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An Ecological Study of the Anurans in Tea Plantations in a Biodiversity Hotspot

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

AN ECOLOGICAL STUDY OF ANURANS IN TEA PLANTATIONS IN A
BIODIVERSITY HOTSPOT

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Lilly Margaret Eluvathingal

2016

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Lilly Margaret Eluvathingal, and entitled An Ecological Study of the Anurans in Tea Plantations in a Biodiversity Hotspot, having been approved in respect to style and intellectual content, is referred to you for judgment.

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

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Florida International University, 2016

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DEDICATION

This dissertation is dedicated to my parents and to the late Dr. M. S. Mayilvahanan.

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I have had the honor of interacting with some of the most inspiring people I have ever met, as a part of this dissertation. To each and every one of them I am very grateful.

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ABSTRACT OF THE DISSERTATION

AN ECOLOGICAL STUDY OF ANURANS IN TEA PLANTATIONS IN A
BIODIVERSITY HOTSPOT

by

Lilly Margaret Eluvathingal

Florida International University, 2016

Miami, Florida

Professor Maureen Donnelly, Major Professor

Increasing human population size is increasing the demand for resources like timber, oil, tea, coffee, and other crops. Plantation crops mimic some aspects of native habitats, and there are studies that report the presence of some native anuran biodiversity in plantations. I focused on tea plantations in the Western Ghats-Sri Lanka Biodiversity Hotspot and studied the diversity and health of anurans in different habitats found within a tea cultivation area, near Munnar region in the Western Ghats, India. The landscape includes tea bushes, native evergreen shola forest patches, and eucalyptus forest stands. I reviewed 40 studies comparing amphibian species richness in plantations and primary forests. The age of the plantation, type of plantation, presence in a biodiversity hotspot, number of species in the dominant plantation type, number of species in the paired forest habitat, and latitudinal zone of the study, did not correlate with species richness, but plantations that had periodic harvesting had higher species richness than plantations that practiced clear-cut harvesting. I tested different methods of standard amphibian sampling

in the field season 2012 in Munnar, and found that Visual Encounter Surveys (VES) in the shola habitat and Stream Transects (ST) were the most efficient. Using the VES and ST methods, I sampled amphibians in three upland habitats (tea, shola, and eucalyptus) at four different sites, and 150m of stream transects at each site, for two consecutive monsoon seasons. Fourteen species were encountered in both years and the community structure was similar across the years. The community structure at the four sites that was driven by the presence of exclusive species at each site and species composition in streams was similar across the landscape and was driven by the presence of similar species in streams across the four sites. Two hundred and sixteen anurans of 17 species, were tested for the presence of the lethal fungus *Batrachochytrium dendrobatidis*. The preliminary results from the Polymerase Chain Reactions were negative. My study provides baseline data for anuran diversity, composition, and health in the Munnar region of India and results of this project can be compared with tea plantations around the world.

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ABBREVIATIONS AND ACRONYMS

ANOSIM	Analysis of Similarity
ASL	Above Sea Level
Bd	<i>Batrachochytrium dendrobatidis</i>
BdGPL	<i>Batrachochytrium dendrobatidis</i> global panzootic lineage
Bsal	<i>Batrachochytrium salamandrivorans</i>
FAO	Food and Agriculture Organization
GAA	Global Amphibian Assessment
HCV	High Conservation Value
KDHP	Kanan Devan Hills Tea Plantation Company Private Limited
MHC	Major Histocompatibility Complexes
nMDS	non-metric Multi-Dimensional Scaling
IACUC	Institutional Animal Care and Use Committee
IUCN	International Union for Conservation of Nature and Natural Resources
PCR	Polymerase Chain Reaction
ST	Stream Transect
VES	Visual Encounter Survey
WG	Western Ghat

I. Introduction

Global flora and fauna face several pressing threats and it is essential to collect data which increases our knowledge of individual species, as well as communities of species in different locations, to make successful conservation plans (Wake and Vredenburg 2008). The threats have been reported to be more severe for amphibians compared to other vertebrates, and some of the threats amphibians face include fragmentation and loss of habitat, pollution and increase in pesticide use, infection by chytrid fungus and other diseases, dam and water management systems, ultraviolet β radiation, and the introduction of alien species (Alford and Richards 1999; Bickford et al. 2010; Blaustein and Kiesecker 2002; Cushman 2006; Hayes et al. 2010; Nowakowski et al. 2016; Stuart et al. 2004; Whitfield et al. 2007; Whitfield et al. 2013). With an increasing human population, our agricultural lands are under constant pressure to increase production and at the same time more land area is being converted to agricultural purposes. Though most biodiversity conservation plans are executed in protected areas, in the past few decades there have been multiple studies to investigate the role played by plantation landscapes in supporting amphibian diversity (Brockerhoff et al. 2008; Felton et al. 2010; Folt and Reider 2013; Gardner et al. 2007).

