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Enigmatic Faunal Declines at La Selva, Costa Rica: Patterns and Processes in a Collapsing Neotropical Herpetofauna

Steven M. Whitfield

Florida International University, steven.whitfield@fiu.edu

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ENIGMATIC FAUNAL DECLINES AT LA SELVA, COSTA RICA: PATTERNS
AND PROCESSES IN A COLLAPSING NEOTROPICAL HERPETOFAUNA

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Steven M. Whitfield

2011

To: Dean Kenneth Furton
College of Arts and Sciences

This dissertation, written by Steven M. Whitfield, and entitled Enigmatic Faunal Declines at La Selva, Costa Rica: Patterns and Processes in a Collapsing Neotropical Herpetofauna, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Michael Heithaus

Joel Heinen

Thomas Philippi

Craig Guyer

Maureen Donnelly, Major Professor

Date of Defense: May 20, 2011

The dissertation of Steven M. Whitfield is approved.

Dean Kenneth Furton
College of Arts and Sciences

Interim Dean Kevin O'Shea
University Graduate School

Florida International University, 2011

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I owe tremendous thanks to a very large number of people for providing support during the completion of this dissertation: my labmates, collaborators, field assistants and volunteers, the Organization for Tropical Studies, and to my friends.

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ABSTRACT OF THE DISSERTATION

ENIGMATIC FAUNAL DECLINES AT LA SELVA, COSTA RICA: PATTERNS
AND PROCESSES IN A COLLAPSING NEOTROPICAL HERPETOFAUNA

by

Steven M. Whitfield

Florida International University, 2011

Miami, Florida

Professor Maureen Donnelly, Major Professor

Amphibian populations are declining even in pristine areas in many parts of the world, and in the Neotropics most such enigmatic amphibian declines have occurred in mid- to high-elevation sites. However, amphibian populations have also declined at La Selva Biological Station in the lowlands of Costa Rica, and similar declines in populations of lizards have occurred at the site as well. To set the stage for describing amphibian declines at La Selva, I thoroughly review knowledge of amphibian decline and amphibian conservation in Central America: I describe general patterns in biodiversity, evaluate major patterns in and ecological correlates of threat status, review trends in basic and applied conservation literature, and recommend directions for future research. I then synthesize data on population densities of amphibians, as well as ecologically similar reptiles, over a 35-year periods using quantitative datasets from a range of studies. This synthesis identifies assemblage-wide declines of approximately 75% for both amphibians and reptiles between 1970 and 2005. Because these declines defy patterns most commonly reported in the Neotropics, it is difficult to assess causality evoking known processes associated with enigmatic decline events. I conduct a 12-month pathogen

surveillance program to evaluate infection of frogs by the amphibian chytrid fungus, an emerging pathogen linked to decline events worldwide. Although lowland forests are generally believed to be too warm for presence or adverse population effects of chytridiomycosis, I present evidence for seasonal patterns in infection prevalence with highest prevalence in the coolest parts of the year. Finally, I conducted a 16-month field experiment to explore the role of changes to dynamics of leaf litter, a critical resource for both frogs and lizards. Population responses by frogs and lizards indicate that litter regulates population densities of frogs and lizards, particularly those species with the highest decline rate. My work illustrates that sites that are assumed to be pristine are likely impacted by a variety of novel stressors, and that even fauna within protected areas may be suffering unexpected declines.

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INTRODUCTION

Amphibian declines are rapidly emerging as a frontier issue in conservation research. While amphibians have been long neglected in basic and applied conservation research compared to other vertebrate groups (Lawler et al. 2006, Gardner et al. 2007), a consensus emerged in the 1990s that amphibians are declining at alarming rates (Pechmann et al. 1991, Pechmann and Wilbur 1994, Alford and Richards 1999, Alford et al. 2001). Approximately 32% of amphibian species are believed to be threatened with extinction, and more than 120 species have disappeared globally – many of these are undoubtedly extinct (Stuart et al. 2004). While amphibian declines in human-dominated landscapes are expected given current understanding of ecological processes, amphibian declines are also widespread in national parks and protected reserves - habitats thought to be among the most pristine sites remaining on the planet; these declines in pristine sites have been dubbed “enigmatic declines” (Alford and Richards 1999, Stuart et al. 2004, Sodhi et al. 2008). A number of factors are undoubtedly implicated in amphibian declines, both enigmatic and conventional – including habitat modification, contamination from pesticides and other xenobiotic compounds, emerging infectious diseases, and climate change (Kiesecker et al. 2001, Collins and Storfer 2003, Stuart et al. 2004, Sodhi et al. 2008). Sorting the relative contributions of these factors has proved a considerable challenge in applied conservation research.

Amphibians in the Neotropics have been affected particularly adversely by enigmatic amphibian declines (Lips 1998, Young et al. 2001, Lips et al. 2005, Lips et al. 2006). Several perceived phenomenological similarities link the majority of observed amphibian decline events in the Neotropics: declines are believed to occur primarily in

montane sites (Lips 1998, Lips 1999); declines are believed to primarily affect riparian species of amphibians (Lips 1998, Lips 1999, Lips et al. 2003); and amphibians are believed to decline while other taxa appear unaffected. However, none of these general rules is absolute. Terrestrial species of amphibians have been observed to decline (Lips and Donnelly 2005, Lips et al. 2006, Rovito et al. 2009). Limited observations of lowland population declines and extinctions have been reported (Stewart 1995, Puschendorf 2003, Puschendorf et al. 2005, Puschendorf et al. 2009, Richards-Zawacki 2010). Populations of lizards have declined at sites where amphibian declines have occurred (Pounds et al. 1999), and also appear to be widespread throughout montane regions of the Neotropics (Huey et al. 2009, Sinervo et al. 2010).

In this dissertation, I describe patterns behind and explore processes responsible for long-term declines in amphibian and reptile populations at a well-studied research reserve: La Selva Biological Station in Costa Rica. These declines are problematic because they appear associated with population declines in amphibian populations occurring throughout Central America, but differ in a number of important ways. These declines are therefore unlike any other declines reported for the Neotropics, and the causal processes responsible for these declines are difficult to identify.

In Chapter 1, I provide a thorough background for understanding such declines by reviewing the extensive body of literature addressing amphibian decline and amphibian conservation in Central America. Chapter 1 reviews amphibian diversity in the region, identifies correlates of threat status among amphibians in the region, provides a detailed review of research on amphibian decline in the region, and highlights important areas for

future research. This chapter was written for a volume in the *Amphibian Biology* series edited by Hal Heatwole.

In Chapter 2, I describe patterns behind long-term declines in populations of amphibians and reptiles at La Selva Biological Station. I synthesize data collected over a 35-year period (1970-2005) using a single standard method for examining population densities of leaf-litter amphibians and reptiles to explore long-term fluctuations or directional change in density. While there was no a priori reason to expect population declines given current knowledge of amphibian declines, data indicate approximately 75% declines in populations of both amphibians and reptiles over this period. A large number of factors may be implicated in these declines (Figure 1) including indirect effects of habitat fragmentation, climate change, the emerging infectious disease chytridiomycosis, and contamination by pesticide drift from nearby agricultural areas. This chapter is published in the *Proceedings of the National Academy of Sciences USA*.

In Chapter 3, I explore temporal dynamics of infection by the amphibian chytrid fungus in three species of frogs at La Selva. The disease chytridiomycosis, a potentially lethal infection by the apparently non-native chytridiomycete fungus *Batrachochytrium dendrobatidis* (Bd), has been implicated in most enigmatic amphibian decline events in the Neotropics. A number of studies have shown that Bd is temperature sensitive, prefers cool climates, and cannot survive warm climates. The inability of Bd to survive warm climates has been used to explain why most enigmatic amphibian declines have occurred in cooler montane regions of the US. It has been suggested that Bd cannot cause declines in warm lowland sites such as La Selva. I address this assumption by describing prevalence of Bd at this site over a 12-month study period.

In Chapter 4, I explore the potential role of shifting dynamics of leaf litter in long-term declines in both amphibian and reptile populations at La Selva. A wide variety of studies have suggested that depth of standing leaf litter is a primary correlate of density for leaf-litter amphibians and reptiles (Scott 1976, Lieberman 1986, Fauth et al. 1989, Whitfield and Pierce 2005). Climate-related shifts in litterfall or litter decomposition rates, which are sensitive to a changing climate e.g. (Clark et al. 2003), may be reducing depth of standing litter on the forest floor and depleting critical microhabitats for amphibians and reptiles. Further, apparent increases in populations of collared peccaries have been suggested to accelerate decomposition of leaf litter by mechanical degradation of litter through trampling effects. To explore impacts of litter depth on amphibians and reptiles, I conduct a 16-month field experiment to relate litter quantity to population densities of amphibians and reptiles.

LITERATURE CITED

- Alford, R. A., P. M. Dixon, and J. H. K. Pechmann. 2001. Global amphibian population declines. *Nature* **412**:499-500.
- Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* **30**:133-160.
- Clark, D. A., S. C. Piper, C. D. Keeling, and D. B. Clark. 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984 - 2000. *Proceedings of the National Academy of Sciences* **100**:5282-5287.
- Collins, J. P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* **9**:89-109.
- Fauth, J. E., B. I. Crother, and J. B. Slowinski. 1989. Elevational patterns of species richness, evenness, and abundance of the Costa Rican leaf-litter herpetofauna. *Biotropica* **21**:178-185.
- Gardner, T. A., J. Barlow, L. W. Parry, and C. A. Peres. 2007. Predicting the uncertain future of tropical forest species in a data vacuum. *Biotropica* **39**:25-30.
- Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. A. Perez, and T. Garland. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B-Biological Sciences* **276**:1939-1948.
- Kiesecker, J. M., A. R. Blaustein, and L. K. Belden. 2001. Complex causes of amphibian population declines. *Nature* **410**:681-684.
- Lawler, J. J., J. E. Aukema, J. B. Grant, B. S. Halpern, P. Kareiva, C. R. Nelson, K. Ohleth, J. D. Olden, M. A. Schlaepfer, B. R. Silliman, and P. Zaradic. 2006. Conservation science: a 20-year report card. *Frontiers in Ecology and the Environment* **4**:473-480.
- Lieberman, S. S. 1986. Ecology of the leaf litter herpetofauna of a neotropical rain forest: La Selva, Costa Rica. *Acta Zoologica Mexicana (ns)* **15**:1-72.
- Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* **12**:106-107.
- Lips, K. R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* **13**:117-125.

- Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* **103**:3165-3170.
- Lips, K. R., P. A. Burrowes, J. R. Mendelson, III, and G. Parra-Olea. 2005. Amphibian population declines in Latin America: a synthesis. *Biotropica* **37**:222-226.
- Lips, K. R., and M. A. Donnelly. 2005. Lessons from the Tropics. Pages 198-205 *in* M. Lannoo, editor. *Amphibian declines : the conservation status of United States species*. University of California Press, Berkeley.
- Lips, K. R., J. D. Reeve, and L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* **17**:1078-1088.
- Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* **253**:892-895.
- Pechmann, J. H. K., and H. M. Wilbur. 1994. Putting declining amphibian populations in perspective: natural fluctuation and human impact. *Herpetologica* **50**:65-84.
- Pounds, J. A., M. P. L. Fogden, and J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. *Nature* **398**:611-615.
- Puschendorf, R. 2003. *Atelopus varius* (harlequin frog). *Herpetological Review* **34**:355-355.
- Puschendorf, R., A. C. Carnaval, J. VanDerWal, H. Zumbado-Ulate, G. Chaves, F. Bolanos, and R. A. Alford. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions* **15**:401-408.
- Puschendorf, R., G. Chaves, A. J. Crawford, and D. R. Brooks. 2005. *Eleutherodactylus ranoides* (NCN). Dry forest population, refuge from decline? *Herpetological Review* **36**:53-53.
- Richards-Zawacki, C. L. 2010. Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proceedings of the Royal Society B-Biological Sciences* **277**:519-528.
- Rovito, S. M., G. Parra-Olea, C. R. Vasquez-Almazan, T. J. Papenfuss, and D. B. Wake. 2009. Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of the National Academy of Sciences of the United States of America* **106**:3231-3236.

- Scott, N. J. 1976. The abundance and diversity of the herpetofaunas of tropical forest litter. *Biotropica* **8**:41-58.
- Sinervo, B., F. Mendez-de-la-Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. V. S. Cruz, R. Lara-Resendiz, N. Martinez-Mendez, M. L. Calderon-Espinosa, R. N. Meza-Lazaro, H. Gadsden, L. J. Avila, M. Morando, I. J. De la Riva, P. V. Sepulveda, C. F. D. Rocha, N. Ibarguengoytia, C. A. Puntriano, M. Massot, V. Lepetz, T. A. Oksanen, D. G. Chapple, A. M. Bauer, W. R. Branch, J. Clobert, and J. W. Sites. 2010. Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science* **328**:894-899.
- Stewart, M. M. 1995. Climate driven population fluctuations in rain forest frogs. *Journal of Herpetology* **29**:437-446.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306**:1783-1786.
- Whitfield, S. M., and M. S. F. Pierce. 2005. Tree buttress microhabitat use by a neotropical leaf-litter herpetofauna. *Journal of Herpetology* **39**:192-198.
- Young, B. E., K. R. Lips, J. K. Reaser, R. Ibanez, A. W. Salas, J. R. Cedeno, L. A. Coloma, S. Ron, E. La Marca, J. R. Meyer, A. Munoz, F. Bolanos, G. Chaves, and D. Romo. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* **15**:1213-1223.

CHAPTER 1: DECLINE AND CONSERVATION OF AMPHIBIANS IN CENTRAL AMERICA

ABSTRACT

I review the conservation status of amphibians in Central America, describing general patterns in biodiversity, evaluate major patterns in and ecological correlates of threat status, review trends in basic and applied conservation literature, and recommend directions for future research. I begin by describing the Central American environment in order to describe patterns of amphibian species richness and patterns of endemism in the context of ecological associations and biogeographic patterns. I use data from the Global Amphibian Assessment (GAA) to explore the conservation status of amphibians in the region. I review conservation threats in light of a meta-analysis of conservation of Central American amphibians; 43 papers out of 401 valid studies of amphibians in Central America from 1967 to 2007 dealt with conservation. My analysis revealed gaps in spatial coverage for the region and a paucity of studies focused on particular processes. Most research in Central America has focused on losses associated with the spread of chytridiomycosis. I also review conservation actions in place and conclude with statements concerning future research in Central America.

INTRODUCTION

Central America, the geographic province that spans seven political entities between southern Mexico and northern South America, is a global hotspot of amphibian biodiversity, a priority region for conservation action, and a site of intensive amphibian research. While Central America comprises only 0.36% of global land area, it harbours 7.2% of extant amphibian species (Myers *et al.* 2000; Stuart *et al.* 2004; Lamoreux *et al.* 2006). In addition to facing conventional threats to biodiversity such as modification and degradation of habitat, Central American amphibians have experienced some of the most severe, rapid population declines and mass-mortality events ever reported (Crump *et al.* 1992; Pounds and Crump 1994; Pounds *et al.* 1997; Lips 1999; Lips *et al.* 2005, 2006, Pounds *et al.* 2006a). The rapid, apparent extinction of the Golden Toad, *Incilius periglenes*, from Monteverde, Costa Rica, helped mobilize herpetologists to consider the existence of the crisis in amphibian decline (Crump *et al.* 1992; Pounds and Crump 1994) and later empirical studies from Central America have helped to convince skeptical biologists of the reality of these declines (Pounds *et al.* 1997; Lips 1998, 1999; Lips *et al.* 2006). Data from Central America have helped steer biologists toward examination of putative causes contributing to declines in Central America and throughout the world (Berger *et al.* 1998, 2008; Pounds 2001; Lips *et al.* 2005, 2006, 2008; Pounds *et al.* 2006a; Whitfield *et al.* 2007, Rohr *et al.* 2008, Rohr and Raffel 2010).

Enigmatic amphibian declines and disappearances were first noticed in Central America in the early 1980s in Guatemala and Honduras when repeat surveys of historic locations failed to yield once-common species (Campbell 1999; McCranie and Wilson 2002; Rovito *et al.* 2009). In the late 1980s, well-reported declines occurred at

Monteverde, Costa Rica; the Golden Toad, the Monteverde Harlequin Frog and numerous other species in the area all declined in 1987-1988 although no field surveys were conducted over that period and no mortality was noted (Crump *et al.* 1992). The declines at Monteverde were initially suggested to be linked to shifting climate – specifically, to increasing temperature and reduced moisture in cloud-forest habitats (Pounds and Crump 1994). Through the 1990s and 2000s, additional declines were reported in upland forests of southern Costa Rica and Panama (Lips 1998, 1999; Lips *et al.* 2006, 2008; Woodhams *et al.* 2008; Richards-Zawacki 2010). These declines were shown to have reduced species richness by 50% and density by 80% within as short a time period as six months (Lips *et al.* 2006). The declines appeared to spread from Monteverde toward the southeast, and spatiotemporal patterns of decline gave rise to the “extinction wave hypothesis” (Lips 1999; Lips *et al.* 2006). This wave was later associated with the invasion front of the emerging amphibian disease chytridiomycosis, producing the “spreading pathogen hypothesis” (Lips *et al.* 2006, 2008). As a consensus arose over the central role of chytridiomycosis in these enigmatic declines, reinterpretation of the data from Monteverde gave rise to the “climate-linked epidemic hypothesis,” followed by the controversial “chytrid thermal-optimum hypothesis” (Pounds 2001; Pounds *et al.* 2006a) and most recently the “climate variability hypothesis” (Rohr *et al.* 2008; Rohr and Raffel 2010). While it appears that the agent of chytridiomycosis, *Batrachochytrium dendrobatidis* (*Bd*) is novel to Central America on the basis of genetic evidence, the relative roles of other stressors in synergy with chytridiomycosis remain highly controversial for amphibians in the region (Pounds and Crump 1994; Pounds 2001; Lips *et al.* 2006, 2008; Pounds *et al.* 2006a; Daly *et al.*

2007b; Whitfield *et al.* 2007, Rohr *et al.* 2008; Anchukatis and Evans 2010; Rohr and Raffel 2010). At the same time as these enigmatic declines occurred – and indeed, starting centuries before the first enigmatic declines were reported, conventional threats to biodiversity such as habitat loss and degradation had begun to encroach on amphibian populations in all areas of Central America; these threats continue to this day. Future threats which have not yet been reported in Central America, or perhaps throughout the world, may yet threaten amphibian populations in the mid-term to long-term.

Herein, the current knowledge of amphibian declines and amphibian conservation in Central America are assessed. The aim is to synthesize available knowledge and data, set a benchmark for current understanding, and provide a springboard for future research. Specifically, this chapter: (1) Describes the environment and amphibian fauna of Central America, (2) uses data generated by the Global Amphibian Assessment (GAA) to summarize the current status of conservation in Central American amphibians, 3) reviews the empirical studies relevant to amphibian conservation in the region (4) identifies current and future actions for conservation in the region, and (5) prioritizes topics for research into the conservation of Central American amphibians.

THE CENTRAL AMERICAN ENVIRONMENT

Central America as a geographic province covers about 51 million hectares and reaches 1,500 km across seven political entities (Guatemala, Belize, El Salvador, Honduras, Nicaragua, Costa Rica, and Panama) (Figure 1.1). Central America is bounded to the west by the Pacific Ocean, to the east by the Caribbean Sea, to the north by Chiapas and Yucatan of Mexico and to the south by the northwestern limit of South

America. At its widest, Central America extends over 400 km in northern Nicaragua and Honduras, and at its narrowest constricts to <65 km near the Panama Canal. In general, Central America is comprised of a lowland area along each coast separated by one or more upland mountain ranges. The region's diverse physiography creates a highly diverse range of ecosystems, from the xeric thorn scrub in the rainshadows of the upper Rio Motagua Valley to lush cloud forests shrouded in epiphytes and perpetual mist on the slopes of the Talamancas, and from lowland rainforests on the Caribbean slopes to fir-dominated forests in the highlands of Guatemala. This diverse group of ecosystems offers a wide range of habitats for amphibians in the region.

The considerable climatic variation is produced by two primary factors. First, because the region lies entirely within the tropics and seasonal variation in temperature is therefore minimal, temperature is predominantly driven by elevational variation (from sea level on either coast to over 4,000 m in southern Guatemala). Second, variation in precipitation is produced largely from trade winds blowing off the Caribbean towards the west. These winds bring moisture-laden air onto the moist to wet forests on the eastern versant of Central America. As the air rises to surmount the mountains, it cools to produce often dramatic orographic precipitation ($>7,000 \text{ mm y}^{-1}$) that fosters wet montane forest and cloud forest. Drier forests that dominate the Pacific versants and interior valleys are produced by rainshadows on the leeward slopes of the higher ranges.

Central America exhibits distinct upland and lowland amphibian faunas resulting from differing environmental conditions and biogeographic associations. Upland faunas show strong latitudinal gradients along a north-south axis with each montane province containing a very high number of endemics. Lowland faunas do not show such strong

latitudinal gradients in turnover but show significant differences between the Pacific and the Caribbean slopes because of reduced interchange and climatic differences.

AMPHIBIAN DIVERSITY IN CENTRAL AMERICA

The amphibian fauna of Central America comprises 451 currently recognized species, distributed unevenly amongst 17 families and 71 genera (Table 1.1). All three extant orders of amphibians are present, with 123 species of salamanders (all plethodontids), 16 species of caecilians (all caeciliids) and 312 species of anurans (split among 15 families and 59 genera). The best-represented families of amphibians include Plethodontidae, Hylidae, Craugastoridae, and Bufonidae (Table 1.1). New species of frogs and salamanders continue to be described each year, and no country is likely to have been exhaustively sampled taxonomically. That such a small region can host such an enormous and varied amphibian fauna is the product of varied climate and topography, a diverse range of amphibian life histories, and a unique historical biogeography.

Part of the reason that Central America is able to possess such high amphibian species richness is that the variety of ecological forms (i.e., niches) of amphibians in the Neotropics is much greater than in temperate areas. Species differ widely in their utilization of habitats, with affinities ranging from dense forest to open ponds to high-elevation paramó. In moist tropical forests – the most species-rich habitats – species may variously inhabit forest strata close to the ground (some craugastorids, bufonids, leptodactylids), or may lead primarily fossorial lives (microhylids, caecilians, *Rhinophrynus*). Many species are primarily, or exclusively, arboreal (hylids, centrolenids, some eleutherodactylids, some plethodontids), occupying and even breeding

in canopy microhabitats such as bromeliads or mats of mosses. Reproductive modes among the amphibians of Central America are especially diverse (Donnelly 1994). Pond-breeding species (many hylids, leptodactylids, microhylids, rhinophrynids) are diverse and particularly common in lowland forests. Stream-breeding species (many hylids, centrolenids, bufonids, and some dendrobatids) are more diverse in the upland forest regions and exhibit some of the highest rates of endemism. Almost half of the amphibians in Central America are terrestrially-breeding species with direct development (craugastorids, eleutherodactylids, strobomantids, plethodontids, and caeciliids), and these species often reach high population densities even far from sources of standing water. The diverse ecological roles played by these amphibians affect the relative strengths of various anthropogenic impacts.

Species Richness and Uniqueness

Regional species richness of amphibians varies strongly throughout Central America, and is greatest in the mid-elevation forests of southern Central America (Figure 1.2). Extremes in richness range from only three species on the highest peaks of Guatemala to over 72 species at El Cope, Panama (Crawford et al. 2010). Species richness is highly correlated with mean annual precipitation, and peaks at intermediate elevations. The wetter regions of eastern Central America, as well as the Golfo Dulcean lowlands of southwestern Costa Rica, show the greatest species richness, with drier regions such as the western lowlands and highest peaks of the Guatemalan Plateau and the upper Rio Motagua valley containing considerably fewer species. Species richness peaks in mid-elevation forests (Wiens *et al.* 2006; Smith *et al.* 2007) (Figure 1.3); many

species also are found in the lowlands but very few species occur at the highest elevations (>2,500 m asl).

The amphibians of Central America have high levels of endemism; two hundred and seven of them (47% of the entire fauna) are endemic to the region, and almost all of these are even more range-restricted within the area. Many of the endemic species are in the following genera: *Craugastor* (47 endemic species), *Bolitoglossa* (38), *Isthmohyla* (14), *Oedipina* (13), and *Nototriton* (13). Because of low tropical intra-annual variability in temperature, many tropical endemics are physiologically restricted to narrow elevational bands (Wake and Lynch 1976; Feder 1982; Wake 1987). Species endemism is correlated with elevation, and virtually all the endemics in Central America are located in upland regions where many of them evolved *in situ* (Savage 1982). Lowland regions harbour relatively few endemics. Not surprisingly, a large number of range-restricted species occur entirely within a single country. Costa Rica has 47 single-country endemics, Honduras 42, Guatemala 40, and Panama 34. Nicaragua has only three endemic species, Belize has a single one, and El Salvador has none at all.

Biogeography and Modern Ecological Associations

Patterns of species richness, uniqueness, and endemism are heavily influenced by biogeographic processes on evolutionary and geological timescales. The amphibian fauna of Central America is composed primarily of three distinct historical units: North American and South American species groups, which largely evolved outside the region and later invaded, and a large group of distinctive Middle American clades that evolved *in situ* (Duellman 1966, 1988; Savage 1966, 1982; Campbell 1999). Previous treatments

of the Central American amphibian fauna have differed in their delimitation of biotic provinces (Duellman 1966, 1988; Savage 1966, 1982; Campbell 1999) but in the present chapter a composite of these classifications are used to describe the environment and the amphibian fauna of the region (Figure 1.1, Table 1.2).

Yucatán Lowlands: This flat, lowland region of Cretaceous-Paleogene limestone deposits extends from northern Guatemala and eastern Belize northward through the Yucatan Peninsula. The region exhibits a strong southward increase in precipitation from <1,500 mm to about 3,000 mm y⁻¹. Within Guatemala and Belize, the vegetation is dominated by deciduous lowland forest in the north, and moist evergreen lowland forests in the south. This region contains approximately 40 species of amphibians, mostly with affinities to extralimital northern groups. The single endemic to this region, *Bolitoglossa yucatana*, is widely distributed throughout the Yucatan Peninsula.

Maya Mountains: This small isolated formation of relatively low mountains (mostly <1,000 m) is located almost exclusively within southern Belize. Annual precipitation ranges up to about 2,500 mm y⁻¹, and vegetation includes premontane forests with tropical broadleaf vegetation, pine forests and oak forests. This region hosts 34 species and a single endemic (*Lithobates juliani*). Most of the amphibians here also occur in the Yucatan Lowlands but several of the species from mid-elevation are shared with the Northern Nuclear highlands.

Northern Nuclear Highlands: This broad upland region in southern Guatemala and extreme western Honduras includes the highest peaks in Central America (Volcán Tajumulco at 4,220 m and Volcán Tacaná at 4,020 m) and is contiguous with the Chiapan highlands of Mexico. The region includes a number of ranges: the Sierra de las Minas, Sierra de los Cuchumatanes, Sierra de Santa Cruz, Sierra de Cuacús, and the extensive Guatemalan Plateau to the south and west. The Caribbean and upper Pacific slopes of these highlands can be quite moist, (with over 4,000 mm y⁻¹), but the Guatemalan plateau and upper Rio Motagua valley are considerably drier (to < 1,000 mm y⁻¹). The slopes are dominated by broadleaf evergreen vegetation at lower elevations, with communities of oak-pine at higher elevations and fir forests on the peaks. One of the most diverse and unique regions in Central America, these highlands host 121 species, including 34 species restricted to the region. The region shares many species with the extralimital Chiapan highlands. The diversity in this region is highest on the eastern slopes and in the Sierra de las Minas. The region has high diversity of *Bolitoglossa* (20 species, 5 endemic), *Craugastor* (22 species, 8 endemic), *Plectrohyla* (11 species, 4 endemic), and *Ptychohyla* (6 species, 4 endemic). The region hosts an especially diverse salamander fauna experiencing high rates of decline (Rovito *et al.* 2009), with members of *Bradytriton*, *Nyctanolis*, and *Pseudoeurycea* reaching the southern end of their ranges there.

Pacific Lowlands: This narrow band of lowland forest extends from southwestern Guatemala along the Pacific Coast to southwestern Costa Rica, with the most expansive contiguous area in northwestern Nicaragua. The vast majority of this area receives a precipitation of <2,000 mm y⁻¹ and is dominated by deciduous broadleaf vegetation and

scrub forests. Two small isolated patches of evergreen broadleaf forests (with up to 6,000 mm precipitation) are present in southwestern Guatemala and the Golfo Dulce region of Costa Rica and adjacent Panama; these areas are characterized by high endemism. The Pacific lowlands share greatest similarity with adjacent montane regions.

Southern Nuclear Highlands: This unit comprises numerous contiguous ranges in the uplands of Honduras, northern El Salvador, and northern Nicaragua (Sierra de Nombre de Dios, Sierra de La Esperanza, and Sierra de Comayagua in Honduras; Sierra de Patuca and Sierra de Isabela in Nicaragua). These ranges are generally lower than the northern nuclear highlands (mostly <2,000 m). The area generally receives 1,000-2,000 mm annual precipitation and vegetation is predominantly pine-oak forests, with higher and moister peaks having montane broadleaf cloud forests. The fauna is diverse and unique with 137 species, including 53 endemics (including, among others, 11 *Bolitoglossa*, 16 *Craugastor*, 4 *Nototriton*, and 4 *Plectrohyla*). Many species groups evolved *in situ*, and the region has many groups at the northernmost (*Isthmohyla*) or southernmost (*Dendrotriton*, *Cryptotriton*) limit of their distributions.

Caribbean Lowlands: This is the largest continuous expanse of lowland forests in Central America, occupying the alluvial slopes of eastern Honduras, Nicaragua, and Costa Rica, and into the Bocas del Toro region of extreme western Panama. This area receives extreme levels of precipitation (2,000-6,000 mm y⁻¹) and vegetation is dominated by moist, evergreen, broadleaf vegetation, with isolated patches of Misquito pine savanna in southeastern Honduras and northeastern Nicaragua. This diverse lowland region holds

112 species, with three endemics (*Ranitomeya claudiae*, *Oedipina martima*, *Lithobates miadis*) occurring on small coastal islands. The Caribbean lowlands are strongly influenced by species derived from South America that likely diversified *in situ* (*Dendropsophus*, *Hypsiboas*, *Scinax*, *Agalychnis*, dendrobatoids, centrolenids) (Savage 1982).

Isthmian Highlands – These highlands are comprised of several cordilleras – the relatively low Cordillera de Tilarán and Cordillera de Guanacaste, and the higher Cordillera Central of Costa Rica, Cordillera de la Talamanca (which includes Cerro Chirripó, at 3,850 m the highest point in lower Central America) and the Cordillera Central of western Panama. These evergreen broadleaf forests are very wet (up to 7,000 mm rainfall y^{-1}), producing cloud forests above about 1,000 m and paramó at the highest elevations. This region supports 203 species, including 55 endemics. Particularly diverse genera include *Bolitoglossa* (23 species, 16 endemic), *Craugastor* (35 species, 8 endemic), *Isthmohyla* (13 species, 10 endemic), and *Nototriton* (7 species, all endemic). Richness and endemism are largely from an influx of taxa from the northern highlands (*Plectrohyla*, *Ptychohyla*, *Oedipina*, *Bolitoglossa*) or the South American highlands (some bufonids and hemiphractids). Many of the South American affiliated species-groups reach their northern limit in this region, including *Atelopus*, many centrolenids, and the hemiphractids.

Panamanian Lowlands – These are moist (< 4,000 mm precipitation), lowland coastal forests on either slope of the Cordillera Central de Panama, and between the Darien

highlands in eastern Panama, with drier regions east of the Panama Canal and on the eastern Azuero Peninsula (precipitation $<1,500 \text{ mm y}^{-1}$). Vegetation ranges from evergreen moist forests to deciduous dry forests according to precipitation regimes. The Panamanian lowlands hold 121 species in total, but only a single endemic (*Osgaecilia elongata*). This region shares great similarity with the Panamanian highlands and is heavily influenced by South American species-groups that have invaded since the uplift of the isthmus (Savage 1966, 1982).

Panamanian Highlands – This area includes a number of low-lying ranges in eastern Panama: Serranía de San Blas ($<1,000 \text{ m}$) and the Serranía de Darién (up to $1,600 \text{ m}$) on the Caribbean, and the Serranía del Sapo ($<1,000 \text{ m}$), Serranía del Majé (up to $1,200 \text{ m}$) on the Pacific. The western Serranía de San Blas and the Serranía del Sapo receive up to $3,000 \text{ mm}$ precipitation, while the remainder of this region receives between $1,500$ and $2,500 \text{ mm}$. Major forest types include broadleaf premontane forests with cloud forests at the highest elevations of the western Serranía de San Blas and Serranía del Sapo. This region hosts 108 species, of which only three are endemics, and the region holds far fewer recognized species than do other highland regions because of its relatively small geographic extent and the limited effort relegated to surveys. The highlands of eastern Panama are much more heavily influenced by South American species-groups (dendrobatids, centrolenids, *Atelopus*) and contain fewer Middle American species (plethodontids) and none of the core Middle-American hylids.

CONSERVATION STATUS OF CENTRAL AMERICAN AMPHIBIANS

The Global Amphibian Assessment (GAA) has assigned IUCN red-list criteria to virtually all of Central American amphibians, and these uniform criteria for assessment of conservation are highly valuable for planning and synthetic analyses (Stuart *et al.* 2004; Hero *et al.* 2005; Rodrigues *et al.* 2006). As of 2010, the GAA listed 88 Central American amphibian species as critically endangered (19% of fauna), 75 (16%) as endangered, 39 (8%) as vulnerable – together these three listings represent those species that are threatened with extinction (Table 1.3). Twenty-six species (5%) are listed near threatened and 150 (33%) as least concern. Fifty-two (11%) species are listed as data deficient, indicating that insufficient knowledge of the species exists to make a robust assessment (Table 1.3). Four species (1%) of Central American amphibians are listed as globally extinct (*Incilius periglenes*, *Incilius holdridgei*, *Craugastor chrysozetetes*, and *Craugastor milesi*) and 31 additional species, officially listed as critically endangered or data deficient, are possibly extinct. Seventeen species have not been assessed because they were formally described after the most recent assessment had been conducted.

The status of threat is divided unevenly among taxonomic groups – over half of the species of plethodontids, brachycephalids, bufonids and hylids are threatened, while smaller proportions of ranids, dendrobatids, and centrolenids are threatened (Table 1.4). There are several genera in which all constituent Central American species are listed as threatened (*Duellmanohyla*, *Ecomiohyla*, *Exerodonta*, *Hyla*, *Hylomantis*, *Plectrohyla*, *Atelopus*, *Pipa*, *Bradytriton*, *Cryptotriton*, *Dendrotriton*, and *Nyctanolis*).

When compared to global trends for all amphibians, Central America contains a higher proportion of species listed as critically endangered and endangered, but fewer

species listed as vulnerable, near threatened, and least concern (Table 1.2). While a high proportion of Central American species are listed as data deficient (11%), this proportion is far lower than for the entire world (25%), underscoring a greater-than-average knowledge of the status of Central American amphibians, particularly in comparison to most tropical regions where amphibians are diverse, yet often poorly known.

Status of threat is also divided unevenly among countries and physiographic provinces (Tables 1.2, 1.3; Figure 1.4). Seventy-eight species in Guatemala are listed as threatened, 60 in Costa Rica, 54 in Honduras, and 50 in Panama. Far fewer species are threatened in Belize (7), El Salvador (10), or Nicaragua (10). Much of this variation has to do with the size of the country, ecological diversity of the area, and the extent of upland habitat in each nation. Far more threatened species occur in upland physiographic provinces than in lowland ones (Figs. 3, 4).

