). In six of the 18 quadrats, leaf litter was removed using rakes and by hand. Care was taken to remove all leaf litter from the quadrats without disturbing the plant life. All leaf litter in these Leaf Litter Removed (LLR) plots was subsequently added to the six Leaf Litter Added (LLA) quadrats. Leaf litter was spread evenly through the plot so as to approximately double the amount of leaf litter in the plots. We maintained sites as LLR by removing leaf litter and LLA by depositing the leaf litter in respective plots monthly during the 12 month period.

Data Analysis

To examine interactions between rearing sites, leaf litter manipulations, and time period, a three-factor analysis of variance (ANOVA) was performed. I examined a variety of factors that would possibly be affected by the manipulations of rearing sites and leaf litter. These factors included capture rate, persistence (defined as proportion of marked to unmarked individuals), and sex. Data were examined in the statistical program R (R Core Development Team 2009).

To examine effects between years, comparisons were performed between June 2009 and June 2010 capture periods for capture rate, and July 2009 and June 2010 for persistence using two three-factor ANOVAs. To examine within year effects and to discern if differences were detectable within months of set up, I did comparisons between June 2009 and August 2009 for capture rate and sexes with a three-factor and four-factor ANOVA, respectively. For persistence, within-year comparisons were performed between May 2010 and June 2010. Persistence was defined as the likelihood that a captured frog would remain in the study area between capture periods. Since persistence was a proportion, I arcsine transformed the data to allow for a normal distribution. Three-factor ANOVAs (Rearing Sites, Leaf Litter, and Year) were performed for both capture rate and persistence to examine any effects of the manipulations between years for the number of frogs encountered and the number of frogs recaptured during the study period, respectively. A Four-Factor ANOVA (Rearing Sites, Leaf Litter, Year, and Sex) was performed for sex differences to examine if any differential reactions to the manipulations could be observed based on sex such as changes in the ratio of males-to-females captured.

Results

I took photographs of 351 frogs in the eighteen quadrats from June 2009 to June 2010. In June 2009, 92 frogs were captured and photographed. In July 2009, 81 frogs were captured and photographed, with 24 of those individuals being recaptures from June 2009. In August 2009, 66 frogs were captured and photographed with 27 of those individuals being recaptured (18 from June 2009 and 9 from July 2009). No frogs were captured in one rearing sites added (RSA) quadrat. One-hundred eighty-eight frogs were captured and photographed in 2009 (Table 4.1).

In May 2010, I captured and photographed 125 frogs with 33 of those individuals being recaptures (14 from June 2009, 11 from July 2009, and 8 from August 2009). In June 2010, I captured and photographed 129 frogs with 58 individuals being recaptures (18 from June 2009, 8 from July 2009, 7 from August 2009, and 25 from May 2010; Table 4.1).

I captured more frogs in 2010 than in 2009 ($F_{1, 24} = 5.21$, $p = 0.031$, Table 4.2) but not between June 2009 and August 2009 ($F_{1, 24} = 3.14$, $p = 0.089$; Table 4.2; Figure 4.4). I did not detect any differences in persistence between years ($F_{1, 24} = 0.22$, $p = 0.641$, Table 4.3) or within the year of 2010 ($F_{1, 24} = 0.01$, $p = 0.911$, Table 4.3).

I found the effect of rearing site addition to be important in all traits examined. More frogs were likely to be captured between-years in the RSA quadrats ($F_{1, 24} = 15.09$, $p = 0.0007$). Additionally, frogs were also more likely to persist between-years in the RSA quadrats ($F_{1, 24} = 5.467$, $p = 0.028$; Table 4.3). Frogs were also more likely to persist in RSA quadrats within the year of 2010 ($F_{1, 24} = 8.044$, $p = 0.009$; Table 4.3) indicating quick reactions to rearing site manipulations. When I added sex ratios to the four-way ANOVA, I also detected that both sexes were more likely to be captured in RSA quadrats between-years ($F_{1, 48}$ = 8.946, p = 0.004), but this increased likelihood of capture did not change the male-to-female ratio between-years ($F_{1, 48}$ = 1.99, p = 0.165) or within-year ($F_{1, 48}$ = 1.542, p = 0.22). Interestingly, both sexes showed increases in captures in RSA quadrats within-years ($F_{1, 48}$ = 1.29, p = 0.044) also indicating very fast reactions to rearing site addition. An interaction between rearing sites addition and year in the between-years analysis was observed for capture rates ($F_{1, 24}$ = 7.03, p = 0.013; Table 4.2; Figure 4.5) showing a distinct difference in the number of captures of frogs in rearing site added quadrats between years.

Discussion

Both males and females displayed some reaction to manipulations of rearing site addition (Figure 4.6A and 4.6B), but no effects of leaf litter addition or removal were detected in these groups (Figure 4.6C and 4.6D). Both males and females showed similar increases in density in RSA quadrats, but male density was slightly higher than that of female density, which is consistent with previous research on rearing site manipulations (Donnelly 1989). As predicted, rearing site addition also allowed habitat patches to support greater densities of frogs. The reaction creates potential for rearing site manipulation to be used on habitat patches to increase capacity for the habitat to support frogs, which could be a valuable conservation tool. Additionally, the addition of these rearing sites suggests that frogs will be more likely to defend their territory over the course of a year, rather than emigrate out of the area in search of better territories since the persistence rate increased after rearing site manipulations were conducted. These reactions to the resource manipulations offer strong evidence that the availability of suitable rearing sites for *O. pumilio* populations appear to be the primary limiting resource in determining population size and demographics.