In India, commercial tea production is spread across 14 states (Shah and Pate 2016). However, there are very few studies that look at the diversity of anurans in tea plantations (Daniels, 2003) despite the fact that many tea plantations occur in the Western Ghats and Sri Lanka biodiversity hotspot. My research examined the role played by tea plantations in supporting anuran diversity in the state of Kerala, India, in the Western Ghats mountain range. India has seen an increase in anuran species descriptions since 2000,

however, not as much work is focused on studying the community level studies of these species, especially in agro-economic landscapes (Bossuyt et al. 2004; Frost 2016). My research was conducted in the Munnar region of the Indian part of the Western Ghats and Sri Lanka biodiversity hotspot; a region designated as such, because of its high biodiversity, and because of the presence of several anthropogenic stressors in the region (Bossuyt et al. 2004; Myers et al. 2000; Myers 2003). The landscape in my study area was modified into tea plantations starting in the late 1870s by the British and has been privately owned since 1878. The current management scheme started in 2005 (Daniels 2003). The Munnar area is lacking in peer-reviewed species lists and information on anuran communities from the tea landscape, though several new species have been described from the region in the past 10 years (Frost 2016).

There are four chapters in the present dissertation: Chapter 1: A global analysis of factors driving amphibian diversity in plantations; Chapter 2: A comparative study of standard methods for surveying anurans in tea plantations in the southern Western Ghats; Chapter 3: Patterns of community structure in tea plantations in Munnar, India; Chapter 4: Testing for chytrid in tea plantations in the southern Western Ghats, India. In Chapter one I reviewed studies comparing amphibian diversity in plantations to amphibian diversity in a natural forest habitat nearby, and a case study from of amphibian species richness from four estates in the tea plantation landscape in southern India. From each study, I extracted information about the age of the plantation, the type of plantation, the type of harvesting employed (complete or periodic), whether it was in a biodiversity hotspot or not, the number of species in the dominant plantation type, the number of species in the paired forest habitat, and which latitudinal zone the study was conducted in. Using data obtained

from the different standard amphibian sampling methods and opportunistic encounters during 2012, at elevations between 1500m and 2000m asl in the Munnar district of Kerala, India, I made a checklist of anurans found in the tea plantations in Munnar between 1500 and 2000m asl and compared these data to the amphibian diversity information obtained from other studies. In Chapter 2, I sampled and used the data obtained from the field year 2012, to rate the efficiencies of different standard methods of surveying amphibians in three different habitats (tea, shola forests, eucalyptus forests). I used nocturnal and diurnal, upland Visual Encounter Surveys (VES), VES in Stream Transects (ST), and funnel traps with drift fences standard methods. Using the most efficient standard amphibian surveying methods obtained from Chapter 2, I surveyed amphibian diversity and composition across four sites in the tea plantation landscape of Munnar, India, during the monsoon seasons of two years: 2013 and 2014. I compared the community composition across the four sites, and across three different habitat types (shola, tea, and eucalyptus) in the landscape using data collected in the years 2013 and 2014. In Chapter four, I focused on testing anurans in the tea plantations of Munnar for the presence of a known amphibian pathogen. Between 2012 and 2015, I swabbed anurans in the tea plantation landscape and tested the swabs for the presence of the global and Asian strain of the fungus *Batrachochytrium dendrobatidis* (*Bd*). The present study provides baseline data about the richness, abundance, and health of anuran communities in a tea-dominated landscape in the Munnar area, of the southern Western Ghats, India.

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II. CHAPTER 1

A Global Analysis of Factors Affecting Amphibian Diversity in Plantations

Abstract

Anthropogenic conversions of natural areas to agriculture and monoculture plantations are a large threat to flora and fauna, especially in biodiversity hotspots which harbor large numbers of endemic species. Plantations in the tropics are established in areas characterized by high species richness. However, studies on the biodiversity present in these systems are only a few decades old. It is becoming clear that plantations can be hospitable to many species, and are used by some native organisms. Plantations in the tropics tend to support some native species that are endemic and have narrow distribution ranges indicating a potential high conservation value. Papers published on amphibian diversity in plantations were examined for factors affecting the number of species, with a focus on tropical plantation systems. Forty papers comparing amphibian species richness in plantations and nearby primary forests are compared with each other and to preliminary data on anurans obtained from an active tea plantation in Kerala, India. The tea plantation study site (1500-2000m asl) is in the Western Ghats and Sri Lanka biodiversity hotspot. Factors like age of the plantation, type of crop, and harvesting regime did not explain the richness of amphibian species in plantations in the tropics, likely because of the variations in crop types, geographic locations of the studies, and the methods used used to harvest crops. Amphibian species occur in the plantations in each study site, and generally plantations are species poor compared to the forested reference sites. Of the 25 species encountered in the tea plantations in Munnar, Kerala, all but one species is endemic to the Western Ghats. Nine species are in the first three threat

categories (Critically endangered, Threatened, and Vulnerable) of the IUCN red list.