Small size of geographic range is a major correlate of proneness to extinction in Central American amphibians (Lips *et al.* 2003b; Smith *et al.* 2009). While the high proportion of endemic amphibians in Central America contributes to the high species richness and distinctiveness of the fauna, it also contributes to the severe conservation status of amphibians in the region (Donnelly and Crump 1998). Very high proportions of all the range-restricted species are listed as threatened (Figure 1.5). Three major factors contribute to the grave conservation status of these endemics. First, an inherent susceptibility of range-restricted species to environmental perturbations makes them vulnerable to any disturbance. Second, the majority of range-restricted species occur at mid-elevations to high-elevations, the very regions that have been most adversely affected by chytridiomycosis. Finally, mountaintop endemics are particularly susceptible

to effects of shifting climate (Donnelly and Crump 1998; Pounds *et al.* 1999; Pounds 2001; Parmesan 2006).

Previous studies have compared ecological characteristics of declining and persisting species from a collection of well-documented sites of declines (Lips *et al.* 2003b). The present authors conducted a region-wide analysis of ecology on the probability of decline in which they compiled life-history characteristics for all Central American species from a variety of sources (Campbell 1998; Lee 2000; McCranie and Wilson 2002; Savage 2002; IUCN *et al.* 2006; Kohler *et al.* 2006). It was possible to compile data both on larval and adult affinities for habitat for over 95% of all taxa, indicating a reasonably comprehensive dataset for these simple analyses. The diverse life-history characteristics – both for larval and adult habitats –strongly influence whether species are threatened (Figure 1.6). Species that breed in streams or rivers or phytotelmata, or species that have direct development, are far more likely to be threatened than are species that breed in ponds. Species whose adults are found near streams are far more likely to be threatened than are those from any other habitat. It was not possible to compile data for Central American taxa that have proved significant in other studies (e.g., ovarian clutch size, body mass) (Hero *et al.* 2005, Sodhi *et al.* 2008), and poor understanding of systematic relationships among species at the present time prevents formal analyses that account for evolutionary history (Corey and Waite 2008). However, there are clearly phylogenetic and biogeographic signatures to species-specific patterns in declines: all *Atelopus* are threatened, as are virtually all of the large and diverse clades of stream-dwelling Middle American hylids (*Isthmohyla*, *Plectrohyla*, *Ptychohyla*) but very few of the predominantly pond-breeding species are threatened.

Future analyses that control for phylogeny, biogeography, and more detailed life-history parameters will likely elucidate further trends.

CONSERVATION THREATS

Trends in Research on Conservation

The present section summarizes general knowledge of known threats to amphibian biodiversity as well as the research on each topic that has been conducted within Central America. A systematic review was conducted to quantitatively evaluate trends in research activity that addressed amphibian conservation in Central America. A search was made of ISI Web of Knowledge (1977-2007) and BIOSIS (1967-2007) with one taxonomic keyword (“amphibian,” “frog,” “salamander,” or “caecilian”) and one geographic keyword (“Central America,” “Guatemala,” “Belize,” “Honduras,” “El Salvador,” “Nicaragua,” “Costa Rica,” or “Panama”). References and abstracts were exported to an external database. Duplicate records, records that did not address amphibian biology, and distributional notes were discarded. Systematic or phylogenetic studies were generally included if they were conducted at the specific or generic level (e.g. Crawford 2003) but not if they were conducted at the familial or ordinal level (e.g., Frost *et al.* 2006). Global review papers that included Central America among other geographic regions (i.e., Alford and Richards 1999) were also excluded but reviews that focused primarily on Central America, Mesoamerica, or the entire Neotropics (i.e., Young *et al.* 2001) were included. Reviews that encompassed all taxa in Central America but did not give special attention to amphibians (e.g., Ellison 2004) were excluded, but reviews that focused substantially on amphibians were included. A large

number of articles were excluded if they dealt primarily with systematics of parasites of amphibians because amphibian biology was peripheral to these studies. While many studies have been conducted since 2007, publication trends since 2007 reflect those from earlier years.

The studies were sorted by country of origin. Those that were primarily focused on conservation or decline were divided by the process of interest (i.e., habitat modification, chytridiomycosis, climatic change). Because of the uncertain processes involved in enigmatic amphibian declines, a large number of conservation studies focused specifically on describing declines did not perform tests of the hypotheses about factors driving population declines, and these studies were listed as general descriptive “reports” (e.g., Lips 1998, 1999; Whitfield *et al.* 2007). A large number of studies reviewed conservation status over a broad area (a national park, biogeographic region, or country) and these were categorized as “assessments” (e.g., Wilson and McCranie 2004a; Greenbaum and Komar 2005). Generally, reports and assessments speculated as to causes, often in detail, but did not seek to test formally whether a specific process was involved.

The resulting database contained 401 valid studies of amphibians in Central America from 1967 to 2007. Of these, 43 studies were determined to be primarily related to conservation. This empirical review certainly did not yield every study conducted on amphibians in Central America, but such an extensive analysis does provide a quantitative and effective procedure to detect broad trends in amphibian research in Central America (Figures 1.7, 1.8). Significant gaps exist in the spatial coverage (Figure 1.7) and processes of interest (Figure 1.8) in Central American amphibian research. The

majority of studies in Central America have been conducted in Costa Rica and Panama, with very few in El Salvador or Nicaragua. Few studies have been conducted on the impacts of pesticides or UV-B radiation, and few on modification and fragmentation of habitats. Much recent attention has been devoted to understanding the role of chytridiomycosis in amphibian declines (Figure 1.8). Because of lack of understanding of certain processes in Central America (e.g., pesticides, UV-B), it remains difficult to rule out their importance in Central America. Nonetheless, this chapter covers the empirical literature on specific threats to amphibians within Central America. When data from the region are not available, data generated elsewhere were used to generate expectations of how specific threats may apply in the region.

Modification of Habitats

Anthropogenic modification of habitats affects 75.2% of amphibians in Central America, a greater proportion of species than are affected by any other threat (Figure 1.9). Habitat loss is believed responsible for the presumed extinction of two Central American salamanders, *Bolitoglossa jacksoni* from Sierra de los Cuchumatanes of Guatemala and *Oedipina paucidentata* from the Cordillera de Talamanca of Costa Rica. It is probable that the proportion of amphibians affected by habitat modification will increase as economic development exerts increased pressure on remaining habitats. The proportion of remaining habitat and the rate of deforestation vary greatly among countries (FAO 2006) (Figure 1.10) and by ecological zone. El Salvador has the lowest remaining forest cover, and remnant old-growth forest is negligible, while Panama retains a high proportion of forest cover and intact primary forest. Honduras shows the most rapid

recent loss in forest cover (Wilson and McCranie 2004a, b). A very small proportion of intact dry forest remains in Central America, while forests at higher elevations on steep elevational gradients have been less influenced by habitat modification (Sanchez-Azofeifa *et al.* 2001). The amount of area covered by secondary and other disturbed forests (~13.3 million ha) in Central America now far exceeds the area of remaining primary forest (~9.1 million ha) (FAO 2006).

Despite considerable past and expected future losses of habitat, very few studies have documented how amphibian populations or communities are affected by habitat modification in Central America. Three studies examined forest floor amphibians in very small patches of cacao plantations (*Theobroma cacao*) at La Selva Biological Station in Costa Rica (Lieberman 1986; Heinen 1992; Whitfield *et al.* 2007). These studies indicated that plantations showed lower amphibian diversity and higher amphibian abundance than did adjacent primary forests. Whitfield *et al.* (2007) compiled data from these cacao plantations over three decades and found decreasing population density of all species of amphibians except for *Oophaga pumilio*, which increased in population density despite declines in adjacent primary forest. Van Wijngaarden and van den Brink (2000) compared the density of *Oophaga pumilio* in various regimes of habitat disturbance in Panama and found that this species is often more abundant in disturbed habitats than in primary forests, a result concordant with studies at La Selva. A comparative study of vertebrate diversity in forests, pasture, and plantations in Nicaragua found that pasture harbours greatly reduced density of amphibians, but was too limited in sample size for inferences to be made about patterns of amphibian diversity in primary and secondary forests or in allspice (*Pimenta dioca*) plantations (King *et al.* 2007). Most

recently, Furlani *et al.* (2010) compared diversity of anurans in primary forests, secondary forests, and pastures in southwestern Costa Rica and found clear subsets of species associated with pastures hosting livestock, and other species with clear preferences for primary forests.

In general, few of these studies have examined dominant categories of land-use in Central America (pasture, active plantations, disturbed or regenerating secondary forests). The single study that examined the effects of secondary regeneration on amphibians was limited by lack of spatial replication and temporal scale (Whitfield *et al.* 2007). The lack of studies on secondary forest is of particular concern considering the large proportion of secondary forests in Central America, and this will continue to increase. None of the studies of amphibians' responses to changes in use of the land has examined effects of land-use on range-restricted species that are inherently vulnerable to changes in habitat because of small ranges. Amphibians may be generally more susceptible to consequences of change in land-use than are other vertebrate groups (Barlow *et al.* 2007), in part because of their dependence on both terrestrial and aquatic habitats, changes to either of which could adversely affect populations (Becker *et al.* 2007).

Although few studies have been conducted within Central America on the impacts of change in land use on amphibians, it is possible to infer broad trends from studies conducted elsewhere on the effects of habitat modification (Lemckert *et al.* 2011). The response of amphibian species to loss of forest is generally individualistic, and difficult to predict for individual species (Tocher *et al.* 2001; Gardner *et al.* 2007b). It is probable that a deterministic subset of species dependent on old-growth forest will be most severely affected by changes in land-use (Pineda *et al.* 2005; Gardner *et al.* 2007b) but

identification of these species is not currently possible. Some studies in South America have shown that direct-developing frogs are particularly susceptible to loss of forest cover (Pearman 1997; Barlow *et al.* 2007; Gardner *et al.* 2007b) but these trends have not been demonstrated for direct-developing frogs in Central America.

Habitat Fragmentation

Even when habitats remain protected in national reserves or in private holdings, remnants may exist in small fragments that may be of insufficient size to harbour intact amphibian faunas. Fundamentally, because of the different processes and spatial scales involved in modification and fragmentation of habitat, the response of amphibians to these two types of habitat disturbance may be quite different. Only two studies have examined fragmentation of forests in Central America. Schlaepfer and Gavin (2001) investigated how forest-pasture edges affected densities of amphibians in southwestern Costa Rica and found that species' responses were individualistic and varied among seasons (Schlaepfer and Gavin 2001). A study of impacts of forest fragmentation surrounding La Selva Biological Station in Costa Rica found that even very small fragments (1-7 ha) may harbour a high proportion of species even after two decades of isolation, but that overall density of amphibians is reduced in fragments relative to continuous forest (Bell and Donnelly 2006). Bell and Donnelly (2006) found evidence for a deterministic subset of species which are particularly vulnerable to loss in small fragments, and that this subset represents all major families and reproductive modes.

Because it is often unclear exactly how amphibian populations are affected by the changes in ecological processes following fragmentation, some expectations are offered

here. Within a single fragment, populations at high densities should be resistant to local extinctions, but species that exist at low densities, or are adversely affected by edges, should be particularly susceptible to inbreeding, demographic stochasticity, or genetic drift, resulting in gradual local extirpations. Understanding persistence of amphibian populations in a fragmented landscape requires a detailed understanding of metapopulation processes such as dispersal potential and matrix-effects, but these factors remain largely overlooked for tropical amphibians (Smith and Green 2005; Watling and Donnelly 2006; Gardner *et al.* 2007a; Robertson *et al.* 2008). The roles of edge effects, corridors that link fragments, and spatial arrangement of fragments remain entirely unexplored in Central America and most of the Neotropics. As for habitat modification, because amphibians often require both aquatic and terrestrial habitats, aquatically breeding species in “dry fragments” lacking suitable aquatic resources should be particularly susceptible to extirpation (Becker *et al.* 2007). Despite these gaps in knowledge, it is clear that some species will be unable to survive in individual fragments, and others will be unable to persist in a fragmented landscape. Identification of these fragmentation-sensitive species will be critical for preserving entire amphibian faunas, but is currently impossible without species-specific or clade-specific data from Central American amphibians, or trends in consensus from studies elsewhere in the Neotropics.

Overharvesting

In some parts of the world there are significant harvests of amphibians for food, pets, and use in research and teaching, either locally or for export (Das 2011; Kusrini 2011; Kusrini *et al.* 2011). However, there appear to be no studies on the impacts of

overharvesting of amphibians in Central America. Human consumption of amphibians in Central America is negligible, and is unlikely to have significant impacts on many populations. Greater impacts of overharvesting are likely to result from trade in captive amphibians, particularly colourful dendrobatid frogs (Gorzula 1996). Regulated exports appear to be minimal, and while it is impossible to estimate the volume of illegal, unpermitted trade, or collections of wild amphibians that are not exported, the impacts of trade on populations or species is likely to be minimal. Brightly-coloured species such as *Atelopus* or dendrobatids may be particularly targeted for collection (La Marca *et al.* 2005). Still, the greatest risk of collections for trade is to small populations, range-restricted species, or species whose populations have been severely affected by other factors.

Invasive Species

Although non-native species have had a significant impact on local populations of amphibians in various parts of the world (Piliod *et al.* 2011), tropical mainland ecosystems are generally thought to be largely resistant to invasions of species because of pre-existing high species richness and being nearly saturated with species (Stachowicz *et al.* 1999; Fine 2002). Accordingly, few invasive predators or competitors have been reported for Central America and only 1.6% of Central American amphibians are threatened by invasive predators or competitors (Figure 1.9). However, one potential invasive threat is the anthropogenic introduction of exotic fishes (primarily rainbow trout, *Oncorhynchus mykiss*) which was suggested as a plausible contributing factor to enigmatic declines in Costa Rica (Lips 1998) before the discovery of chytridiomycosis. Trout have

been introduced into streams in montane Central America where no native fishes occur (Bussing 1998); introduced trout have been shown to negatively affect amphibian populations in the western United States (Knapp and Matthews 2000; Vredenburg 2004; Knapp *et al.* 2007). However, the first introductions of trout occurred long before the first enigmatic declines were reported, and established populations of trout are not widespread (Bussing 1998). The spatial and temporal patterns in the introduction and establishment of trout are therefore not concordant with a significant role for introduced fishes in montane declines elsewhere. Other fish, such as tilapia (*Tilapia* and *Oreochromis*), have been introduced into lakes and streams in Central America – where other native fish do occur - but no studies have examined the impacts of these introduced predators on amphibian populations.

The globally widespread invasive competitor *Lithobates catesbeianus* has recently been reported in Costa Rica (Savage and Bolanos 2009) but does not appear to be widespread in Central America. The widespread tropical invasive *Rhinella marinus* is a natural element of the Central American herpetofauna. A few other amphibians introduced into Central America, *Eleutherodactylus johnstonei*, *Eleutherodactylus antillensis*, *Eleutherodactylus planirostris*, and *Osteopilus septentrionalis*, generally occur in highly disturbed sites such as urban areas and do not appear to have adverse direct effects on populations of native amphibians (Kaiser 1997; Savage 2002), but may serve as important vectors for long-distance transport of disease (Schloegel *et al.* 2009).

Pollution

The presence of intensive agricultural activity in Central America (e.g., bananas, sugarcane, cotton, coffee, pineapples) and heavy use of agrochemical pesticides by plantations leads to a high potential for environmental impacts by pesticides (Castillo *et al.* 1997). Numerous studies in Central America have demonstrated that pesticides applied in agricultural areas enter aquatic systems (reviewed by Castillo *et al.* [1997]), and at times may occur at concentrations that may prove lethal to amphibians. Recent research has identified aerial transport of pesticides in Costa Rica from application sites at low elevations to higher montane forests, where wet deposition stimulates accumulation of the pesticide (Daly *et al.* 2007 a,b), a spatial pattern coincident with that of declines in the region. Concentrations of agrochemicals in montane forests are likely far below lethal levels for amphibians, but may be accumulated if they persist in the environment or they may act as novel stressors contributing to weakened immune response (Carey *et al.* 1999).

Only two studies have examined relationships between pesticides and amphibians in Central America. In one study, breakdown products from long-lasting organochlorine pesticides were found in *Rhinella marinus*, *Lithobates forreri*, and *Rhinophrynus dorsalis* at the Guanacaste Conservation Area in the dry forests of western Costa Rica. However, five other species of anurans (*Smilisca baudinii*, *Scinax boulengeri*, *Phrynohyas venulosa*, *Leptodactylus melanonotus*, and *Hypopachus variolosus*) did not contain such residues (Klemens *et al.* 2003) and none of these species have been noted to suffer population declines. Tests of water quality, conducted in Sierra de las Minas, Guatemala, at a site where enigmatic declines of a number of species had occurred (Mendelson *et al.*

2004), demonstrated elevated levels of nitrogen associated with upstream cultivation of ornamental plants, but no detectable pesticides or residues. The primary cause of declines at this site appeared to have been Bd but the authors noted the presence of tadpoles with white tips to the tail and disoriented swimming behaviours, symptoms not generally associated with chytridiomycosis.

While studies on pesticides are limited within Central America, studies from outside the region may help inform about potential impacts of pesticides within the region (Boone *et al.* 2009). Pesticides, or their breakdown products, may cause direct mortality (Relyea and Mills 2001; Relyea 2003; Sparling and Fellers 2007), may alter physiological processes that can reduce population sizes (Hayes *et al.* 2002), may stress amphibians so that they become vulnerable to other factors (Carey *et al.* 1999; Forson and Storfer 2006), or may alter community dynamics causing a variety of individual and population responses (Relyea and Diecks 2008). Correlative studies have linked extirpations of amphibians with upwind use of pesticide in California (Lenoir *et al.* 1999; Davidson *et al.* 2002; Davidson 2004) but the role of pesticides in amphibian declines at sites far from those where agricultural pesticides are applied is less clear and highly controversial. Research on the effects of pesticides on amphibians in Central America is warranted because these factors are known to have an impact on amphibians in other parts of their global range.

UV-B radiation

Biologically-damaging UV-B radiation (285-315 nm) has been increasing globally in recent decades primarily because of depletion of stratospheric ozone by

anthropogenic release of chlorofluorocarbons and other compounds (Kerr and McElroy 1993; Marco *et al.* 2009). Montane areas of Central America have among the highest baseline levels of UV-B radiation anywhere on earth (Middleton *et al.* 2001) and these are increasing, a broad-scale pattern consistent with declines (Blaustein *et al.* 2003). Middleton *et al.* (2001) examined patterns in UV-B radiation at nine sites in Costa Rica, Panama, and Honduras where amphibian declines have occurred. Using satellite data, they indicated significant increases in UV-B exposure for all nine sites over the period from 1979 to 1998 and they show that the number of days with the greatest UV-B exposure ($\geq 6.75 \text{ kJ/m}^2$) had increased. Increases in UV-B have been linked with reduction in cloud cover associated with changing climate, and to global losses in ozone.

Only one study has examined the relationships between UV-B and amphibians in Central America (Han *et al.* 2007). These authors experimentally demonstrated behavioural avoidance by two species of dendrobatid frogs (*Oophaga pumilio* and *Dendrobates auratus*) to elevated levels of UV-B radiation, and also demonstrated that male *O. pumilio* select calling microsites in the understory that receive lower than average levels of UV-B radiation. While this study suggests that some species may be able to detect and respond to UV-B radiation, it does not make an explicit link to conservation or declines, and was conducted in a lowland site where ambient baseline levels of UV-B are much lower than at montane sites where declines generally occur.

The impacts of biologically-damaging UV-B radiation may be broadly inferred from studies conducted outside of Central America, primarily in the western United States. Increased levels of exposure of amphibian eggs to UV-B radiation may decrease hatching success (Blaustein *et al.* 1994, 1998) or increase frequency of deformities

(Blaustein *et al.* 1997). Exposure of post-metamorphic anurans to UV-B decreased survival in some species (Blaustein *et al.* 2005). Ultraviolet-B has never been associated with mass mortality events such as those that have been documented in Central America (Lips 1999; Lips *et al.* 2006) and no deformed amphibians have been documented in Central American countries, but UV-B may serve as an important stressor when acting synergistically with other factors (Kiesecker *et al.* 2001). Still, many amphibians exhibit behavioural mechanisms that allow them to avoid UV-B exposure (Han *et al.* 2007) and possess physiological repair mechanisms that reduce damage to affected tissues (Blaustein *et al.* 1999).

Pathogens

Chytridiomycosis is a skin disease caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). This disease has been implicated in mass mortality of amphibians at sites worldwide (Berger *et al.* 2009). Research in Central America has greatly contributed to an understanding of the role that disease plays in amphibian population declines, especially *Bd* (Lips *et al.* 2003a, 2006, 2008). *Bd* is increasingly recognized as an invasive species because no studies detected it at Central American sites until just before the onset of declines, and molecular evidence (Morehouse *et al.* 2003; Morgan *et al.* 2007) indicates that it is globally distributed and has been introduced into the Neotropics multiple times over the past 30 years. *Bd* has been reported in Guatemala, Honduras, El Salvador, Costa Rica, and Panama, but sampling has not been conducted in Belize or Nicaragua (Table 1.5). Lips *et al.* (2004, 2006, 2008) hypothesized that *Bd* was introduced into Mexico from the north and had

caused declines in upland amphibians there by the late 1970s (Figure 1.4, Table 1.5). From Mexico, they hypothesized it spread into Guatemala (Mendelson *et al.* 2004) and into Honduras in the 1980s (McCranie and Wilson 2002; Wilson and McCranie 2004a) (Table 1.5). *Bd* was first reported in northern Costa Rica in 1986 (Puschendorf *et al.* 2006a). The spread of *Bd* southeastward through multiple upland sites in Costa Rica and Panama has been well-documented (Lips *et al.* 2008). *Bd* has been detected at almost all sites where searches have been conducted, except for a few sites in Panama east of the Panama Canal (Lips, personal observations). As of 2010, far eastern Panama is believed to be the only *Bd*-free zone in all of Central America, but *Bd* is expected to make its way through the remaining upland regions of eastern Panama within the next 1-3 years if it continues to spread from the west at its current rate of movement of 22 km y⁻¹ (Lips *et al.* 2008). *Bd* might also enter eastern Panama from Colombia, where it has been reported from multiple sites and is traveling at a comparable 25 km y⁻¹ (Lips *et al.* 2008).

Bd has had a significant impact on upland amphibian populations throughout the Neotropics (Stuart *et al.* 2004; Lips *et al.* 2008) because the environmental conditions that contribute to the abundance and diversity of amphibians also contribute to the growth and survival of *Bd*. In particular, the cool moist environment present year-round at many upland (>500 m elevation) tropical areas offer an ideal incubator for *Bd*, which requires moisture to survive and to spread, and which thrives at temperatures between 17-24°C. Species that inhabit niches that fit this profile best (e.g., cloud forests, upland streams) tend to show the greatest and most noticeable losses, while terrestrial species (Lips *et al.* 2006) and those at lowland sites, and even some pond species from mid-elevation, show

lower prevalence of infection and consequently if they do decline, they do so at a slower rate (Brem and Lips 2008).

Species vary in their response to exposure to *Bd* (Rowley and Alford 2009; Crawford *et al.* 2010), with some species disappearing completely and instantly (e.g., *Craugastor punctariolus*) (Ryan *et al.* 2008), others declining more slowly to extirpation (e.g., *Hyloscirtus colymba*), others declining but persisting at reduced abundance (e.g., *Espadarana prosoblepon*) (Robertson *et al.* 2008), and some not seeming to decline at all (e.g., *Smilisca phaeota*, *Agalychnis callidryas*). Variation in susceptibility may also result from population and individual factors, including secretion of cutaneous antimicrobial peptides (e.g., Woodhams *et al.* 2006), commensal bacterial species (Harris *et al.* 2006), and many aspects of amphibian ecology and behaviour (Lips *et al.* 2003b; Brem and Lips 2008).

Bd may persist in the environment for several days independent of the host (Lips *et al.* 2006), allowing transmission from the environment as well as from other frogs, a characteristic of diseases that is known to increase the risk of extinction of the host (de Castro and Bolker 2005). *Bd* also occurs at low levels in many tadpoles and in adults of some species of amphibians that decline very little (Brem and Lips 2008). These species and stages are likely reservoirs for the disease and may contribute to extirpation of other species at the site (Brem and Lips 2008). Adults of these species are also likely to function as vectors and spread the disease to other sites because of dispersal across metapopulations (Robertson *et al.* 2008).

Another emerging infectious disease, ranavirus is a widespread pathogen predominantly infecting species from the temperate zone (Daszak *et al.* 2000; Green *et*

al. 2002; Daszak *et al.* 2003; Jancovich *et al.* 2005; Storfer *et al.* 2007; Hemingway *et al.* 2009). While ranavirus can cause high mortality and has been implicated in some population declines, it has not been attributed as the primary cause of any major population declines or extinctions. Only Picco and Collins (2007) have systematically searched for ranavirus in Central America, testing 84 individuals of 16 amphibian species at two sites in Costa Rica; none were infected. Ranavirus was detected in two *Lithobates warszewitschii* tadpoles collected during a die-off at Fortuna, Panama (Lips' personal observations). These tadpoles were swimming erratically and had open, bloody lesions (D. E. Green, personal communication). Other evidence of ranavirus in Latin America comes from dead *Atelognathus patagonicus* collected at Laguna Blanca, Argentina (Fox *et al.* 2006), from non-native *Lithobates catesbeianus* in Uruguay and Brazil (Galli *et al.* 2006) and *Rhinella marinus* in Venezuela (Zupanovic *et al.* 1998) and possibly in Costa Rica (Speare *et al.* 1991). In Argentina, ranavirus and *Bd* co-occur (Fox *et al.* 2006).

Trematodes (e.g., *Ribeiroia* sp.) may cause infections and deformities in amphibians (Lannoo 2009; Rohr *et al.* 2009) but there do not appear to be any reports of this from Central America.

Climate Change

Significant local and regional shifts in climate over recent decades have been recorded within Central America, mirroring global changes attributed to anthropogenic activities (IPCC 2007). Because of climatic variation across the region, the local or sub-regional effects of climate will differ strongly among localities according to their elevation and current climatic regimes (Malhi and Wright 2004; Aguilar *et al.* 2005).

Region-wide, there have been significant increases in daytime and night-time maximum and minimum temperatures (Aguilar *et al.* 2005). There is no region-wide directional trend in total annual precipitation (unlike the drying trends found in most other tropical forest regions) (Malhi and Wright 2004) and on a local scale, both significant increases and decreases in precipitation regimes have occurred. Still, there is a non-significant regional trend for increased precipitation, and a significant region-wide trend for increasing intensity of rain, with a greater proportion of yearly rainfall derived from intense rainfall (Aguilar *et al.* 2005).

Few studies have examined the impacts of changing climate on amphibian declines and extinctions in Central America, and fewer still have presented a mechanistic basis for climate-induced declines. Before the recognition of chytridiomycosis as a major factor in amphibian declines, Pounds and Crump (1994) related the extinctions of *Incilius periglenes* and *Atelopus varius* in Monteverde, Costa Rica, to the unusually warm and dry El Niño-Southern Oscillation event of 1987. In a later and more sophisticated analysis, Pounds *et al.* (1999) suggested that regional warming has raised the elevation at which orographic cloud formation occurs (the lifting cloud-base hypothesis), reducing the frequency of mist in this cloud forest and increasing the number of days with zero precipitation. These climatic changes at Monteverde are correlated with widespread amphibian declines and extirpations, and changes in assemblages of lizards and birds as well (Pounds *et al.* 1999). More recently, Anachatakis and Evans (2010) reconstructed a climatic record based on isotopic signatures of samples from trees and provided additional support for a strong ENSO event just preceding the decline of *I. periglenes*. On a sub-regional scale, an analysis of climatic records in Costa Rica and Panama found that

amphibian extinctions tended to occur after particularly dry years, although these dry years were not exceptionally strong compared to historic trends in temperature (Alexander and Eischeid 2001). In another study from the lowlands of Costa Rica, Whitfield *et al.* (2007) correlated local, but taxonomically widespread, population declines in terrestrial amphibians and lizards to warming and increased frequency of precipitation. They hypothesized that increased temperature and moisture could affect the dynamics of leaf litter by reducing the rate of litterfall, or by increasing the rate of decomposition of litter, both of which are sensitive to climate. Because the terrestrial amphibians and lizards they examined are highly sensitive to depth of standing litter, reductions in the volume of standing litter could deplete necessary microhabitats

In the absence of sufficient regional information about the impacts of changing climate on amphibians, other studies can be used to infer expected broad patterns for the future. The greatest concern of changing climate would be a regional or widespread local decrease in precipitation, to which amphibians are particularly sensitive. While drying trends have been reported in Central America (Pounds *et al.* 1999; Aguilar *et al.* 2005; Burrowes 2009), drying is not a predominant regional trend. Sufficient analyses are not currently available to determine whether the lifting cloud-base seen at Monteverde is representative of all Central American cloud forests. Significant future warming may drive elevational or latitudinal migration and affect species' elevational distributions (Parmesan 2006); these shifts may adversely affect amphibians particularly harshly because of their poor ability to disperse (Marsh and Trenham 2001). Mountaintop endemics are likely to suffer extinction because of the lack of cooler and wetter refuges upslope. Tropical amphibians, which experience a much narrower range in yearly

temperature variation than do temperate amphibians, may have more narrow thermal envelopes than do amphibians in temperate regions (Janzen 1967, Feder 1982, Donnelly and Crump 1998). Brattstrom (1968) examined this hypothesis and found in general, thermal tolerances of tropical species were similar to closely related temperate species, but also that species with narrow ranges do tend to have narrow thermal tolerance. A wealth of studies from the temperate zone has demonstrated significant shifts in phenology of breeding or metamorphosis with warming, but comparable long-term datasets essential for detecting such changes do not appear to be available for the Neotropics. Phenological shifts in the region should not be expected because of earlier rainfall or earlier onset of spring, but increased temperature (and associated increases in evaporation rates) could shorten hydroperiod in seasonal ponds or swamps, thereby reducing the available time for development of larval amphibians (Donnelly and Crump 1998). While studies to date have yet to establish a firm direct link between amphibian declines and climate, there is little doubt that future climatic shifts will produce dramatic changes in amphibian biology in the region.

Synergistic Interactions

Synergistic interactions among stressors have been investigated widely outside of Central America (Kiesecker *et al.* 2001; Forson and Storfer 2006) but few studies have explored synergisms among factors in Central American declines (Pounds and Puschendorf 2004; Pounds *et al.* 2006a, Rohr *et al.* 2008, Rohr and Raffel 2010). Pounds and Puschendorf (2004) proposed the climate-linked epidemic hypothesis, that emergence of *Bd* is ultimately triggered by changing climate. In support of this

hypothesis, Pounds *et al.* (2006a) correlated extinctions of *Atelopus* throughout Central and South America with exceptionally warm years. They used climatic data from Monteverde, Costa Rica, to demonstrate higher night-time temperatures and lower daytime temperatures, and suggested the “chytrid thermal optimum hypothesis” – that changes in montane forests shift climate toward the ideal growing conditions for *Bd*. The data of Pounds *et al.* (2006a) have been criticized (Skerratt *et al.* 2007; Lips *et al.* 2008, Rohr *et al.* 2008), although the climate-linked epidemic hypothesis has also been invoked to explain outbreaks in Spain (Bosch *et al.* 2007). Morphological asymmetry (shown to be correlated with increased stress) in amphibian populations preceded *Bd*-induced extinctions in Australia (Alford *et al.* 2007). Lips *et al.* (2008) conducted a more sophisticated analysis of the data used by Pounds *et al.* (2006a) and concluded that there was no evidence to support the climate-linked epidemic hypothesis. Rohr *et al.* (2008) demonstrated that the data of Pounds *et al.* (2006a) were temporally confounded, and concluded that correlations between mean climate and extinctions are specious. Rohr and Raffel (2010) further analyzed data on *Atelopus* declines and found evidence that *Atelopus* extinctions occur more frequently following ENSO events, and proposed the “climate variability hypothesis:” that disease dynamics are at least in part determined by variability in temperature. While evidence for the specific hypothesis of Pounds *et al.* (2006a) are limited, there is no doubt that temperature and moisture affect not only amphibian biology and behaviour, but also the growth, survival, and spread of *Bd*; or that changes in temperature and moisture will influence amphibians not only directly, but will also affect them indirectly through effects on *Bd* and other disease-causing agents.

CONSERVATION ACTIONS

The Amphibian Conservation Action Plan (ACAP) recently outlined a set of recommendations for a global, integrated amphibian conservation and management strategy for the near future, and recognized that the challenges of such an undertaking were unprecedented (Gascon *et al.* 2007). Central America's role in the ACAP will be particularly challenging because of high diversity, high levels of threat, and limited local resources. Immediate conservation action is required to continue monitoring, conduct further research on rapid enigmatic declines, conduct surveillance of disease, and to establish captive assurance populations of critically threatened species. Longer-term solutions will require innovative conservation strategies for abatement of threat from *Bd* (such as design of effective vaccines, genetic engineering of the fungus, selection for resistance among amphibian populations, or biological control using cutaneous bacteria with anti-*Bd* properties), action against climatic change, maintenance of captive assurance populations, and eventual reintroductions. Advancement on this agenda will necessitate a profoundly enhanced in-country capacity to handle amphibian research, conservation, and management.

Effective conservation of Central American amphibian diversity will not be possible without first fully documenting species diversity in the region. This work will require description of new taxa, completion of local and national inventories, and updating distributional records for all species as knowledge increases. Amphibian diversity in Central America is likely significantly underestimated. Globally, new species of amphibians continue to be described at a rate greater than that for any other group of vertebrates (Kohler *et al.* 2005). Even for countries that are relatively well-

surveyed, such as Costa Rica, new forms continue to be described (Hanken *et al.* 2007). Areas of Central America that have been largely unexplored (Nicaragua and eastern Panama) are likely to hold many new species. Lags between discovery and recognition, and limited taxonomic expertise (particularly for diverse and challenging lineages) threaten to hamper efforts to fully document amphibian diversity in the region before these species are lost. It is likely that amphibian diversity in Central America will be permanently underestimated because of the high probability that many species in remote and poorly-surveyed sites suffered extinction before comprehensive surveys were initiated (Crawford *et al.* 2010). Furthermore, amphibian species richness may prove much greater than currently recognized if genetic techniques reveal multiple cryptic species within complexes currently recognized as a single taxon (Bickford *et al.* 2007; Fouquet *et al.* 2007; Crawford *et al.* 2010). Indeed, genetic studies in Central America have produced evidence for cryptic species-complexes that are overlooked by taxonomy based on morphology alone (Crawford 2003; Crawford and Smith 2005; Grant *et al.* 2006; Richards and Knowles 2007), and further still, Crawford *et al.* (2010) used DNA barcoding from El Cope, Panama, to identify 11 cryptic species, five of which were extirpated upon arrival of *Bd* to the site. If genetic studies divide cryptic complexes into separate species, the conservation status of the resulting species will likely be more severe than currently recognized because of inherent risks associated with smaller size of geographic ranges.

Monitoring

Field monitoring programs are a key component of a conservation agenda to prevent further losses, and to assess future changes to currently unanticipated threats (Dodd *et al.* 2011). Several monitoring programs in Central America are currently underway (Young *et al.* 2001) but increased spatial extent, taxonomic coverage, and temporal resolution of monitoring efforts are essential for effective conservation. Long-term datasets have identified systematic declines that would not otherwise have been expected (Whitfield *et al.* 2007) and the probability of detecting and identifying declines increases with the duration of monitoring efforts. Distinguishing real declines from background fluctuations in population size is often difficult from limited datasets, and virtually no long-term datasets are available to assess background fluctuation in population size for amphibians in Central America; such time-series may be impossible to collect because no sites in Central America are now free from known amphibian stressors (Table 1.5). Probabilities of detecting subtle changes (such as those expected for climate) may be low, and changes in population density may be impossible to detect without quantitative data, as opposed to presence-absence data (e.g., Lips and Donnelly 2005; Whitfield *et al.* 2007). Monitoring programs should incorporate extensive metadata and detailed sampling protocols to ensure potential for long-term replication. Clearly, realistic baselines for amphibian assemblages have been lost in much of Central America because of *Bd*, but monitoring should not be restricted to those regions that have not yet been affected by *Bd*. In regions where *Bd* has already produced widespread declines and extinctions, monitoring will provide evidence of population dynamics post-decline (Robertson *et al.* 2008). Collection of voucher specimens from monitoring

efforts will also be critical for assessing long-term change (for example, histological examination of disease status or genetic composition pre-decline).