Resource limitation may be important for sexual selection in this species. Males of this species defend territories which include foraging sites as well as rearing sites (McVey et al. 1981). Due to the unique energetic requirements of females (including feeding infertile ova to larvae), female selection is important in the evolution of this species (Summers et al. 1999, Maan and Cummings 2009). While call quality is known to influence female mate choice in anurans (Ryan 1983, Forester and Czarnowsky 1985), territorial quality of males also likely plays a role in female choice as seen in other anurans (Wells 1977). Since populations show such a strong reaction to rearing site addition, resource, and specifically rearing site, limited populations likely show stronger selective pressure than those not rearing site limited. As such, addition of artificial rearing sites should lessen the selective pressure on these frogs.

Given the density of these frogs and the level of parental care displayed by this species, I hypothesize that the availability of rearing sites to these frogs may be an important limiting resource. If rearing sites are limiting, the possibilities for active management of this species may be enhanced, as rearing sites can likely be more easily added to habitat patches than can food or leaf litter. This species appears flexible in regards to the selection of rearing sites, having been known to deposit tadpoles in bromeliad and *Heliconia* axils, tree holes, and artificial structures, and consequently, the manipulation of rearing sites as a conservation tool is a very real possibility.

Active management of *O. pumilio* has not been a high conservation priority to date due to their historical abundance and extensive range through Central America (Lötters et al. 2007). However, future conservation initiatives may be needed if we wish to preserve the phenotypic and genetic diversity seen in this species in the Bocas del Toro archipelago. The present study demonstrates that *O. pumilio* populations are at least locally sensitive to the availability of rearing sites. Additionally, this species appears to be highly tolerant of disturbed and secondary forest. Given these traits, this species may be a good candidate for *in situ* conservation of declining populations. Since habitat destruction affects amphibians (Young et al. 2001) and other taxa, this species could act as an umbrella species for conserving lowland rainforests in which other endangered taxa reside. Additionally, the use of artificial rearing sites could reduce the pressure local populations receive from commercial harvest (Nijman and Shepherd 2010).

Future research could provide data on whether rearing-site augmentation might enhance the detection and conservation of other arboreal amphibian species, including other species within the genus *Oophaga*. The canopy is the last frontier in understanding tropical ecology due to its general inaccessibility (Perry 1978, Lowman and Moffett 1993). Many species of invertebrates (Ellwood and Foster 2004) and amphibians (Good and Wake 1993) have been recently described after canopy surveys. However, detection of arboreal species likely remains low due to the accessibility problem. Given that many arboreal amphibian species, including *Bolitoglossa* salamanders (Jimenez 1994), will utilize bromeliads and other similar structures for shelter and ova/larval deposition (Wake 1987, Galindo-Leal et al. 2003), the use of artificial rearing sites for detection of these species could prove successful. *Oophaga vicenti* and *O. arborea* are two small arboreal dendrobatids that are particularly difficult to study since they are rarely, if ever, observed on the forest floor (Myers et al. 1984, Lötters et al. 2007). Due to the poor accessibility of their preferred habitat as well as the declining populations due to habitat loss (Lips et al. 2005) and the spread of chytridiomycosis in the region (Lips et al. 2006), relatively few studies have thoroughly examined population trends in these species. Similarly, dendrobatids in the genus *Ranitomeya* are a primarily South American radiation of small, largely arboreal species (Lötters et al. 2007). Many of these species are popular in the pet trade (Lötters et al. 2007), and similarly, are often poorly known. Many new species of *Ranitomeya* have been described in recent years (Brown et al. 2008), and the detection of new species as well as existing species might be facilitated by the recognition and manipulation of limiting resources, such as rearing sites. It seems likely that *Ranitomeya* species may also be rearing site limited noting similarities in parental investment in larval care with *Oophaga* (Brown et al. 2010). Perhaps the use of artificial rearing sites, such as large diameter drinking straws, would be of use in better understanding, detecting, and conserving such secretive species.