Conservation practices that can be implemented to protect amphibians without affecting tea production, including selective protection of breeding microhabitats, may increase the attractiveness of tea to an increasingly socially conscious, international market. With the global area under plantations increasing annually, understanding how wildlife uses plantations can provide managers with information on the biodiversity value of their property.

Key words: Biodiversity Hotspots, conservation, plantations, tea, tropics

Introduction

Increasing industrialization and globalization are the leading drivers of several anthropomorphic changes on earth (Felton et al. 2010; Gardner et al. 2007). These alterations are in turn linked with increased logging, cattle ranching, and plantations, which are among the main drivers of conversion of tropical forests to other uses (Brockerhoff et al. 2008; Laurance 2014). According to the Oxford Dictionary there are two definitions of the word plantation: 1) An estate on which crops such as coffee, sugar, and tobacco are cultivated by resident labor, and 2) An area in which trees have been planted, especially for commercial purposes (Oxforddictionaries.com 2016). The term plantation is now used to describe stands of pine, eucalyptus; cash-crops like tea, cashew, coffee; spices such as cinnamon, and cardamom; oil producing crops like groundnuts, and oil palm, and other crops such as sugar cane, banana, and pineapple. Data collected by

the Food and Agriculture Organisation of the United Nations (FAO) show that the global annual rate of change to planted forests in the world has increased by 2.09% in the past 10 years, up from 1.88% in the decade before (Table 1.1; Global Forest Resources Assessment 2005; FAO 2011). With a growing human population, it is clear that existing plantations will not suffice, which had resulted in the establishment of additional plantations. Many of the world's plantations are found in Asia, Central America, and South America in the tropics, which have climatic conditions that are conducive to the management of large plantations (Felton et al. 2010; Gardner et al. 2007). Most of the land that is considered to be promising for conversion to plantations is located in the tropics (Figure 1.1; IIASA 2002), and overlaps with several 'Biodiversity Hotspots' (Myers et al. 2000). In the past, biologists have often considered plantations to be "green deserts" with a hostile environment for flora and fauna, and yet in the past few decades there has been a change in this attitude because of several studies showing that plantations around the world provide refuge for a subset of the native biota (Brockerhoff et al. 2008; Felton et al. 2010; Folt and Reider 2013; Gardner et al. 2007). Of all the extant amphibian species described, Nori et al. (2015) reported that approximately 25% are found outside protected areas, and an added 18% have less than 5% of their population in protected areas. Asia had the fastest rate of increasing land under plantation cover of 2.8% from 2000 to 2005 (FAO 2006). From Asia, the two families Nasikabatrachidae and Micrixalidae, (both endemic to the Western Ghats), had the largest percentage of species found outside protected areas (Nori et al. 2015).

Plantations bring to mind clearing of vast expanses of land, monocultures, non-native plants, genetically modified crops, complete harvesting, soil abuse, substantial additions of chemicals, disease breakouts, and an increasing demand for various products, fueling an increasing pressure on the lands that are already under cultivation. Wilcove and Koh (2010) warned that plantations like oil palm are very profitable for the poor communities in Southeast Asia, as they are used in several different products, and that demand for organically grown palm oil is very low, with countries like China and India being the largest markets. With these negative effects, and the global area under plantation increasing and with exponential human growth, the rate of increase in area converted to plantations is only going to increase annually (FAO 2009; FAO and JRC 2012; FAO 2015). The controlled application of various agrochemicals is a factor to be taken into consideration while conducting any ecological study in a plantation setting (Ghose et al. 2014; Johnson et al. 2013). With the increase in area under plantations, it is important to understand how different life history stages of amphibians are affected by different concentrations and combinations of commonly used herbicides, weedicides, pesticides, and fertilizers.

The value of plantations versus the value of the diversity that is still supported by the area under plantation is difficult to estimate, since most plantations are usually areas that are written off as green deserts, and because it is hard to estimate the value of an individual species (Brockerhoff et al. 2008; Gardner et al. 2007; Hudson et al. 2014). The economic value of the ecological role of biodiversity and of individual species is largely unquantified and even if valued, these valuation methods are sometimes controversial

(Allsop et al. 2008; Edwards and Laurance 2012; Ghazoul 2005; Winfree et al. 2011). In several situations, plantations are less severe in their manipulation of nature than is a complete urban environment. As the total area under cultivation continues to increase, the effects on biodiversity should be documented, and the present study compares diversity in altered tropical ecosystems to nearby patches of remnant forests.