Surveillance of disease and monitoring programs are also desperately needed throughout the region. Data can help track the spread of disease into naïve populations or monitor temporal fluctuations in populations where *Bd*, or other diseases, are already endemic. These data could identify potential vectors, reservoirs, and temporal changes in incidence, and will greatly contribute to improved understanding of the dynamics of transmission in wild populations that is necessary to design effective conservation measures.

Network of Protected Areas

Protection of habitats is the most straightforward means of preventing loss of biodiversity and is valuable not only to amphibians but also to other native organisms and to ecosystem services (Hecnar and Lemckert 2011). Habitat protection can provide an effective deterrent to habitat loss, deforestation, urban encroachment, and direct impacts of the modification of habitats (Bruner *et al.* 2001; Sanchez-Azofeifa *et al.* 2003).

Central America contains an extensive network of national parks, biosphere reserves, wildlife refuges, forest reserves and similar protected areas that encompass 411 reserves covering over 92,000 km² (17.7% of all land area in the region) (IUCN 2006) and the number of protected areas has increased rapidly over the past three decades. However, the number of reserves and amount of area protected varies by country and region. Belize protects 36.7% of its area, and Guatemala 26.9%, while Honduras protects only 7.5%, and El Salvador only 0.5%. Only about 7% of the Pacific Lowlands is protected

within reserves, about 8% of Southern Nuclear highlands, about 11% of Northern Nuclear highlands, but about 50% of Yucatan Lowlands and about 75% of the Maya Mountains are protected. Except for the diverse and relatively well-protected Isthmian Highlands (about 30% protected), upland regions with high amphibian richness and endemism are less well protected than are lowland regions with lower richness and endemism.

Approximately 50 species of Central American amphibians have ranges that are completely outside of protected areas. Virtually all of these are range-restricted endemics, and the majority is found within the Northern and Southern Nuclear Highlands – where coverage within protected areas is clearly insufficient. Smaller numbers of species occur entirely outside of protected areas in the Isthmian Highlands, Panamanian lowlands and Panamanian highlands. These trends are explained largely by the richness and endemism of these regions, as well as by the insufficient spatial coverage of the network of protected areas.

While establishment and recognition of protected areas by governmental agencies is a requisite step, effective protection of habitat is dependent on active prevention of encroachment by anthropogenic activities. For protected areas to serve as more than “paper parks” recognized only by governmental agencies and not by local communities, national environmental regulatory institutions must be strengthened and must provide active protection from human impacts (Smith 2003; Nygren 2004). Additionally, even small private reserves may have significant benefits for preservation of endemics with narrow ranges if those small reserves provide protection for critical habitat (Laurance 2008)

The requirements for protected areas for amphibians differ strongly from the needs of other vertebrate groups, which are often given primacy in the establishment of reserves (Caro *et al.* 2004). Instead of investing resources in large protected areas often chosen for the protection of umbrella or flagship species, multiple small reserves protecting non-overlapping distributions of range-restricted endemics would be optimal for endemic amphibians in Central America.

Captive Assurance Populations

Protected areas alone will clearly be insufficient for safeguarding amphibian diversity in Central America. A large number of declines and extinctions have occurred in protected areas (Pounds and Crump 1994; Lips 1999; Mendelson *et al.* 2004; Lips *et al.* 2006; Puschendorf *et al.* 2006a; Whitfield *et al.* 2007). Global threats such as emerging infectious diseases and climatic change do not recognize sociopolitical boundaries or limits of protected areas, and long-term solutions will require innovative approaches to controlling the spread of diseases or allowing for adaptation and migration of amphibians in response to climatic change. In the short-term, the only immediately available means of preventing extinction may be establishment of captive-breeding assurance populations –animals from wild populations brought to breeding facilities to ensure that captive populations exist for eventual reintroduction and for use in necessary research efforts (Mendelson *et al.* 2006; Griffiths and Kuzmin 2011). In-situ preservation presents enormous challenges, because most species have never been kept in captivity and there are no protocols. Methods of husbandry are being developed and refined in the field as these activities continue. The breeding habitats and basic biology of these

species remain largely unknown, presenting formidable challenges to husbandry of animals in captivity. Captive assurance populations must be maintained indefinitely, because the identification of, and solutions to, environmental problems may be decades away, and even then habitats must be protected from future, unknown threats in order to maintain environments suitable for reintroductions. For example, animals collected at sites with *Bd* cannot be released until effective control of *Bd* is possible. There are 244 amphibian species in Central America listed as threatened or data deficient; establishment and maintenance of captive assurance populations of as many of these as possible will require an enormous investment in finances, human resources, and infrastructure.

A pilot program for the establishment of captive assurance populations was recently attempted for the amphibian assemblage at El Valle, Panama (Gagliardo *et al.* 2008). Just before the onset of a *Bd*-associated decline, hundreds of individuals of 30 species were brought into captive assurance populations in a nearby facility that was constructed *de novo*, as well as a second facility in the United States. The El Valle program met significant short-term challenges in the location of space to house collections and in providing food for captive animals. Longer-term challenges include maintaining quarantine against *Bd*, assuring successful breeding, and maintaining genetic diversity within captive populations. While several species in this pilot program have been bred successfully in captivity since taken from the wild, other species have proved difficult to maintain in captivity. Funding and local expertise in amphibian husbandry is likely to prove limited if programs such as this become more widespread.

In Central America, establishment of captive assurance populations for the subset of most sensitive species will be particularly difficult; most of the region has already

suffered profound losses associated with chytridiomycosis. Many of the highly threatened, endemic species occurring in Central America have already disappeared from the major part of their ranges, and have possibly gone extinct, making the establishment of captive populations impossible.

Remnant Populations

Encouragingly, a few species for which widespread and massive declines have been reported have recently been found to occur in single, isolated populations (*i.e.*, Lazarus taxa - those taxa believed to be extinct or nearly so, but that have since been rediscovered). *Atelopus varius* was thought globally extinct, but a single remnant population has since been located. *Craugastor ranoides*, which has vanished from much of its range, may exist in a single population in Costa Rica (Puschendorf *et al.* 2005). *Isthmohyla rivularis*, which disappeared from most of Costa Rica in the 1990s, has recently been rediscovered in the area surrounding Monteverde, Costa Rica. Detecting remnant populations, if and when they exist, represents the only hope for recovery for those species that have been most devastated. Remnant populations may represent the difficulty in locating individuals when populations are small, and may provide important insight into the mechanisms and routes of the spread of disease or into potential differences in synergistic interactions of multiple factors. Genetic studies may elucidate mechanisms for persistence or resistance, and possibly illustrate potential for selection for resistance.

Challenges for Reintroductions

Establishment of captive assurance populations will make eventual reintroduction possible. Reintroductions currently face overwhelming challenges; reintroduction on a community level have never been attempted for any vertebrate group worldwide, and only one reintroduction of a tropical amphibian has ever been attempted (the Puerto Rican toad, *Peltophryne lemur*) (A. Lind, personal communication). Most reintroductions have been of European, North American or Australian species, and while some have been successful (Denton *et al.* 1997) most have not been met with success (Dodd and Seigel 1991). Before reintroductions work as effective strategies, new approaches for controlling the environmental changes affecting amphibians – emerging infectious diseases and any synergisms – must be established. Recent research into anti-*Bd* effects of symbiotic bacteria on the skin of amphibians perhaps provides one avenue of investigation into biological control of *Bd*. Any strategy for reintroduction may take years or decades to implement, and captive assurance populations must persist until these environmental factors have been resolved. Simultaneously, future and perhaps unanticipated environmental issues must be averted, and adequate habitats must remain protected from future impacts by humans at eventual sites for reintroductions.

Reintroductions of amphibians will be an enormous challenge for conservation. For most species, habitat associations and requirements are not understood. In the absence of pre-decline data for most species on basic biology (e.g., population parameters such as survival and fecundity), it will be difficult to evaluate the success of re-established populations because there will be no historic baselines to use in evaluating the health of populations. Genetic diversity of re-established populations may be severely

compromised; initially, re-established populations will be small and susceptible to even the smallest stochastic disturbances. Monitoring and assessments of re-established populations will be essential to gauge effectiveness of reintroduction.

Building In-Country Capacity

All of the conservation actions needed in Central America (enhanced monitoring efforts, establishment of reserves, improving taxonomy and systematics, establishment and maintenance of captive assurance populations, eventual efforts to re-introduce and to monitor post-recovery) will require greatly augmented in-country human and institutional capacity and associated investments in financial resources and infrastructure. The need for increased capacity is immediate for conservation actions such as surveillance of disease and monitoring programs, establishment of captive populations, identifying target sites for protected areas, research into the causes of declines, and detecting remnant populations of critically endangered species. Particularly important is in-country, rapid-response capacity – the ability to quickly respond to population crashes with immediate attention to research and to efforts to establish captive assurance populations. In the long term, conservation in-country will require maintaining captive assurance populations and maintaining research on husbandry, and eventual reintroduction and post-reintroduction monitoring.

Despite the number of published studies produced on amphibians in Central America, a very small percentage of these studies included authors affiliated with in-country institutions (Figure 1.7). The overwhelming majority of research published on Central American amphibians is the work of scientists based at institutions in North

America and Europe. Yet encouragingly, the proportion of studies that involve local authors is higher for conservation-oriented work than for amphibian studies in general (Figure 1.7). Despite a number of prolific herpetologists and conservation biologists working in Central America, the human resources required to implement a comprehensive strategy for amphibian conservation clearly are not available currently. Specific training programs dealing with conservation of amphibians, such as the successful Research and Analysis Network for Neotropical Amphibians (RANA), will be immensely helpful in increasing the capacity for amphibian research and conservation in Central America. Grants targeted to scientists in Central America – and other developing nations – hold much potential to augment greatly the in-country capacity for amphibian research in the region. A very small number of major research organizations (principally the Universidad de Costa Rica and Organization for Tropical Studies in Costa Rica and the Smithsonian Tropical Research Institute in Panama) are largely responsible for the volume of research on amphibians produced in their host countries. These organizations facilitate research and often provide financial support to researchers. Institutions of similar strength are lacking in other Central American countries. These organizations should serve as models for developing a capacity for research and conservation in countries where the fauna is less well known.

An additional problem inhibiting progress in amphibian conservation and biology in Central America is the lack of communication and dissemination of research results. Scientists at Latin American universities are not faced with the pressure to publish as are scientists based at universities in North America, and language barriers may prevent widespread dissemination of work. Much research on, and knowledge about, amphibians

in Central America therefore remains unpublished. Development of journals dealing specifically with amphibian conservation (such as *FrogLog* and *Phyllomedusa*), particularly those that accept manuscripts in Spanish, could positively affect the dissemination of results. Similarly, encouragement of editorial boards of English-language herpetological journals to accept manuscripts in Spanish would be likely to significantly increase publication by Latin American scientists. *Biotropica* and *Revista de Biología Tropical* are good examples of successful, international, multi-lingual journal that publishes many papers on conservation in the tropics, including several on amphibian declines. Volumes 9 and 11 of the series *Amphibian Biology*, of which this chapter is a part, taps the expertise of local authors from many countries and presents to the international scientific community a number of unpublished governmental reports, papers in different languages from local, hard-to-access journals, and other relatively inaccessible but valuable information. Finally, web-based databases, listservers, and electronic journals are often more accessible to Latin American scientists than are printed media (Young *et al.* 2001) and these can rapidly distribute information, a critical factor when dealing with crises and rapidly changing field conditions.

Research and Conservation Actions

A bold research agenda will be required to safeguard the remaining amphibian diversity in Central America. The following are among the high priority actions for research and conservation in Central America:

(1) Basic biology and ecology of Central American amphibians, including research on physiology and evolution. Applied conservation programs are often inhibited by lack of basic information on natural history.

(2) *Research into the Ecology of Bd*. Important topics are how and where *Bd* persists in the environment, whether it can persist as a saprobe, whether it can infect non-amphibian species, how it is dispersed from site to site, whether any museum specimens from pre-decline populations were infected, and population genetic studies to identify where *Bd* originated and how it has spread.

(3) *Research into Bd-frog interactions*. Research is needed on interactions between amphibians and pathogens to better understand the mechanisms that produce variation in susceptibility among species (*i.e.*, Richmond *et al.* 2009), populations and individuals; identify species that serve as reservoirs and vectors, assess direct impacts on individuals and populations (*i.e.*, Pilliod *et al.* 2010) and how much transmission is frog-to-frog and how much is mediated by the environment.

(4) *Study the few remaining intact montane amphibian populations in eastern Panama*. As the only Central American region free from *Bd*, amphibian species and populations in eastern Panama deserve special attention.

(5) *Explore synergistic interactions between Bd and other stressors*. Especially important are the synergistic interactions of other pathogens, climate, and pesticides.

(6) *Develop a capacity for amphibian research and management in Central America.*

(7) *Bolster monitoring efforts in Central America to assess prior loss, and collect baseline data to detect further change.*

(8) *Establish captive assurance populations of sensitive species.* Such populations will be essential for research efforts aimed towards investigating novel stressors and recovery efforts.

(9) *Establish protected areas that include populations of sensitive amphibian species.*

(10) *Research the impacts of change in land-use.* Important topics are the modification and fragmentation of habitat and how amphibians respond to them.

(11) *Improve the understanding of the long-term impacts of climatic change on amphibians.* In particular, studies should test mechanistic hypotheses of how climatic change will affect animals in the field, and go beyond correlational studies of past change.

(12) *Develop amphibian conservation policy.* Nations, both within Central America and abroad, should restrict and monitor trade of amphibians to reduce

transmission of pathogens, and develop wildlife policy for addressing issues of emerging infectious diseases.

CONCLUSIONS

A substantial proportion of Central American amphibians are presently threatened with extinction under current conditions. Dozens of extinctions have almost certainly already occurred, and environmental conditions are only likely to worsen in the short term. Intact amphibian assemblages have essentially disappeared from virtually all montane areas of the region except for eastern Panama in the past 30 years, as *Bd* has spread through Central America (Lips *et al.* 2006, 2008). At the same time, loss of habitat and other more subtle anthropogenic factors have continuously eroded amphibian populations throughout the region.

Crippling gaps persist in the understanding of the extent of, causes for, and conservation solutions for, these declines. While *Bd* is clearly a key factor in enigmatic declines, it is currently difficult to rule out climate or other stressors as important co-factors, primarily because all declines in Central America have occurred against a backdrop of diverse and significant anthropogenic impacts (Table 1.5). Virtually all studies in Central America have taken a single-factor approach to research on amphibian declines, although potential for interactions among factors is high (Table 1.5).

A massive effort to bolster the capacity for conservation in Central American nations must be initiated. Governmental environmental regulatory institutions will require assistance in the selection, establishment, and protection of biological preserves. In-country organizations fostering research, education, and conservation must train

biologists to conduct monitoring studies, address causality for declines, and initiate solutions for managing threatened amphibians. Captive assurance populations and eventual release programs are desperate, expensive, controversial, and human-intensive strategies for management, but they present the only currently available viable strategy standing between persistence and extinction for Central America's most threatened species. In developed nations, conservation strategies directed toward imperiled species have been successful largely through employing species-specific management plans; such options may be successful in Central America if the funding, institutional capacity, and human resources become available to implement them. Biodiversity is a collective global resource, and the responsibility for protection of biodiversity is ultimately a global responsibility, not one for the host nations alone. Protection of biodiversity in species-rich, developing nations should be a priority, not just for nations in Central America, but for all nations.

Clearly, the severity, immediacy, and challenges presented by enigmatic and conventional declines vary greatly. Disturbance of habitats threatens more species, the impacts and global scale of change in land-use (loss of genetic diversity; broad-scale eradication of sensitive species and populations; loss of cryptic species) are clearly severe and the management strategies required to prevent these losses have long been available. Enigmatic declines are dramatic, immediate, and produce widespread, irreversible extinctions. As the world becomes increasingly populated, global threats such as climatic change and emerging infectious diseases are likely to increase in extent and severity. Undoubtedly, known threats that have not been reported for Central America (deformities, parasitic trematodes, invasive competitors or predators) may pose future

problems, and globally unrecognized threats may yet emerge. There is a great need for research not only on conventional threats, but also on enigmatic ones that are well-recognized (*Bd*) as well as those that have received less attention in Central America (e.g., pesticides, ranavirus, UV-B).

Population declines of amphibians have numerous and significant impacts on other components of ecosystems because of the central roles amphibians play in food webs (Whiles *et al.* 2006; Colon-Gaud *et al.* 2008; Connelly *et al.* 2008). The loss of adult and larval amphibian biodiversity in upland streams will have an impact on terrestrial habitats and downstream sites. Not only are species and populations of amphibians lost, but ecosystem structure and function are compromised, with changes in nutrient cycling, primary productivity, quality and quantity of seston, and the composition of algal, invertebrate and snake assemblages (Whiles *et al.* 2006). Most of the ecosystem-level consequences of amphibian declines are likely to be unrecognized.

Research into amphibian decline has been particularly active in Central America and observations of declines in the region helped to confirm the nature of the global crisis. Links between declines and chytridiomycosis were in part forged in Central America. While much diversity has undoubtedly been irrevocably lost, Central America has the opportunity to continue pioneering work in research on amphibians and on their conservation and management. In coming years, amphibian conservation in the region must move beyond identifying which factors have caused declines. Biologists must look ahead with a bold agenda of preventing further loss, mitigating environmental deterioration, and restoring the diversity that has not been irretrievably lost.

LITERATURE CITED

- Aguilar, E., Peterson, T. C., Obando, P. R., Frutos, R., Retana, J. A., Solera, M., Soley, J., Garcia, I. G., Araujo, R. M., Santos, A. R., Valle, V. E., Brunet, M., Aguilar, L., Alvarez, L., Bautista, M., Castanon, C., Herrera, L., Ruano, E., Sinay, J. J., Sanchez, E., Oviedo, G. I. H., Obed, F., Salgado, J. E., Vazquez, J. L., Baca, M., Gutierrez, M., Centella, C., Espinosa, J., Martinez, D., Olmedo, B., Espinoza, C. E. O., Nunez, R., Haylock, M., Benavides, H., and Mayorga, R., 2005. Changes in precipitation and temperature extremes in Central America and northern South America, 1961-2003. *Journal of Geophysical Research-Atmospheres* **110**: D23107, doi:10.1029/2005JD006119.
- Alexander, M. A. and Eischeid, J. K., 2001. Climate variability in regions of amphibian declines. *Conservation Biology* **15**: 930-942.
- Alford, R. A., Bradfield, K. S. and Richards, S. J., 2007. Ecology - Global warming and amphibian losses. *Nature* **447**: E3-E4.
- Alford, R. A. and Richards, S. J., 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* **30**: 133-160.
- Anchukaitis, K. J. and Evans, M.N., 2010. Tropical cloud forest climate variability and the demise of the Monteverde golden toad. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 5036-5040.
- Barlow, J., Gardner, T. A., Araujo, I. S., Avila-Pires, T. C., Bonaldo, A. B., Costa, J. E., Esposito, M. C., Ferreira, L. V., Hawes, J., Hernandez, M. I. M., Hoogmoed, M. S., Leite, R. N., Lo-Man-Hung, N. F., Malcolm, J. R., Martins, M. B., Mestre, L. A. M., Miranda-Santos, R., Nunes-Gutjahr, A. L., Overal, W. L., Parry, L., Peters, S. L., Ribeiro-Junior, M. A., da Silva, M. N. F., da Silva Motta, C. and Peres, C. A., 2007. Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. *Proceedings of the National Academy of Sciences USA* **104**: 18555-18560.
- Becker, C. G., Fonseca, C. R., Haddad, C. F. B., Batista, R. F. and Prado, P. I., 2007. Habitat split and the global decline of amphibians. *Science* **318**: 1775-1777.
- Bell, K. E. and Donnelly, M. A., 2006. Influence of forest fragmentation on community structure of frogs and lizards in northeastern Costa Rica. *Conservation Biology* **20**: 1750-1760.
- Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G. and Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and

Central America. *Proceedings of the National Academy of Sciences USA* **95**: 9031-9036.

- Berger, L., Longcore, J. L., Speare, R., Hyatt, A. and Skerratt, L. F., 2009. Fungal diseases of amphibians. Chapter 2 (pp. 2986-3052) in "Amphibian Decline: Diseases, Parasites, Maladies and Pollution", ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K. and Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* **22**: 148-155.
- Blaustein, A. R., Hays, J. B., Hoffman, P. D., Chivers, D. P., Kiesecker, J. M., Leonard, W. P., Marco, A., Olson, D. H., Reaser, J. K. and Anthony, R. G., 1999. DNA repair and resistance to UV-B radiation in western spotted frogs. *Ecological Applications* **9**: 1100-1105.
- Blaustein, A. R., Hoffman, P. D., Hokit, D. G., Kiesecker, J. F., Walls, S. C. and Hays, J. B., 1994. UV repair and resistance to solar UV-B in amphibian eggs: a link to possible declines? *Proceedings of the National Academy of Sciences USA* **91**: 1791-1795.
- Blaustein, A. R., Kiesecker, J. M., Chivers, D. P. and Anthony, R. G., 1997. Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Sciences USA* **94**: 13735-13737.
- Blaustein, A. R., Kiesecker, J. M., Chivers, D. P., Hokit, D. G., Marco, A., Belden, L. K. and Hatch, A., 1998. Effects of ultraviolet radiation on amphibians: field experiments. *American Zoologist* **38**: 799-812.
- Blaustein, A. R., Romansic, J. M., Kiesecker, J. M. and Hatch, A. C., 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distributions* **9**: 123-140.
- Blaustein, A. R., Romansic, J. M. and Scheessle, E. A., 2005. Ambient levels of ultraviolet-B radiation cause mortality in juvenile western toads, *Bufo boreas*. *American Midland Naturalist* **154**: 375-382.
- Boone, M. D., Davidson, C. and Bridges-Britton, C., 2009. Evaluating the impact of pesticides in amphibian declines. Chapter 8 (pp. 3186-3207) in "Amphibian Decline: Diseases, Parasites, Maladies and Pollution", ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.

- Bosch, J., Carrascal, L. M., Duran, L., Walker, S. and Fisher, M. C., 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? *Proceedings of the Royal Society of London Series B-Biological Sciences* **274**: 253-260.
- Brattstrom, B. H. 1968. Thermal acclimation in anuran amphibians as a function of latitude and altitude. *Comparative Biochemistry and Physiology* **24**:93-111.
- Brem, F. and Lips, K. R., 2008. Patterns of infection by *Batrachochytrium dendrobatidis* among species, habitats, and elevations during epidemic and endemic stages. *Diseases of Aquatic Organisms. Diseases of Aquatic Organisms* **81**:189-202.
- Bruner, A. G., Gullison, R. E., Rice, R. E. and da Fonseca, G. A. B., 2001. Effectiveness of parks in protecting tropical biodiversity. *Science* **291**: 125-128.
- Burrowes, P. A., 2009. Climatic change and amphibian declines. Chapter 12 (3268-3279) in "Amphibian Decline: Diseases, Parasites, Maladies and Pollution", ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Bussing, W. A., 1998. "Peces de las Aguas Continentales de Costa Rica." 2nd edition, Editorial de la Universidad de Costa Rica. San Jose, Costa Rica.
- Campbell, J. A., 1998. "Amphibians and Reptiles of Northern Guatemala, the Yucatan, and Belize". University of Oklahoma Press, Norman, Oklahoma.
- Campbell, J. A., 1999. "Distribution Patterns of Amphibians in Middle America". Pp. 111-210 in Patterns of distribution of amphibians: a global perspective, ed by W. E. Duellman. Johns Hopkins University Press, Baltimore.
- Campbell, J. A. and Smith, E. N., 1992. A new frog of the genus *Ptychohyla* (Hylidae) from the Sierra de Santa Cruz, Guatemala, and description of a new genus of Middle American stream-breeding treefrogs. *Herpetologica* **48**: 153-167.
- Carey, C., Cohen, N. and Rollins-Smith L., 1999. Amphibian declines: an immunological perspective. *Developmental and Comparative Immunology* **23**: 459-472.
- Caro, T., Engilis, A., Fitzherbert, E. and Gardner, T., 2004. Preliminary assessment of the flagship species concept at a small scale. *Animal Conservation* **7**: 63-70.
- Castillo, L. E., de la Cruz, E. and Ruepert, C., 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. *Environmental Toxicology and Chemistry* **16**: 41-51.

- Colon-Gaud, J. C., Peterson, S., Whiles, M. R., Kilham, S. S., Lips, K. R. and Pringle, C. M., 2008. Allochthonous litter inputs, organic matter standing stocks, and organic seston dynamics in upland Panamanian streams: potential effects of larval amphibians on organic matter dynamics. *Hydrobiologia* **603**:301-312
- Connelly, S., Bixby, R. J., Pringle, C. M., Brenes, R., Whiles, M. R., Lips, K. R., Kilham, S. S. and Huryn, A. D., 2008. Changes in stream primary producer communities resulting from loss of tadpoles: can small-scale experiments predict effects of large-scale catastrophic amphibian declines? *Ecosystems* **11**: 1262-1276
- Corey, S. J. and Waite, T. A., 2008. Phylogenetic autocorrelation of extinction threat in globally imperiled amphibians. *Diversity and Distributions* **14**:614-629.
- Crawford, A. J., 2003. Huge populations and old species of Costa Rican and Panamanian dirt frogs inferred from mitochondrial and nuclear gene sequences. *Molecular Ecology* **12**: 2525-2540.
- Crawford, A. J. and Smith, E. N., 2005. Cenozoic biogeography and evolution in direct-developing frogs of Central America (Leptodactylidae: *Eleutherodactylus*) as inferred from a phylogenetic analysis of nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution* **35**: 536-555.
- Crawford A.J., Lips K.R. and Bermingham E., 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proceedings of the National Academy of Sciences USA* **107**, 13777-13782.
- Crump, M. L., Hensley, F. R. and Clark, K. L., 1992. Apparent decline of the golden toad: Underground or extinct? *Copeia* **1992**:413-420.
- Daly, G. L., Lei, Y. D., Teixeira, C., Muir, D. C. G., Castillo, L. E., Jantunen, L. M. M. and Wania, F., 2007a. Organochlorine pesticides in the soils and atmosphere of Costa Rica. *Environmental Science & Technology* **41**: 1124-1130.
- Daly, G. L., Lei, Y. D., Teixeira, C., Muir, D. C. G., Castillo, L. E. and Wania, F., 2007b. Accumulation of current-use pesticides in neotropical montane forests. *Environmental Science & Technology* **41**: 1118-1123.
- Das, I., 2011. Man meets frog: Perceptions, use and conservation of amphibians by indigenous people. Chapter 3 (pp. 3383-3468) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.

- Daszak, P., Cunningham, A. A. and Hyatt, A. D., 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* **287**: 443-449.
- Daszak, P., Cunningham, A. A. and Hyatt, A. D., 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* **9**: 141-150.
- Davidson, C., 2004. Declining downwind: amphibian population declines in California and historical pesticide use. *Ecological Applications* **14**: 1892-1902.
- Davidson, C., Shaffer, H. B. and Jennings, M. R., 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conservation Biology* **16**: 1588-1601.
- de Castro, F. and Bolker, B., 2005. Mechanisms of disease-induced extinction. *Ecology Letters* **8**: 117-126.
- Denton, J. S., Hitchings, S. P., Beebee, T. J. C. and Gent, A., 1997. A recovery program for the natterjack toad (*Bufo calamita*) in Britain. *Conservation Biology* **11**: 1329-1338.
- Dodd, C. K. and Seigel, R. A., 1991. Relocation, repatriation, and translocation of amphibians and reptiles - are they conservation strategies that work? *Herpetologica* **47**: 336-350.
- Dodd, C. K. Jr., Loman, J., Cogalniceanu, D. and Puky, M., 2011. Monitoring amphibian populations. Chapter 11 (pp. 3577-3635) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Donnelly, M. A., 1994. Amphibian diversity and natural history. Pp 199-209 in "La Selva: Ecology and Natural History of a Neotropical Rainforest", ed by L. McDade, K. Bawa, H. Hespeneheide and G. Hartshorn. University of Chicago Press, Chicago.
- Donnelly, M. A. and Crump, M. L., 1998. Potential effects of climate change on two neotropical amphibian assemblages. *Climatic Change* **39**: 541-561.
- Duellman, W. E., 1966. The Central American herpetofauna: an ecological perspective. *Copeia* **1996**: 700-719.
- Duellman, W. E., 1988. Patterns of species diversity in anuran amphibians in the American tropics. *Annals of the Missouri Botanical Garden* **75**: 79-104.

- Ellison, A. M., 2004. Wetlands of Central America. *Wetlands Ecology and Management* **12**: 3-55.
- Faivovich, J., Haddad, C. F. B., Garcia, P. C. A., Frost, D. R., Campbell, J. A. and Wheeler, W. C., 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: Phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History* **294**: 1-240.
- FAO., 2006. "Global Forest Resources Assessment 2005". Food and Agriculture Organization of the United Nations, Rome.
- Feder, M. E., 1982. Thermal ecology of neotropical lungless salamanders (Amphibia: Plethodontidae): environmental temperatures and behavioral responses. *Ecology* **63**: 1665-1674.
- Fine, P. V. A., 2002. The invasibility of tropical forests by exotic plants. *Journal of Tropical Ecology* **18**: 687-705.
- Forson, D. D. and Storfer, A., 2006. Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. *Ecological Applications* **16**: 2325-2332.
- Fouquet, A., 2007. Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. *PLoS ONE* **2**: e1109.
- Fox, S. F., Greer, A. L., Torres-Cervantes, R. and Collins, J. P., 2006. First case of ranavirus-associated morbidity and mortality in natural populations of the South American frog *Atelognathus patagonicus*. *Diseases of Aquatic Organisms* **72**: 87-92.
- Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., de Sa, R. O., Channing, A., Wilkinson, M., Donnellan, S. C., Raxworthy, C. J., Campbell, J. A., Blotto, B. L., Moler, P., Drewes, R. C., Nussbaum, R. A., Lynch, J. D., Green, D. M. and Wheeler, W. C., 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* **297**: 1 - 370.
- Furlani, D., Francesco Ficetola, G., Colombo, G., Ugurlucan, M. and De Bernardi, F., 2009. Deforestation and the structure of frog communities in the Humedale Terraba-Sierpe, Costa Rica. *Zoological Science* **26**: 197-202.
- Gagliardo, R., Crump, P., Griffith, E., Mendelson, J. R., Ross, H. and Zippel, K., 2008. The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook* **42**: 125-135.

- Galli, L., Pereira, A., Marquez, A. and Mazzoni, R., 2006. Ranavirus detection by PCR in cultured tadpoles (*Rana catesbeiana* Shaw, 1802) from South America. *Aquaculture* **257**: 78-82.
- Gardner, T. A., Barlow, J., Parry, L. W. and Peres, C. A., 2007a. Predicting the uncertain future of tropical forest species in a data vacuum. *Biotropica* **39**: 25-30.
- Gardner, T. A., Ribeiro-Junior, M. A., Barlow, J., Cristina, T., Avila-Pires, S., Hoogmoed, M. S. and Peres, C. A., 2007b. The value of primary, secondary, and plantation forests for a neotropical herpetofauna. *Conservation Biology* **21**: 775-787.
- Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E. and Mendelson, J. R., 2007. "Amphibian Conservation Action Plan." World Conservation Union (IUCN), Gland, Switzerland.
- Gorzula, S., 1996. The trade in dendrobatid frogs from 1987 to 1993. *Herpetological Review* **27**: 116-123.
- Grant, T., Frost, D. R., Caldwell, J. P., Gagliardo, R., Haddad, C. F. B., Kok, P. J. R., Means, D. B., Noonan, B. P., Schargel, W. E. and Wheeler, W. C., 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia : Athesphatanura : Dendrobatidae). *Bulletin of the American Museum of Natural History* **299**: 1-262.
- Green, D. E., Converse, K. A. and Schrader, A. K., 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996-2001. *Annals of the New York Academy of Sciences* **969**: 323-339.
- Greenbaum, E., and Komar, O., 2005. Threat assessment and conservation prioritization of the herpetofauna of El Salvador. *Biodiversity and Conservation* **14**: 2377-2395.
- Griffiths, R. A. and Kuzmin, S. L., 2011. Captive breeding of amphibians for conservation. Chapter 14 (pp. 3687-3703) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Han, B. A., Kats, L. B., Pommerening, R. C., Ferrer, R. P., Murry-Ewers, M. and Blaustein, A. R., 2007. Behavioral avoidance of ultraviolet-B radiation by two species of neotropical poison-dart frogs. *Biotropica* **39**: 433-435.
- Hanken, J., Wake, D. B. and Savage, J. M., 2007. Montane Salamanders from the Costa Rica-Panama Border Region, with Descriptions of Two New Species of *Bolitoglossa*. *Copeia* **2007**: 556-565.

- Harris, R. N., James, T. Y., Lauer, A., Simon, M. A. and Patel, A., 2006. Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of amphibian species. *Ecohealth* **3**: 53-56.
- Hayes, T. B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A. A. and Vonk, A., 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences of the USA* **99**: 5476-5480.
- Hecknar, S. J. and Lemckert, F., 2011. Habitat protection: Refuges and reserves. Chapter 12 (pp. 3636-3676) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Heinen, J. T., 1992. Comparisons of the leaf-litter herpetofauna in abandoned cacao plantations and primary rain forest in Costa Rica: some implications for faunal restoration. *Biotropica* **24**:431-439.
- Heinicke, M. P., Duellman, W. E. and Hedges, S. B., 2007. Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences of the USA* **104**: 10092-10097.
- Hemingway, V., Brunner J., Speare, R. and Berger, L., 2009. Viral and bacterial diseases of amphibians. Chapter 1 (pp. 2963-2985) in "Amphibian Decline: Diseases, Parasites, Maladies and Pollution", ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Hero, J. M., Williams, S. E. and Magnusson, W. E., 2005. Ecological traits of declining amphibians in upland areas of eastern Australia. *Journal of Zoology* **267**: 221-232.
- IPCC, 2007. "Climate Change 2007 - The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change." Cambridge University Press, Cambridge.
- IUCN., 2006. World Database of Protected Areas. World Commission on Protected Areas. <<http://www.unep-wcmc.org/wdepa/index.htm>> Accessed 31 October 2007.
- IUCN, Conservation International, and NatureServe, 2006. Global Amphibian Assessment. <<http://www.globalamphibians.org/>>. Accessed 30 November 2007.

- Jancovich, J. K., Davidson, E. W., Parameswaran, N., Mao, J., Chinchar, J., Collins, J. P., Jacobs, B. L. and Storfer, A., 2005. Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. *Molecular Ecology* **14**: 213-224.
- Janzen, D. H., 1967. Why mountain passes are higher in the tropics. *American Naturalist* **101**: 233-249.
- Kaiser, H., 1997. Origins and introductions of the Caribbean frog, *Eleutherodactylus johnstonei* (Leptodactylidae): management and conservation concerns. *Biodiversity and Conservation* **6**: 1392-1407.
- Kerr, J. B. and McElroy, C. T., 1993. Evidence for Large Upward Trends of Ultraviolet-B Radiation Linked to Ozone Depletion. *Science* **262**: 1032-1034.
- Kiesecker, J. M., Blaustein, A. R. and Belden, L. K., 2001. Complex causes of amphibian population declines. *Nature* **410**: 681-684.
- King, D. I., Hernandez-Mayorga, M. D., Trubey, R., Raudales, R. and Rappole, J. H., 2007. An evaluation of the contribution of cultivated allspice (*Pimenta dioca*) to vertebrate biodiversity conservation in Nicaragua. *Biodiversity and Conservation* **16**: 1299-1320.
- Klemens, J. A., Wieland, M. L., Flanagin, V. J., Frick, J. A. and Harper, R. G., 2003. A cross-taxa survey of organochlorine pesticide contamination in a Costa Rican wildland. *Environmental Pollution* **122**: 245-251.
- Knapp, R. A., Boiano, D. M. and Vredenburg, V. T., 2007. Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). *Biological Conservation* **135**: 11-20.
- Knapp, R. A. and Matthews, K. R., 2000. Non-Native Fish Introductions and the Decline of the Mountain Yellow-Legged Frog from within Protected Areas. *Conservation Biology* **14**: 428-438.
- Kohler, G., Vesely, M. and Greenbaum, E., 2006. "The Amphibians and Reptiles of El Salvador." Krieger Publishing Company, Malabar, Florida.
- Kohler, J., Vieites, D. R., Bonett, R. M., Garcia, F. H., Glaw, F., Steinke, D. and Vences, M., 2005. New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *BioScience* **55**: 693-696.