APPENDIX 4

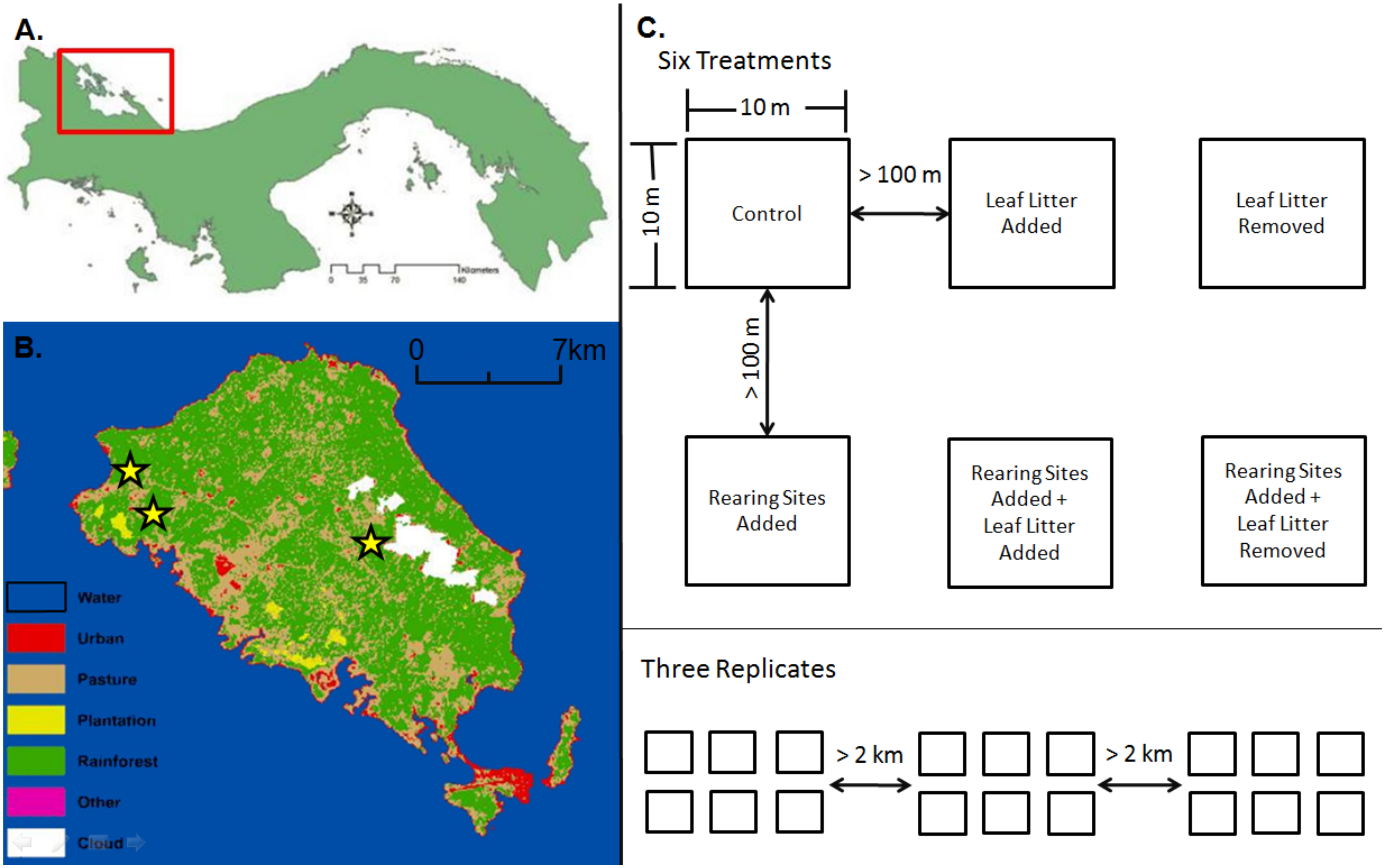

Figure 4.1: In Bocas del Toro, Panama (A.) on Isla Colon (B.), six experimental quadrats were set up with three replicates spread throughout the island represented by stars. Quadrats manipulations included a control, leaf litter added and removed, rearing sites added, and combinations of the two resources (C.).