There have been several studies conducted in plantations around the world that examine the diversity of a variety of plants and animals in these manipulated systems (Brockerhoff et al. 2008; Felton et al. 2010; Gardner et al. 2007; Palacios et al. 2013; Thompson et al. 2003; Thompson et al. 2015). Hudson et al. (2014) started the PREDICT database of global biodiversity data with over 28,000 species from 78 countries, to develop models that estimate effects of humans on local biodiversity. Lieberman (1986) recorded that disturbed cacao plantations had low species richness, high abundance, and low diversity, compared to natural forests of the La Selva Biological Station in Costa Rica. Slowinski et al. (1987), reported more individuals of some species of leaf-litter amphibians and reptiles, in abandoned cacao plantations during nocturnal surveys, than during daytime surveys, suggesting that these species showed a diel pattern in their use of leaf litter. Heinen (1992), found that old growth cacao plantations were intermediate, to younger cacao plantations and primary forests at La Selva Biological Station, in Costa Rica, both in herpetofaunal diversity as well as in leaf litter depth, that supports a positive relationship between litter depth and species richness. Gardner et al. (2007) reported that 23 out of 157 studies examined herpetological diversity in plantations between 1945 and 2006 and the majority of which are from the New World (Gardner et al. 2007). In a study

of *Physalaemus lisei*, Becker et al. (2007), reported that the abundance of the species in monocultures of *Araucaria angustifolia*, *Pinus*, and eucalyptus, was similar to that in natural *Araucaria* forests. In Canada, Thompson et al. (2003), reported that amphibians were poorly represented in studies of plantations. In a review of global studies comparing diversity across plantations and pasture land, Felton et al. (2010), found that amphibians and reptiles were more diverse in plantations than in pasture land, however they warned against making this general assumption as there were several other factors that needed to be accounted for, such as, management practices, field methods, and the kind of crop in question. Sreekar et al. (2013), using photographs, captured 45 out of 59 marked lizards (*Draco dussumieri*), from a two-hectare areca-nut plantation to estimate the population density of the lizards in the plantation. Rathod and Rathod (2013) reported 19 species of amphibians from organic coffee plantations, and 15 species from protected sacred groves from the Kodogu region of Karnataka, India, with comparable abundances in the two habitats. A study comparing the reproductive habitats available for a frog from Ireland in conifer plantations, bogs, roadsides drains, and clear cut areas showed that the highest use and output in reproductive activity was seen in pools that were near the roadside and the clear felled areas (Dibner et al. 2014). Popescu and Hunter (2011), reported that the mature forests, recent clear cut forests, 11-year-old, and 20-year-old regenerated forests of conifer plantations offered different levels of resistance to the migration of the juvenile wood frogs (*Lithobates sylvaticus*), with the anurans showing a strong preference to forests with the thickest canopy cover. While the presence of diversity in plantations is important, it is also important to study of the health of the individuals encountered. After three years of sampling, Gurushankara et al. (2007) reported 35 individual frogs with

visible abnormalities from 754 frogs encountered in coffee plantations. Abnormalities observed, included, missing digits, missing eyes, and reduced limb length (Gurushankara et al. 2007).

On the other hand, some species are more abundant in plantations. Species like *Geobatrachus walkeri*, a leaf-litter specialist, seem to thrive in plantations, with much higher abundances in the plantations than in natural forests (Martínez Baños et al. 2011). The thick leaf-litter microhabitat available to them in the plantation seems comparable to the litter habitat which they usually use, and the chemical manipulations did not appear to have any observable affect on the reproductive health measured by testicular and follicular growth (Martínez Baños et al. 2011). A pit-fall trap study showed the presence of four, four, and three species of common anurans in agro-systems of corn, rubber, and soy fields, in Southern Brazil, respectively (da Silva et al. 2009). All the species found in these plantations were known generalists that feed on a wide variety of insects that are usually associated with the agricultural fields (da Silva et al. 2009). The presence of nine species of anurans in rice field quadrats near the Pantanal wetland in Brazil, also shows that monoculture agro-systems seems to provide a refuge to a subset of the native anuran fauna (Piatti et al. 2010). However, all the nine species reported in these rice fields are known to be habitat generalists, suggesting that rice fields offer a homogenous habitat that only supports generalist species (Piatti et al. 2010). Populations of *Rhinella schneideri* in Brazil showed no reduction in gene flow and migrations rates even in study sites with sugarcane plantations as the dominant habitat (Arruda et al. 2011). A single study from Santiago Jalahui, Oaxaca reports the presence of five species of amphibians

from a shaded coffee plantations landscape (Moguel and Toledo 1999) which is quite poor compared to amphibian diversity (23 species) reported by Lazcano-Barrero et al. (1992), from the Lacandon Rainforests. Adults of the Coqui Frog (*Eleutherodactylus coqui*) were equally abundant in native forests and eucalyptus plantations, and their abundance seemed to depend more on the structural complexity of the plants than on the specific plant species (Fogarty and Vilella 2003). In a 2013 review, 37 cases (in 279 publications) of amphibian diversity in forests versus plantations were compared from 14 countries (mostly from Latin America and the Caribbean) and in 25 of these cases, the species diversity in plantations was reported to be reduced to that of native forests (Palacios et al. 2013). The same study reported that there was no significant difference in the abundance of amphibians between plantations and forests, suggesting that the though in poor in species richness, plantations provided a reasonable refuge for the species that were present. Murrieta-Galindo et al. (2013), found 46% of the amphibian in the cloud forests, represented in nearby shaded and non-shaded coffee plantations in the highly agricultural Veracruz region of Mexico. The direct developing family of anurans, Craugastoridae was found in all the sites and the authors speculated that independence from aquatic breeding sites might be the reason that species from this family are found in plantations where the water bodies might be contaminated (Murrieta-Galindo et al. 2013).