- Kusrini, M. D., 2011. International trade in amphibians. Chapter 5 (pp. 3494-3504) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Kusrini, M. D., Heatwole, H. and Davenport, D., 2011. Harvesting of amphibians for food. Chapter 4 (pp. 3469-3493) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- La Marca, E., Lips, K. R., Lotters, S., Puschendorf, R., Ibanez, R., Rueda-Almonacid, J. V., Schulte, R., Marty, C., Castro, F., Manzanilla-Puppo, J., Garcia-Perez, J. E., Bolanos, F., Chaves, G., Pounds, J. A., Toral, E. and Young B. E., 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae : *Atelopus*). *Biotropica* **37**: 190-201.
- Lamoreux, J. F., Morrison, J. C., Ricketts, T. H., Olson, D. M., Dinerstein, E., McKnight, M. W. and Shugart, H. H., 2006. Global tests of biodiversity concordance and the importance of endemism. *Nature* **440**: 212-214.
- Lannoo, M. J., 2009. Amphibian malformations. Chapter 5 (pp. 3089-3111) in "Amphibian Decline: Diseases, Parasites, Maladies and Pollution", ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Laurance, W.F., 2008. Adopt a forest. *Biotropica*, **40**: 3-6
- Lee, J. C., 2000. "A Field Guide to the Amphibians and Reptiles of the Maya World." Cornell University Press, Ithaca, New York.
- Lemckert, F., Hecnar, S. J. and Pilioud, D. S., 2011. Loss and modification of habitat. Chapter 1 (pp. 3292-3342) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Lenoir, J. S., McConnell, L. L., Fellers, G. M., Cahill, T. M. and Seiber, J. N., 1999. Summertime transport of current-use pesticides from California's central valley to Sierra Nevada mountain range, USA. *Environmental Toxicology and Chemistry* **18**: 2715-2722.
- Lieberman, S. S., 1986. Ecology of the leaf litter herpetofauna of a neotropical rain forest: La Selva, Costa Rica. *Acta Zoologica Mexicana (ns)* **15**: 1-72.

- Lips, K. R., 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* **12**: 106-107.
- Lips, K. R., 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* **13**: 117-125.
- Lips, K. R., Brem, F. , Brenes, R., Reeve, J. D., Alford, R. A., Voyles, J., Carey, C., Livo, L., Pessier, A. P. and Collins, J. P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* **103**: 3165-3170.
- Lips, K. R., Burrowes, P. A., Mendelson, J. R. III and Parra-Olea, G., 2005. Amphibian population declines in Latin America: a synthesis. *Biotropica* **37**: 222-226.
- Lips, K. R., Diffendorfer, J. , Mendelson, J. R. and Sears, M., 2008. Riding the wave: Climate change, emerging infectious disease and amphibian declines. *PLOS Biology* **6**: e72.
- Lips, K. R. and Donnelly, M. A., 2005. Lessons from the tropics. Pp 198-205 in "Amphibian Declines: the Conservation Status of United States Species", ed by M. Lannoo. University of California Press, Berkeley.
- Lips, K. R., Green, D. E., and Papendick, R., 2003a. Chytridiomycosis in wild frogs from southern Costa Rica. *Journal of Herpetology* **37**: 215-218.
- Lips, K. R., Mendelson, J. R. III, Munoz-Alonso, A., Canseco-Marquez, L. and Mulcahy, D., 2004. Amphibian population declines in montane southern Mexico: resurveys of historical localities. *Biological Conservation* **119**: 555-564.
- Lips, K. R., Reeve, J. D. and Witters, L. R., 2003b. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* **17**: 1078-1088.
- Malhi, Y. and Wright, J., 2004. Spatial patterns and recent trends in the climate of tropical rainforest regions. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**: 311-329.
- Marco, A., Bancroft, B. A., Lizana, M. and Blaustein, A. R., 2009. Ultraviolet-B radiation and amphibians. Chapter 6 (pp. 3112-3144) in "Amphibian Decline: Diseases, Parasites, Maladies and Pollution", ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Marsh, D. M. and Trenham, P. C., 2001. Metapopulation dynamics and amphibian conservation. *Conservation Biology* **15**: 40-49.

- McCallum, H., 2005. Inconclusiveness of chytridiomycosis as the agent in widespread frog declines. *Conservation Biology* **19**: 1421-1430.
- McCranie, J. R. and Wilson, L. D., 2002. "The Amphibians of Honduras". Society for the Study of Amphibians and Reptiles, Ithaca.
- Mendelson, J. R., Brodie, E. D., Malone, J. H., Acevedo, M. E., Baker, M. A., Smatresk, N. J. and Campbell, J. A., 2004. Factors associated with the catastrophic decline of a cloudforest frog fauna in Guatemala. *Revista de Biología Tropical* **52**: 991-1000.
- Mendelson, J. R., Lips, K. R., Gagliardo, R. W., Rabb, G. B., Collins, J. P., Diffendorfer, J. E., Daszak, P., Ibanez, R., Zippel, K. C., Lawson, D. P., Wright, K. M., Stuart, S. N., Gascon, C., da Silva, H. R., Burrowes, P. A., Joglar, R. L., La Marca, E., Lotters, S., du Preez, L. H., Weldon, C., Hyatt, A., Rodriguez-Mahecha, J. V., Hunt, S., Robertson, H., Lock, B., Raxworthy, C. J., Frost, D. R., Lacy, R. C., Alford, R. A., Campbell, J. A., Parra-Olea, G., Bolanos, F., Domingo, J. J. C., Halliday, T., Murphy, J. B., Wake, M. H., Coloma, L. A., Kuzmin, S. L., Price, M. S., Howell, K. M., Lau, M., Pethiyagoda, R., Boone, M., Lannoo, M. J., Blaustein, A. R., Dobson, R. A., Griffiths, A., Crump, M. L., Wake, D. B. and Brodie, E. D., 2006. Biodiversity - Confronting amphibian declines and extinctions. *Science* **313**: 48-48.
- Middleton, E. M., Herman, J. R., Celarier, E. A., Wilkinson, J. W., Carey, C. and Rusin, R. J., 2001. Evaluating ultraviolet radiation exposure with satellite data at sites of amphibian declines in Central and South America. *Conservation Biology* **15**: 914-929.
- Morehouse, E. A., James, T. Y., Ganley, A. R. D., Vilgalys, R., Berger, L., Murphy, P. J. and Longcore, J. E., 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology* **12**: 395-403.
- Morgan, J. A. T., Vredenburg, V. T., Rachowicz, L. J., Knapp, R. A., Stice, M. J., Tunstall, T., Bingham, R. E., Parker, J. M., Longcore, J. E., Moritz, C., Briggs, C. J. and Taylor, J. W., 2007. Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences of the USA* **104**: 13845-13850.
- Myers, N., Mittermeier, R. A., Mittermeier, G. G., da Fonseca, G. A. B. and Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853-838.

- Nygren, A., 2004. Contested lands and incompatible images: The political ecology of struggles over resources in Nicaragua's Indio-Maiz reserve. *Society & Natural Resources* **17**: 189-205.
- Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* **37**: 637-669.
- Pearman, P. B., 1997. Correlates of amphibian diversity in an altered landscape Amazonian Ecuador. *Conservation Biology* **11**: 1211-1225.
- Picco, A. M. and Collins, J. P., 2007. Fungal and Viral Pathogen Occurrence in Costa Rican Amphibians. *Journal of Herpetology* **41**: 746-749.
- Pilliod, D.S., Griffiths, R. A. and Kuzmin, S. L., 2011. Ecological impacts of non-native species. Chapter 2 (3343-3382) in "Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans, and Management", ed by H. Heatwole and J. W. Wilkinson. Vol. 10 in "Amphibian Biology", ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Pilliod, D.S., Muths, E., Scherer, R.D., Bartelt, P.E., Corn, P.S., Hossack, B.R., Lambert, B.A., McCaffery, R. and Gaughan, C., 2010. Effects of amphibian chytrid fungus on individual survival probability in wild boreal toads. *Conservation Biology* **24**: 1259-1267.
- Pineda, E., Moreno, C., Escobar, F. and Halfpeter, G., 2005. Frog, bat, and dung beetle diversity in the cloud forest and coffee agroecosystems of Veracruz, Mexico. *Conservation Biology* **19**: 400-410.
- Pounds, J. A., 2001. Climate and amphibian declines. *Nature* **410**: 639-640.
- Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sanchez-Azofeifa, G. A., Still, C. J. and Young, B. E., 2006a. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**: 161-167.
- Pounds, J. A., Carnaval, A. C., Puschendorf, R., Haddad, C. F. B. and Masters, K. L., 2006b. Responding to amphibian loss. *Science* **314**: 1541-1541.
- Pounds, J. A. and Crump, M. L., 1994. Amphibian declines and climate disturbance: the case of the Golden Toad and the Harlequin Frog. *Conservation Biology* **8**: 72-85.
- Pounds, J. A., Fogden, M. P. L. and Campbell, J. H., 1999. Biological response to climate change on a tropical mountain. *Nature* **398**: 611-615.

- Pounds, J. A., Fogden, M. P. L., Savage, J. M. and Gorman, G. C., 1997. Tests of null models for amphibian declines on a tropical mountain. *Conservation Biology* **11**: 1307-1322.
- Pounds, J. A. and Puschendorf, R., 2004. Clouded futures. *Nature* **427**: 107-109.
- Puschendorf, R., Bolanos, F. and Chaves, G., 2006a. The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biological Conservation* **132**: 136-142.
- Puschendorf, R., Castaneda, F. and McCranie, J. R., 2006b. Chytridiomycosis in wild frogs from Pico Bonito National Park, Honduras. *Ecohealth* **3**: 178-181.
- Puschendorf, R., Chaves, G., Crawford, A. J. and Brooks, D. R., 2005. *Eleutherodactylus ranoides* (NCN). Dry forest population, refuge from decline? *Herpetological Review* **36**: 53-53.
- Puschendorf, R., Carnaval, A. C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolanos, F. and R. A. Alford. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions* **15**: 401-408.
- Relyea, R. A., 2003. Predator cues and pesticides: A double dose of danger for amphibians. *Ecological Applications* **13**: 1515-1521.
- Relyea R. A. and Diecks, N. 2008. An unforeseen chain of events: lethal effects of pesticides on frogs at sublethal concentrations. *Ecological Applications* **18**: 1728-1742.
- Relyea, R. A. and Mills, N., 2001. Predator-induced stress makes the pesticide carbaryl more deadly to grey treefrog tadpoles (*Hyla versicolor*). *Proceedings of the National Academy of Sciences USA* **98**: 2491-2496.
- Richards, C. L. and Knowles, L. L., 2007. Tests of phenotypic and genetic concordance and their application to the conservation of Panamanian golden frogs (Anura, Bufonidae). *Molecular Ecology* **16**: 3119-3133.
- Richards-Zawacki, C. L., 2010. Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proceedings of the Royal Society B-Biological Sciences* **277**: 519-528.
- Richmond, J.Q., Savage, A.E., Zamudio, K.R. and Rosenblum, E.B. 2009. Toward immunogenetic studies of amphibian chytridiomycosis: linking innate and acquired immunity. *Bioscience* **59**:311-320.

- Robertson, J. M., Lips, K. R. and Heist, E., 2008. Fine scale gene flow and individual movements among subpopulations of *Centrolene prosoblepon* (Anura: Centrolenidae). *Revista de Biologia Tropical* **56**: 13-26.
- Rodrigues, A. S. L., Pilgrim, J. D., Lamoreux, J. F., Hoffmann, M. and Brooks, T. M., 2006. The value of the IUCN Red List for conservation. *Trends in Ecology & Evolution* **21**: 71-76.
- Rohr, J., Raffel, T. and Sessions, S. K., 2009. Digenetic trematodes and their relationship to amphibian declines and deformities. Chapter 4 (pp. 3067-88) in *Decline: Diseases, Parasites, Maladies and Pollution*, ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in *Amphibian Biology*, ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Rohr, J. R., Raffel, T. R., 2010. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 8269-8274
- Rohr, J. R., Raffel, T. R., Romansic, J. M., McCallum, H. and Hudson, P. J., 2008. Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 17436-17441.
- Rovito, S. M., Parra-Olea, G., Vasquez-Almazan, C. R., Papenfuss, T. J. and Wake, D. B., 2009. Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of the National Academy of Sciences USA* **106**: 3231-3236.
- Rowley, J. L. and Alford, R. A., 2009. Factors affecting interspecific variation in susceptibility to disease in amphibians. Chapter 3 (pp. 3053-3066) in *Amphibian Decline: Diseases, Parasites, Maladies and Pollution*, ed by H. Heatwole and J. W. Wilkinson. Vol. 8 in *Amphibian Biology*, ed by H. Heatwole. Surrey Beatty & Sons Pty. Ltd, Baulkham Hills.
- Sanchez-Azofeifa, G. A., Daily, G. C., Pfaff, A. S. P. and Busch, C., 2003. Integrity and isolation of Costa Rica's national parks and biological reserves: examining the dynamics of land-cover change. *Biological Conservation* **109**: 123-135.
- Sanchez-Azofeifa, G. A., Harriss, R. C. and Skole, D. L., 2001. Deforestation in Costa Rica: a quantitative analysis using remote sensing imagery. *Biotropica* **33**: 378-384.
- Savage, J. M., 1966. The origins and history of the Central American herpetofauna. *Copeia* **1966**: 719-766.

- Savage, J. M., 1982. The Enigma of the Central American Herpetofauna: Dispersals or Vicariance? *Annals of the Missouri Botanical Garden* **69**: 464-547.
- Savage, J. M., 2002. "The Amphibians and Reptiles of Costa Rica." University of Chicago Press, Chicago.
- Savage, J. M. and Bolaños, F., 2009. A checklist of the Amphibians and Reptiles of Costa Rica: Additions and nomenclatural revisions. *Zootaxa* **2005**:1-23.
- Schlaepfer, M. A. and Gavin, T. A., 2001. Edge effects on lizards and frogs in tropical forest fragments. *Conservation Biology* **15**: 1079-1090.
- Schloegel, L. M., Picco, A. M., Kilpatrick, A. M., Davies, A. J., Hyatt, A. D. and Daszak, P., 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation* **142**:1420-1426.
- Skerratt, L. F., Berger, R., Speare, R., Cashins, S., McDonald, K. R., Philpott, A. D., Hines, H. B. and Kenyon, N., 2007. Spread of chytridiomycosis has caused rapid global decline and extinction of frogs. *Ecohealth* **4**: 125-134.
- Smith, A. M. and Green, D. M., 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**: 110-128.
- Smith, K. G., Lips, K. R. and Chase, J. M., 2009. Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. *Ecology Letters* **12**: 1069-1078.
- Smith, J. H., 2003. Land-cover assessment of conservation and buffer zones in the BOSAWAS Natural Resource Reserve of Nicaragua. *Environmental Management* **31**: 252-262.
- Smith, S. A., de Oca, A. N. M., Reeder, T. W. and Wiens, J. J., 2007. A phylogenetic perspective on elevational species richness patterns in Middle American treefrogs: Why so few species in lowland tropical rainforests? *Evolution* **61**: 1188-1207.
- Sparling, D. W. and Fellers, G. M., 2007. Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. *Environmental Pollution* **147**: 535-539.
- Sodhi, N. S., Bickford, D., Diesmos, A.C., Lee, T. M., Koh, L.P., Brook, B. W., Sekercioglu, C. H. and Bradshaw, C. J. A., 2008. Measuring the meltdown: drivers of global amphibian extinction and decline. *PLoS ONE* **3**: e1636.

- Speare, R., Freeland, W. J. and Bolton, S. J., 1991. A possible iridovirus in erythrocytes of *Bufo marinus* in Costa Rica. *Journal of Wildlife Diseases* **27**: 457-462.
- Stachowicz, J. J., Whitlatch, R. B. and Osman, R. W., 1999. Species diversity and invasion resistance in a marine ecosystem. *Science* **286**: 1577-1579.
- Storfer, A., Alfaro, M. E., Ridenhour, B. J., Jancovich, J. K., Mech, S. G., Parris, M. J. and Collins, J. P., 2007. Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic. *Ecology Letters* **10**: 1075-1083.
- Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L. and Waller, R. W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306**: 1783-1786.
- Tocher, M. D., Gascon, C. and Meyer, J., 2001. Community composition and breeding success of Amazonian frogs in continuous forest and matrix habitat aquatic sites". Pp 235-247 in "Lessons from Amazonia: The Ecology and Conservation of a Fragmented Forest", ed by R. O. Bierregaard Jr., C. Gascon, T. E. Lovejoy and R. C. G. Mesquita. Yale University Press, New Haven.
- van Wijngaarden, R. P. A. and Van den Brink., P. J., 2000. Abundanz des Pfeilgiftfrosches *Dendrobates pumilio* in verschiedenen Biotopen. *Salamandra* **36**: 223-232.
- Vredenburg, V. T., 2004. Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. *Proceedings of the National Academy of Sciences of the USA* **101**: 7646-7650.
- Wake, D. B., 1987. Adaptive radiation of salamanders in Middle American cloud forests. *Annals of the Missouri Botanical Garden* **74**: 242-264.
- Wake, D. B. and Lynch, J. F., 1976. The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. *Natural History Museum of Los Angeles County Science Bulletin* **25**: 1-65.
- Watling, J. I. and Donnelly, M. A., 2006. Fragments as islands: a synthesis of faunal responses to habitat patchiness. *Conservation Biology* **20**: 1016-1025.
- Whiles, M. R., Lips, K. R., Pringle, C. M., Kilham, S. S., Bixby, R. J., Brenes, R., Connelly, S., Colon-Gaud, J. C., Hunte-Brown, M., Huryn, A. D., Montgomery, C. and Peterson, S., 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology and the Environment* **4**: 27-34.

- Whitfield, S. M., Bell, K. E., Philippi, T., Sasa, M., Bolanos, F., Chaves, G., Savage, J. M. and Donnelly, M. A., 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences of the USA* **104**: 8352-8356.
- Wiens, J. J., Graham, C. H., Moen, D. S., Smith, S. A. and Reeder, T. W., 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. *American Naturalist* **168**: 579-596.
- Wilson, L. D. and McCranie, J. R., 2004a. The conservation status of the herpetofauna of Honduras. *Amphibian and Reptile Conservation* **3**: 6-33.
- Wilson, L. D. and McCranie, J. R., 2004b. The herpetofauna of Parque Nacional El Cusuco, Honduras (Reptilia, Amphibia). *Herpetological Bulletin* **87**: 13-24.
- Woodhams, D. C., Voyles, J., Lips, K. R., Carey, C. and Rollins-Smith, L. A., 2006. Predicted disease susceptibility in a Panamanian amphibian assemblage based on skin peptide defenses. *Journal of Wildlife Diseases* **42**: 207-218.
- Woodhams, D. C., Kilburn, V. L., Reinert, L. K., Voyles, J., Medina, D., Ibanez, R., Hyatt, A. D., Boyle, D. G., Pask, J.D., Green, D. M. and Rollins-Smith, L. A., 2008. Chytridiomycosis and Amphibian Population Declines Continue to Spread Eastward in Panama. *Ecohealth* **5**: 268-274.
- Young, B. E., Lips, K. R., Reaser, J. K., Ibanez, R. D., Salas, A. W., Cedeno, J. R., Coloma, L. A., Ron, S., La Marca, E., Meyer, J. R., Munoz, A., Bolanos, F., Chaves, G. and Romo, D., 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* **15**: 1213-1223.
- Zupanovic Z., Lopez G., Hyatt A. D., Green, B., Bartran, G., Parkes, H., Whittington, R. J., Speare, R., 1998. Giant toads *Bufo marinus* in Australia and Venezuela have antibodies against 'ranaviruses'. *Diseases of Aquatic Organisms* **32**: 1-8

Table 1.1. Taxonomic overview of the amphibians of Central America. Taxonomy follows (Faivovich *et al.* (2005); Frost *et al.* (2006); Grant *et al.* (2006); Heinicke *et al.* (2007).

Taxon	Genera	Species
ANURA		
Rhinophrynidae	1	1
Pipidae	1	1
Hemiphractidae	2	3
Craugastoridae	1	85
Eleutherodactylidae	2	11
Strobomantidae	2	14
Hylidae	21	95
Centrolenidae	5	14
Leiuperidae	2	2
Aromobatidae	1	1
Dendrobatidae	8	18
Bufo	5	40
Leptodactylidae	1	7
Microhylidae	6	9
Ranidae	1	11
CAUDATA		
Plethodontidae	8	123
GYMNOPHIONA		
Caeciliidae	4	16
Total	71	451

Table 1.2. Similarity in species composition between Central American physiographic provinces. Values are expressed as symmetric Bray-Curtis compositional similarity; higher values indicate higher proportions of shared species. The number of endemic species confined in each region is provided in italics along the diagonal.

	YL	MM	NNCA	PacL	SNCA	CL	IH	PanL	PH
Yucatan Lowlands (YL)	<i>1</i>	91.9	44.7	22.8	36.2	21.1	11.5	9.9	10.8
Maya Mountains (MM)		<i>1</i>	41.3	21.1	35.1	20.5	11.0	9.0	9.9
NNCA									
Highlands (NNCA)			<i>34</i>	28.5	39.5	17.2	10.5	6.6	7.0
Pacific Lowlands (PacL)				<i>1</i>	43.9	58.3	58.6	45.2	38.9
SNCA Highlands (SNCA)					<i>53</i>	38.6	25.9	21.7	18.8
Caribbean Lowlands (CL)						<i>3</i>	66.0	51.5	46.4
Isthmian Highlands (IH)							<i>55</i>	50.6	44.4
Panamanian Lowlands (PanL)								<i>1</i>	86.5
Panamanian Highlands (PH)									<i>6</i>

Table 1.3. The IUCN threat status of Central American amphibians by country. Number of species listed in each category is provided, and percentage of species in each threat category is given in parentheses. Threatened species are listed as vulnerable, endangered, or critically endangered. Species listed as “Not Assessed” are recently reported species whose IUCN status has not yet been evaluated by the Global Amphibian Assessment. Number of “Not Assessed” species is not available for global trends. Species can occur in more than one country. Regions are abbreviated as follows: GT – Guatemala, BZ- Belize, ES – El Salvador, HO – Honduras, NI – Nicaragua, CR – Costa Rica, PA - Panama, CA = Central America, G = Global.

IUCN Red List Category	GT	BZ	ES	HO	NI	CR	PA	CA	G
Least Concern (LC)	37 (26%)	25 (64%)	52 (42%)	22 (64%)	57 (79%)	98 (51%)	109 (54%)	150 (33%)	2376 (37%)
Near Threatened (NT)	10 (7%)	5 (12%)	6 (4%)	1 (2%)	0 (0%)	10 (5%)	10 (5%)	26 (5%)	387 (6%)
Vulnerable (VU)	21 (15%)	3 (7%)	4 (3%)	2 (5%)	5 (6%)	13 (6%)	5 (2%)	39 (8%)	654 (10%)
Endangered (EN)	25 (17%)	3 (7%)	20 (16%)	5 (14%)	3 (4%)	24 (12%)	22 (11%)	75 (16%)	758 (12%)
Critically Endangered (CR)	32 (22%)	1 (2%)	30 (24%)	3 (8%)	2 (2%)	23 (11%)	23 (11%)	88 (19%)	486 (7%)
Extinct (EX)	0 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	3 (1%)	0 (0%)	4 (0%)	37 (0%)
Data Deficient (DD)	13 (9%)	1 (2%)	4 (3%)	0 (0%)	1 (1%)	16 (8%)	26 (13%)	52 (11%)	1596 (25%)
Not Assessed	2 (1%)	1 (2%)	5 (4%)	1 (2%)	4 (5%)	5 (2%)	4 (2%)	17 (3%)	NA

Table 1.4. Conservation status by family for the amphibians of Central America. The families with the most threatened species include Plethodontidae, Bufonidae, Craugastoridae, and Hylidae. Abbreviations for IUCN threat categories are as listed in Table 1.3.

Family	LC	NT	<i>VU</i>	<i>EN</i>	<i>CR</i>	EX	DD	NA	Total
Rhinophrynidae	1								1
Pipidae				1					1
Hemiphractidae	1	1		1					3
Craugastoridae	18	5	10	15	23	2	11	1	85
Eleutherodactylidae	8		2				1		11
Strabomantidae	8	3		1			2		14
Hylidae	36	5	4	14	33		3		95
Centrolenidae	11	1					1	1	14
Leiuperidae	2								2
Aromobatidae	1								1
Dendrobatidae	8	1	1	3			5		18
Leptodactylidae	6				1				7
Microhylidae	8		1						9
Bufonidae	13	1	2	7	7	2	3	5	40
Ranidae	6	1	2		1			1	11
Plethodontidae	18	8	16	33	23		16	9	123
Caeciliidae	5		1				10		16
Total	150	26	39	75	88	4	52	17	451

Table 1.5. Environmental change at sites from which amphibian declines have been reported in Central America. Y = Present; N = Not present; “-“=no data.

Country	Site	Labeled as Figure 2	Endo- parasites	Mass Mortality Events	Reproductive Diversity	Chytrid	Reservoirs	Predictable Pollutants	UV-B Radiation	Climate Change	References
Costa Rica	San Marcos	1	Y	-	Y	Y	-	-	-	-	Korbel et al. 2009
Costa Rica	El Cedral, Sierra de las Mulas	2	Y	Y	Y	Y	-	Y	-	-	Morvelson et al. 2004
Costa Rica	Puntilla, Elajó Verapaz	3	Y	-	Y	Y	-	-	-	-	Campbell 1998, Morvelson et al. 2004
Costa Rica	El Cedral, Sierra de las Mulas	4	Y	-	Y	Y	-	-	-	-	Campbell 1998, Campbell and Smith 1992
Costa Rica	El Cedral, Sierra de las Mulas	5	Y	-	Y	Y	-	-	-	-	Mitchell 2003
Costa Rica	El Cedral, Sierra de las Mulas	6	Y	Y	Y	Y	-	-	-	-	Puschendorf et al. 2006
Costa Rica	Volcán Chiriquí	7	Y	-	Y	Y	-	Y	-	-	Puschendorf et al. 2006 Puschendorf et al. 1997, 1999, Pico and Collins 2007
Costa Rica	Monteverde	8	Y	-	Y	Y	N	Y	Y	Y	Wetzel et al. 2007
Costa Rica	La Selva	9	N	N	Y	Y	-	Y	-	Y	Puschendorf et al. 2006a
Costa Rica	San Ramón	10	Y	-	Y	Y	-	-	-	-	Puschendorf et al. 2006a
Costa Rica	Zona Protectora Tapanti	11	Y	-	Y	Y	-	Y	-	-	D. White pers. comm.
Costa Rica	Tapanti	12	Y	-	Y	Y	-	Y	-	-	Kipo and Diamond 2005
Costa Rica	Cerro de la Muñeca	13	Y	-	Y	Y	-	-	-	-	Kipo et al. 2003; Pico and Collins 2007
Costa Rica	Las Chumas	14	Y	-	Y	Y	N	-	-	-	Kipo et al. 2003a
Costa Rica	Las Alturas	15	Y	-	Y	Y	-	-	-	-	Kipo 1998, Kipo et al. 2003
Costa Rica	Las Tablas	16	Y	Y	Y	Y	-	-	Y	-	Kipo unpublished data, Mitchell 2001
Panama	Las Tablas	17	Y	-	Y	Y	-	Y	-	-	Kipo 1999, Kipo unpublished data
Panama	Barro Colorado	18	Y	Y	Y	Y	Y	Y	Y	-	Brown and Kipo 2008, Kipo unpublished data
Panama	Barro Colorado	19	Y	Y	Y	Y	-	Y	-	-	Kipo et al. 2006; Kipo unpublished data
Panama	Barro Colorado	20	Y	Y	Y	Y	-	Y	-	-	Craighead et al. 2008
Panama	Barro Colorado	21	Y	Y	Y	Y	-	Y	-	-	Woodhouse et al. 2008
Panama	Barro Colorado	22	Y	-	Y	Y	-	-	-	-

Figure 1.1. Political entities and physiographic entities of Central America. Highland areas are shaded

Figure 1.2. Amphibian species richness patterns of amphibians in Central America. Greatest species richness occurs in mid-elevation forests of lower Central America.

Figure 1.3. Elevational distributions and threat status of Central American amphibians. The greatest proportion of threatened species occurs at high-elevation sites.

Figure 1.4. Threat status (number of species listed as Critically Endangered, Endangered, or Vulnerable by the IUCN) of Central American amphibians. Data are derived from the Global Amphibian Assessment. Numbers identifying decline locations are as listed in Table 1.5.

Figure 1.5. Threat status is highly correlated with range size for amphibians in Central America.

Figure 1.6. Life history characteristics and threat status of Central American amphibians. Species that associate with more than one habitat may be included in more than one category

Figure 1.7. Trends in amphibian research in Central America. In both figures, gray bars indicated all studies, and the subset of each bar in black indicates the number of studies containing at least one author at an affiliation from the country of origin. A) includes all studies of amphibian biology in Central America; and B) includes only studies with direct conservation application. The vast majority of work conducted in Central America –for amphibians as a whole and for conservation studies in particular – has occurred in Costa Rica and Panama. A small proportion of research in Central America is conducted by in-country researchers.

Figure 1.8. Research activity in conservation by study topic. Studies may belong to more than one category.

Figure 1.9. Specific threats to the amphibians of Central America from GAA data. Habitat loss threatens the greatest proportion of species, but species threatened by global warming, pathogens, or natural disasters are most likely to be at risk of extinction.

Figure 1.10. Recent trends in loss of forest cover, 1990-2005. Proportion of the bar with diagonal hatching represents the amount of primary forest lacking obvious human impacts. The portion not hatched indicates secondary or disturbed forests or cultivated forest plantations. Data in this figure are derived from FAO (2006).

CHAPTER 2: AMPHIBIAN AND REPTILE DECLINES OVER 35 YEARS AT LA SELVA, COSTA RICA

ABSTRACT

Amphibians stand at the forefront of a global biodiversity crisis. More than a third of amphibian species are globally threatened, and over 120 species have likely suffered global extinction since 1980. Most alarmingly, many rapid declines and extinctions are occurring in pristine sites lacking obvious adverse effects of human activities. The causes of these ‘enigmatic’ declines remain highly contested. Still, lack of long-term data on amphibian populations severely limits our understanding of the distribution of amphibian declines – and therefore the ultimate causes of these declines. Here, I identify a systematic community-wide decline in populations of terrestrial amphibians at La Selva Biological Station, a protected old-growth lowland rainforest in lower Central America. I use data collected over 35 years to show that population density of all species of terrestrial amphibians has declined by ~75% since 1970, and I show identical trends for all species of common reptiles. The trends I identify are neither consistent with recent emergence of chytridiomycosis nor the climate-linked epidemic hypothesis – two leading putative causes of enigmatic amphibian declines – and instead suggest changes to ecosystem-level processes driven by changing climate. My results raise further concerns about global persistence of amphibian populations by identifying widespread declines in species and habitats that are not currently recognized as susceptible to such risks.

INTRODUCTION

Global declines in amphibian populations rank among the most critical issues in conservation biology (Stuart et al. 2004, Mendelson et al. 2006). Over one third of amphibian species are globally threatened, and over 120 amphibian species have likely become extinct since 1980 (Stuart et al. 2004). Amphibian populations worldwide are negatively affected by anthropogenic factors such as habitat modification or invasive predators, yet many rapid population declines and extinctions have occurred even in habitats lacking obvious anthropogenic disturbances. Such ‘enigmatic’ declines have aroused particular alarm (Stuart et al. 2004, Pounds et al. 2006), and much research and controversy have surrounded factors contributing to these declines (Blaustein et al. 1994, Hayes et al. 2002, Lips et al. 2006, Pounds et al. 2006).

The impacts of enigmatic declines have been particularly problematic in Central and South America (Lips et al. 2006, Mendelson et al. 2006, Pounds et al. 2006). Within the Neotropics – and elsewhere – the primary large-scale trend observed surrounding enigmatic declines is that population declines and extinctions occur almost exclusively in regions >400 m asl (Lips et al. 2003, Pounds et al. 2006). These montane declines have been associated with outbreaks of chytridiomycosis, a lethal infection of the amphibian epidermis by the chytrid fungal pathogen *Batrachochytrium dendrobatidis* (hereafter *Bd*). Chytridiomycosis is now widely viewed as the leading causative factor of enigmatic amphibian declines (Berger et al. 1998, Lips et al. 2006, Pounds et al. 2006). There are no reports of *Bd* in Central America before 1986, and first emergence of *Bd* at a site has been linked with rapid extirpations of up to 50% of resident amphibian species and total declines in amphibian density of 80% (Lips et al. 2006). Both laboratory and field

evidence indicate that growth and pathogenicity of *Bd* are inhibited by warm temperatures (Kriger and Hero 2006, Berger et al. 1998, Berger et al. 2004, Piotrowski et al. 2004), potentially explaining why most observed amphibian decline events have occurred in montane areas. The recently proposed “climate-linked epidemic hypothesis” suggests that severe outbreaks of chytridiomycosis are triggered by extreme climatic events (Pounds et al. 2006).

Despite the growing amount of research investigating ultimate causes of amphibian declines, a pronounced lack of long-term data still makes it impossible to determine the status of most populations. Approximately 22.5% of amphibian species are listed as data-deficient by IUCN, and this knowledge deficit is concentrated in tropical regions where the overwhelming proportion of biodiversity is located (Stuart et al. 2004). Those declines that are generally reported are strongly biased toward “rapid” declines, which may include populations that decline from stable to extinct in a matter of months (Lips et al. 2006).

In contrast to the lack of long-term data for most tropical amphibian assemblages, amphibians have been sampled since the 1950s at La Selva Biological Station, a 16 km² primarily old-growth wet forest reserve in the Caribbean lowlands of Costa Rica (Guyer and Donnelly 2005). I compiled data obtained from several studies (1970 to 2005) that estimated density of amphibians and reptiles at La Selva using a standard method (Jaeger and Inger 1994). The available data focused specifically on the leaf-litter herpetofauna, which at La Selva includes approximately 26 species of frogs and 2 species of salamanders, but also 13 species of lizards and many species of snakes (Guyer and Donnelly 2005). The leaf-litter guild of the lizard fauna represents the vertebrate group

most ecologically similar to litter amphibians, because both terrestrial frogs and terrestrial lizards use similar habitats, microhabitats, and prey (Lieberman 1986, Whitfield and Donnelly 2006). But because these two groups differ in physiological susceptibility to factors associated with amphibian declines (e.g. pesticide exposure or emerging infectious diseases), syntopic lizards provide an invaluable contrast for sorting hypotheses about mechanisms driving amphibian declines.

Our primary goals were to determine whether amphibians at La Selva show evidence of population declines, whether population trends vary between terrestrial amphibians and terrestrial lizards, and whether population trends vary among habitat disturbance regimes. Given current knowledge of global trends in amphibian population declines (Stuart et al. 2004), as well as detailed patterns for montane regions in the Neotropics (Lips et al. 2003, Lips et al. 2005, Pounds et al. 2006), there is no *a priori* reason to expect widespread population declines to have occurred for the site or assemblage I examine.