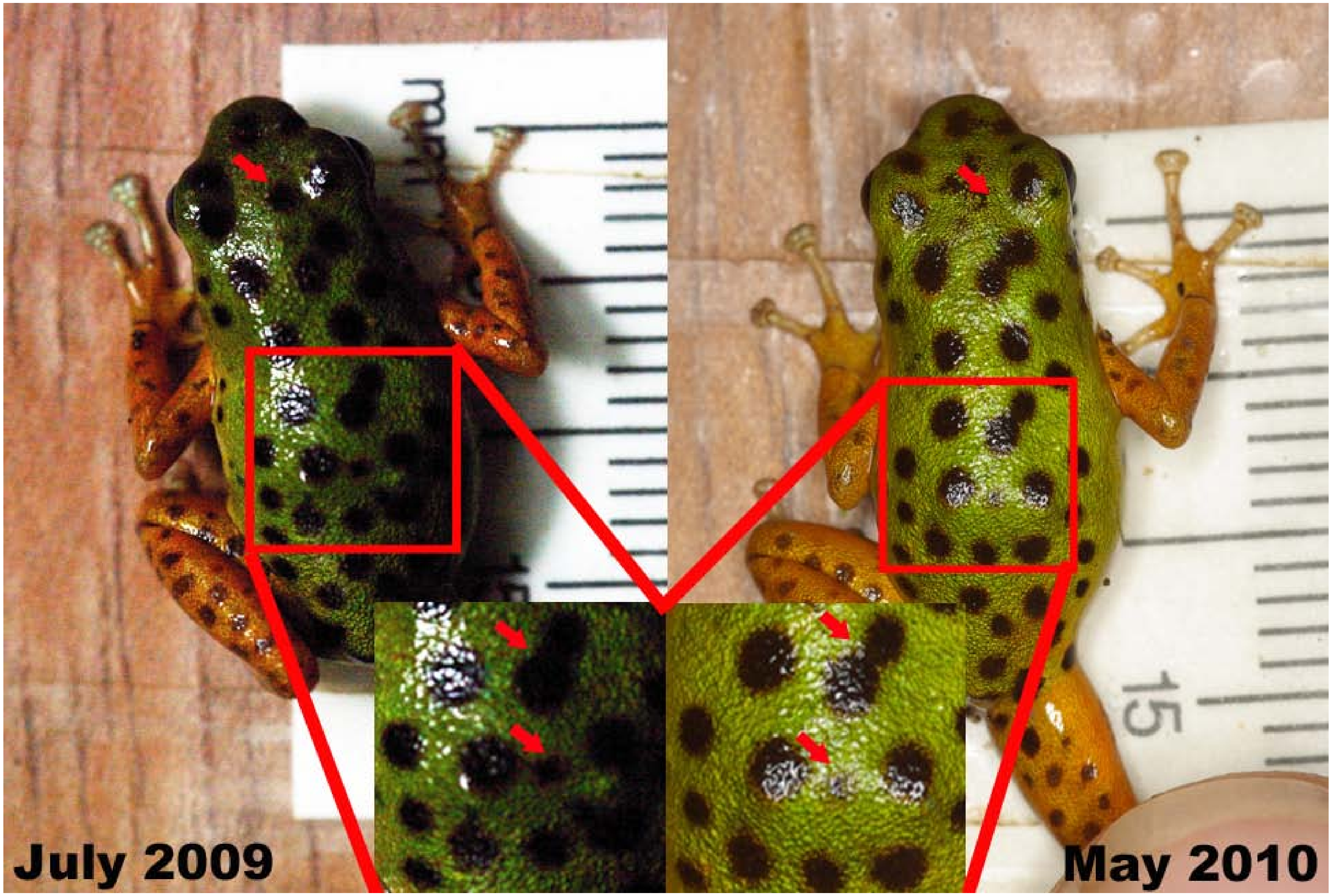

Figure 4.2: Spot comparison between an individual frog between July 2009 and May 2010. A spot was lost on the head and dorsum, as well as a spot starting to split into two. While these changes affect the overall spotting pattern, the rest of the spotting pattern makes it unmistakably the same frog.

Figure 4.3: Straws were cut to create a tip that would aid in capturing rain water. Straws were attached to woody vegetation using zip-ties and fence staples taking care not to crush the straw.

Table 4.1: Number of frogs captures and recaptures over the course of the study. Accumulated are the total number of marked individuals until that period.

	Capture Period				
	June 2009	**July 2009**	**August 2009**	**May 2010**	**June 2010**
Captures	92	81	66	125	129
Recaptures	0	24	27	33	58
Accumulated	92	149	188	280	351

Table 4.2: Results of a Three-Factor ANOVA for Capture Rate for all treatments and interactions. Between years were comparisons between June 2009 and June 2010, and within years were comparisons between June 2009 and August 2009. Residuals degrees of freedom were 24. Values in bold indicate a significant interaction with that treatment (α = 0.05).

Treatment	**Degrees of Freedom**	**Between Years**		**Within Years**	
		F	***P***	***F***	***P***
Rearing Sites	1	15.091	**0.0007**	2.251	0.147
Leaf Litter	2	0.688	0.512	0.354	0.706
Year	1	5.205	**0.031**	3.144	0.089
Rearing Sites x Leaf Litter	2	0.833	0.447	0.019	0.982
Rearing Sites x Year	1	7.03	**0.013**	0.019	0.893
Leaf Litter x Year	2	0.278	0.76	0.13	0.879
Rearing Sites x Leaf Litter x Year	2	0.346	0.711	0.074	0.929