Tea is popular global beverage and is grown in several countries (China, India, Turkey, and Tanzania) around the world in monoculture plantations. China produces the largest quantity of tea globally, with an output of 1.9 million tons, which is over 38% of the world total, and is followed by India, producing 1.2 million tons (Chang 2015). Eighty

percent of global tea export is from the top five highest tea producing countries: China, India, Kenya, Sri Lanka, and Turkey (Shah and Pate 2016). Commercial tea production in India occurs in 14 states (Shah and Pate 2016). Tea, is a tree which grows in the tropics and sub tropics, and all commercial tea is produced from a single species, *Camellia sinensis* (Chang 2015; Shalleck 1972). The ideal temperature range for growing tea is between 20° and 30° C and the plant requires around 150-300 cm of annual rainfall which is ideally distributed evenly throughout the year (Chang 2015; Shalleck 1972). Even though high rainfall is beneficial for the tea bushes, stagnation of water is not, therefore tea is usually grown along the sides of mountain where the rainwater can run off. Tea thrives in soils which are slightly acidic and are rich in organic matter (Shalleck 1972). High humidity and morning mist encourage the growth of new leaves but they also make the plant susceptible to several fungi (Shalleck 1972). Tea plants are regularly sprayed with chemicals to keep fungi and other diseases at bay (Chang 2015; Shalleck 1972). Short term observations reveal changes in distribution patterns of amphibians because of harsh pesticide applications in tea plantations in South India (Daniels 1991, 1992, 2003). Daniels (2003) reported 13 amphibians from a short 11 day study in a tea plantation in Vaalparai, Tamil Nadu, India. Eleven species were reported from streams flowing into grassy swamps that were created during the rains (Daniels 2003). Krishna et al. (2005) reported six species of anurans from native forests streams, eight species from cardamom forests streams, and six species from streams in coffee plantations. *Micrixalus saxicola* was most abundant in native forests, *Nyctibatrachus* sp. in cardamom plantations, and *Rana temporalis* was the most common frog in coffee plantations. A month-long study from a tea plantation which shifted to organic cultivation five years ago in Sri-Lanka,

observed ten species of amphibians, including six endangered species in tea bushes (Kottawa-Arachchi et al. 2014). At elevations above 2000m asl, the productivity of tea is modelled to increase up to 37% with the doubling of carbon dioxide from 370ppm to 600ppm as result of global warming (Wijeratne et al. 2011).

I review studies in plantations where amphibian diversity was measured and compared to that of nearby natural forests. I also examined these data to determine what might explain why some plantations appear to be better refuges than others. I predicted that older plantations would support higher species richness compared to younger plantations. I also predicted that factors like harvest methodology, and organic management practices would support high species richness. A list of suggestions for why some species survive in plantations may help create a set of guidelines for managers to design biodiversity-friendly plantations without compromising their productivity and in the process gaining green certifications from Non-Governmental Organizations. I compared my results from two different manipulated habitats: eucalyptus stands and tea fields to natural shola forests, in an actively managed tea plantation in Munnar, located in the southern Western Ghats. I also provided an updated version of an existing species list from a nearby National park. Finally, I examined my case study in the context of the data obtained from reviewing published papers comparing amphibian species richness between plantations and primary habitats.

Literature Survey

In order to find drivers for anuran species richness in plantations across the globe, a search was conducted with the Web of Science™ core collection search engine for the key words amphibian + plantations. The data set was queried in May 2015 and then in February 2016. Out of 97 papers obtained by the search only 36 papers compared amphibian diversity between a primary forest and a plantation of some sort and also provided species richness information from both habitat types. I also looked at all the papers in the literature cited in each of the 36 papers and expanded the data set to 40 papers. These 40 papers fit the initial search criteria and (Appendix 1.1) compared amphibian diversity between a plantation area and a primary habitat. Information on the location of the plantation, country, type of crop, method of harvesting, type of management, year of the start of the plantation, the earliest year of harvesting and species richness in each habitat type was collected from each paper.

From each of the 40 papers, location, year of the establishment of the plantation, year of earliest sampling, number of amphibian species in the dominant plantation, number of amphibian species in primary forest, intensity of harvesting (Periodic or complete), and type of plantation (organic or not) was recorded and summarized.