METHODS

La Selva Biological Station (10° 25'N, 84° 00' W) is a 1615 ha evergreen tropical wet forest reserve in the Caribbean lowlands of Heredia Province, Costa Rica (McDade and Hartshorn 1994). Elevation ranges from 30 – 135 m asl. Mean monthly temperature ranges 24.7° in January to 27.1° in August, and annual precipitation averages ~4000mm with a short mild dry season from January to April (Sanford et al. 1994). Approximately 53% of the La Selva reserve consists of old-growth forests, and <4% of the reserve consists of several small plantations of abandoned cacao plantations. These former

plantations of cacao (*Theobroma cacao*) were abandoned in part in 1963 and the remainder in 1986 (McDade and Hartshorn 1994). Secondary succession followed abandonment of these plantations. Though many *Theobroma* trees remain in these sites, in some cases there were efforts to remove trees from former plantation sites. Our data from former cacao plantations, thus, track complicated histories of anthropogenic forest disturbance.

I compiled published and unpublished reports of density of leaf-litter amphibians and reptiles at La Selva (sources used in this study are summarized in Table 2.1). I included only data collected using a single standard method, day-sampled litter quadrats (Jaeger and Inger 1994). Briefly, sampling litter quadrats involves the demarcation of an area of the forest floor of fixed size followed by an exhaustive search for all amphibians and reptiles (Jaeger and Inger 1994). This method is widely used for sampling of terrestrial amphibians and reptiles throughout the tropics (Allmon 1991, Vonesh 2001). Size of quadrats used in this study range from 4m² to 144m². Plots were sampled in both primary forest and in abandoned cacao plantations undergoing secondary succession. In the case of studies that conducted experimental manipulations of study quadrats, only unmanipulated control quadrats were used. A component of these data (~11% of area sampled) was collected by students in graduate and undergraduate courses and represent small studies with limited sample sizes. These student studies were supervised by experienced professional ecologists or herpetologists. Inclusion of these studies may increase variance due to observer effects, but are the only data available for many of the years in which data were collected.

The fundamental data from each census are the numbers of individuals of each species captured in exhaustive sampling of a plot ranging in size from 4-144m². Total amphibians included 93 unidentified frogs (e.g., *Eleutherodactylus* sp.) and 17 salamanders (*Oedipina* sp.); total reptiles included 27 snakes. For tests of frogs and lizards, only species with at least 5 individuals were included. Individual studies included from 1 to 98 separate plots, typically within the same month but occasionally spread over an entire year. Not all studies recorded data separately by plot. Therefore, studies or plots were weighted by area sampled rather than equally by study, which produced estimates unaffected by the level of aggregation.

These data were analyzed with generalized linear mixed models with Poisson error and log-link, with log(quadrat size) included as an offset to correct for varying quadrat size (McCullagh and Nelder 1989). Temporal trends were estimated as linear slopes across years; with the log-link, these parameter estimates translate to multiplicative changes in abundance per year. For each species and habitat with 5 or more individuals, I report the multiplicative change per year as a percent: $(1 - \beta_{\text{year}}) * 100\%$. Habitat (cacao v. forest) and taxon (frog v. lizard) were treated as fixed effects; species within taxon were considered random effects. Differences between taxa or habitats in temporal trends were tested as taxon $\times$ year or habitat $\times$ year interactions. Denominator degrees of freedom were computed by the containment method, so the taxon $\times$ year term was tested against the species(taxon) $\times$ year random effect. Preliminary analyses including wet season v. dry season found no interactions of season with other factors, and there was no trend in season of sampling across years, so season was dropped from the analyses reported. The overdispersion parameter of the overall

model including species, habitat, and year was 0.85, indicating reasonable fit with the Poisson error. Similar results to those I report were obtained when OTS course data were excluded from the analyses.

Climate data

Data on precipitation and daily temperature were collected throughout the study period by meteorological equipment at La Selva Biological Station (1). Daily minimum temperature data were collected manually at La Selva from the automated meteorological station beginning in 1992. The daily temperature record was extended back to 1984 by regressing data from the La Selva record against data from MOLA, a nearby meteorological station (2).

Until 1992, precipitation data were collected manually from a standard rain gauge. Starting in 1992, an automated meteorological station was installed which collected daily precipitation data with a tipping-bucket system. Precipitation data that I report are daily precipitation data from the automated meteorological station from 1992 – 2004, and data before 1992 were adjusted to correct for the change in data collection methodology and a move of rain gauge data collection location in 1982. Because manual checking of rain gauge may not differentiate between days with no rainfall and days the gauge was not checked, this dataset is only reliable after 1992. However, number of dry days also decreased in the period 1992-2005. To test if these differences in data collection details may bias trends in number of dry days that I report, I conducted analyses using only data collected from the automated meteorological station from 1992

– 2005. Even using this subset of the data, there was a decrease in the number of dry days ($r^2 = 0.310$, $F = 6.393$, $P = 0.028$).

RESULTS

Our data indicate a 75% decline in total densities of leaf-litter amphibians and reptiles in primary forest since 1970 (Figure 2.1). Total frog density has declined by an average rate of 4.1% per year since 1970 and, unexpectedly, total lizard density has decreased by an average of 4.5% per year over the same period. Every individual species of frog or lizard for which I detected a significant trend ($n=17$) decreased in density over the study period in primary forest (Table 2.2). Population trends differed markedly between primary forest and abandoned cacao plantations ($F_{1,12}=104.6$, $P<0.0001$). In cacao, total density of amphibians and reptiles increased by an annual average of 4.0% and 2.7%, respectively. While all species declined significantly in primary forest, in cacao seven species declined and four increased in density; this habitat by taxon interaction was significant ($F_{1,12}=17.44$, $P=0.001$). Even species that increased in density in cacao declined significantly in forest. Temporal trends in density did not differ between frog species and lizard species in forest ($F_{1,19}=0.05$, $P=0.82$), in cacao ($F_{1,13}=0.95$, $P=0.35$), or when habitat types were pooled ($F_{1,12}=0.001$, $P=0.95$). While individual species' temporal trends were statistically significant only for common species with adequate statistical power, there was no association between ranked abundance and slope of trend ($r=0.029$, $P=0.801$, $N=77$).

DISCUSSION

The data indicate dramatic declines in total density of terrestrial amphibians since 1970, and nearly identical trends for lizards, but also indicate opposite trends in adjacent abandoned cacao groves. Declines of similar magnitude have been widely reported for other tropical sites in Central and South America and Australia, but the trends I identify are distinct from all other recorded population declines in at least three major ways. First, community-wide amphibian population declines of the magnitude that I report herein have only been reported from cool climates – temperate regions or montane regions of the tropics (Young et al. 2001, Lips et al. 2005, Pounds et al. 2006). Second, amphibian decline events in the montane tropics have occurred rapidly, often in as little as 6 months (Lips et al. 2006). Third, declines in montane sites are only rarely reported to accompany declines in populations of other faunal elements such as reptiles or birds (Pounds et al. 1999, Whiles et al. 2006). Hence, the widespread declines I describe appear fundamentally different from all other enigmatic population declines that have been reported previously.

Differentiating problematic declines from natural fluctuations in populations is an issue of particular difficulty in applied ecology (Pechmann et al. 1991, Alford and Richards 1999), yet community-wide unidirectional declines of the duration and intensity I report here are not consistent with reported data on population fluctuations at other tropical sites (Andrews 1991, Stewart 1995). Dramatic fluctuations in amphibian population density may occur, but appear to be driven primarily by unpredictable aquatic environments (Alford and Richards 1999, Marsh 2001, Daszak et al. 2005). However, amphibian species with terrestrial direct-development, including the majority of this

assemblage (Guyer and Donnelly 2005), are independent from standing water and do not show such dramatic fluctuations in population size (Marsh 2001, Green 2003). Finally, our data for primary forest suggest simultaneous declines among all common species without apparent intermittent years of high recruitment, an unlikely pattern for stochastic population fluctuations.

Habitat modification cannot provide a satisfactory explanation because these declines have occurred in protected old-growth rainforests (McDade and Hartshorn 1994). Habitat fragmentation is a plausible, but unlikely, mechanism for these declines. Extensive deforestation has decreased forest in the landscape surrounding La Selva from 55% in 1976 to 34% in 1996 (Sanchez-Azofeifa et al. 1999). The La Selva Biological Station (35 - 135 m) is connected to the Braulio Carrillo National Park (135 - 2400 m), but is essentially isolated from other lowland forests on three of its borders. The isolation of La Selva from other large forest patches has been invoked to explain the extirpation of several species of insectivorous understory birds (Sigel et al. 2006). Yet in contrast to these birds, most species of declining amphibians and reptiles at La Selva historically existed in very large populations that are unlikely to be susceptible to fragmentation-driven processes such as genetic drift, inbreeding, or demographic stochasticity. Furthermore, La Selva is bordered on two sides by rivers large enough to form significant historical barriers to population connectivity, and it is across these rivers that the majority of deforestation in the region has taken place.

Major processes currently proposed to explain enigmatic declines also fail to provide a parsimonious explanation for trends reported for La Selva. A consensus is emerging that chytridiomycosis is the cause many of these enigmatic declines, either

acting alone or synergistically with other factors (Berger et al. 1998, Lips et al. 2003, Lips et al. 2006, Pounds et al. 2006). However, no studies have yet reported widespread declines in lowland populations of amphibians. Laboratory and field evidence consistently suggest that high temperatures inhibit growth and pathogenicity of *Bd* (Kriger and Hero 2006, Berger et al. 1998, Longcore et al. 1999, Piotrowski et al. 2004, Pounds et al. 2006), and it has been suggested that declines driven by *Bd* cannot occur in lowland habitats. Furthermore, recent reports have demonstrated that amphibian populations of some species may coexist with *Bd* with no apparent decline (Daszak et al. 2005, Kriger and Hero 2006). Critically, there is no evidence that *Bd* can affect reptiles and chytridiomycosis cannot provide a straightforward explanation of why population densities of the same species declining in primary forest have increased in adjacent abandoned cacao plantations. The declines I report are inconsistent with any reported declines attributable to chytridiomycosis, because of recent invasion of *Bd* (Lips et al. 2006) or emergence of *Bd* to shifting climate (Pounds et al. 2006).

Pollutants from commercial agriculture that involve frequent aerial applications of pesticides have also been implicated as factors driving enigmatic amphibian declines (Davidson 2004). Banana and pineapple plantations in particular have proliferated in the eastern Costa Rican lowlands upwind from La Selva (Sanchez-Azofeifa et al. 2001). Agrochemical pesticides may travel long distances (Sparling et al. 2001), and upwind pesticide use has been correlated significantly with declines in populations of ranid frogs in the western United States (Davidson 2004). Pesticides may increase mortality directly by disrupting physiological processes (Hayes et al. 2002), but may also reduce densities of arthropod prey that have been demonstrated to limit population sizes of lizards at this

site (Guyer 1988). Pesticide-mediated declines in arthropod density offer a reasonable explanation for faunal declines among amphibians, reptiles, and insectivorous birds, but cannot explain population increases in abandoned cacao groves among the same species experiencing declines in adjacent primary forest.

I suggest that the most parsimonious explanation for declines at La Selva is that climate shifts in the past 35 years have affected standing litter mass, a major proximate determinant of abundance for leaf-litter amphibians and reptiles (Lieberman 1986). The annual mean of daily minimum temperature increased between 1982 and 2004 (Figure 2.2A). Although total rainfall did not increase, the proportion of days with no rainfall decreased between 1970 and 2004 (Figure 2.2B; climate data described in supplemental material). The annual mean daily minimum temperature is negatively correlated with tree growth during the same period (Clark et al. 2003), indicating that these climatic shifts are of sufficient intensity to influence ecosystem processes. The increasingly warm and wet conditions of the past two decades could negatively influence standing litter mass by affecting rates of litterfall or litter decomposition (Aerts 1997). Standing leaf litter depth has been used to explain density of leaf-litter amphibians and reptiles among microhabitats (Whitfield and Pierce 2005), forest types (Lieberman 1986, Heinen 1992), biomes (Fauth et al. 1989), and between seasons (Lieberman 1986). Furthermore, litter dynamics can explain the difference in trends between habitat types because cacao trees have several leaf-flushing events each year, therefore litter accumulation is greater in cacao plantations than it is in old-growth forests (Lieberman 1986). Further, rates of litterfall may increase as early succession progresses in abandoned plantations (Reed and Lawrence 2001). Because depth of leaf litter appears to strongly influence the density of

both terrestrial amphibians and terrestrial reptiles, and because litter decomposition may be constrained by dry season moisture limitation (Wieder and Wright 1995), an increase in precipitation frequency could decrease litter mass and reduce critical microhabitat resources for amphibians and reptiles.

Regardless of which factor - or combination of factors - has contributed to La Selva declines, I identify what I believe to be the strongest available evidence that amphibian declines in pristine habitats may be accompanied by simultaneous declines in other taxa. Cross-taxa declines have been documented elsewhere, yet not emphasized. Declines in populations of anoline lizards and forest birds accompanied well-documented amphibian declines in Monteverde, Costa Rica (Pounds et al. 1999). Populations of reptiles declined in conjunction with amphibians in the Western United States (Matthews et al. 2002) and in Panama (Whiles et al. 2006). These simultaneous declines have been attributed either to wide-acting environmental changes (Pounds et al. 1999), or as indirect consequences of amphibian declines manifested through trophic links (Whiles et al. 2006). Yet because very few studies have documented population trends of other taxa when reporting amphibian declines, it is impossible to determine how frequently simultaneous declines of several taxa occur. These data urge that attention be devoted to understanding how often other faunal groups decline in conjunction with amphibian population, and what processes cause these simultaneous declines.

Amphibian decline events reported in the literature – particularly those from the Neotropics – are dominated by mass mortality events and rapid extirpations that occur over a period of a few months (Lips et al. 2006, Pounds et al. 2006). In contrast to these sudden decline events, I demonstrate in this report that community-wide gradual declines

may occur. Sadly, the dramatic declines I report here can only be considered slow in comparison to such instantaneous extinction events. It is currently impossible to determine how widespread gradual community-wide declines such as the one I report here are occurring, because trends such as those I report are impossible to detect without long-term abundance-based data on population densities collected using consistent methodology. While such datasets are exceptionally rare, I suggest that these datasets will be critical to understanding the full extent of the amphibian decline crisis.

Further, the lack of historic data on population densities may lead to naïve or inappropriate assessments of conservation status, a phenomenon known as shifting baselines syndrome (Dayton et al. 1998). Even in 2005, the last year for which I include data, population densities of terrestrial amphibians and reptiles at La Selva may be greater than densities from sites where similar methodologies have been used (Allmon 1991, Vonesh 2001). Without robust historical datasets indicating precipitous declines, current densities of amphibians and reptiles could be used to suggest that amphibian and reptile populations at La Selva are free from conservation risks. Indeed, all but one of the amphibian species for which I report persistent decade-long declines in protected old-growth rainforests are listed as “Least Concern” by the IUCN Red List (IUCN 2006).

These data raise the worrying possibility that systematic declines in amphibian populations do not only occur in cool climates, but that because declines occurring in cooler sites occur more quickly, these are the only habitats where they are detected. These data also indicate that even populations of amphibians for which specific threats have not been identified may nonetheless be suffering dramatic decline, and that such populations may be considered stable due to lack of long-term data, not lack of threats.

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LITERATURE CITED

- Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* **79**:439-449.
- Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* **30**:133-160.
- Allmon, W. D. 1991. A plot study of forest floor litter frogs, Central Amazon, Brazil. *Journal of Tropical Ecology* **7**:503-522.
- Andrews, R. M. 1991. Population stability of a tropical lizard. *Ecology* **72**:1204-1217.
- Bell, K. E., and R. K. Barrett. 2003. Reinventing the wheel: day vs. night sampling for leaf litter herpetofauna. *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.
- Bell, K. E., and M. A. Donnelly. 2006. Influence of forest fragmentation on community structure of frogs and lizards in northeastern Costa Rica. *Conservation Biology* **20**:1750-1760.
- Bennett, S., M. A. Donnelly, K. Etheridge, C. Guyer, R. Loflin, L. Rayor, and J. Tsuji. 1982. Comparison of the leaf litter herpetofauna of primary forest and cacao. Pages 327-328 *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.
- Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences* **95**:9031-9036.
- Berger, L., R. Speare, H. B. Hines, G. Marantelli, A. D. Hyatt, K. R. McDonald, L. F. Skerratt, V. Olsen, J. M. Clarke, G. Gillespie, M. Mahony, N. Sheppard, C. Williams, and M. J. Tyler. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal* **82**:434-439.
- Black, R. W., E. P. Flint, D. J. Futuyma, B. C. Lewis, M. N. Melampy, M. K. Palmer, W. R. Teska, W. Vandevender, and J. Talbot. 1974. Litter herps. Pages 143-147 *Tropical Biology Course*. Organization for Tropical Studies.
- Blaustein, A. R., P. D. Hoffman, D. G. Hokit, J. F. Kiesecker, S. C. Walls, and J. B. Hays. 1994. UV repair and resistance to solar UV-B in amphibian eggs: a link to possible declines? *Proceedings of the National Academy of Science* **91**:1791-1795.

- Buttenhoff, P., and D. Nicolson. 1996. A comparison of leaf litter herpetofauna at Volcan Cacao, Corcovado and La Selva. Pages 327-330 Tropical Biology: An Ecological Approach. Organization for Tropical Studies.
- Clark, D. A., S. C. Piper, C. D. Keeling, and D. B. Clark. 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984 - 2000. Proceedings of the National Academy of Sciences **100**:5282-5857.
- Daszak, P., D. E. Scott, A. M. Kilpatrick, C. Faggoni, J. W. Gibbons, and D. Porter. 2005. Amphibian population declines at Savannah River Site are linked to climate, not chytridiomycosis. Ecology **86**:3232-3237.
- Davidson, C. 2004. Declining downwind: amphibian population declines in California and historical pesticide use. Ecological Applications **14**:1892-1902.
- Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1998. Sliding baselines, ghosts, and reduced expectations in kelp forest communities. Ecological Applications **8**:309-322.
- Fauth, J. E., B. I. Crother, and J. B. Slowinski. 1989. Elevational patterns of species richness, evenness, and abundance of the Costa Rican leaf-litter herpetofauna. Biotropica **21**:178-185.
- Futuyma, D. J., R. Bush, M. Keller, J. Evans, J. Burcham, and S. C. Adolph. 1984. The abundance of litter herps. Pages 36-38 Tropical Biology: An Ecological Approach. Organization for Tropical Studies.
- Green, D. M. 2003. The ecology of extinction: population fluctuation and decline in amphibians. Biological Conservation **111**:331-343.
- Grossman, E., K. Bakkegard, M. A. Donnelly, R. Fredrickson, P. Herron, and L. Schreeg. 2000. To boldly herp where no one has herped before: A survey of the leaf litter herpetofauna of La Selva's Sarapiquí Annex. Pages 181-185 Tropical Biology: An Ecological Approach. Organization for Tropical Studies.
- Guyer, C. 1988. Food supplementation in a tropical mainland anole, *Norops humilis*: demographic effects. Ecology **69**:350-361.
- Guyer, C., and M. A. Donnelly. 2005. Amphibians and Reptiles of La Selva, Costa Rica, and the Caribbean Slope. University of California Press, Berkeley.

- Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. A. Stuart, and A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences of the United States of America* **99**:5476-5480.
- Heinen, J. T. 1992. Comparisons of the leaf-litter herpetofauna in abandoned cacao plantations and primary rain forest in Costa Rica: some implications for faunal restoration. *Biotropica* **24**:431-439.
- Hernandez-Huerta, A., C. Ortiz, O. Montenegro, R. Cordero, C. Garcia-Mendez, and B. Garcia. 1994. Comparacion de dos metodos de monitoreo en anfibios y reptiles. Pages 31-33 *Ecologia Tropical y Conservacion*. Organization for Tropical Studies.
- Hufft, R., S. M. Whitfield, J. Carlson, M. Coelho, and V. Gowda. 2003. The Neotropical leaf litter herpetofauna and the litter shadow of large canopy trees. Pages 71-73 *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.
- Hughes, M., A. Edwards, M. A. Donnelly, T. C. Bukowski, T. R. Schultz, and M. A. Wohlenberg. 1991. Leaf litter herpetofauna on residual soils at La Selva. Pages 155-158 *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.
- IUCN. 2006. 2006 IUCN Red List of Threatened Species. Downloaded on 01 October 2006.
- Jaeger, R. G., and R. F. Inger. 1994. Quadrat sampling. Pages 97-103 *in* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek, and M. S. Foster, editors. *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington.
- Kruger, K. M., and J.-M. Hero. 2007. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology* **271**: 352-359.
- Kruger, K. M., and J. M. Hero. 2006. Survivorship in wild frogs infected with chytridiomycosis. *Ecohealth* **3**:171-177.
- Lieberman, S. S. 1986. Ecology of the leaf litter herpetofauna of a neotropical rain forest: La Selva, Costa Rica. *Acta Zoologica Mexicana (ns)* **15**:1-72.
- Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* **103**:3165-3170.

- Lips, K. R., P. A. Burrowes, J. R. Mendelson, III, and G. Parra-Olea. 2005. Amphibian population declines in Latin America: a synthesis. *Biotropica* **37**:222-226.
- Lips, K. R., J. D. Reeve, and L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* **17**:1078-1088.
- Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et. sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91**:219-227.
- Marsh, D. M. 2001. Fluctuations in amphibian populations: a meta-analysis. *Biological Conservation* **101**:327-335.
- Matthews, K. R., R. A. Knapp, and K. L. Pope. 2002. Garter snake distributions in high-elevation aquatic ecosystems: Is there a link with declining amphibian populations and nonnative trout introductions? *Journal of Herpetology* **36**:16-22.
- McCullagh, P., and J. A. Nelder. 1989. *Generalized Linear Models*. 2nd edition. Chapman and Hall, London.
- McDade, L. A., and G. S. Hartshorn. 1994. La Selva Biological Station. Pages 6-14 in L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, editors. *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. University of Chicago Press, Chicago.
- Mendelson, J. R., K. R. Lips, R. W. Gagliardo, G. B. Rabb, J. P. Collins, J. E. Diffendorfer, P. Daszak, R. Ibanez, K. C. Zippel, D. P. Lawson, K. M. Wright, S. N. Stuart, C. Gascon, H. R. da Silva, P. A. Burrowes, R. L. Joglar, E. La Marca, S. Lotters, L. H. du Preez, C. Weldon, A. Hyatt, J. V. Rodriguez-Mahecha, S. Hunt, H. Robertson, B. Lock, C. J. Raxworthy, D. R. Frost, R. C. Lacy, R. A. Alford, J. A. Campbell, G. Parra-Olea, F. Bolanos, J. J. C. Domingo, T. Halliday, J. B. Murphy, M. H. Wake, L. A. Coloma, S. L. Kuzmin, M. S. Price, K. M. Howell, M. Lau, R. Pethiyagoda, M. Boone, M. J. Lannoo, A. R. Blaustein, A. Dobson, R. A. Griffiths, M. L. Crump, D. B. Wake, and E. D. Brodie. 2006. Biodiversity - Confronting amphibian declines and extinctions. *Science* **313**:48-48.
- Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* **253**:892-895.
- Piotrowski, J. S., S. L. Annis, and J. E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* **96**:9-15.

- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**.
- Pounds, J. A., M. P. L. Fogden, and J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. *Nature* **398**:611-615.
- Reed, L., and D. Lawrence. 2001. Litter nutrient dynamics during succession in dry tropical forests of the Yucatan: regional and seasonal effects. *Ecosystems* **6**:747-761.
- Sanchez-Azofeifa, G. A., R. C. Harriss, and D. L. Skole. 2001. Deforestation in Costa Rica: a quantitative analysis using remote sensing imagery. *Biotropica* **33**:378-384.
- Sanchez-Azofeifa, G. A., C. Quesada-Mateo, P. Gonzalez-Quesada, S. Dayanandan, and K. S. Bawa. 1999. Protected areas and conservation of biodiversity in the tropics. *Conservation Biology* **13**:407-411.
- Sanford, R. L., Jr., P. Paaby, J. C. Luvall, and E. Phillips. 1994. Climate, geomorphology, and aquatic systems. Pages 19-33 *in* L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, editors. *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. University of Chicago Press, Chicago.
- Scott, N. J. 1976. The abundance and diversity of the herpetofaunas of tropical forest litter. *Biotropica* **8**:41-58.
- Sigel, B. J., T. W. Sherry, and B. E. Young. 2006. Avian community response to lowland tropical rainforest isolation: 40 years of change at La Selva Biological Station, Costa Rica. *Conservation Biology* **20**:111-121.
- Sparling, D. W., G. M. Fellers, and L. L. McConnell. 2001. Pesticides and amphibian population declines in California, USA. *Environmental Toxicology and Chemistry* **20**:1591-1595.
- Sorenson, A. M. 2002. Leaf litter herpetofauna and leaf litter depth in four different habitats. Pages 199-202 *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.
- Sorhaindo, R., S. Cachel, V. Leftwich, D. Fenner, J. Rankin, and N. J. Scott. 1972. Tropical litter herpetofauna in cacao groves at la Selva. Pages 1-3 *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.

- Stewart, M. M. 1995. Climate driven population fluctuations in rain forest frogs. *Journal of Herpetology* **29**:437-446.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306**:1783-1786.
- Ugarte, C. A. 1999. Herpetofaunal species within two quadrat shapes: a comparison of methods. Pages 147-150 *Tropical Biology: An Ecological Approach*. Organization for Tropical Studies.
- Vonesh, J. R. 2001. Patterns of richness and abundance in a tropical African leaf-litter herpetofauna. *Biotropica* **33**:502-510.
- Whiles, M. R., K. R. Lips, C. M. Pringle, S. S. Kilham, R. J. Bixby, R. Brenes, S. Connelly, J. C. Colon-Guad, M. Hunte-Brown, A. D. Huryn, C. Montgomery, and S. Peterson. 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology and the Environment* **4**:27-34.
- Whitfield, S. M., and M. A. Donnelly. 2006. Ontogenetic and seasonal variation in diets of a Costa Rican leaf-litter herpetofauna. *Journal of Tropical Ecology* **22**:409-417.
- Whitfield, S. M., and M. S. F. Pierce. 2005. Tree buttress microhabitat use by a neotropical leaf-litter herpetofauna. *Journal of Herpetology* **39**:192-198.
- Wieder, R. K., and S. J. Wright. 1995. Tropical forest litter dynamics and dry season irrigation on Barro Colorado Island, Panama. *Ecology* **76**:1971-1979.
- Young, B. E., K. R. Lips, J. K. Reaser, R. D. Ibanez, A. W. Salas, J. R. Cedeno, L. A. Coloma, S. Ron, E. La Marca, J. R. Meyer, A. Munoz, F. Bolanos, G. Chaves, and D. Romo. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* **15**:1213-1223.

Table 2.1. Sources used in this analysis. Studies marked with an asterisk are derived from unpublished reports in the so-called grey literature, generally reports of student studies conducted during courses administered by the Organization for Tropical Studies. Total density refers to all reptiles and amphibians.

Source	Dates	Forest Type	Number of Quadrats	Total Sample Area (m ²)	Total Density 100m ⁻²
Scott (1976) (3)	Mar, Aug 1970	Forest	9	522	22.03
Scott (1976) (3)	Mar, Jul 1971	Forest	10	580	12.93
	Dec 1973 - Dec				
Lieberman (1986) (4)	1974	Forest	27	1728	21.12
Black et al. (1974)* (5)	Jan - Feb 1974	Forest	4	232	11.64
Bennett et al (1982)* (6)	Jan 1982	Forest	3	150	14.67
Futuyma et al. (1984)* (7)	Jan 1984	Forest	2	122	11.48
Heinen (1992) (8)	Jan - Mar 1990	Forest	25	625	14.72
Hughes et al. (1991) * (9)	Feb 1991	Forest	8	200	15.00
Savage, unpubl.	Jul 1994 - Jun 1995	Forest	88	5632	8.90
Buttenhoff and Nicolson (1996)*(10)	Jan 1996	Forest	2	32	15.63
Ugarte (1999)* (11)	July 1999	Forest	8	512	4.69
Whitfield and Pierce (2005) (12)	Apr - May 2000	Forest	32	512	6.25
Grossman et al. (2000)* (13)	Jan 2000	Forest	20	1280	3.36
Sasa, unpubl.	Mar 2002	Forest	10	640	6.88
Sorenson (2002)* (14)	Jun 2002	Forest	1	16	6.25
Sasa, unpubl.	Nov 2003	Forest	8	512	8.01
Bell and Barrett (2003)* (15)	Jun 2003	Forest	3	75	4.00
Hufft et al. (2003)* (16)	Jun 2003	Forest	10	40	17.50
Sasa, unpubl.	Oct 2004	Forest	18	1152	4.69
Bell and Donnelly (in press) (17)	Oct 2003 - Apr 2004	Forest	98	2450	3.80
Sasa, unpubl.	Apr 2005	Forest	19	475	6.74
	Dec 1973 - Dec				
Lieberman (1986) (4)	1974	Cacao	16	1024	62.50
Sorhaindo et al. (1972)* (18)	Aug 1972	Cacao	4	232	18.97
Bennett et al (1982)* (6)	Jan 1982	Cacao	3	75	41.33
Fauth et al. (1989) (19)	Aug 1985	Cacao	10	250	22.40
Heinen (1992) (8)	Jan - Mar 1990	Cacao	50	1250	30.88
Hernandez-Huerta et al. (1994)* (20)	Jan 1994	Cacao	6	384	9.64
Donnelly, unpubl.	Sep 1996	Cacao	10	1440	51.39
Donnelly, unpubl.	Mar 1998	Cacao	10	1440	60.21
Donnelly, unpubl.	May 1999	Cacao	10	1440	55.56
Whitfield and Pierce (2005) (12)	Apr - May 2000	Cacao	22	352	16.48
Sorenson (2002)* (14)	Jun 2002	Cacao	1	16	6.25

Table 2.2. Population trends for leaf-litter amphibians and reptiles at La Selva Biological Station. Density (1970s) for forest and cacao represent mean number of individuals per 100 m² derived from studies in the early 1970s for forest and cacao habitats, respectively. Mean percent change indicates mean yearly percentage change in population status for forest and cacao habitats, respectively; trends in bold are significant at the 0.05 significance level. Confidence limits indicate 95% confidence limits about the mean percent change. *F* and *P* values indicate differences in trends between forest and cacao habitats. Entire herpetofauna includes frogs, lizards, salamanders, and snakes.

Taxon	1970s Forest Density (individuals 100 m ⁻²)	Mean Yearly Percent Change	95% Confidence Limits	1970s Cacao Density (individuals 100 m ⁻²)	Mean Yearly Percent Change	95% Confidence Limits	F	P
Entire herpetofauna	19.01	-4.10	(-4.6, -3.6)	54.46	3.42	(2.0, 4.9)	180.6	<0.0001
All frogs	14.24	-4.01	(-4.5, -3.5)	38.30	3.99	(2.5, 5.5)	168.6	<0.0001
All lizards	4.57	-4.54	(-5.4, -3.7)	14.81	2.68	(1.0, 4.5)	79.4	<0.0001
All salamanders	0.13	-14.52	(-17.0, -12.2)	0.64	-17.10	(-19.0, -15.2)	2.3	0.13
All snakes	0.03	1.90	(-0.1, 4.1)	0.72	-2.95	(-6.0, 0.5)	8.1	0.0047
Frogs:								
<i>Rhaebo haematiticus</i>	0.23	-5.93	(-7.4, -4.4)	0.32	-5.69	(-8.4, -2.6)	0.03	0.86
<i>Chaunus marinus</i>	0.00			0.00	3.19	(-1.1, 8.0)		
<i>Oophaga pumilio</i>	3.20	-1.18	(-2.0, -0.4)	14.33	8.75	(7.3, 10.3)	146.8	<0.0001
<i>Craugastor</i>								
<i>bransfordii</i>	7.41	-5.22	(-6.0, -4.5)	20.46	-4.45	(-6.0, -2.8)	1.2	0.27
<i>C. cerasinus</i>	0.10	-0.74	(-2.4, 1.0)	0.00	-1.27	(-4.5, 2.4)	0.11	0.74
<i>C. fitzingeri</i>	0.00	1.95	(-0.1, 4.1)	0.00				
<i>C. megacephalus</i>	0.56	-9.97	(-11.2, -8.8)	1.27	-7.35	(-9.5, -5.0)	5.9	0.016
<i>C. mimus</i>	0.39	-13.49	(-15.7, -11.5)	0.00				
<i>C. noblei</i>	0.13	-10.42	(-13.3, -7.7)	0.00				
<i>C. talamancae</i>	0.42	-6.78	(-8.2, -5.4)	0.00				
<i>Eleutherodactylus</i>								
<i>caryophyllaceus</i>	0.16	-2.27	(-3.8, -0.6)	0.00				
<i>E. cruentus</i>	0.10	-3.51	(-5.2, -1.8)	0.00				
<i>E. diastema</i>	0.46	-6.69	(-8.0, -5.4)	0.64	-5.41	(-9.5, -0.4)	0.58	0.45
<i>E. ridens</i>	0.20	-5.77	(-7.3, -4.2)	0.72	-14.44	(-19.1, -9.6)	22.3	<0.0001
<i>Gastrophryne</i>								
<i>pictiventris</i>	0.26	-2.10	(-4.7, 0.7)	0.08	-3.52	(-6.4, -0.3)	0.54	0.46
<i>Lithobates</i>								
<i>warszewitschii</i>	0.16	-5.06	(-6.7, -3.3)	0.00				
Lizards:								
<i>Ameiva festiva</i>	0.13	-3.05	(-4.9, -1.1)	0.40	6.14	(-2.9, 17.4)	8.5	0.0037
<i>Lepidoblepharis</i>								
<i>xanthostigma</i>	0.82	-8.05	(-9.4, -6.8)	5.41	-9.05	(-10.8, -7.2)	0.78	0.38
<i>Norops capito</i>	0.07	-0.73	(-2.5, 1.1)	0.08	-0.24	(-4.0, 4.1)	0.08	0.78
<i>N. humilis</i>	2.12	-4.44	(-5.5, -3.4)	6.13	5.68	(3.9, 7.5)	116.2	<0.0001
<i>N. limifrons</i>	0.85	-3.05	(-4.4, -1.7)	1.27	5.73	(2.7, 9.0)	43.3	<0.0001
<i>Sphenomorphus</i>								
<i>cherriei</i>	0.42	-10.03	(-12.2, -7.9)	1.27	6.51	(2.8, 10.6)	69.0	<0.0001

Figure 2.1. Amphibian and reptile density over 35 years at La Selva Biological Station, Costa Rica. Each point indicates mean density for all quadrats in a given year. Closed symbols and solid line indicate data from primary forest. Open symbols and dashed line indicate data from abandoned cacao plantations. A. Trends for all terrestrial amphibians and reptiles. B. Trends for frogs only. C. Trends for lizards only.

Figure 2.2. Climate data for 35 years at the La Selva Biological Station indicates that the climate is getting warmer and consistently wetter. The annual mean of daily mean temperature (A) has increased and the number of days with zero rainfall has decreased since 1970 (B).