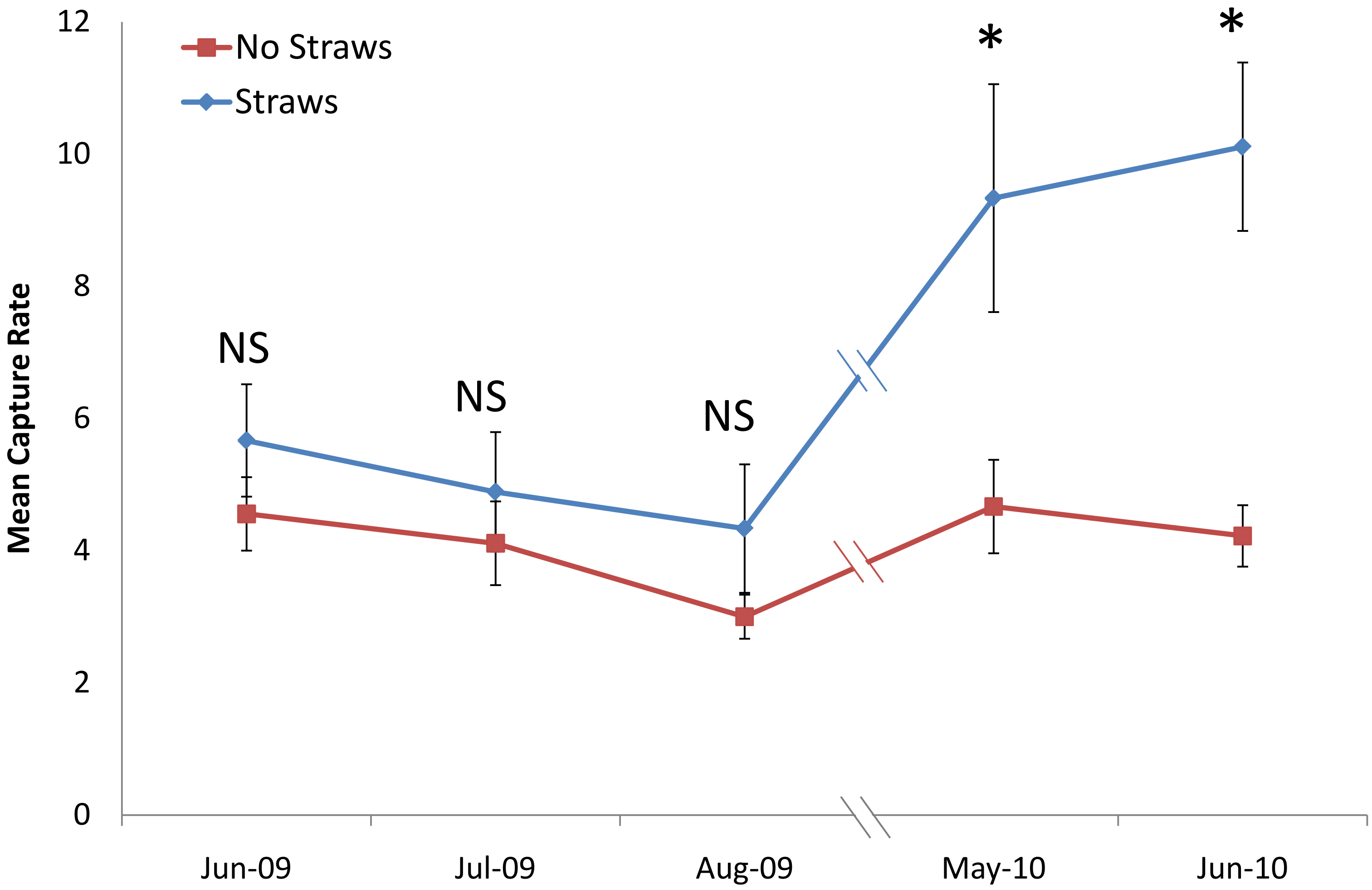

Figure 4.4: Effects of rearing site (straws) addition capture rate for *Oophaga pumilio*. Mean capture rate for each capture period was plotted with ± 1 SE. Distinct differences in capture rate were observed 12 months after the manipulation. NS – Nonsignificant; * - Significant (α = 0.05).

Table 4.1: Results of a Three-Factor ANOVA for differences in persistence for all treatments and interactions. Between years comparisons were done between July 2009 and June 2010, and within years were comparisons between May 2010 and June 2010. Values in bold indicate a significant interaction with that treatment ($\alpha = 0.05$).

Treatment	**Degrees of Freedom**	**Between Years**		**Within Years**	
		F	***P***	***F***	***P***
Rearing Sites	1	5.738	**0.025**	8.044	**0.009**
Leaf Litter	2	0.486	0.621	0.399	0.675
Year	1	0.224	0.641	0.013	0.911
Rearing Sites x Leaf Litter	2	0.013	0.903	2.322	0.119
Rearing Sites x Year	1	0.241	0.628	1.049	0.315
Leaf Litter x Year	2	0.758	0.479	0.928	0.409
Rearing Sites x Leaf Litter x Year	2	2.664	0.09	0.775	0.472