Study Site

A survey of anurans was study was conducted near the town of Munnar, in the state of Kerala in the Southern Western Ghats of India from 2012-2014 across three habitat types:

tea, shola forest, and eucalyptus stands. The Munnar mountain range in the Munnar Forest Division has over 106 Km² of reserve forest, most of which is interspersed among tea plantations (Figure 1.2). Munnar receives rainfall both during the Southwest and the Northeast monsoons. The Southwest monsoons account for over 80% of the annual precipitation which is usually between 1200-1500mm and occurs during June and July. The rest of the precipitation (400-800mm) is received during Northeast monsoon which from October to November (weather-and-climate.com). I conducted amphibian surveys using standard methods in the Kanan Devan Hills Tea Plantation Company Private Limited (henceforth KDHP; 10° N and 77° E) located in the Idukki district, of Kerala, in the Southern Western Ghats.

Field Methodology

At the field site in Munnar, all anuran species encountered along transects in different habitats were recorded, in order to find the species richness supported by the tea plantation landscape. Nocturnal Visual Encounter Surveys (VES) and Stream transect standard methods were used for sampling amphibians (Crump and Scott 1994). I set up 25m transects in all three habitat types. Each transect was marked every five meters using flagging tape. The VES method involved two people walking along the transect and searching carefully for frogs, 1m on either side of the transect line and up to 2m above the ground for 15 minutes (Crump and Scott 1994). The transects were sampled at night (19:00 hrs-00:00hrs). The starting point of each transect within a particular habitat, was at least five meters away from a different habitat type. Twenty-five meter stream transects

were set up in the tea and shola habitats. Stream transects were marked every 5m using flagging tape on vegetation in the riparian zone. Two people walked in the middle of the stream searching carefully for frogs, 1m on either side of the transect line and up to 2m above the ground for 15 minutes (Crump and Scott 1994). The transects were conducted during the day (09:00 hrs-12:00hrs) and at night (19:00 hrs-00:00hrs using head lamps). All streams were from independent drainages. I also added to this list generated by standard sampling, all the amphibian species that were opportunistically encountered in the tea plantations that were being surveyed. All the habitats (shola, tea, and eucalyptus) that each species was spotted in, even if just an opportunistic observation, were included.

Results

Of the 40 studies included in my sample, 30 of the study sites were in biodiversity hotspots and 30 studies were in the tropics (Figure 1.3). The studies were distributed across 19 countries with the largest number of studies in Costa Rica, followed by Mexico (Figure 1.4). Australia, Canada, Colombia, Ecuador, India, Madagascar, Nicaragua, Nigeria, Borneo, and Uganda had only one study that compared amphibian from a plantation to a native forest (Figure 1.5). The most common kind of plantations were eucalyptus, coffee, and various kinds of pines and conifers. The other plantation types included in the review were cacao, oil palm, rubber, corn, sugarcane, coconut, cardamom, cashew, and tea (Figure 1.6). The 40 papers were published between 1992 and 2016 with an increase in the number of publications after 2011 (Figure 1.7). Some of the studies (13) involved complete one-time harvesting of the crop (e.g., eucalyptus, sugarcane) and

in many others (27) periodic harvesting of plant products occurred (e.g., coffee, tea). Only three (cardamom and cacao) of the 40 plantations in the study followed organic practices (Deheuvels, et al., 2014; Krishna, et al., 2005). In two of the studies of organic farming, the native forests had greater species richness than the organic plantations. The age of a plantation was calculated as the year of start of the plantation to the time of the study and the difference in species richness was calculated as the number of species in a nearby primary forest and the number of species in the dominant plantation in the study. In four of the 40 studies, there was no difference in species richness between the plantation and native forests, and in four studies, the species richness was greater in the plantations than in the forest and in the remaining 31 studies the species diversity was greater in the forest than in plantations. After standardizing (dividing total number of excess species by the total number of studies with high species richness in each case) for the number of plantations, primary forests showed 7.55 species more than plantations. The four studies in which plantations had higher species richness harbored 2.75 species more than the primary forest. A paired t-test for the number of total number of species in plantations versus the total number of species in native forests indicated higher species richness in native forests ($p > 0.003$). When studies were divided into those with complete harvesting ($n=13$) and periodic harvesting ($n=27$), and there was no difference in the difference in species richness between the two harvest patterns ($p=0.48$). However, three (two pine, and one black spruce plantation) out of the four studies in which the total number of species was greater in the plantation than in the nearby native forest were from the complete harvesting regime. These three plantations were in the temperate zones and from areas with low amphibian species diversity (Mitchell et al. 1997; Vonesh 2001;

Waldick et al. 2014). The Pearson's correlation coefficients between age of plantation and number of species in the plantation, as well as, the difference in number of species between primary forest and dominant plantation are -0.056 and 0.103, respectively. A generalized linear model showed that only the number of species in the control forest sites and the harvest method were significant factors that correlated with the number of species in plantations. Location in a biodiversity hotspot, and age of the plantation were not significantly correlated with the number of species in each plantation (Table 1.2). Thus, plantations practicing periodic harvesting appear to be slightly better for amphibian species diversity than those practicing complete harvesting in the tropics.