CHAPTER 3: TEMPORAL VARIABILITY IN PREVALENCE OF INFECTION BY THE AMPHIBIAN CHYTRID FUNGUS IN THREE SPECIES OF FROGS AT LA SELVA, COSTA RICA

ABSTRACT

The emerging infectious disease chytridiomycosis, caused by the Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis* (Bd), is implicated in widespread population declines, extirpations, and extinctions of amphibians in the Neotropics and throughout the world. While most tropical amphibian declines have occurred in mid- to high-elevation sites (>400m asl), populations of amphibians have also declined at the lowland (30-140m asl) La Selva Biological Station in northeastern Costa Rica. The role of Bd in these declines has not been assessed. Here, I report the results of a 12-month pathogen surveillance program for three common species of frogs at La Selva. I combine standard non-invasive skin swabbing techniques with a quantitative polymerase chain reaction assay to analyze the prevalence and load of Bd across an annual cycle. My data indicate an overall Bd infection rate of 6.1% for frogs at this site and an average Bd load among infected animals of 1020 zoospore equivalents (range: 0.08 to 22418 zoospore equivalents). My data indicate strong intra-annual variation in the prevalence of Bd: prevalence of infection increases in months with coolest air temperatures, and coolest temperatures experienced by the site appear to drive large variation in prevalence. I find no difference among species in prevalence of infection, despite considerable differences in affiliation of these species with water. While Bd is one of several potential stressors

affecting the amphibians of La Selva, it remains unclear whether Bd has a significant role in declines at this site.

INTRODUCTION

Amphibian assemblages in the highly diverse New World tropics have been decimated in the past three decades as so-called "enigmatic decline events" have swept through the region - causing rapid population declines, mass mortality events, and numerous extinctions (Kilpatrick et al.; 2006; Lips et al. 2005; Lips et al. 2008; Whitfield et al. in press; Young et al. 2001). These declines are particularly problematic because they have occurred even in protected areas – the last refuges for biodiversity not eroded by widespread habitat loss and modification, and environments thought to be relatively pristine (Whitfield et al. in press; Young et al. 2001). Enigmatic declines in many Neotropical localities have been associated with the emerging infectious disease chytridiomycosis, caused by the apparently non-native Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis* (hereafter, “Bd,” Berger et al. 1998; Lips et al. 2006; Lips et al. 2008). On a local scale, enigmatic declines result in rapid reductions in population density and species richness (Lips 1998; Lips et al. 2006), but across large spatial scales the sum of these decline events has produced widespread extinction events, rapid and severe range contractions, and the homogenization of remnant amphibian faunas (Smith et al. 2009; Whitfield et al. in press).

Severe declines in amphibian populations have been documented throughout the Neotropics, including at La Selva Biological Station, a lowland site in northeastern Costa Rica (Whitfield et al. 2007; Whitfield et al. in press). However, the declines at this site

are atypical of enigmatic decline events for a number of reasons. First, La Selva is located below 150m asl, whereas most reported enigmatic declines in amphibian populations in the Neotropics have taken place in mid- to high-elevation sites (>400m asl). Second, declines at La Selva were gradual, occurring over four decades rather than within a period of weeks or months (Lips et al. 2006, Whitfield et al. 2007, Richards-Zawacki 2010). Third, no mass mortality events have ever been witnessed at this well-observed research outpost; and lastly, simultaneous population declines in small lizards (which are not susceptible to infection by Bd) accompanied declines in amphibian populations (Whitfield et al. 2007). The principal factor or factors contributing to population declines at La Selva have not been established.

The Amphibian Chytrid Fungus was formally described and first linked to amphibian declines in the late 1990s (Berger et al. 1998; Kilpatrick et al. 2010; Longcore et al. 1999; Rosenblum et al. 2010). The life cycle of Bd consists of two stages: thalli that develop in keratinized epithelial tissues of amphibians, and flagellated zoospores that are the propagules for dispersal. Zoospores are believed to be dependent on water for dispersal, a fact that has been used to explain higher rates of infection and decline among amphibian species with aquatic life stages (Kriger and Hero 2007b; Lips et al. 2006; Lips et al. 2003). Amphibians infected with Bd may eventually die of cardiac arrest triggered by disruption of electrolyte transport across amphibian skin (Voyles et al. 2007; Voyles et al. 2009). Evidence suggests that Bd is introduced to the Neotropics (Lips et al. 2008; Rohr et al. 2008, but see Pounds et al. 2006) possibly with an origin in Africa (Soto-Azat et al.; Weldon et al. 2004). The first records of Bd in Costa Rica are from 1986, and the

projected current distribution of Bd covers most of the country (Puschendorf et al. 2009; Ron 2005).

A growing body of evidence, from both lab experiments and empirical field data, has attempted to explain why Bd-associated declines generally occur in cool climates. Evidence from physiological studies of Bd in culture indicates that growth of Bd is temperature-sensitive. Piotrowski et al.(2004) found that growth in culture peaks at 15-23°C, that growth stops at 28°C, and that 50% of cultures died after 8d at 30°C. Johnson et al. (2003) found that Bd cultures in the lab survive well at 26°C, but 100% mortality of Bd cultures occurs after 4d at 32°C. Berger et al. (2004) found that experimentally infected frogs experienced 100% mortality at 23°C, but only 50% mortality at 27°C. Field studies have also confirmed temperature-sensitivity of Bd. Kriger and Hero (2007c) demonstrated a negative correlation between prevalence of chytridiomycosis and mean air temperature (range: 12 °C to 22 °C) at a subtropical site in Queensland, Australia. Brem and Lips (2008) conducted surveys for Bd along two elevational gradients in Panama and found highest prevalence at high elevations. Richards-Zawacki (2010) demonstrated that amphibians can use behavioral thermoregulation to increase body temperatures and fight infection by Bd. Cumulatively, these studies suggest that intolerance of Bd to warm climates may explain why the vast majority of observed enigmatic declines - as well as the greatest density of threatened and possibly extinct species - occurs above 400m asl in the tropics (Lips et al. 2005; Whitfield et al. in press). Nevertheless, the precise thermal limits of Bd in the field have not been rigorously established.

It is not currently possible to determine whether chytridiomycosis is a factor contributing to population declines of amphibians at La Selva, or whether Bd should be seen as a threat to lowland populations of amphibians elsewhere in the neotropics. Whitfield et al. (2007) failed to detect Bd at La Selva based on a preliminary analysis of 140 individuals of *Oophaga pumilio*, *Craugastor bransfordii*, and *Dendropsophus ebraccatus*. However, Bd has been present in the region at least since 1986 (Puschendorf et al. 2006), and enigmatic decline events consistent with our knowledge of chytridiomycosis have occurred directly upslope from La Selva in the Parque Nacional Braulio Carrillo (Puschendorf et al. 2006). Further, there exist scattered reports of Bd in lowland tropical forests (Brem and Lips 2008; Puschendorf and Bolanos 2006; Puschendorf et al. 2006; Sanchez et al. 2008; Woodhams et al. 2008). Given current knowledge of thermal physiology of Bd, it appears that average temperatures in lowland wet forests of Costa Rica, such as La Selva, are within the range under which Bd can persist, but are beyond the optimal range of temperatures for growth of Bd. Without a basic understanding of the prevalence of chytridiomycosis on amphibians at this site, it will not be possible to evaluate: 1) the role of chytridiomycosis in population declines at La Selva; 2) the threat posed to lowland amphibian faunas by Bd; or 3) the relation of La Selva declines to those in montane regions of the neotropics.

Herein, I report the results of a 12-month pathogen surveillance program at La Selva Biological Station for three common species of frogs that represent a gradient from aquatic to terrestrial reproduction. Our primary goals were to assess the overall prevalence of infection by Bd on frogs at this site, and to relate infection prevalence to

temperature and precipitation. I expect that prevalence of infection will be very low at this site because temperatures are above those at which most Bd-associated declines have occurred, and I expect little intra-annual variation in prevalence because lowland tropical ecosystems show little seasonal variation in temperature. Further, I expect that prevalence should be restricted to – or greatest in – species most strongly associated with aquatic resources.

METHODS

Study Site

La Selva Biological Station is a 16 km² private biological reserve in the northeastern lowlands of Sarapiquí, Heredia Province, Costa Rica (10° 26' N, 83° 59' W). The site has been protected and managed by the Organization for Tropical Studies since 1968 (Clark 1990), and has been described as lowland wet forest, with elevation ranging from 35m to 137m asl. The site receives on average 4000mm annual precipitation, with no month on average receiving <100mm rainfall. There is little intra-annual variation in temperature (Figure 3.1), as is typical for tropical ecosystems. The majority of the site is primary forest, apparently with only small-scale direct human impacts since the European invasion of the Americas; other parts of the reserve include forests regenerating from pasture and other agricultural uses (McDade and Hartshorn 1994). The reserve rests at the foot of 460km² Parque Nacional Braulio Carrillo, a national protected area which extends up to ~2900m in elevation. Since the 1950s, the lowland regions surrounding La Selva have largely been converted from a continuous expanse of rainforest into heterogeneous patches of pasture, agricultural plantations (typically cacao, palm heart,

bananas, and pineapple), and residual forest fragments (Butterfield 1994). The amphibian fauna of La Selva is very well-known, with 52 amphibian species known to occur at the site (Donnelly 1994; Guyer 1990; Guyer and Donnelly 2005, Whitfield pers. obs.). The site has experienced a prolonged, gradual decline in total density of terrestrial amphibians (Whitfield et al. 2007), but with little or no evident species loss.

Field sampling

I sampled three common species of frogs at La Selva Biological Station that represent a range of life histories. *Rhaebo* (= *Bufo*) *haematiticus* is a bufonid toad with stream-dwelling tadpoles that passes the adult phase in forests or along rocky beaches. *Oophaga pumilio* is a dendrobatid poison frog with terrestrial eggs, tadpoles that are deposited in phytotelmata, and terrestrial forest-dwelling adults. *Craugastor bransfordii* is a direct-developing craugastorid frog that deposits eggs under soil away from aquatic habitats and that lacks free-living tadpoles but instead has eggs that hatch directly into small froglets that spend the entirety of their lives in the leaf-litter on the forest floor. These species represent points along a gradient ranging from the generalized amphibian life cycle with aquatic reproduction towards more derived terrestrial forms of reproduction that are common in humid tropical forests. Each species studied here is the most abundant representative of its reproductive mode found at La Selva.

To determine whether amphibians were infected with Bd at La Selva, I combined a non-invasive quantitative swabbing technique (Hyatt et al. 2007; Kriger and Hero 2007a) with quantitative (real-time) PCR ("qPCR," Boyle et al. 2004; Hyatt et al. 2007). Between May 2007 and April 2008, I captured frogs in the field by hand, using either

latex gloves or unused plastic bags. For all captured frogs, I swabbed surfaces of the body with sterile Fisher brand cotton fiber tipped wooden applicators (Fisher# 14-959-96B, hereafter "swabs"). A small number of amphibian skin cells adhere to the swab, and because thalli of Bd infect the amphibian dermis and epidermis, thalli or zoospores of Bd are collected in the swab sample. I swabbed frogs 10 times on the venter, 5 times on each side of the abdomen, and twice on each hand or foot (with the exception of the December 2007 and early January 2008 sampling sessions, in which hands and feet were swabbed five times each). The venter, hands, and feet are among the body surfaces most likely to be infected with Bd (Puschendorf and Bolanos 2006). The slight methodological difference in December 2007 and January 2007 and the other months was likely to produce a bias in Bd load, but less so in prevalence. To determine whether such biases exist, I re-analyzed data while reducing our estimates of Bd load by 20% in these months and found no substantial difference in the results. In addition to the swab sample for each captured frog, I collected data on species, snout-vent length, body mass, and age-sex class (juvenile, male, or female for *D. pumilio* and *C. bransfordii*; *R. haematiticus* were categorized as juvenile or adult).

Our sampling consisted of thirteen sessions over the period between May 2007 and April 2008. Sampling sessions generally lasted up to five days, and were usually conducted at four week intervals (see Table 3.1). I attempted to sample 30 individuals per species in each of these sampling sessions. Swab samples were stored at room temperature until July 2008, when they were placed into a -20° C freezer where they remained until processing.

Pathogen quantification

I used qPCR to evaluate infection status and to quantify Bd loads of sampled individuals. I extracted DNA from swabs using Qiagen DNeasy kits following manufacturer's procedures. While studies using qPCR to quantify Bd infection in amphibians generally use a different extraction protocol (PrepMan Ultra), I encountered difficulties with inhibition of PCR using the PrepMan Ultra extraction techniques, presumably because of secondary compounds present in the wooden handles of our swabs. I confirmed that issues with PCR inhibition were not problematic with Qiagen extraction protocols by comparing extractions of PrepMan Ultra and Qiagen techniques of swabs spiked with culture broth containing an isolate of Bd. These trials indicated no evidence of inhibition using the Qiagen extraction protocols.

I ran qPCR reactions based on methodology from Boyle et al. (2004). I used 10 μ L reactions containing 3 μ L of extracted DNA template, 900-nmol forward primer, 900-nmol reverse primer, 250 nmol probe, and 2X Taqman Fast Universal Master Mix (Applied Biosystems, Foster City, CA). I ran plates for one cycle of 95° C (20 sec) and 50 cycles of 95° C (3 sec) and 60° C (20 sec) on a StepOnePlus qPCR machine (Applied Biosystems, Foster City, CA). Each plate included a negative control and standard curve from 0.06 - 60 zoospore equivalents. I obtained standards from an Australian diagnostic lab (CSIRO, Livestock Industries). I ran reactions in triplicate, initially with DNA pooled from three swabs in a single reaction to save time and cost. Reactions from pooled swabs that showed positive results in any of the three wells were subsequently broken up separately and run in triplicate as individual swab samples. I considered samples to be positive if assays tested positive for Bd in two of the three triplicates.

Samples that tested positive in only one well of the three triplicates were considered suspicious and run in triplicate a second time. I used StepOne software v2.1 (Applied Biosystems, Foster City, CA) to analyze the qPCR results.

Weather and climate data

To examine relationships between prevalence and climatic variables, I used meteorological data from La Selva Biological Station's on-site meteorological station. I chose to examine relationships between average daily temperature and precipitation during the study period from May 2007 - April 2008 as they relate to prevalence and infection intensity. To illustrate long-term patterns in climate, I used a historical database of temperature records from April 1982 - December 2008. A more detailed summary of La Selva's climate has been provided previously (Sanford et al. 1994).

Statistical analysis

I used generalized additive models (“GAMs”) to evaluate temporal variation in prevalence of infection by Bd (Wood 2006; Zuur et al. 2009). Additive modeling makes no assumption of a linear relationship between predictor and response variables, but produces non-parametric smoothed curves that identify a relationship between such variables; smoothed curves generated by such GAMs are subject to tests to assess significance. Generalized additive modeling allows for the analysis of data with non-normal distributions (*i.e.*, binary data). I used GAMs to describe relationships between date and prevalence and between date and Bd load because exploratory analyses indicated that these relationships were nonlinear, and could not be linearized. I

conducted GAMs using the gam procedure in the *mgcv* package in *R* (Wood 2006), using date as a predictor variable and infection status as a binary response variable and specifying binomial error distributions and a logit link. To model infection intensity over time, I also used the gam procedure, used quantitative Bd load (number of zoospore equivalents) as a response variable; Bd load was log-transformed for analysis, and I specified Gaussian error distributions for this analysis because the underlying assumptions for normality.

Exploratory analyses indicated that all predictor variables except date allowed for linear modeling with infection prevalence and intensity. I therefore used generalized linear mixed modeling to explore relationships among infection status and the following predictor variables: frog age-sex class (juvenile, female, or male), species, snout-vent length (SVL), and index of body condition (IBC). To obtain values for IBC, I performed species-specific regressions between (square-root transformed) mass and SVL. The resulting model was highly significant ($F_{2,773}=1971$, $r^2=0.927$, $P<0.0001$), and I used the residuals from this model as our values for IBC. I were also interested in modeling effects of two climatic variables: T_{air} , the average of daily average air temperature from the 30 days preceding each swab sample; and P_{30} , the cumulative precipitation over the 30 days preceding each swab sample. Preliminary analyses using different metrics for temperature (daily minimum temperature, daily maximum temperature) or precipitation (daily average rainfall) and using different temporal scales (averaged temperature and cumulative precipitation over 7, 15, or 30 days) produced trends similar to those for our chosen climatic metrics. Because there were a considerable number of missing values for mass and IBC, I performed two separate GLMs for each fundamental response variable.

One included species and climatic variables (which were available for all samples in the dataset) and another that included species, sex, SVL, and IBC. Our prevalence analysis was conducted with a generalized linear mixed model using the lmer procedure in the *lme4* package of *R*, again with binomial error distributions and a logit link for prevalence as a response variable, and with sampling session included as a random factor. Modeling of infection intensity was performed as above, but with log-transformed number of zoospore equivalents as a response variable, and with specification of Gaussian error distributions.

RESULTS

I examined prevalence and infection intensity for 836 frogs between May 2007 and April 2008 (Table 3.1). Of these, 51 frogs tested positive for Bd, yielding an overall prevalence rate of 6.1%. For individuals that tested positive for Bd, the Bd load ranged from 0.08 to 22,417 zoospore equivalents (mean = 1020; sd = 3414; median = 52.7).

I detected the presence of Bd at La Selva throughout the year, although prevalence was very low from May 2007 through November 2007. Prevalence increased rapidly from November 2007 until January 2008, and decreased thereafter through the end of our study period (Table 3.1). Our GAM confirmed strong evidence of variation in infection prevalence over time (Figure 3.2, statistics for smoothing term: $\chi^2 = 27.01$, ref. df = 4.724, $P < 0.0005$). I detected no temporal trend in Bd load among infected animals ($F = 1.64$, ref. df = 1.76, $P < 0.203$).

There was a strong negative relationship between prevalence and temperature, and no direct relationship between prevalence and precipitation (Figure 3.3). However,

the analyses indicate an interaction between species and precipitation: prevalence in *O. pumilio* and *R. haematiticus* is positively correlated with precipitation, while *C. bransfordii* shows a slight negative relationship with precipitation. Our analysis of individual covariates indicated no effect of species, age-sex class, or IBC (Table 3.2). While I found no main effect of SVL, there was a significant interaction between SVL and species: *C. bransfordii* with Bd infections tended to be larger than those without ($F_{1,201}=15.97$, $P<0.001$); in contrast *O. pumilio* with Bd tended to be smaller than those without ($F_{1,359}=11.42$, $P<0.0008$); there was no difference in SVL between infected and non-infected *R. haematiticus* ($F_{1,253}=0.061$, $P=0.8$; Figure 3.4).

Our analyses of Bd load both indicated differences among species in infection intensity. Specifically, *O. pumilio* showed higher Bd loads than either *C. bransfordii* or *R. haematiticus*, but that there was no difference in Bd loads between *R. haematiticus* and *C. bransfordii* (Table 3.2, Figure 3.5). I detected no association between Bd load and temperature or precipitation, or with any of the individual covariates I examined (age-sex class, SVL, or IBC; Table 3.2).

DISCUSSION

My results indicate a generally low prevalence of infection by Bd for species examined in this study, but also considerable variation in prevalence over the course of an annual cycle. While prevalence was extremely low during the warmer months of our study period (May - October 2007), prevalence was much higher in the coolest months of the study (December 2007 until March 2008). I detected no variation over time in Bd load of infected animals; however, because only 51 individuals in our dataset tested

positive for Bd, I do not have strong evidence against seasonal variation in infection intensity.

Prevalence was indeed strongly negatively correlated with temperature, confirming well-established trends detected by others (Brem and Lips 2008; Kriger and Hero 2007a; Piotrowski et al. 2004). The model indicates that the relationship between prevalence and temperature is not linear, but that prevalence increases exponentially as temperature decreases (Figure 3.3). Two non-mutually exclusive hypotheses may account for these trends. First, at the coolest times of the year, temperatures at La Selva may be compatible with growth of Bd, but in warmer months temperature acts as a control on growth or survival of the fungus. Alternatively, cooler temperatures may be adversely affecting the frog immune system of our study animals. In either case, the relationship between prevalence and temperature is generalizable across the three species in this study.

I found no difference among species in prevalence, in spite of pronounced differences in these species' associations with water or streams. However, our models did consistently indicate species-level variation in Bd loads, with *O. pumilio* demonstrating higher Bd loads than the other two species. At least one other study has demonstrated species-level variation in infection prevalence, a result that was attributed to differential associations with flowing water and reproductive mode (Kriger and Hero 2007b). However, of the three species in our study *O. pumilio* is intermediate in its dependence on water. While the number of species examined in our study is limited, the data do not support the idea that infection prevalence is correlated with flowing water or reproductive mode.

While details of field swabbing techniques and lab methodology among studies of Bd are likely to confound efforts to directly compare the results of this study to others, our estimates of prevalence are concordant with limited data on Bd that exist for our region. Using histological techniques on specimens collected between March and April 1986, Puschendorf et al. (2006) detected Bd in 1 of 13 *C. bransfordii* and in 1 of 6 *O. pumilio* at nearby sites, though these positive samples were detected at a site with slightly higher elevation. Puschendorf et al. (2006) also reported 1 of 4 *C. bransfordii* positive for Bd in March 2002 at Reserva Forestal Escalera del Mono at Earth University, a site ~65km from our site and at a similar elevation. Puschendorf et al. (2009) failed to detect Bd using histology in the majority of specimens from the lowlands of northeastern Costa Rica.

My data indicate significant temporal variation in the prevalence of chytridiomycosis. This pattern can be interpreted in at least three ways: as the initial invasion of Bd to this site, as a periodic but temperature-independent outbreak of chytridiomycosis, or as a recurring seasonal disease driven by slight changes in temperature. Because Bd has been present at a site 12km from La Selva since 1986, and is rapidly increasing its range across Central America (Lips et al. 2008; Woodhams et al. 2008), it seems unlikely that our data represent the initial introduction of Bd to this site, and it is reasonable to assume that Bd has been present at La Selva at least since the mid-1980s. Given the low intra-annual variation in temperature at La Selva, it is plausible to assume that Bd occurs in episodic – though not necessarily regularly-occurring or predictably seasonal – outbreaks. However, non-quantitative pathogen surveillance efforts between June and November 2006 failed to find any evidence of Bd (Whitfield et

al. 2007), though continued disease monitoring efforts at the site indicated presence of Bd in February and March 2007 (Whitfield et al. unpubl.). These data are consistent with the hypothesis of regularly occurring outbreaks triggered by slightly lowered temperatures in cooler months.

Except for the consistent link between temperature and prevalence, details of the three species' responses to climate and individual covariates were individualistic - trends could not be generalized across all three species. Both *R. haematiticus* and *O. pumilio* showed weak positive correlations between precipitation and infection probability, but *C. bransfordii* showed a weak negative correlation between precipitation and infection probability. The flagellated zoospores of Bd require water for dispersal, and thus the trends for *R. haematiticus* and *O. pumilio* are relatively easy to interpret. It is at least plausible that in drier months, *C. bransfordii* are attracted to moister microclimates where Bd may persist, or where other *C. bransfordii* aggregate – thereby facilitating disease transfer. Further, because the climate at La Selva is quite consistently wet and has no regularly occurring extremely dry periods, I would expect that moisture limitation should not limit reproduction of Bd through most of the year.

While infected *C. bransfordii* tended to be larger than uninfected individuals, *O. pumilio* showed an opposite trend and *R. haematiticus* showed no evident association between size and infection status. At least one other study has indicated that smaller individuals are more likely to be infected (Kriger and Hero 2007a), suggesting that Bd may adversely affect growth. In the case of *O. pumilio*, the smaller individuals that have recently metamorphosed may be more likely to be infected because they have more exposure to water (in this case, phytotelmata) than do adults. Alternatively, dispersal in

O. pumilio primarily occurs among juveniles, as adults are philopatric and have small home ranges (Donnelly 1989). It is possible that dispersal events cause juvenile *O. pumilio* to cross small streams where Bd may be more prevalent. Although the patterns in size of infected individuals can plausibly be explained species by species, I do not have data for sufficient numbers of species to generate robust hypotheses about relationships between body size and infection.

My data indicate a clear association between Bd prevalence and monthly temperature, and suggest that La Selva's climate is amenable to infection by Bd in cooler months, but not in warmer months. While studies attempting to predict the geographic range of Bd suggest that lowland forests are amenable to Bd (Puschendorf et al. 2009; Ron 2005), relatively little attention is given to Bd in lowland ecosystems. My data provide valuable information about the occurrence of Bd in lowland forests, and suggest that attempts to model the occurrence of Bd should rely on estimates of lowest temperatures found at a site, not necessarily average temperatures. Our results also indicate that during cool periods, Bd can occur at relatively high prevalence in forests that are generally thought to be too warm for persistence of Bd.

While our study expands the understanding of Bd in the lowland tropics, I ultimately remain unable to determine whether chytridiomycosis is responsible for – in whole or in part – observed population declines of amphibians at La Selva. While on one extreme, arrival of Bd at a site can cause immediate and dramatic impacts on amphibian assemblages (Lips et al. 2006; Richards-Zawacki 2010), other studies have shown that amphibians infected with Bd persist in the wild. Kriger (2006) found that Bd-infected frogs appear to show no reduced survival compared with uninfected individuals.

However, Murray et al. (2009) demonstrated the Bd may continue to cause increased mortality – though not pronounced declines – in amphibians long after the initial invasion of Bd to a site; such a pattern may be consistent with trends for La Selva.

Chytridiomycosis may be acting as a novel stressor on amphibian populations at La Selva, yet it is difficult to attribute all trends reported from La Selva to chytridiomycosis. Whitfield et al. (2007) used long-term data to demonstrate parallel declines in small terrestrial lizards and small terrestrial amphibians, though lizards are not susceptible to Bd. Further, Whitfield et al. (2007) reported increased amphibian density in disturbed forests of La Selva, which is again difficult to reconcile with current knowledge of Bd.

In any case, Bd is at best one of a complex suite of novel stressors likely to impact amphibians at this site. Fragmentation effects have been felt on the birds and large mammals (Sigel et al. 2006; Young et al. 2008). Pesticide residues, presumably carried by air from agricultural plantations upwind from La Selva have been detected in soils and air of La Selva (Daly et al. 2007a; Daly et al. 2007b). Climate change is a known stressor, likely to significantly affect ectotherms in the near term if it is not affecting ectotherms at this site already. Severe El Niño-Southern Oscillation events have pronounced effects on the forests (Clark et al. 2003), although no data exist on their impacts on amphibians. There is little doubt that La Selva, like most tropical ecosystems – even those thought to be pristine – is impacted by a variety of novel stressors that may be adversely affecting amphibian populations. Until more data are available on impacts of each of these stressors on amphibian populations, it will be impossible to isolate any role of Bd in La Selva's declines.

My data provide the first robust estimates of prevalence of Bd at a lowland tropical site and I illustrate relatively high prevalence of chytridiomycosis in northern winter, despite low variation in temperature between coolest and warmest months at this site. These data are important for a number of reasons. First, our data indicate that efforts to detect Bd in lowland forests should be concentrated in the coolest months of the year even if those months are only slightly cooler than the yearly average. Similarly, efforts to model the distribution of Bd should rely on coolest temperatures in determining the geographic range of Bd. Indeed, Ron et al. (2005) found that minimum temperature of the coldest month was one of the strongest predictors in efforts to predict occurrence of Bd for the New World, but Puschendorf et al. (2009) found that minimum temperature of the coldest month was a relatively weak predictor of Bd occurrence for modeling efforts in Costa Rica.

Our study also underscores the importance of relatively long-term disease surveillance efforts. Because prevalence was extremely low in most months, and our estimate of prevalence is zero for several sampling periods, sampling efforts that are restricted to a single point in time, or that are limited in sample size, may fail to detect Bd at sites where it occurs. Such false negatives will impact efforts to understand the dynamics of chytridiomycosis as well as efforts to model the geographic distribution of Bd (Puschendorf et al. 2009; Ron 2005).

The climate at La Selva appears to be at the warm edge of Bd's distributional limit, and La Selva's climate is warming (Clark et al. 2003; Whitfield et al. 2007). Clark et al. (2003) indicated that the greatest recent climate change at La Selva has been an increase in daily minimum temperature, while the average and daily maximum

temperatures have remained relatively stable. Recent climate change may impact Bd in several ways. First, upon initial invasion to La Selva, Bd may have encountered a climate with somewhat cooler minimum temperatures than those found during our study period. While the recent increase in daily minimum temperatures is small ($<1^{\circ}\text{C}$), our study clearly indicates that relatively minor variability at the lower range of temperatures found at La Selva may have a strong impact on the ability of Bd to survive there (Figure 3.3). Second, if warming trends continue – or accelerate – La Selva may eventually become uninhabitable for Bd. However, continued increases in temperature are also likely to make La Selva uninhabitable for those species of amphibians at the warm edge of their thermal limit (Colwell et al. 2008; Deutsch et al. 2008; Huey et al. 2009). While it is likely that warming will make lowland forests less hospitable to Bd, it is also unlikely that warming will be a wholesale gain for lowland amphibian faunas.

A number of studies have now indicated presence of Bd at lowland sites (Brem and Lips 2008; Frias-Alvarez et al. 2008; Kriger and Hero 2008; Sanchez et al. 2008). None of the lowland sites in these studies appear to have suffered massive species loss characteristic of upland sites, though it is impossible to determine whether these sites suffer decline. A multi-decade dataset of quantitative population densities is available for none of these lowland sites, and without such a dataset, declines even at our lowland site would be nearly impossible to detect. If Bd is causing La Selva's declines, and Bd is widespread in lowland tropical forests – as data seem to suggest – then gradual population declines such as those at La Selva may be widespread as well.

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LITERATURE CITED

- Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences* 95, 9031-9036.
- Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonald, K.R., Skerratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G., Mahony, M., Sheppard, N., Williams, C., Tyler, M.J., 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal* 82, 434-439.
- Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T., Hyatt, A.D., 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60, 141-148.
- Brem, F.M.R., Lips, K.R., 2008. *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic. *Diseases of Aquatic Organisms* 81, 189-202.
- Butterfield, R.P., 1994. The regional context: land colonization and conservation in Sarapiquí, In *La Selva: ecology and natural history of a neotropical rain forest*. eds L.A. McDade, K.S. Bawa, H.A. Hespenheide, G.S. Hartshorn. University of Chicago Press, Chicago.
- Clark, D.A., Piper, S.C., Keeling, C.D., Clark, D.B., 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984 - 2000. *Proceedings of the National Academy of Sciences* 100, 5282-5857.
- Clark, D.B., 1990. La Selva Biological Station: a blueprint for stimulating tropical research, In *Four Neotropical Forests*. ed. A.H. Gentry, pp. 9-27. Yale University Press, New Haven.
- Colwell, R.K., Brehm, G., Cardelus, C.L., Gilman, A.C., Longino, J.T., 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* 322, 258-261.
- Daly, G.L., Lei, Y.D., Teixeira, C., Muir, D.C.G., Castillo, L.E., Jantunen, L.M.M., Wania, F., 2007a. Organochlorine pesticides in the soils and atmosphere of Costa Rica. *Environmental Science & Technology* 41, 1124-1130.

- Daly, G.L., Lei, Y.D., Teixeira, C., Muir, D.C.G., Castillo, L.E., Wania, F., 2007b. Accumulation of current-use pesticides in neotropical montane forests. *Environmental Science & Technology* 41, 1118-1123.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* 105, 6668-6672.
- Donnelly, M.A., 1989. Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. *Oecologia* 81, 212-218.
- Donnelly, M.A., 1994. Amphibian Diversity and Natural History, In *La Selva: Ecology and Natural History of a Neotropical Rainforest*. eds L. McDade, K. Bawa, H. Hespenheide, G. Hartshorn, pp. 199-209. University of Chicago Press, Chicago.
- Frias-Alvarez, P., Vredenburg, V.T., Familiar-Lopez, M., Longcore, J.E., Gonzalez-Bernal, E., Santos-Barrera, G., Zambrano, L., Parra-Olea, G., 2008. Chytridiomycosis survey in wild and captive Mexican amphibians. *Ecohealth* 5, 18-26.
- Guyer, C., 1990. The herpetofauna of La Selva, Costa Rica, In *Four Neotropical Forests*. ed. A.H. Gentry, pp. 371-385. Yale University Press, New Haven.
- Guyer, C., Donnelly, M.A., 2005. *Amphibians and Reptiles of La Selva, Costa Rica, and the Caribbean Slope*. University of California Press, Berkeley.
- Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Perez, H.J.A., Garland, T., 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B-Biological Sciences* 276, 1939-1948.
- Hyatt, A.D., Boyle, D.B., Olsen, V., Berger, L., Obendorf, D., Dalton, A., Kriger, K.M., Hero, J.M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Fleason, F., Colling, A., 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73, 175-192.
- Johnson, M.L., Berger, L., Philips, L., Speare, R., 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57, 255-260.
- Kilpatrick, A.M., Briggs, C.J., Daszak, P., 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. *Trends in Ecology & Evolution* 25, 109-118.

- Kruger, K.M., Hero, J.-M., 2007a. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology* 271, 352-359.
- Kruger, K.M., Hero, J.M., 2006. Survivorship in wild frogs infected with chytridiomycosis. *Ecohealth* 3, 171-177.
- Kruger, K.M., Hero, J.M., 2007b. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions* 13, 781-788.
- Kruger, K.M., Hero, J.M., 2007c. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology* 271, 352-359.
- Kruger, K.M., Hero, J.M., 2008. Altitudinal distribution of chytrid (*Batrachochytrium dendrobatidis*) infection in subtropical Australian frogs. *Austral Ecology* 33, 1022-1032.
- Lips, K.R., 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* 12, 106-107.
- Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* 103, 3165-3170.
- Lips, K.R., Burrowes, P.A., Mendelson, J.R., Parra-Olea, G., 2005. Amphibian declines in Latin America: Widespread population declines, extinctions, and impacts. *Biotropica* 37, 163-165.
- Lips, K.R., Diffendorfer, J., Mendelson, J.R., Sears, M., 2008. Riding the wave: Climate change, emerging infectious disease and amphibian declines. *Plos Biology* in press.
- Lips, K.R., Reeve, J.D., Witters, L.R., 2003. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17, 1078-1088.
- Longcore, J.E., Pessier, A.P., Nichols, D.K., 1999. *Batrachochytrium dendrobatidis* gen. et. sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91, 219-227.
- McDade, L.A., Hartshorn, G.S., 1994. La Selva Biological Station, In *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. eds L.A. McDade, K.S. Bawa, H.A. Hespenheide, G.S. Hartshorn, pp. 6-14. University of Chicago Press, Chicago.

- Murray, K.A., Skerratt, L.F., Speare, R., McCallum, H., 2009. Impact and Dynamics of Disease in Species Threatened by the Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*. *Conservation Biology* 23, 1242-1252.
- Piotrowski, J.S., Annis, S.L., Longcore, J.E., 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96, 9-15.
- Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sanchez-Azofeifa, G.A., Still, C.J., Young, B.E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439, 161-167.
- Puschendorf, R., Bolanos, F., 2006. Detection of *Batrachochytrium dendrobatidis* in *Eleutherodactylus fitzingeri*: Effects of skin sample location and histologic stain. *Journal of Wildlife Diseases* 42, 301-306.
- Puschendorf, R., Bolanos, F., Chaves, G., 2006. The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biological Conservation* 132, 136-142.
- Puschendorf, R., Carnaval, A.C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolanos, F., Alford, R.A., 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions* 15, 401-408.
- Richards-Zawacki, C.L., 2010. Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proceedings of the Royal Society B-Biological Sciences* 277, 519-528.
- Rohr, J.R., Raffel, T.R., Romansic, J.M., McCallum, H., Hudson, P.J., 2008. Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences of the United States of America* 105, 17436-17441.
- Ron, S.R., 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. *Biotropica* 37, 209-221.
- Rosenblum, E.B., Voyles, J., Poorten, T.J., Stajich, J.E., 2010. The Deadly Chytrid Fungus: A Story of an Emerging Pathogen. *Plos Pathogens* 6, 3.
- Sanchez, D., Chacon-Ortiz, A., Leon, F., Han, B.A., Lampo, M., 2008. Widespread occurrence of an emerging pathogen in amphibian communities of the Venezuelan Andes. *Biological Conservation* 141, 2898-2905.