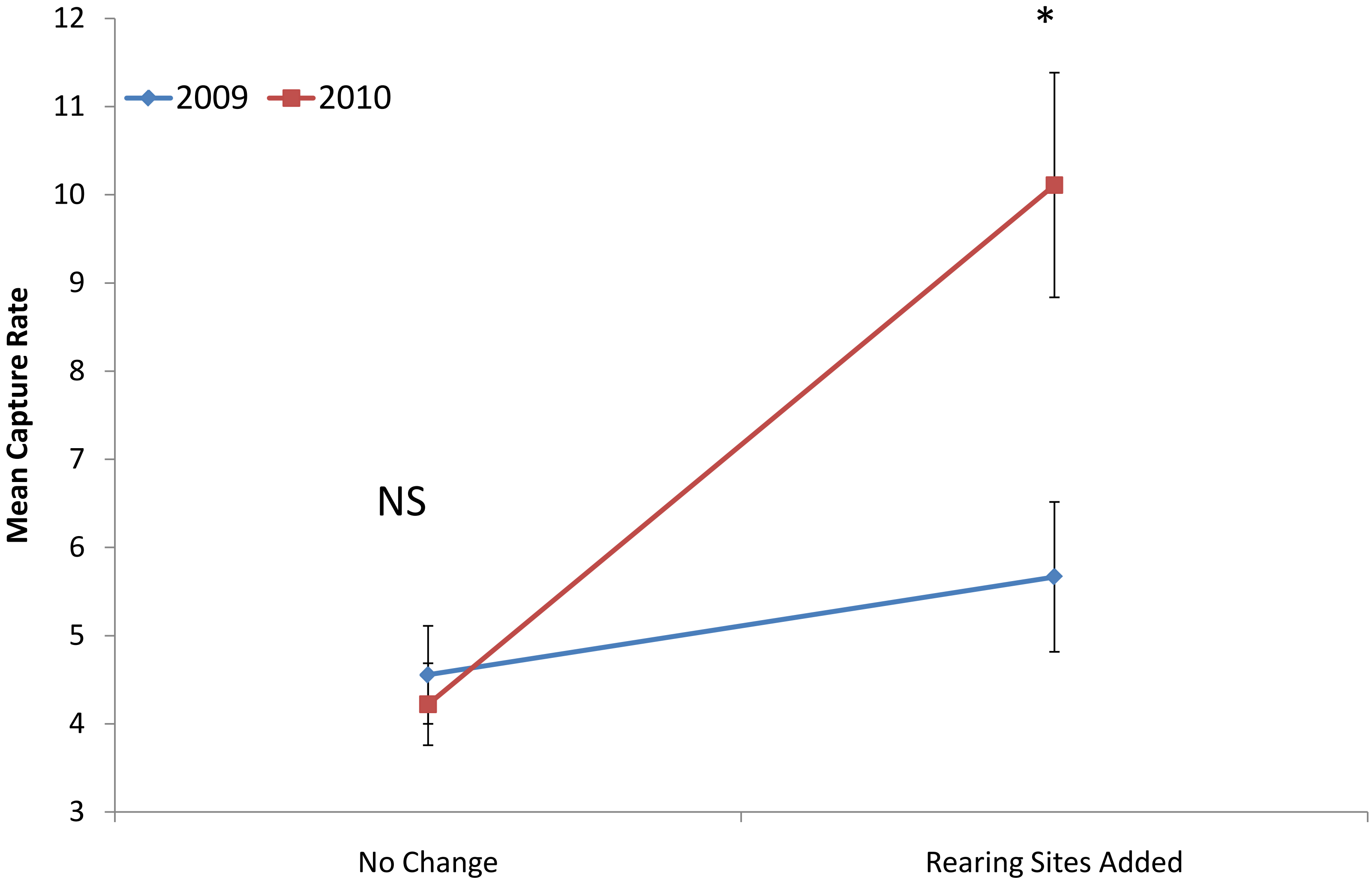

Figure 4.5: Interactions between rearing sites and years for capture rate. Mean capture rate is plotted for each year for rearing sites added and no change. NS – Nonsignificant; * - Significant (α = 0.05).

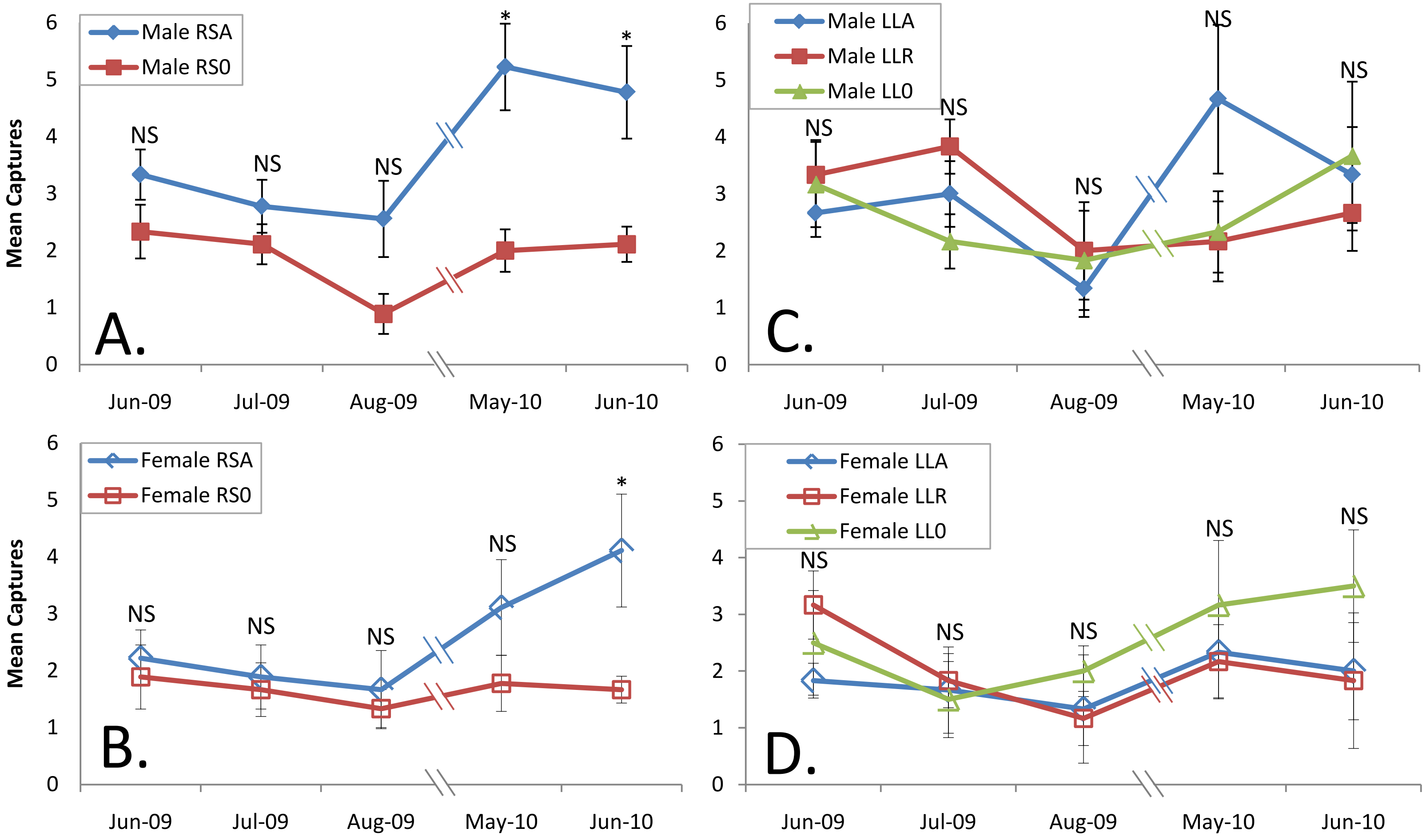

Figure 4.6: Mean capture rate responses of both males (A.) and females (B.) to rearing site manipulations as compared to male (C.) and female (D.) responses to the various leaf litter manipulations. Differences were observed in the RSA quadrats between years, but not between the LL quadrats. RSA – Rearing Sites Added; LLA – Leaf Litter added; LLR – leaf litter removed; LL0 – No change in leaf litter. Error bars represent ±1 SE. NS – Nonsignificant; * - Significant ($\alpha = 0.05$).