In my study of amphibian diversity in Munnar, India, 25 species were found in the tea plantation landscape between 1500 and 2000m asl. Eighteen species were found in remnant shola forest patches (Table. 1.3). *Beddomixalus bijui* was found exclusively on tea bushes. One individual of *Nyctibatrachus acanthodermis* was found in a stream flowing through a eucalyptus forest patch. Five species were found exclusively in patches of shola forest. Of the 25 species I encountered, nine species are in the most endangered categories of the IUCN red list (IUCN 2016), and all except one (*Duttaphrynus melanostictus*), are endemic to the Western Ghats. *Raorchestes chlorosomma*, *Raorchestes munnarensis*, *Raorchestes griet*, *Rhacophorus pseudomalabaricus* are Critically Endangered species. All of the four species of anurnas were described since the year 2000. Another nine species are designated as either data deficient or not evaluated by the IUCN (Table. 1.3; IUCN 2016).

Discussion

My literature review revealed that no single driver other than harvest method drove species richness in plantations around the world. There were few studies directly comparing amphibian species richness in plantations and primary forests. The 40 studies conducted globally were from several different countries and ten countries only had a single study. Barring Costa Rica, most of the countries which had more than four studies were conducted in North America. There are many plantations in tropical biodiversity hotspots, which are not represented in biodiversity studies, and these are areas that are already known to harbor a high number of species which make them high priority areas for conservation (Myers 2000). There were fourteen different crops under cultivation across these 40 studies, and of these, 14 were designated as forestry plantations that planted a mix of several different kinds of conifers. The studies reviewed in the chapter are representative of the diversity of the plantation industry, also reflected in earlier reviews (Felton et al. 2010; Palacios et al. 2013). The harvesting methods vary among plantations and in several papers specific details about the harvesting process are missing. I expected the scale of scale of harvesting to have a strong effect on the species numbers and their abundances. However, this review showed no significant difference in the number of species from plantations that were periodically harvested or completely harvested at fixed intervals. However, three of the four studies that reported increased amphibian richness in the plantations compared to forests were from the temperate zone, had long growing periods, and were from areas with low species richness (Mitchell et al. 1997; Vonesh 2001; Waldick et al. 1999). There was no significant correlation between

the age of a plantation and the number of amphibian species found in it. There was no significant relationship between the age of the plantation and the species richness found exclusively in the primary forests. The result suggests that the factors I addressed in my review do not affect species richness in plantations. The different sampling protocols used in each of the plantations with different crops and harvesting characteristics make it difficult to group studies together, though plantations practicing periodic harvesting supported slightly more amphibian species than plantations which practiced clear-cut harvesting (Table. 1.2). Only four studies showed higher species diversity in plantations compared to adjacent primary forest. Gardner et al. (2007) reported the same trend with most studies showing a reduced species richness measure in plantations compared to native forests with two exceptions to the pattern. Loehle et al. (2005) and Vonesh (2001) advised taking into account factors like complexity of the habitat available to anurans as a key factor supporting diversity found in plantations. None of the studies showed the complete absence of amphibians in plantations, and all plantations in the papers reviewed supported a subset of native species. As suggested in several publications, researchers need to provide plantation managers with information that encourages them to run their operations in a manner that can help sustain the biodiversity is remaining on their property (Felton et al. 2010; Gardner et al. 2007; Palacios et al. 2013). The information provided to managers needs to include checklists of species found on their property and the microhabitats within the plantations that species use. Advice on sustainable techniques may appear obvious to the research community but can be ignored in profit-oriented industries. Das et al. (2006) reported that the Munnar grid was an irreplaceable site for species conservation, in a study modelling the importance of an area determined

on surrogates, such as the presence of endemic plants and vertebrates. From the tea plantations sampled in Munnar, Kerala, we report 25 species of anurans, of which nine are endangered according to the IUCN Red List (IUCN 2016). The Munnar tea plantations are over 140 years old and this might explain why the tea harbors several anuran species. Sixteen of the species at our site have been described since the year 2000, and there is not much published information about their basic biology. All, but one of the 25 species are endemic to the Western Ghats, and several of them are currently known to be endemic to Munnar. Ten of these 25 species are from the genus of direct developing frogs, *Raorchestes* that do not require an aquatic habitat for breeding and of the 14 species found in the tea bushes, seven species belong to the genus *Rarochestes*. In both cases roughly 40% of the species are from the genus, *Raorchestes*. My results in Munnar are similar to the percentage of direct developers reported Murrieta-Galindo et al. (2013), in coffee plantations in Mexico. The direct developing species, *Eleutherodactylus bransfordii*, has been reported to be the most common species at La selva in Costa Rica from several studies (Folt and Reider, 2013; Lieberman et al. 1986; Heinen 1992). The endemic anurans with a specialized life history have shifted to use an altered landscape in India. The tea plantations sampled were between 1500 and 2000m in elevation and during my study, no visible deformed individuals of any species were encountered. In a recent study, Wordley et al. (2015) recorded seven species of bats in tea plantations, especially close to wooded areas along riparian zones. Though the density of bats is poorer in tea than it is in coffee plantations, it shows that tea plantations with small patches of native forests provide some refuge for volant mammals. The authors recommend maintenance of native trees along streams in these tea plantations landscapes (Wordley et al. 2015).