- Sanford, R.L., Jr., Paaby, P., Luvall, J.C., Phillips, E., 1994. Climate, geomorphology, and aquatic systems, In *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. eds L.A. McDade, K.S. Bawa, H.A. Hespenheide, G.S. Hartshorn, pp. 19-33. University of Chicago Press, Chicago.
- Sigel, B.J., Sherry, T.W., Young, B.E., 2006. Avian community response to lowland tropical rainforest isolation: 40 years of change at La Selva Biological Station, Costa Rica. *Conservation Biology* 20, 111-121.
- Smith, K.G., Lips, K.R., Chase, J.M., 2009. Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. *Ecology Letters* 12, 1069-1078.
- Soto-Azat, C., Clarke, B.T., Poynton, J.C., Cunningham, A.A., 2010. Widespread historical presence of *Batrachochytrium dendrobatidis* in African pipid frogs. *Diversity and Distributions* 16, 126-131.
- Voyles, J., Berger, L., Young, S., Speare, R., Webb, R., Warner, J., Rudd, D., Campbell, R., Skerratt, L.F., 2007. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. *Diseases of Aquatic Organisms* 77, 113-118.
- Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W.F., Dinudom, A., Cook, D., Webb, R., Alford, R.A., Skerratt, L.F., Speare, R., 2009. Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines. *Science* 326, 582-585.
- Weldon, C., du Preez, L.H., Hyatt, A.D., Muller, R., Speare, R., 2004. Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10, 2100-2105.
- Whitfield, S.M., Bell, K.E., Philippi, T., Sasa, M., Bolanos, F., Chaves, G., Savage, J.M., Donnelly, M.A., 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences of the United States of America* 104, 8352-8356.
- Whitfield, S.M., Lips, K.R., Donnelly, M.A., in press. Decline and conservation of amphibians in Central America, In *Status of Conservation and Declines of Amphibians: Western Hemisphere*. eds H.H. Heatwole, C. Barrio-Amoros, J.W. Wilkenon. Surrey Beatty and Sonos, Pty. Ltd., Sydney.
- Wood, S.N., 2006. Generalized additive models: An introduction with R. R. Chapman and Hall/CRC.

- Woodhams, D.C., Kilburn, V.L., Reinert, L.K., Voyles, J., Medina, D., Ibanez, R., Hyatt, A.D., Boyle, D.G., Pask, J.D., Green, D.M., Rollins-Smith, L.A., 2008. Chytridiomycosis and Amphibian Population Declines Continue to Spread Eastward in Panama. *Ecohealth* 5, 268-274.
- Young, B.E., Lips, K.R., Reaser, J.K., Ibanez, R.D., Salas, A.W., Cedeno, J.R., Coloma, L.A., Ron, S., La Marca, E., Meyer, J.R., Munoz, A., Bolanos, F., Chaves, G., Romo, D., 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15, 1213-1223.
- Young, B.E., Sherry, T.W., Sigel, B.J., Woltmann, S., 2008. Nesting success of Costa Rican lowland rain forest birds in response to edge and isolation effects. *Biotropica* 40, 615-622.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.Z., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

Tables

Table 3.1. Prevalence of infection for three species of common frogs.

Sampling Dates	<i>Rhaebo haematiticus</i>		<i>Craugastor bransfordii</i>		<i>Oophaga pumilio</i>		Total	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
8-11 May 2007	51 (100%)	0 (0%)	11 (100%)	0 (0%)	54 (100%)	0 (0%)	116 (100%)	0 (0%)
30 May - 8 Jun 2007	6 (100%)	0 (0%)	36 (97.3%)	1 (2.7%)	53 (100%)	0 (0%)	95 (99.0%)	1 (1.0%)
30 Jul – 2 Aug 2007	2 (100%)	0 (0%)	4 (80%)	1 (20%)	30 (100%)	0 (0%)	36 (96.4%)	1 (3.6%)
27 Aug - 6 Sep 2007	15 (100%)	0 (0%)	1 (100%)	0 (0%)	13 (100%)	0 (0%)	29 (100%)	0 (0%)
24-27 Sep 2007	29 (100%)	0 (0%)	11 (84.6%)	2 (15.4%)	14 (100%)	0 (0%)	54 (96.4%)	2 (3.6%)
22-25 Oct 2007	31 (100%)	0 (0%)	7 (100%)	0 (0%)	24 (96.0%)	1 (4.0%)	62 (98.4%)	1 (1.6%)
21-24 Nov 2007	17 (85.0%)	3 (15.0%)	11 (100%)	0 (0%)	27 (100%)	0 (0%)	55 (94.8%)	3 (5.2%)
24 Dec 2007	-	-	2 (100%)	0 (0%)	5 (100%)	0 (0%)	7 (100%)	0 (0%)
3-7 Jan 2008	13 (65.0%)	7 (35.0%)	6 (100%)	0 (0%)	13 (56.5%)	10 (43.5%)	32 (65.3%)	17 (34.7%)
14-17 Jan 2008	14 (77.8%)	4 (22.2%)	24 (92.3%)	2 (7.7%)	28 (90.3%)	3 (9.7%)	66 (88.0%)	9 (12.0%)
10-13 Feb 2008	30 (96.8%)	1 (3.2%)	24 (82.8%)	5 (17.2%)	27 (90.0%)	3 (10.0%)	81 (90.0%)	9 (10.0%)
10-13 Mar 2008	11 (84.6%)	2 (15.4%)	28 (93.3%)	2 (6.7%)	31 (100%)	0 (0%)	70 (94.6%)	4 (5.4%)
8-11 Apr 2008	25 (100%)	0 (0%)	25 (92.6%)	2 (7.4%)	30 (93.8%)	2 (6.3%)	80 (95.2%)	4 (4.8%)
TOTAL	246 (93.5%)	17 (6.5%)	190 (92.7%)	15 (7.3%)	349 (94.8%)	19 (5.2%)	785 (93.9%)	51 (6.1%)

Table 3.2. Summary statistics for linear models for prevalence and infection intensity.**Prevalence**

Prevalence x climate

Generalized linear model	Df	Deviance	Resid. Df	Resid. Dev.	P(> Chi)
Null			831	383.59	
Species	2	1.16	829	382.43	0.561
Precipitation	1	26.58	828	355.85	<0.0001
Temperature	1	26.24	827	329.61	<0.0001
Species x precipitation	2	12.94	825	316.67	0.002

Prevalence x individual covariates

Generalized linear model	Df	Deviance	Resid. Df	Resid. Dev.	P(> Chi)
NULL			746	340.08	
Species	2	1.501	744	338.58	0.4721
SVL	1	0.431	743	338.15	0.5115
IBC	1	0.002	742	338.15	0.9683
Age-sex class	3	5.785	739	332.36	0.1225
Species x SVL	2	29.644	737	302.72	<0.0001

Bd load (positives only)

Bd load x climate

Analysis of covariance	Df	Sum Sq	Mean Sq	F	Pr(>F)
Species	2	57.487	28.7437	4.6946	0.0140
30-Day Precipitation	1	3.400	3.3998	0.5553	0.4600
30-Day Temperature	1	5.736	5.736	0.9368	0.3382
Residuals	46	281.648	6.1228		

Bd load x individual covariates

Analysis of covariance	Df	Sum Sq	Mean Sq.	F	Pr(>F)
Species	2	53.990	26.995	4.200	0.0227
SVL	1	10.772	10.772	1.676	0.2035
IBC	1	17.369	17.369	2.702	0.1087
Age-sex class	3	2.059	0.686	0.107	0.9556
Residuals	37	237.833	6.428

Figure 3.1. Annual variability in air temperature at La Selva Biological Station and temperature data for the study period. The solid black line indicates the weekly average of mean daily temperature (average of "long-term" data: Apr 1982-Dec 2008); the shaded area represents the range between weekly averaged daily maximum temperature and weekly averaged daily minimum temperature (both based on long-term data). The broken black line indicates the weekly-averaged mean daily temperature for the period of this study (May 2007 - Apr 2008); the broken red line and broken blue lines indicate weekly averaged daily maximum temperature and weekly averaged daily minimum temperature, respectively, over the period of this study.

Figure 3.2. Estimated infection prevalence among three species of common frogs between May 2007 and April 2008. Shaded areas indicate 95% confidence intervals for infection prevalence by Bd. The broken line indicates weekly average of daily average air temperature during the study period.

Figure 3.3. Estimated relationship between 30-day air temperature and prevalence of infection by Bd. Prevalence increases with cooler temperatures, and most variability in prevalence is driven by the lowest temperatures experienced at the site.

Figure 3.4. Associations between body length (SVL) and infection by Bd for three species of frogs.

Figure 3.5. Bd load given by species. Individual points represent outliers beyond the 90% percentile. *Oophaga pumilio* showed higher Bd loads than either *R. haematiticus* or *C. bransfordii*.

CHAPTER 4: LITTER DYNAMICS REGULATE POPULATION DENSITIES OF
AMPHIBIANS AND REPTILES IN A DECLINING TROPICAL HERPETOFAUNA
ABSTRACT

Populations of terrestrial amphibians and reptiles have declined dramatically over the past four decades at La Selva Biological Station, a protected rainforest reserve in lowland Costa Rica. Because the quantity of standing leaf litter on the forest floor is the predominant correlate of abundance for the litter herpetofauna, it was suggested that changes in litter dynamics may be related to faunal declines for both amphibians and reptiles. I conducted a 16-month experimental investigation of the relationship between litter depth and herpetofaunal density. I established nine 15x15m capture-recapture plots in fall 2006, and sampled frogs and lizards on each plot on 99 separate occasions during which all encounters of frogs and lizards were recorded, and captured animals were assigned unique marks. After a 6-mo pre-treatment period, plots were assigned to three treatments: litter addition (L_+), litter removal (L_-), or sham treatment controls (C). Total encounters of pooled amphibians and reptiles were higher in the L_+ treatment than in controls in the post-treatment period, while total encounters were fewer in L_- treatments, consistent with *a priori* expectations. Species-level response to treatments were individualistic; some common species increased in density in L_+ treatments and reduced density in L_- treatments while other species showed no effects of litter manipulation. The greatest response to manipulations was by the fastest-declining species encountered at our study site. Further, synthesis of datasets on litter depth from separate studies over four decades indicates interannual variability in standing litter quantity, and suggests possible long-term reductions in standing litter depth.

INTRODUCTION

In the past three decades, amphibian assemblages across the world have suffered rapid, unexpected population declines and widespread extinctions (Alford and Richards 1999, Young et al. 2001, Stuart et al. 2004). Approximately 33% of amphibian species are currently threatened with extinction (Stuart et al. 2004), and while conventional threats such as habitat loss and modification are directly responsible for many of these declines, much research attention has been focused on so-called “enigmatic declines” (Lips et al. 2006, Pounds et al. 2006, Lips et al. 2008). Enigmatic declines are particularly concerning for conservation biology because these declines may occur in protected areas – refuges for biodiversity from direct anthropogenic threats, and habitats generally thought to be relatively pristine (Lips et al. 2006, Whitfield et al. 2007, Smith et al. 2009). In the highly diverse neotropical realm, enigmatic declines often manifest as sudden population reductions and extirpations on a local scale; yet on a regional scale these decline events are producing severe contractions in species’ ranges and widespread extinctions (Young et al. 2001, Lips et al. 2005a, Lips et al. 2005b, Smith et al. 2009, Whitfield et al. in press).

Most reported enigmatic decline events appear similar in a number of underlying characteristics: amphibians decline while other components of biodiversity appear unaffected; declines generally occur in cool climates (generally above 400m asl in the Neotropics); and declines occur suddenly – over a period of weeks to months (Lips et al. 2005a, Lips et al. 2006, Whitfield et al. in press). Populations of amphibians have also declined at La Selva Biological Station – a protected lowland (<150m asl) biological reserve in northeastern Costa Rica (Whitfield et al. 2007). While the declines at La Selva

may be considered enigmatic because they are not caused by obvious direct anthropogenic effects (habitat loss, overexploitation), in a number of ways the declines at this site are inconsistent with other reported decline events in Central America. First, declines of forest-inhabiting amphibians at La Selva occurred gradually – over a period of four decades rather than within a single year. Second, populations of terrestrial lizards accompanied declines in amphibians. Additionally, enigmatic declines in the neotropics are believed to differentially affect stream-inhabiting amphibian species; in contrast, amphibians at La Selva known to have declined are predominantly terrestrial species which have little or no contact with streams. Finally, virtually all other sites in the New World tropics where widespread amphibian declines have been reported have occurred in cooler climates (generally >400m asl). The mechanistic factors responsible for faunal declines at La Selva have not been established.

A number of novel stressors may be implicated in faunal declines at La Selva, including habitat fragmentation (Bell and Donnelly 2006, Sigel et al. 2006), pesticide contamination (Daly et al. 2007a, Daly et al. 2007b), emerging infectious diseases (Puschendorf et al. 2009), and climate change (Clark et al. 2003, Aguilar et al. 2005, Whitfield et al. 2007). However, most processes generally emphasized in enigmatic decline research are not parsimonious explanations for faunal declines at La Selva. Enigmatic declines caused by chytridiomycosis – the emerging infectious disease caused by chytridiomycete fungus *Batrachochytrium dendrobatidis* (Bd) – are predominant in the literature, yet Bd has not been reported to cause widespread declines in lowland amphibian faunas, Bd-driven declines are sudden rather than gradual, and Bd does not affect lizards.

Whitfield et al. (2007) proposed that declines at La Selva may be at least partially attributable to reductions in standing quantity of leaf-leaf litter on the forest floor. Both the terrestrial amphibians and terrestrial reptiles which have suffered declines at La Selva are highly dependent on leaf litter as a microhabitat (Scott 1976, Lieberman 1986). Leaf litter also serves as the trophic base of litter arthropods in brown food webs (Kaspari et al. 2008, Kaspari and Yanoviak 2008, 2009), upon which terrestrial amphibians and reptiles prey (Lieberman 1986, Whitfield and Donnelly 2006). Leaf litter provides shelter and refuge from predation (Cooper et al. 2008a, b, 2009). Finally, leaf litter creates a moist microhabitat on the forest floor, upon which amphibians depend to prevent desiccation, and upon which both lizards and terrestrially-breeding amphibians are critically dependent to prevent desiccation of vulnerable egg stages (Schlaepfer 2003, Succi et al. 2005).

Every major study that has examined the leaf litter herpetofauna at La Selva has demonstrated positive correlations between density of amphibians and reptiles and standing quantity of leaf litter. Scott (1976) was among the first to suggest that litter quantity relates to abundance of terrestrial amphibians and reptiles – specifically that upland sites in Costa Rica have both much greater litter quantity and much greater amphibian and reptile densities in this litter. Lieberman (1986) reported data from an extensive sampling of litter amphibian and reptile communities from La Selva, and identified litter volume as a primary correlate of amphibian density. Specifically, Lieberman (1986) demonstrated that both litter volume and amphibian and reptile densities at La Selva are higher during the dry season than during the wet season, and that both litter volume and herpetofaunal densities are greater in plantations of cacao

(*Theobroma cacao*) than in primary forest. Fauth (1989) surveyed ten Costa Rican sites across a broad elevational gradient and found that density was positively – though not significantly – correlated with litter quantity. Heinen (1992) sampled amphibians and reptiles at La Selva in primary forest and in abandoned cacao plantations and found positive correlations between litter quantity and herpetofaunal density across and within forest types. Whitfield and Pierce (2005) compared density and litter quantity between microhabitats, and found generally higher densities of amphibians and reptiles in microhabitats with greater litter volume.

However, Whitfield and Pierce (2005) argued for caution in interpretation of the relationship between litter depth and amphibian or reptile densities because all links between the two have been correlative. Litter quantity and herpetofaunal densities may covary because both are related to productivity, soil type, edaphic variability, tree species composition, or moisture (Meentemeyer 1978, Lieberman 1986, Couteaux et al. 1995, Wieder and Wright 1995, Aerts 1997, Heneghan et al. 1998, Vonesh 2001, Watling 2005, Kaspari et al. 2008, M. Kaspari 2008). Because quantity of standing leaf litter has been suggested repeatedly to be the primary factor determining population densities of terrestrial amphibians and reptiles at this site, an empirical demonstration that litter depth regulates population densities of amphibians and reptiles is could greatly improve our understanding of the ecology of the leaf-litter herpetofauna, with strong implications for our understanding of declines in this assemblage.

Herein, I report the results of two tests to evaluate the role of leaf-litter in reductions in population density of amphibians and reptiles. First, I conducted a 16-month experimental manipulation of the quantity of standing litter on the forest floor, and

measured the response of amphibians and reptiles to this manipulation. Second, I compile data from multiple studies, spanning 1973-2008, to evaluate evidence for changes in litter dynamics associated with the litter depletion hypothesis for declines at this site.

METHODS

Study Site

La Selva Biological Station is a 16 km² private biological reserve in the northeastern lowlands of Sarapiquí, Heredia Province, Costa Rica (10° 26' N, 83° 59' W). The site has been protected and managed by the Organization for Tropical Studies since 1968 (Clark 1990), and has been described as lowland wet forest, with elevation ranging from 35m to 137m asl. The site receives on average 4000mm annual precipitation, with no month on average receiving <100mm rainfall. The majority of the site is primary forest, apparently with only small-scale direct human impacts since the European invasion of the Americas; other parts of the reserve include forests regenerating from pasture and other agricultural uses (McDade and Hartshorn 1994). The reserve rests at the foot of 460km² Parque Nacional Braulio Carrillo, a national protected area which extends to ~2900m in elevation. Since the 1950s, the lowland regions surrounding La Selva have largely been converted from a continuous expanse of rainforest into heterogeneous patches of pasture, agricultural plantations (typically cacao, palm heart, bananas, and pineapple), and residual forest fragments (Butterfield 1994). The amphibian fauna of La Selva is very well-known, with 52 amphibian species known to occur at the site (Guyer 1990, Donnelly 1994, Guyer and Donnelly 2005), Whitfield pers.

obs.). The site has experienced a prolonged, gradual decline in total density of terrestrial amphibians (Whitfield et al. 2007), but with little or no evident species loss.

Study plots

Within old-growth forest in the La Selva reserve, I established nine 15 x 15m permanent study plots as the experimental units in this study. I judged these plots to be of sufficient size for this study because of effective use of plots of this size for similar manipulations at this site (Donnelly 1987, Guyer 1988b, a, Donnelly 1989c, a, Donnelly 1989b). Plots were arranged in three spatial blocks to maximize spatial extent of the study and to encompass variation in habitat characteristics, but to minimize spatial heterogeneity within replicates. Each spatial block contained three plots separated by between 34 and 103 m (mean = 59.8m). I placed each spatial block on a different soil type, but all plots were established in mature forests on low-grade terrain. I marked each plot with an array of 36 grid tubes spaced at 3m intervals.

Current litter dynamics

At the onset of the study, I erected a single 50x50cm litter trap at each plot. Litter traps were located 1m from the ground, and consisted of a suspended mesh basket into which litter fell; the trap design prevented leaf loss. I placed litter traps haphazardly at one point along the edge of each plot, and moved the traps at irregular intervals throughout the duration of the study. I collected all litter from each trap biweekly, then dried the litter at 60°C for at least 72 hrs until thoroughly dry and weighed the total dry litter mass.

At irregular intervals throughout the study period, I also measured the quantity of standing leaf litter on the study plots. I used a geographic information system (ESRI ArcGIS) to generate random points from each study plot with reference to the existing grid posts. At each standing leaf-litter measurement period, I calculated the amount of standing leaf litter at twenty random points in each plot, for a total of 2965 points where I conducted measurements of standing leaf litter. At each point, I measured leaf litter using three metrics: first, I measured the distance between the top of the soil and the top of the leaf litter to the nearest 0.1mm with a dial calipers (“measured depth”). Second, at the same point where the depth was taken in mm, I counted the number of leaves pierced by the probe on the dial calipers (“count depth”). Finally, I use presence or absence of leaf litter at these random points as a third metric of litter quantity (“litter cover”). While these three metrics are correlated, they provide different information about the quantity of standing leaf litter. In the post-treatment period, I measured standing litter quantity immediately before litter manipulation; thus, our measurements of litter quantity provide a conservative estimate of litter quantity because they represent two weeks of relaxation following manipulation.

Amphibian and reptile sampling

I sampled amphibians and reptiles on each plot every other week from 01 September 2006 to 14 December 2007, for a total of 33 sampling “sessions” per plot. Each sampling session consisted of three samples of each plot, generally on three subsequent days, but in a few instances (35 of 890 total sampling sessions) sampling occurred over three days within a four-day period. The three plots within each sampling

block were always sampled subsequently to control for variation in weather or amphibian and reptile activity, and the team of observers was almost always fixed among plots within a block. I randomized the sampling sequence of spatial blocks, and the sampling sequence of treatments within each block in advance of that sampling session. With a single exception, I always sampled the three treatments within each sampling block within the same day.

During each sampling event, between one and three observers (generally one or two observers) walked slowly along grid posts, at a rate of approximately 3m min^{-1} , for a total search time for each plot of approximately 30min. During searches, observers carefully scanned the ground and all vegetation to a height of $\sim 2\text{m}$ for any amphibians and reptiles, and leaf litter was gently agitated with a probe to elicit movement by hidden or cryptic species. For any amphibians or reptiles detected during sampling events, I recorded species and location with reference to the nearest grid post. I measured all frogs and lizards captured to the nearest 0.1mm, measured animals with a spring scale to the nearest 0.05g, and marked all animals upon first capture using a unique combination of toe-clips (Donnelly et al. 1994).

Microclimate

To determine whether standing leaf-litter affects microhabitat characteristics that may regulate population densities of litter amphibians and reptiles, I deployed a network of small temperature and humidity dataloggers (Embedded Data Systems Hygrochron iButtons). These data loggers were deployed between 25 and 29 January 2007, during the pre-treatment phase, and collected data nearly continuously until the termination of

the experiment. I programmed dataloggers to record temperature and humidity data every 30 minutes, and one data logger was placed under the leaf litter at the base of a randomly selected grid post; a paired datalogger was placed approximately 30cm above the soil on the same grid post. Two pairs of dataloggers were placed in each plot.

Litter Manipulation

Between 12 March 2007 and 31 March 2007 (after 14 pre-treatment sampling periods on each plot), I randomly assigned plots to one of three treatments: litter addition (L_+), litter removal (L_-), and sham-treatment control (L_0). At the initial manipulation of litter quantity, I removed virtually all standing leaf litter from the L_- plots, inspected leaf litter by hand to remove any amphibians or reptiles, and applied the entire quantity of leaf litter to the adjacent L_+ plot in the same spatial block. For our sham treatment, I removed all leaf litter from the L_0 plot and immediately replaced the leaf litter on the same plot. To maintain these treatments, I continued to remove recently senesced leaf litter every two weeks from all L_- plots, and applied this leaf litter to adjacent L_+ plots, but did not remove and replace litter from L_0 treatments. Litter removal and addition was always conducted immediately after sampling from that two-week period. Treatments were stratified by spatial blocks so that each block received one treatment plot of each type.

Long-term Litter Dynamics

To determine whether substantial changes have occurred in quantity of leaf litter on the forest floor, I compiled two historic datasets that had measured standing litter depth from previous decades (Lieberman 1986, Whitfield et al. 2007), Bolaños et al. unpublished),

and replicated these methods over a nearly two-year period (May 2006 – March 2008).

Both historic studies and our replicated methodology used the three metrics of litter depth described previously (measured depth, count depth, and litter cover); measurements of litter depth were taken from the corners of litter quadrats which were primarily sampled for amphibians and reptiles.

Analysis

Current litter dynamics

I analyzed quantity of standing litter on plots with generalized linear mixed effects models using the lmer procedure in the lme4 package of R, specifying treatment and pre-treatment/post-treatment as fixed effects and block, plot, and sample as random effects. I specified Gaussian errors for depth in mm, Poisson errors for count depth, and binomial errors for litter cover. I compared rates of litterfall between treatments and pre/post treatment periods and correlations between precipitation and litterfall with a generalized linear mixed model. I specified Gaussian distributions on log(litterfall mass) and log(precipitation) and used treatment and pre/post treatment period as fixed effects, and block, plot, and location of litter trap within plots as random effects.

Long-term litter dynamics

To analyze change in measured depth I used a linear mixed effects model with decade (1970s, 1980s, 2000s) as factors and month and quadrat identity as random factors. To analyze change in count depth and litter cover, I used generalized linear models using

Poisson and binomial error distributions and the random factors specified as for measured depth.

Encounters

I evaluated changes in number of animals encountered using a generalized linear mixed effects model with Poisson errors in lmer. I used number of encounters per sample session as a response variable, specified treatment and treatment period as fixed factors and included block and plot as random factors. I conducted this analysis for all amphibians and reptiles, for pooled frogs, pooled lizards, and for each of the six most commonly encountered species in this study (the frogs *Oophaga pumilio*, *Craugastor bransfordii*, *Craugastor mimus*; and the lizards *Norops humilis*, *Norops limifrons*, and *Sphenomorphus cherriei*).

Microclimate

I analyzed microclimate data (temperature and relative humidity) with linear mixed models with treatment (L_0 , L_- , L_+), period (pre-treatment, post treatment), and datalogger location (under leaf litter or in air) as fixed factors and with datalogger location as a random factor.

RESULTS

Current litter dynamics

In the pre-treatment period, count depth and litter cover did not differ among experimental treatments (all $P > 0.1$), but measured depth was greater in both L_+ plots

($z=2.14$, $P=0.03237$) and in L_- ($z=2.19$, $P=0.02856$) than in L_0 plots (Fig. 1).

Experimental manipulations of litter depth caused a dramatic reduction in quantity of standing litter on L_- plots (measured depth: $z=-7.83$, $P<0.0001$; count depth: $z=-10.506$, $P<0.0001$; litter cover: $z=-6.763$, $P<0.0001$). Litter addition produced a dramatic increase in depth of standing leaf litter (measured depth: $z=2.75$, $P=0.00593$; count depth: $z=6.911$, $P<0.0001$; litter cover: $z=4.516$, $P<0.0001$). There was a much less severe increase in quantity of standing litter on L_0 plots during the post-treatment period according to leaf counts and measured litter depth, but not litter cover (measured depth: $z=2.88$, $P=0.00402$; count depth: $z=2.564$, $P=0.0103$; litter cover: $z=1.449$, $P=0.147330$; Fig. 1A-C).

There were no effects of treatment ($F_{2,4}=1.4489$, $P=0.3363$), treatment period ($F_{1,250}=1.6996$, $P=0.1935$), or precipitation ($F_{1,234}=0.0903$, $P=0.7641$) on rates of litterfall. However, there was a significant interaction between treatment and treatment period on rates of litterfall: while there were no differences in litter fall among treatments in the pre-treatment period, both L_+ and L_- plots received more litterfall in the post-treatment period than L_0 plots ($F_{2,250}=3.8314$, $P=0.0230$).

Encounters

I encountered a total of 4192 amphibians and reptiles, including 3020 frogs representing 20 species, 1152 lizards representing 13 species, and 18 snakes among 7 species (Table 1). For all amphibians and reptiles pooled, there were increases in total encounters in both L_0 ($z=6.694$, $P<0.0001$) and L_+ plots in the post-treatment period (although no difference in size of increase between L_0 and L_+ ($z=0.265$, $P<0.791$), and a

strong decrease in total encounters in the L. plots ($z = -3.758$, $P < 0.002$, Figure 2A). For all frogs, there was a similar pattern, with increases in number of encounters in L₀ ($z = 6.700$, $P < 0.0001$) and L₊ plots, and a strong decrease in number of encounters for L. plots ($z = -3.522$, $P = 0.0004$), but no difference in size of increase between L₀ and L₊ plots ($z = 1.878$, $P = 0.0604$; Table 2B). For all lizards, while there was an increase in number of encounters for L₀ plots ($z = 2.680$, $P = 0.0073$), there was a decrease in total number of encounters for both L. ($z = -2.387$, $P = 0.0170$) and L₊ ($z = -2.578$, $P = 0.0099$; Figure 2C) plots relative to controls.

Responses to manipulation by the most common species were individualistic. Encounters of *Oophaga pumilio* did not change on L₀ plots ($z = 1.326$, $P = 0.1850$) or L. plots ($z = -1.620$, $P = 0.1052$), but increased on L₊ plots ($z = 2.147$, $P < 0.0318$, Figure 3A). Encounters of *Craugastor bransfordii* increased on control plots ($z = 6.289$, $P < 0.0001$), but decreased on L. plots relative to controls ($z = -1.912$, $P = 0.0558$) and the increase on L₊ plots was comparable to increases for controls ($z = 0.648$, $P = 0.5169$; Figure 3B). Encounters of *Craugastor mimus* increased on L₀ ($z = 3.147$, $P = 0.0017$), and relative to controls, decreased on L. plots ($z = -2.242$, $P = 0.02497$) but response on L₊ plots did not differ from controls and L₊ plots ($z = 0.543$, $P < 0.5862$, Figure 3C). Encounters of *Norops humilis* showed no change in L₀ ($z = 0.668$, $P = 0.5042$), and L₊ treatments did not differ from controls ($z = -1.780$, $P = 0.0750$), but there was a decrease on L. plots relative to controls ($z = -2.489$, $P = 0.0128$, Figure 4A). Encounters of *Norops limifrons* showed no change in the post-treatment period in controls ($z = 1.897$, $P = 0.5778$), but there were fewer encounters relative to controls on both L. plots ($z = -0.608$, $P = 0.5429$) and L₊ plots ($z = -3.606$, $P = 0.0003$, Figure 4B). Encounters of *Sphenomorphus cherriei*

increased on both L_0 plots ($z= 2.372$, $P=0.0178$), and while the increase on L_+ plots was no different than controls, ($z= 0.245$, $P=0.8062$) encounters decreased strongly on L_- plots relative to controls ($z= -3.592$, $P=0.0003$, Figure 4C). Effect sizes for pooled and individual taxa are given in Table 2.

Microclimate

Average daily air temperatures in the pre-treatment period (23.0°C) were slightly higher than average soil temperatures (22.8°C, $F_{1,7429}=19.34$, $P<0.0001$), maximum daily temperatures were higher in air (26.0°C) than in soil (25.0°C, $F_{1,7429}=1236.31$, $P<0.001$), and minimum daily temperatures were lower in air (20.9°C) than in soil (22.2°C, $F_{1,7434}=607.63$, $P<0.001$). In the pre-treatment period, there was no effect of treatment on average daily temperature ($F_{2,63}=0.12$, $P=0.8881$), maximum daily temperature ($F_{2,63}=0.30$, $P=0.7419$), or minimum daily temperature ($F_{2,63}=0.09$, $P=0.9183$). Both air and soil temperatures in the post-treatment period were warmer than in the pre-treatment period (average temperature: $F_{1,7249}=5.52$, $P<0.0001$; maximum temperature: $F_{1,7429}=170.94$, $P<0.0001$, minimum temperature: $F_{1,7429}=800.19$, $P<0.0001$). There was no interaction between treatment and treatment period for average daily temperature ($F_{2,7429}=1.32$, $P=0.2669$), but there were significant interactions between treatment and treatment period for maximum daily temperatures ($F_{2,7249}=10.97$, $P<0.0001$) and for minimum daily temperatures ($F_{2,7429}=7.76$, $P=0.0004$). These interactions between treatment and treatment period for air temperature are attributable to a smaller increase in average air temperatures in the L_- treatment compared to the L_0 treatment, higher increases in maximum air temperatures than in L_0 or L_+ treatments, a larger increase in

maximum air temperatures in the L₋ treatment than in L₀ or L₊ treatments, and a smaller increase in minimum air temperatures in the L₋ treatment than in L₀ or L₊ treatments (Figure 5). For soil temperatures, there was a higher increase in average soil temperatures in the post-treatment period in L₋ treatment than in L₀ or L₊ treatments and a higher increase in maximum daily soil temperatures in the L₋ treatment than in L₀ or L₊ treatments, but no difference in the rate of change for minimum temperatures (Figure 5).

Long-term litter dynamics

There was no change in measured litter depth between the three study periods (1973-1974, 1994-1995, 2006-2008; $F_{2,989}=0.40331$, $P=0.6682$). There was a difference in count depth between decades ($\chi^2_5=25.913$, $P<0.0001$) attributable to a sharp decrease in number of leaves between the 1995-1996 sampling period and the 2006-2008 sampling period, but no change between the 1973-1974 and 1995-1996 sampling periods (Figure 6). There was also a change in litter cover ($\chi^2_5=43.563$, $P<0.0001$) attributable to a sharp decrease between the 1995-1996 sampling period and the 2006-2008 sampling period, but no change between the 1973-1974 and 1995-1996 sampling periods (Figure 6).

DISCUSSION

This study demonstrates that approximately 50% reductions in standing litter mass have dramatic effects on number of encounters for both amphibians and reptiles, confirming correlations detected by others (Scott 1976, Lieberman 1986, Fauth et al. 1989, Heinen 1992). For all sampled species, effect sizes for litter removal treatments were negative, consistent with our expectations, although the size of the effect varied

widely among the six focal taxa I studied. In general, there were increases in number of encounters in the post-treatment period in both control and litter addition treatments. Further, increasing litter depth produced a range of responses for focal species, including either increases or decreases in number of encounters. Only one species, *Oophaga pumilio* significantly increased in litter addition treatments relative to controls, although non-significant effect sizes for common species were positive with the exception of two species of *Norops* (Table 2).

A number of important factors greatly complicate efforts such as this one to conduct large-scale manipulations in complex environments such as tropical forests (particularly microhabitat characteristics such as soil composition, canopy cover and sun specks, plant species composition, aspect, slope, etc.). Perhaps most importantly for microhabitat variation is that characteristics of leaf litter vary greatly across short distances – defined by the spatial distribution of individual trees or lianas. Guyer (1988a,b) proposed that populations of *Norops humilis* vary in relation to phenological patterns of leaf fall on the scale of individual trees. While some dominant canopy species have very small leaflets that provide virtually no refuge from predation or (e.g., *Pentaclethra macroloba*, the dominant tree species at La Selva), other species produce large, lignin-rich leaves which accumulate in thick mats and decompose slowly (i.e., the lianas *Doliocarpus multiflorus* and *Pinzonia coriacea* and tree species in the genera *Sloanea* and *Lecythis*). In contrast to the structural microhabitat provided by large leaves, litter is the predominant source of nutrients incorporated into tropical forest soils (Wood et al. 2005). While slow-decomposing leaves may provide more structural microhabitat,

rapidly-composing leaves may provide more immediate bursts of nutrients into brown food webs upon which litter amphibians and reptiles rely.

Unfortunately, issues of detection probability complicate our efforts to relate number of encounters to population density of amphibians and reptiles on our study plots. I expect that because litter is a common refuge from predators, increased litter depth reduces detection probability for most species of leaf-litter amphibians and reptiles. Consequently, I expect detection probability to be highest on litter removal treatments, intermediate on control treatments, and lowest on litter addition treatments. Such biases in detection probability are likely to considerably underestimate the effect sizes I report herein. While such biases could be accounted for using mark-recapture analyses, our recapture rates for most species in this study were too low to estimate robustly either detection probability, or ultimately population size, on individual plots.

The rates at which frog and lizard species have declined at La Selva vary considerably among species (Whitfield et al. 2007). If reductions in standing litter quantity are at least in part responsible for long-term declines in amphibian and reptile populations at La Selva, I may expect effect sizes for litter manipulation treatments to correlate with decline rate for sampled taxa. Unfortunately, the relatively small number of regularly-encountered species complicates efforts to directly correlate decline rate with effect size of experimental manipulations. However, the two species with the largest effect size in litter removal treatments were *Craugastor mimus* and *Sphenomorphus cherriei* – the frog and lizard species with the highest decline rate for those amphibians and reptiles sampled in the present study (Whitfield et al. 2007). Whitfield et al. (2007) indicated that only two La Selva taxa have declined at a higher rate than *C. mimus* and *S.*

cherriei: salamanders of the genus *Oedipina*, which were not encountered during the current study, and to our knowledge have only been observed on one occasion at La Selva since 2000; and the gecko *Lepidoblepharis xanthostigma*, which was formerly the second-most common lizard at La Selva (Scott 1976, Lieberman 1986), but which was only observed on one occasion during this study.