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LITERATURE CITED

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GENERAL CONCLUSIONS

The Bocas del Toro archipelago in Panama is an ideal area for study and research of human interactions with the surrounding environment. Christopher Columbus first landed in this area in 1502, and since then, this area has been inhabited by humans in some capacity. For many years, this area was popular for banana plantations, and still is today, with several fruit companies occupying large tracts of land in mainland areas surrounding the archipelago. In recent years, the area has garnered increased interest in the tourism industry as a Caribbean paradise for tourists to escape to. Consequently, the effects of human interaction with the surrounding environment are a complex problem.

Amphibian populations are declining all across the world (Stuart et al. 2004), and while some species are more threatened than others, all deserve attention, especially when in a landscape such as Bocas del Toro. Bocas del Toro, and much of Central America, is considered to be a biodiversity hotspot (Myers et al. 2000), with this area having some of the highest amphibian diversity on the globe (Grenyer et al. 2006). These areas are largely threatened by local human populations converting rainforest to other habitat types such as plantations (e.g., banana, oil palm, teak, and cacao) or pastureland for cattle ranching (see Chapter 2). The amphibians in these areas, as a result, are at risk of local population declines and extirpations. Bocas del Toro has the added risk of being an island complex, so loss or conversion of habitat means the genetically unique and diverse fauna living are at larger risk than mainland areas due to size of these islands (ranging from $2km^2$ to $61km^2$).

The Strawberry Poison Dart Frog (*Oophaga pumilio*) occurs throughout the Bocas del Toro archipelago, and in this area shows a large divergence in color pattern and morphotype from island to island; a pattern that extends to the surrounding mainland areas. While this species has a large range from Nicaragua to Panama (Lötters et al. 2007) and has “least concern” status by the International Union for the Conservation of Nature (IUCN; Sólis et al. 2008), this species also shows one of the most diverse vertebrate polymorphism seen on the planet. This species has remarkable ability to tolerate perturbations and conversions to the surrounding habitat, with populations doing well in organic or unused banana and cacao plantations (J. Lawrence, personal observation), but intensely managed areas, such as teak plantations and commercial banana plantations, offer little to no habitat for this species, even though these areas mimic a closed canopy forest required for this species. While the species as a whole is of little concern for management action, populations in Bocas del Toro represent a divergence necessary to maintain an evolutionarily unique phenomenon.

From 1986 to 1999, rainforests across this region showed annual declines of 0.73 ± 0.18% and conversions of these habitats to colonized (0.99 ± 0.4%), pastureland (3.45 ± 1%), and plantation (14.84 ± 6.6%) habitats; a trend that is likely continuing from 1999 to present (see Chapter 2). Such conversion to unsuitable habitats likely means that *O. pumilio* have shown declines as a result of habitat loss. Populations have variable population densities, with some populations having much lower densities than previously reported. Both the Uyama River (239 ± 72 frogs/ha) and the Isla Colon (246 ± 40 frogs/ha) have densities nearly a quarter the size of those reported for Costa Rican populations (Savage 2002), while the Pastores population (1352 ± 314 frogs/ha) are exceptionally large populations despite a small area of suitable habitat (16.08ha). Not only does the small area threaten this particular population from extirpation from habitat loss, but this population, as well as Isla Colon and Loma Partida, also show negative reactions to forest edges (see Chapter 2). Both Loma Partida and Pastores are small islands that could show fast population declines should deforestation increase in these areas. Isla Colon has the largest human populations (~ 3,500) of the islands in the archipelago and has experienced habitat conversion, with the largest increase being in pastureland (22.11% per year). These populations in particular could be especially negatively impacted by such conversion that is continuing through the region.

In light of such variable population densities across the region as well as variable reactions to forest edges, a method is necessary for monitoring these populations. Resource managers in temperate regions have often solicited the use of call surveys to analyze the population densities as well as trends in anuran populations (Lepage et al. 1997, Mossman et al. 1998, Genet and Sargent 2003). The use of these, however, in a tropical landscape largely seems to be underutilized. Some populations of anurans show the same periodicity to breeding behavior that temperate species do (Gottsberger and Gruber 2004), so use of call surveys could be of benefit to resource managers in tropical areas. Unfortunately, not all species exhibit predictable trends based on calling density of males. *Oophaga pumilio* shows a fairly constant rate of calling regardless of the population density, which, unfortunately, precludes the use of this traditional method for population monitoring. This may be due to the fact that this species is spread throughout the forest landscape (McVey et al. 1981), and does not show the marked periodicity to breeding behavior that other species of anurans show.

While this survey technique is not suitable for population monitoring in *O. pumilio*, it does offer some insight to population function in this species. Due to the territorial nature of this species (McVey et al. 1981), one would expect calling density to increase as population density increases as well. Despite this, *O. pumilio* populations show a relatively constant rate of calling, which suggests that this species may be showing acoustic niche partitioning. Other species of anurans, such as *Dendropsophus* (=*Hyla*) *microcephalus*, have been known to alter their calls to fall between competing males' calls in order to reduce confusion that females may incur (Bourne and York 2001). Due to the long duration of the call of *O. pumilio*, overlap and confusion could result in female mate choice if too many males call at the same time. Call quality is often an important factor in female choice in anurans (Wilbur et al. 1978, Forester and Czarnowsky 1985), and female mate choice has been implicated in driving the evolution of *O. pumilio* (Summers et al. 1999, Maan and Cummings 2008), so presumably, call quality also is an important factor in determining female choice.