Wanger et al. (2009) manipulated leaf litter depth in a study exploring microhabitat determinants of amphibian and reptile diversity in cacao plantations in Sulawesi. They found no significant general increases in abundance of amphibians or reptiles in response to either litter addition or litter removal, and found that individual species' responses to manipulations were highly species specific. Yet they found that manipulation of quantity of leaf litter affected reptile species richness, but not amphibian species richness. However, direct comparisons between the Wanger et al. (2009) study and ours are complicated because of two primary factors: enormous differences between species characteristics of Neotropical versus southeast Asian herpetofaunas (Scott 1976); and because primary tropical forests encompass enormous spatial variation in structural habitat complexity (including complexity of leaf litter), much of which is lost in monodominant stands of cacao (which generally flush multiple times in a year and have year-round abundant leaf-litter (Lieberman 1986).

Wanger et al. (2009) also found that the species that responded most intensely to manipulations were those species that were tolerant to disturbance, a finding not entirely consistent with our own. *Sphenomorphus cherriei*, a rapidly-declining species which responded strongly to our manipulations, is rather tolerant to some types of disturbance such as cacao plantations (Heinen 1992), litter-rich secondary forests (Whitfield et al.

2007), and forest fragments (Bell and Donnelly 2006) does not appear resistant to warmer disturbed microhabitats such as very young secondary forests or cattle pastures (Whitfield, pers. obs). *Craugastor mimus*, another rapidly-declining species which responded strongly to the manipulations does not appear resistant to any type of forest disturbance (Bell and Donnelly 2006, Whitfield et al. 2007). Other species that are known to be tolerant to disturbance either responded to manipulations as expected (*O. pumilio*) or responded in unexpected ways (i.e., the lizards *N. humilis* and *N. limifrons*). In general, our findings on the relationship between response to manipulations and disturbance tolerance do not agree with those of Wanger et al. (2009) and could reflect the difference between intact forest ecosystems and the ecosystems that develop with plantations of a single dominant species.

Thick leaf litter appears to provide a thermal buffer of relative stability in comparison to either air above leaf litter, or to soil where leaf litter is very thin. Further, it appears that removal of leaf litter contributed to increased variability in air temperatures up to 20cm above the soil. The thermal buffer may be important for both amphibians and reptiles, as recent studies have indicated that ectotherms that are thermoconformers in lowland forests often experience ambient temperatures near or above their thermal critical maxima (Colwell et al. 2008, Huey et al. 2009). Perhaps even more important than stable temperatures is increased humidity found in deep leaf-litter. Amphibians are particularly sensitive to drought, and experimental studies have indicated that eggs of lizards of the genus *Norops* also experience high mortality associated with desiccation (Schlaepfer 2003, Socci et al. 2005). While I originally sought to measure humidity in

association with temperature, I experienced very high fail rate for humidity dataloggers within weeks of deployment.

Our compilation and replication of historic measurements of litter depth provides some evidence of long-term change in quantity of standing leaf litter. The measured depth of leaf litter did not change, but depth in number of leaves and litter cover were both significantly lower in 2006-2008 than in 1973-1974 and 1995-1996. The difference in response metrics is likely to the result of the high variability in measured depth on short timescales: dry litter may be rather voluminous, yet measured litter depth may be greatly reduced at the same point after compaction of litter by heavy rains. Therefore the measured depth at a single point may vary considerably over very short intervals, such as upon the onset of rain after only a few dry days. Because of this variation, I argue that depth in number of leaves represents a more stable metric for assessing litter quantity with considerably less variation attributable to weather or observer effects. Quantity of standing litter is notoriously difficult to accurately measure, and because the methodological techniques used by early studies are not consistent with preferred modern methodologies. However, effective replication of historic methodologies is the only way to produce directly comparable estimates of change in standing litter quantity, even if historic studies use methodology that is no longer favorable. While these datasets suggest a possibility of reduced standing litter over time, I would refrain from interpreting these data as strong evidence of a sustained directional trend until more complete historical datasets become available. However, I do believe that these data should encourage more attention to the possibility of long-term changes in litter

dynamics, and at least underscore substantial inter-annual variation in litter quantity on the forest floor.

Litter dynamics in tropical forests are extremely complex and there are few empirical studies of these dynamics (Meentemeyer 1978, Vitousek 1984, Parker 1994, Couteaux et al. 1995, Byard et al. 1996, Aerts 1997, Scott and Binkley 1997, Cuevas and Lugo 1998, Heneghan et al. 1998, Lawrence and Foster 2002, Vasconcelos and Laurance 2005, Kaspari 2008). Both litterfall and litter decomposition rates vary spatially and temporally, among plant species and forest types and ages. Further, periodic climatic events such as El Niño/La Niña events may have profound impacts on either litterfall or litter decomposition. Uninhibited by predation from mesopredators, rapid apparent population growth of collared peccaries at La Selva has been suggested to impact litter quantity through mechanical trampling and through foraging activities in leaf-litter. Further, non-native earthworms have become established at La Selva in recent decades, and are well-known to accelerate decomposition in other sites (Holdsworth et al. 2008, Belote and Jones 2009, Forster et al. 2009, Gomez-Brandon et al. 2010, Huang et al. 2010).

The long-term litter data indicate that there was no substantial difference in litter depth between 1973-1974 and 1994-1995, though there was considerably less litter from 2006-2008 than in past sampling. This timescale corresponds both to apparent increase in population size of collared peccaries, as well as increased frequency of ENSO events. However, Whitfield et al. (2007) report that declines in populations of amphibians and reptiles were well underway by the mid-1990s. While changes to standing litter quantity may be one factor contributing to declines in populations of amphibians and reptiles, it is

at best one of a suite of long-term changes at La Selva which may be affecting populations of frogs, lizards, and other vertebrates.

This study confirms widespread and long-standing correlational studies that have indicated that litter depth has profound effects on density of most common litter amphibians and reptiles at La Selva. In particular, I found evidence of particular dependence upon leaf litter among those species that had declined most rapidly as determined from long-term data. Further, while our evidence for long-term change in leaf-litter is limited because of the paucity of historical data on standing litter depth, I did find some evidence that litter depth may have decreased since the earliest samples in the 1970s. Together, these results indicate that leaf litter may be critical for regulating population densities of litter amphibians and reptiles, and may be part of a suite of factors contributing to long-term declines in amphibian and reptile populations at this site.

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LITERATURE CITED

- Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* **79**:439-449.
- Aguilar, E., T. C. Peterson, P. R. Obando, R. Frutos, J. A. Retana, M. Solera, J. Soley, I. G. Garcia, R. M. Araujo, A. R. Santos, V. E. Valle, M. Brunet, L. Aguilar, L. Alvarez, M. Bautista, C. Castanon, L. Herrera, E. Ruano, J. J. Sinay, E. Sanchez, G. I. H. Oviedo, F. Obed, J. E. Salgado, J. L. Vazquez, M. Baca, M. Gutierrez, C. Centella, J. Espinosa, D. Martinez, B. Olmedo, C. E. O. Espinoza, R. Nunez, M. Haylock, H. Benavides, and R. Mayorga. 2005. Changes in precipitation and temperature extremes in Central America and northern South America, 1961-2003. *Journal of Geophysical Research-Atmospheres* **110**.
- Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* **30**:133-160.
- Bell, K. E., and M. A. Donnelly. 2006. Influence of Forest Fragmentation on Community Structure of Frogs and Lizards in Northeastern Costa Rica. *Conservation Biology* **20**:1750-1760.
- Belote, R. T., and R. H. Jones. 2009. Tree leaf litter composition and nonnative earthworms influence plant invasion in experimental forest floor mesocosms. *Biological Invasions* **11**:1045-1052.
- Butterfield, R. P. 1994. The regional context: land colonization and conservation in Sarapiquí. *in* L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, editors. *La Selva: ecology and natural history of a neotropical rain forest*. University of Chicago Press, Chicago.
- Byard, R., K. C. Lewis, and F. Montagnini. 1996. Litter decomposition and mulch performance from mixed and monospecific plantations of native tree species in Costa Rica. *Agriculture, Ecosystems and Environment* **58**:145-155.
- Clark, D. A., S. C. Piper, C. D. Keeling, and D. B. Clark. 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984 - 2000. *Proceedings of the National Academy of Sciences* **100**:5282-5287.
- Clark, D. B. 1990. La Selva Biological Station: a blueprint for stimulating tropical research. Pages 9-27 *in* A. H. Gentry, editor. *Four Neotropical Forests*. Yale University Press, New Haven.

- Colwell, R. K., G. Brehm, C. L. Cardelus, A. C. Gilman, and J. T. Longino. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* **322**:258-261.
- Cooper, W. E., J. P. Caldwell, and L. J. Vitt. 2008a. Effective crypsis and its maintenance by immobility in Craugastor frogs. *Copeia*:527-532.
- Cooper, W. E., J. P. Caldwell, and L. J. Vitt. 2008b. Escape responses of cryptic frogs (Anura : Brachycephalidae : Craugastor) to simulated terrestrial and aerial predators. *Behaviour* **145**:25-38.
- Cooper, W. E., J. P. Caldwell, and L. J. Vitt. 2009. Conspicuousness and vestigial escape behaviour by two dendrobatid frogs, *Dendrobates auratus* and *Oophaga pumilio*. *Behaviour* **146**:325-349.
- Couteaux, M.-M., P. Bottner, and B. Berg. 1995. Litter decomposition, climate, and litter quality. *Trends in Ecology and Evolution* **10**:63-66.
- Cuevas, E., and A. E. Lugo. 1998. Dynamics of organic matter and nutrient return from litterfall in stands of ten tropical tree plantation species. *Forest Ecology and Management* **112**:263-279.
- Daly, G. L., Y. D. Lei, C. Teixeira, D. C. G. Muir, L. E. Castillo, L. M. M. Jantunen, and F. Wania. 2007a. Organochlorine pesticides in the soils and atmosphere of Costa Rica. *Environmental Science & Technology* **41**:1124-1130.
- Daly, G. L., Y. D. Lei, C. Teixeira, D. C. G. Muir, L. E. Castillo, and F. Wania. 2007b. Accumulation of current-use pesticides in neotropical montane forests. *Environmental Science & Technology* **41**:1118-1123.
- Donnelly, M. A. 1987. Territoriality in the poison-dart frog *Dendrobates pumilio* (Anura: Dendrobatidae). PhD Dissertation. University of Miami, Miami.
- Donnelly, M. A. 1989a. Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. *Ecological Monographs* **59**:207-221.
- Donnelly, M. A. 1989b. Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. *Oecologia (Berlin)* **81**:212-218.
- Donnelly, M. A. 1989c. Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. *Oecologia* **81**:212-218.

- Donnelly, M. A. 1994. Amphibian Diversity and Natural History. Pages 199-209 in L. McDade, K. Bawa, H. Hespenheide, and G. Hartshorn, editors. *La Selva: Ecology and Natural History of a Neotropical Rainforest*. University of Chicago Press, Chicago.
- Donnelly, M. A., C. Guyer, E. J. Juterbock, and R. A. Alford. 1994. Techniques for marking amphibians. *in* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, and L. C. Hayek, editors. *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution Press, Washington, D. C.
- Fauth, J. E., B. I. Crother, and J. B. Slowinski. 1989. Elevational patterns of species richness, evenness, and abundance of the Costa Rican leaf-litter herpetofauna. *Biotropica* **21**:178-185.
- Forster, B., M. Garcia, H. Hofer, E. Morgan, and J. Rombke. 2009. Tropical terrestrial model ecosystems for evaluation of soil fauna and leaf litter quality effects on litter consumption, soil microbial biomass and plant growth. *Pesquisa Agropecuaria Brasileira* **44**:1063-1071.
- Gomez-Brandon, M., C. Lazcano, M. Lores, and J. Dominguez. 2010. Detritivorous earthworms modify microbial community structure and accelerate plant residue decomposition. *Applied Soil Ecology* **44**:237-244.
- Guyer, C. 1988a. Food supplementation in a tropical mainland anole, *Norops humilis*: demographic effects. *Ecology* **69**:350-361.
- Guyer, C. 1988b. Food supplementation in a tropical mainland anole, *Norops humilis*: effects on individuals. *Ecology* **69**:362-369.
- Guyer, C. 1990. The herpetofauna of La Selva, Costa Rica. Pages 371-385 in A. H. Gentry, editor. *Four Neotropical Forests*. Yale University Press, New Haven.
- Guyer, C., and M. A. Donnelly. 2005. *Amphibians and Reptiles of La Selva, Costa Rica, and the Caribbean Slope*. University of California Press, Berkeley.
- Heinen, J. T. 1992. Comparisons of the leaf-litter herpetofauna in abandoned cacao plantations and primary rain forest in Costa Rica: some implications for faunal restoration. *Biotropica* **24**:431-439.
- Heneghan, L., D. C. Coleman, X. Zou, D. A. Crossley, Jr., and B. L. Haines. 1998. Soil microarthropod community structure and litter decomposition dynamics: a study of tropical and temperate sites. *Applied Soil Ecology* **9**:33-38.
- Holdsworth, A. R., L. E. Frelich, and P. B. Reich. 2008. Litter decomposition in earthworm-invaded northern hardwood forests: Role of invasion degree and litter chemistry. *Ecoscience* **15**:536-544.

- Huang, C. Y., P. F. Hendrix, T. J. Fahey, P. J. Bohlen, and P. M. Groffman. 2010. A simulation model to evaluate the impacts of invasive earthworms on soil carbon dynamics. *Ecological Modelling* **221**:2447-2457.
- Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. A. Perez, and T. Garland. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B-Biological Sciences* **276**:1939-1948.
- Kaspari, M., M. N. Garcia, K. E. Harms, M. Santana, S. J. Wright, and J. B. Yavitt. 2008. Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters* **11**:35-43.
- Kaspari, M., and S. P. Yanoviak. 2008. Biogeography of litter depth in tropical forests: evaluating the phosphorus growth rate hypothesis. *Functional Ecology* **22**:919-923.
- Kaspari, M., and S. P. Yanoviak. 2009. Biogeochemistry and the structure of tropical brown food webs. *Ecology* **90**:3342-3351.
- Lawrence, D., and D. Foster. 2002. Changes in forest biomass, litter dynamics, and soils following shifting cultivation in southern Mexico: an overview. *Interscincia* **27**:400-408.
- Lieberman, S. S. 1986. Ecology of the leaf litter herpetofauna of a neotropical rain forest: La Selva, Costa Rica. *Acta Zoologica Mexicana (ns)* **15**:1-72.
- Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* **103**:3165-3170.
- Lips, K. R., P. A. Burrowes, J. R. Mendelson, III, and G. Parra-Olea. 2005a. Amphibian population declines in Latin America: a synthesis. *Biotropica* **37**:222-226.
- Lips, K. R., P. A. Burrowes, J. R. Mendelson, and G. Parra-Olea. 2005b. Amphibian declines in Latin America: Widespread population declines, extinctions, and impacts. *Biotropica* **37**:163-165.
- Lips, K. R., J. Diffendorfer, J. R. Mendelson, and M. Sears. 2008. Riding the wave: Climate change, emerging infectious disease and amphibian declines. *Plos Biology* **6**:441-454.
- M. Kaspari, S. P. Y. 2008. Biogeography of litter depth in tropical forests: evaluating the phosphorus growth rate hypothesis. Pages 919-923.

- McDade, L. A., and G. S. Hartshorn. 1994. La Selva Biological Station. Pages 6-14 *in* L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, editors. *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. University of Chicago Press, Chicago.
- Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecology* **59**:465-472.
- Parker, G. G. 1994. Soil fertility, nutrient acquisition, and nutrient cycling. Pages 54-63 *in* L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, editors. *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. University of Chicago Press, Chicago.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**:161-167.
- Puschendorf, R., A. C. Carnaval, J. VanDerWal, H. Zumbado-Ulate, G. Chaves, F. Bolanos, and R. A. Alford. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions* **15**:401-408.
- Schlaepfer, M. A. 2003. Successful lizard eggs in a human-disturbed habitat. *Oecologia* **137**:304-311.
- Scott, N. A., and D. Binkley. 1997. Foliage litter quality and annual net N mineralization: comparison across North American forest sites. *Oecologia* **111**:151-159.
- Scott, N. J. 1976. The abundance and diversity of the herpetofaunas of tropical forest litter. *Biotropica* **8**:41-58.
- Sigel, B. J., T. W. Sherry, and B. E. Young. 2006. Avian community response to lowland tropical rainforest isolation: 40 years of change at La Selva Biological Station, Costa Rica. *Conservation Biology* **20**:111-121.
- Smith, K. G., K. R. Lips, and J. M. Chase. 2009. Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. *Ecology Letters* **12**:1069-1078.
- Socci, A. M., M. A. Schlaepfer, and T. A. Gavin. 2005. The importance of soil moisture and leaf cover in a female lizard's (*Norops polylepis*) evaluation of potential oviposition sites. *Herpetologica* **61**:233-240.

- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* **306**:1783-1786.
- Vasconcelos, H. L., and W. F. Laurance. 2005. Influence of habitat, litter type, and soil invertebrates on leaf-litter decomposition in a fragmented Amazonian landscape. *Oecologia* **144**:456-462.
- Vitousek, P. M. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* **65**:285-298.
- Vonesh, J. R. 2001. Patterns of richness and abundance in a tropical African leaf-litter herpetofauna. *Biotropica* **33**:502-510.
- Watling, J. I. 2005. Edaphically-biased distributions of amphibians and reptiles in a lowland tropical rainforest. *Studies on Neotropical Fauna and Environment* **40**:15-21.
- Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolanos, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences of the United States of America* **104**:8352-8356.
- Whitfield, S. M., and M. A. Donnelly. 2006. Ontogenetic and seasonal variation in the diets of a Costa Rican leaf-litter herpetofauna. *Journal of Tropical Ecology* **22**:409-417.
- Whitfield, S. M., J. Kerby, L. R. Gentry, and M. A. Donnelly. In prep. Temporal Variation in Prevalence of Chytridiomycosis in Three Species of Frogs at La Selva, Costa Rica
- Whitfield, S. M., K. R. Lips, and M. A. Donnelly. in press. Decline and conservation of amphibians in Central America. *in* H. H. Heatwole, C. Barrio-Amoros, and J. W. Wilkenson, editors. *Status of Conservation and Declines of Amphibians: Western Hemisphere*. Surrey Beatty and Sonos, Pty. Ltd., Sydney.
- Whitfield, S. M., and M. S. F. Pierce. 2005. Tree buttress microhabitat use by a neotropical leaf-litter herpetofauna. *Journal of Herpetology* **39**:192-198.
- Wieder, R. K., and S. J. Wright. 1995. Tropical forest litter dynamics and dry season irrigation on Barro Colorado Island, Panama. *Ecology* **76**:1971-1979.
- Wood, T. E., D. Lawrence, and D. A. Clark. 2005. Variation in leaf litter nutrients of a Costa Rican rain forest is related to precipitation. *Biogeochemistry* **73**:417-437.

Young, B. E., K. R. Lips, J. K. Reaser, R. Ibanez, A. W. Salas, J. R. Cedenio, L. A. Coloma, S. Ron, E. La Marca, J. R. Meyer, A. Munoz, F. Bolanos, G. Chaves, and D. Romo. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* **15**:1213-1223.

Table 4.1. Encounters by treatment period and manipulation type.

Taxon	Pre-Treatment			Post-Treatment			Total
	C	L-	L+	C	L-	L+	
Frogs							
<i>Rhaebo haematiticus</i>		1	2	3	2		8
<i>Bufo melanochlorus</i>		3	1		5	1	10
<i>Craugastor bransfordii</i>	109	90	136	299	178	411	1223
<i>C. crassidigitus</i>				1			1
<i>C. fitzingeri</i>	1		1	5		3	10
<i>C. megacephalus</i>	13		4	2	13		32
<i>C. mimus</i>	5	8	7	31	11	61	123
<i>C. talamancae</i>	3	2		2		4	11
<i>Pristimantis cerasinus</i>		5	1	21	15	11	53
<i>P. cruentus</i>						1	1
<i>Diaspora diastema</i>	2	10	14	10	11	14	61
<i>P. ridens</i>						1	1
<i>Lithobates warszewitchii</i>				1	1	1	3
<i>Oophaga pumilio</i>	177	201	169	273	249	348	1417
<i>Leptodactylus savagei</i>					6		6
<i>Gastrophryne pictiventris</i>	1						1
<i>Hyalinobatrachium fleishmanii</i>						1	1
<i>Scinax boulengeri</i>		1					1
<i>S. eleaochroa</i>			5			7	12
<i>Smilisca baudinii</i>			1				1
Unidentified frog	5		4	9	10	16	44
Lizards							
<i>Corytophanes cristatus</i>		5		1	7	1	14
<i>Norops biporcatus</i>					2	2	4
<i>N. capito</i>	1	2		1	3		7
<i>N. carpenteri</i>	2				1	1	4
<i>N. humilis</i>	60	67	83	91	55	84	440
<i>N. lemurinus</i>		1	2	4	5	5	17
<i>N. limifrons</i>	36	45	103	72	76	83	415
<i>Ameiva festiva</i>	12	9	13	18	27	32	111
<i>Lepidophyma flavimaculata</i>			1			1	2
<i>Lepidoblepharis xanthostigma</i>			1				1
<i>Gonatodes humeralis</i>			3			2	5
<i>Thecadactylus rapicaudus</i>					1		1
<i>Sphenomorphus cherriei</i>	5	12	12	22	2	61	114
Unidentified lizard	1	2	1	7	1	5	17
Snakes							
<i>Coniophanes fissidens</i>						2	2
<i>Imantodes cenchoa</i>	2						2
<i>Leptophis mexicanus</i>			1				1
<i>Rhadinea decorata</i>		1	1			1	3
<i>Pseustes poeciloichilonotus</i>					1		1
<i>Bothrops asper</i>	1						1
<i>Porthidium nasutum</i>			2	1		1	4
Unidentified snake		1		1		2	4
							0
Unidentified				1	1	2	4
Total	436	466	568	876	683	1165	4194

Table 4.2. Estimates of effect size (and 95% CIs in parentheses) for litter removal and litter addition on encounters of amphibians and reptiles.

Taxon	L-	L+
	-0.3154	0.0206
All amphibians and reptiles	(-0.48, -0.15)	(-0.13, 0.17)
	-0.3520	0.1771
All frogs	(-0.55, -0.16)	(-0.01, 0.36)
	-0.3830	-0.3772
All lizards	(-0.7, -0.07)	(-0.66, -0.09)
	-0.2192	0.2890
<i>Oophaga pumilio</i>	(-0.48, 0.05)	(0.03, 0.55)
	-0.3271	0.0969
<i>Craugastor bransfordii</i>	(-0.66, 0.01)	(-0.2, 0.39)
	-1.5061	0.3403
<i>Craugastor mimus</i>	(-2.82, -0.19)	(-0.89, 1.57)
	-0.6139	-0.4046
<i>Norops humilis</i>	(-1.1, -0.13)	(-0.85, 0.04)
	-0.1691	-0.9091
<i>Norops limifrons</i>	(-0.071, 0.38)	(-1.4, -0.41)
	-3.273	0.1443
<i>Sphenomorphus cherriei</i>	(-5.06, -1.49)	(-1.01, 1.3)

Table 4.3. GLM results for microclimate data. A: Average temperature, B: Daily maximum temperature, C: Daily minimum temperature.

A: Average Temperature	df	F	P
(Intercept)	1, 7429	135873.4	<.0001
Sensor location (Air vs. Soil)	1, 7429	19.34	<.0001
Treatment period (Pre vs. Post)	1, 7429	995.52	<.0001
Treatment Type (L ₀ , L ₊ , L ₋)	2, 63	0.12	0.8881
Location x Treatment period	1, 7429	0.26	0.6131
Location x Treatment	2, 7429	4.81	0.0081
Treatment period x Treatment	2, 7429	1.32	0.2669
Location x Treatment Period x Treatment	2, 7429	2.45	0.0867
B: Maximum Temperature	df	F	P
(Intercept)	1, 7429	61091.43	<.0001
Sensor location (Air vs. Soil)	1, 7429	613.19	<.0001
Treatment period (Pre vs. Post)	1, 7429	170.94	<.0001
Treatment Type (L ₀ , L ₊ , L ₋)	2, 63	0.3	0.7405
Location x Treatment period	1, 7429	1.8	0.1799
Location x Treatment	2, 7429	35.47	<.0001
Treatment period x Treatment	2, 7429	10.97	<.0001
Location x Treatment Period x Treatment	2, 7429	0.95	0.3862
C: Minimum Temperature	df	F	P
(Intercept)	1, 7429	201192.09	<.0001
Sensor location (Air vs. Soil)	1, 7429	1236.31	<.0001
Treatment period (Pre vs. Post)	1, 7429	800.19	<.0001
Treatment Type (L ₀ , L ₊ , L ₋)	2, 63	0.09	0.9183
Location x Treatment period	1, 7429	19.42	<.0001
Location x Treatment	2, 7429	21.47	<.0001
Treatment period x Treatment	2, 7429	7.76	0.0004
Location x Treatment Period x Treatment	2, 7429	7.95	0.0004

FIGURES

Figure 4.1. Effects of litter manipulation on metrics of litter depth in the pre-and post treatment periods.

Figure 4.2. Number of encounters per sampling session in response to manipulations and controls for A) total amphibian and reptile encounters, B) encounters for frogs alone and C) encounters for lizards alone.

Figure 4.3. Number of encounters per sampling session in response to manipulations and controls for the three most commonly-encountered amphibians in this experiment.

Figure 4.4. Number of encounters per sampling session in response to manipulations and controls for the three most commonly-encountered lizards in this experiment.

Figure 4.5. Microclimate results from experimental manipulations of leaf litter. Bars indicate maximum daily average temperature (highest cross mark), average daily temperature (middle cross mark) and average daily minimum temperature (lowest cross mark).

Figure 4.6. Results of our comparisons of multi-decade metrics of litter depth.

CONCLUSIONS

My research presented in this dissertation challenges several fundamental assumptions in ecology of enigmatic faunal declines, with implications for applied conservation work. Historically, most work on enigmatic decline events in tropical America has focused on rapid declines in montane forests, and much controversy has arisen over whether such declines are caused by climate change or the emerging infectious disease chytridiomycosis (Pounds and Crump 1994, Pounds 2001, Lips et al. 2005, Lips et al. 2006, Pounds et al. 2006, Lips et al. 2008).

Chapter 1 of this dissertation illustrates that across Central America, a large number of conservation risks threaten amphibian species, challenges the perception that riparian species are particularly extinction-prone are not entirely warranted, and illustrate great need for capacity building in the region. While it is clear that that chytridiomycosis-related research dominates the literature, habitat modification is a principal threat to amphibians, and one that likely has already caused extinctions. Further, many amphibian stressors go almost completely unstudied in the region – including threats from other emerging infectious diseases such as Ranavirus, as well as pollution from a broad range of poorly-regulated pesticides. Historically, much emphasis has been placed on correlations between associations with flowing water and rapid decline associated with chytridiomycosis (Lips et al. 2003, Lips et al. 2005, Kriger and Hero 2007), however data from across the region on conservation status shows that terrestrial species with no association with water are as likely to face high threat as those that live in streams, suggesting that other important factors may be at play (Whitfield et al. 2007, Rovito et al. 2009). Finally, my literature analysis indicates that while scientists

from Central America are poorly represented in amphibian research at large, they are much better represented in conservation-focused research programs – such strong interest in conservation work may greatly assist capacity building programs.

In Chapter 2, I illustrate that population data collected over 35 years at La Selva Biological Station show that even species with no *a priori* reason to expect population declines. These declines are concerning because they illustrate the phenomenon of shifting baselines, and that even in the absence of known conservation threats, anthropogenic impacts may be felt even in reserves thought to be among the most pristine on the planet. Further, declines at La Selva are not only occurring for amphibians, but for forest lizards as well. Further studies have indeed suggested that forest lizards may be at risk from globally diffuse threats (Huey et al. 2009), and that declines in lizard populations may be widespread in the Neotropics (Sinervo et al. 2010). Finally, this dataset on amphibian and reptile populations supplements work from other researchers that highlights long-term change in bird populations (Sigel et al. 2006) and growth and mortality of large trees (Clark et al. 2003) – suggesting broad scale ecological change at this site despite consistent habitat protection.

In Chapter 3, I evaluate the potential role of the emerging infectious disease chytridiomycosis in declines at La Selva. Using field sampling and molecular techniques, I find that Bd exists at this site, often at high prevalence. While Bd is intolerant to warm temperatures, and for this reason it has been suggested that Bd cannot exist or cause declines in lowland forests (Longo et al. 2010). While my study shows that Bd infects frogs at La Selva nearly exclusively in cooler months, and I expect that Bd is not native to Costa Rica, there is no robust data linking Bd to declines. Despite this, it is

very easy to find highly infected frogs during mid-winter cold fronts, and morbid and lethargic frogs infected with Bd can be found at La Selva, but only in the coolest times of the year. It thus appears that Bd is very likely a novel cause of seasonal mortality for La Selva amphibians, and may well be one contributor to population declines. However, Bd is not believed to have the capacity to infect lizards, and the first records of Bd in Costa Rica date to the mid 1980s (Puschendorf 2003), well after declines at La Selva were underway.

In Chapter 4, I provide evidence that a second factor, dynamics of leaf litter, may be implicated in declines. With a long-term replicated field experiment, I show dependence upon leaf litter by a suite of terrestrial amphibians and reptiles. Critically, I also show that the two species that respond most severely to manipulations are those species that declined most rapidly in the long-term. By synthesizing historic datasets on standing litter quantity and replicating these experiments over a two year period, I illustrate that standing litter quantity is at least highly variable between years, and that this evidence does not rule out long-term directional reductions in standing litter quantity.

In addition to the factors examined in this dissertation, there are almost certainly other factors likely involved in declines at La Selva. The emerging pathogen Ranavirus – which was believed to be absent from Central America (Picco and Collins 2007), is present at La Selva, but I have few data on prevalence, number of species affected; and it is unclear whether Ranavirus is native or recently introduced. Preliminary work with amphibian tolerance to pesticides suggests that while pesticide contamination is present at La Selva in low concentrations (Daly et al. 2007a, Daly et al. 2007b), these concentrations certainly not high enough to cause direct mortality, and possibly too low

to affect amphibians in any way. Many of the thermoconforming amphibians and reptiles at La Selva may be very close to the warm edge of their distributional limit (Huey et al. 2009), and further increases in temperature may drive these species out of lowland habitats (Colwell et al. 2008).

This study directly addresses evidence for two factors associated with declines at La Selva. Unfortunately, most studies that attempt to explain enigmatic decline events focus on a single factor rather than investigating multiple overlapping factors, and conclusions of these studies are often used to guide conservation actions. While such single-factor studies are critical for understanding how lone factors operate, they risk ignoring other potential factors or cofactors. As I illustrate in Table 1.5, most amphibian decline sites in Central America have experienced a number of forms of environmental change. Thus, the potential for additive or multiplicative effects (i.e. synergistic interactions) is of these factors high. Until more studies like this one evaluate the role of multiple threats to amphibian populations undergoing declines, it will be impossible to robustly evaluate which factors or co-factors are important conservation threats, and thus effective conservation actions will remain elusive.

LITERATURE CITED

- Clark, D. A., S. C. Piper, C. D. Keeling, and D. B. Clark. 2003. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984 - 2000. *Proceedings of the National Academy of Sciences* **100**:5282-5857.
- Colwell, R. K., G. Brehm, C. L. Cardelus, A. C. Gilman, and J. T. Longino. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* **322**:258-261.
- Daly, G. L., Y. D. Lei, C. Teixeira, D. C. G. Muir, L. E. Castillo, L. M. M. Jantunen, and F. Wania. 2007a. Organochlorine pesticides in the soils and atmosphere of Costa Rica. *Environmental Science & Technology* **41**:1124-1130.
- Daly, G. L., Y. D. Lei, C. Teixeira, D. C. G. Muir, L. E. Castillo, and F. Wania. 2007b. Accumulation of current-use pesticides in neotropical montane forests. *Environmental Science & Technology* **41**:1118-1123.
- Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. A. Perez, and T. Garland. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B-Biological Sciences* **276**:1939-1948.
- Kruger, K. M., and J. M. Hero. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions* **13**:781-788.
- Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* **103**:3165-3170.
- Lips, K. R., P. A. Burrowes, J. R. Mendelson, III, and G. Parra-Olea. 2005. Amphibian population declines in Latin America: a synthesis. *Biotropica* **37**:222-226.
- Lips, K. R., J. Diffendorfer, J. R. Mendelson, and M. W. Sears. 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *Plos Biology* **6**:441-454.
- Lips, K. R., J. D. Reeve, and L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* **17**:1078-1088.

- Longo, A. V., P. A. Burrowes, and R. L. Joglar. 2010. Seasonality of *Batrachochytrium dendrobatidis* infection in direct-developing frogs suggests a mechanism for persistence. *Diseases of Aquatic Organisms* **92**:253-260.
- Picco, A. M., and J. P. Collins. 2007. Fungal and Viral Pathogen Occurrence in Costa Rican Amphibians. *Journal of Herpetology* **41**:746-749.
- Pounds, J. A. 2001. Climate and amphibian declines. *Nature* **410**:639-640.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**:161-167.
- Pounds, J. A., and M. L. Crump. 1994. Amphibian declines and climate disturbance: the case of the Golden Toad and the Harlequin Frog. *Conservation Biology* **8**:72-85.
- Puschendorf, R. 2003. *Atelopus varius* (harlequin frog). *Herpetological Review* **34**:355-355.
- Rovito, S. M., G. Parra-Olea, C. R. Vasquez-Almazan, T. J. Papenfuss, and D. B. Wake. 2009. Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of the National Academy of Sciences of the United States of America* **106**:3231-3236.
- Sigel, B. J., T. W. Sherry, and B. E. Young. 2006. Avian community response to lowland tropical rainforest isolation: 40 years of change at La Selva Biological Station, Costa Rica. *Conservation Biology* **20**:111-121.
- Sinervo, B., F. Mendez-de-la-Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. V. S. Cruz, R. Lara-Resendiz, N. Martinez-Mendez, M. L. Calderon-Espinosa, R. N. Meza-Lazaro, H. Gadsden, L. J. Avila, M. Morando, I. J. De la Riva, P. V. Sepulveda, C. F. D. Rocha, N. Ibarguengoytia, C. A. Puntriano, M. Massot, V. Lepetz, T. A. Oksanen, D. G. Chapple, A. M. Bauer, W. R. Branch, J. Clobert, and J. W. Sites. 2010. Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science* **328**:894-899.
- Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolanos, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences of the United States of America* **104**:8352-8356.

VITA

STEVEN M. WHITFIELD

1996-2000 B.A, B.S, Biology
 The Evergreen State College
 Olympia, Washington

2002-2005 M.S., Biology
 Florida International University
 Miami, Florida

PUBLICATIONS

Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolaños, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences USA* 104(20):8352-8356.

Whitfield, S. M. and M. A. Donnelly. 2006. Ontogenetic and seasonal variation in the diets of a Costa Rican leaf-litter herpetofauna. *Journal of Tropical Ecology*. 22(4): 409-417.

Whitfield, S. M. and M. S. F. Pierce. 2005. Tree buttress microhabitat use by a neotropical leaf litter herpetofauna. *Journal of Herpetology* 39(2): 192-198.

Whitfield, S. M., K. R. Lips, M. A. Donnelly. In press. Decline and conservation of amphibians in Central America. Pp. XX–XX in H.H. Heatwole, C. Barrios-Amorós, and J. W. Wilkenson (eds.), *Status of Conservation and Decline of Amphibians: Western Hemisphere*, Volume 8B in: *Amphibian Biology*, series editor, H. Heatwole. Surrey Beatty and Sons, Pty. Ltd., Sydney.

GRANTS AND AWARDS

University Graduate School Provost Award for Outstanding Graduate Student Paper. Florida International University. 2011.

Dissertation Year Fellowship. Florida International University. 2010-2011.

Greater Research Opportunities Fellowship. U.S. Environmental Protection Agency. 2008-2010.

Presidential Fellowship. Florida International University. 2005-2008.