Call surveys offer resource managers unique opportunity to potentially and quickly assess population trends in many anuran species, but in species that are showing population declines, active management is necessary to prevent loss of the population. Such management actions can include anything from habitat protection (Denton et al. 1997) to reintroduction programs (Griffiths and Pavajeau 2008) to resource manipulations. These actions, however, are all grounded in sound ecological knowledge of the species of interest. Manipulating resources available to *O. pumilio* populations can offer some insight to population function, and offer resource managers additional methods for supplementing threatened populations of *O. pumilio.*

By altering the availability of rearing sites and leaf litter to the Isla Colon population, I examined how the population reacts to the addition or removal of such resources. As with some species of birds (Newton 1994), *O. pumilio* shows a strong reaction to the manipulation of rearing sites. Populations approximately doubled in areas where rearing sites were added, but showed no reaction to the addition or removal of leaf litter, which acted as a surrogate for food resources. Frogs were also more likely to persist in rearing site quadrats. These reactions offer resource managers critical insight to population function. While the addition or removal of resources can be a largely be unreasonable methods for resource managers to pursue (e.g., addition/removal of leaf litter across a landscape), addition of rearing sites does offer a reasonable method for increasing or stabilizing threatened populations.

Management Recommendations

Oophaga pumilio has great ability to adapt to the changing landscape that almost certainly will dominate Bocas del Toro in the future. This ability to cope with changes to the habitat is not all-encompassing, however. The loss of mature forest through the region is of concern, not only for this species, but for the many of the species that occur in the tropical rainforests in the region. The Three-Wattled Bellbird (*Procnias tricarunculatus*), for example, is a species of large rainforest bird that is commonly found through the archipelago. The Three-Wattled Bellbird is listed as vulnerable by the IUCN and the population is declining (BirdLife International 2008). Protection of habitat should be of primary concern to resource managers in the area. A large amount of forest in the area is being fragmented and replaced by pastureland. While people in this region need to support themselves, the growing fragmentation of the forest needs to be addressed and minimized. Ecotourism is a very viable industry in Costa Rica (Tobias and Mendelsohn 1991), and with the increased interest in the Bocas del Toro archipelago for the tourism industry, the locals in the area could adopt an environment-protection view and sustain themselves by protecting the land that houses such unique animals such as *Oophaga pumilio* or the Three-Wattled Bellbird (Kruger 2005). Due to its large range, *O. pumilio* can act as an umbrella species for protection of other species in these areas.

Oophaga pumilio is of minor concern for resource managers for conservation action, which is understandable given the density of populations through the archipelago and their range (see Chapter 2; Lötters et al. 2007). Unfortunately, this lack of interest for conservation can result in the loss of genetic diversity seen in this species in Bocas del Toro. Between loss of habitat (see Chapter 2) and exploitation (Nijman and Shepherd 2010) of this species, populations of *O. pumilio* can face a number of pressures that can lead to declines. Current importation procedure does not recognize this diversity in Bocas del Toro, and that partly could be the result of these different populations being described as morphs. In numerous other species, subspecies recognition indicates the importance for conserving distinct populations, with tigers possibly being the most well known (Lynam 2010). In the Galapagos Tortoise (*Geochelone nigra*), subspecies designations have been given to different island populations (Caccone et al. 1999). Populations of *O. pumilio* in Bocas del Toro represent a similar phenomenon as seen in the Galapagos Tortoises, so as a result, the island populations, at minimum, should garner subspecies recognition. This will aid resource managers in identifying the distinct populations in need of conservation as well as allow for population separation in the importation process.

Many populations are popular for collection for captivity, and because of the adaptability of this species, *O. pumilio* can be sustainably harvested if monitored carefully. The method of rearing site addition I present here (see Chapter 4) offers resource managers an effective method of supplementing the populations such that these populations can support reduction of habitat or exploitation from collectors. These methods are ideal for populations of low density, such as Isla Colon, or populations in small areas, such as Pastores and Loma Partida (see Chapter 2). Unfortunately, populations in Bocas del Toro appear to show no relationship between call density and population density, so the use of call surveys are not an ideal method of tracking population trends over time and identifying at-risk populations (see Chapter 3). Consequently, resource managers should focus on other methods to track population trends, such as visual encounter surveys on transects. Use of these, should, however, also be used with caution as some population do show negative relationships to edges.

Conservation of *O. pumilio* in Bocas del Toro is of great importance. This species has been of great interest to researchers for the evolution of aposematic coloration and toxicity (Summers et al. 2003, Siddiqi et al. 2004, Maan and Cummings 2009), which will offer insight into the functioning of natural systems across the globe. This species is very similar to "Darwin's Finches" in diversification and, unlike the finches, these populations are on the cusp of speciation (Hagemann and Prohl 2007). In order to understand such processes, populations need to be protected into the future. Additionally, these frogs are very abundant in these Caribbean rainforest systems, and undoubtedly aid in ecosystem functioning. Consequently, while most populations are not likely of critical concern for resource managers, populations in this area should be closely monitored to ensure the persistence of these populations into the future.

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