These are the types of suggestions that need to reach the management committees of plantations areas. If implemented on recommendation for one group of animals, these suggestions are likely to be beneficial to several other flora and fauna, directly or indirectly.

With several studies showing the presence of amphibians and other vertebrates surviving in tea plantations, it is important to consider the role that these less than perfect habitats play in the long term conservation of species richness (Brockerhoff et al. 2008; Felton et al. 2010; Kumara et al. 2004; Palacios et al. 2013). Ferreira et al. (2015) found 37 species of amphibians in a heterogeneous cacao growing area in south-east Brazil. In some cases, these small populations may act as source populations for the protected areas nearby, or the plantations may themselves act as buffer zones around protected areas to provide barriers to them from additional urban developments (Brockerhoff et al. 2008; Kumara et al. 2004). Whitfiedl et al. (2007) reported that while all common leaf-litter species declined in old-growth forests in a Selva, one frog and two lizards did not decline in an adjacent abandoned cacao plantation, and they argue that thick leaf-litter may have supported these species. While the research community is aware of the conservation roles of plantations, it is key to involve the management staff of plantations in the process of sustainable design and management of plantations (Brockerhoff et al. 2008).

Organizations like Rainforest Alliance and Conservation International Foundation have several certifications that can help these industries sell their produce at increased prices to a socially conscious market (Edwards and Laurance 2012). The term 'High Conservation Value' (HCV), initially used in the logging industry is now used to assess areas for

conversion to plantations, as well as for species of high conservation priority (Edwards and Laurance 2012). However, several managers are unaware of these schemes, and others believe that it is difficult to obtain these certifications (Mudappa and Raman 2012; Shankar and Mudappa 2003). The difficulty is the variation in each plantation type, location, harvesting methods, and the use of different chemicals that make drafting a generalized document for green certification quite challenging and often intimidating for the average plantation owner, as well as for the research community (Brockerhoff et al. 2008; Edwards and Laurance 2012; Gardner et al. 2007). The absence of information on the viable population sizes, and population health of the HCV species also makes it difficult to justify certifications (Edwards and Laurance 2012). Whatever be the methods, once large plantations in an area are able to get certified, they should be able to encourage and help small private plantation owners get certified as well.

Ryan et al. (2002) reported 14, two, and two, species of amphibians, respectively from three different standard methods (drift fences with funnel traps, cover board method and time constrained searches) in different kinds of plantation landscapes (recent clear-cut area, pine plantation, and mixed pine-hardwood forest) from South Carolina. The study showed that drift fences with funnel traps captured more species and individuals of amphibians than the other two methods (Ryan et al. 2002). Thus, it is not just the type of plantation but also the methods used in the study that can affect the diversity results.

Most researchers are limited by several constraints, both financial, temporal and logistical and thus each study is very unique in the methods used making the studies difficult to compare (Brockerhoff et al. 2008; Gardner et al. 2007). In some rare cases where old plantations are abandoned, recolonization studies have shown that with time, the

biodiversity of the area recovers but the rate of recovery can vary drastically depending on the location (Hilje and Aide 2012). Secondary forests in Costa Rica showed original species richness in 10 to 16 years, whereas in Puerto Rico the recovery took one to five years, and in Brazil only 60% of the original species diversity was recovered after 14 to 19 years (Hilje and Aide 2012). Though the recovery time in some cases may be rather long, and incomplete, it is still important to monitor the rates of recovery and recolonization of species in abandoned plantations to determine their importance for biodiversity because plantation acreage continues to increase.

There has been a continuous increase in the area that is being converted to plantations over the years (FAO 2011; FAO and JRC 2012) and this area is going to keep increasing in light of the United Nation's prediction that the human population will increase to nine billion by the end of this century. The consumption of black tea is modeled to grow at 3% annually to reach by 4.14 million tons by 2023 (Chang 2015; Gerland et al. 2014). At the end of their review, Felton et al. (2010) conclude that it is not accurate to make conclusions about whether there was higher diversity in plantations or pastures without accounting for several variations which come with each of the studies. Looking at several single studies of diversity and abundances in streamside management zones in pine forests, Jones et al. (2010) suggest that in the long run it is more important to look at fitness coefficients, not just richness of species in plantations, to gauge the actual long term effects of plantations on native fauna. Brockerhoff et al. (2008), presented several suggestions at different landscape scales for improving the presence of biodiversity in plantations. Some of the small scale suggestions included discussions about the

composition and structure of plantations, prevention of intensive site manipulations in biodiversity rich regions, increasing the intervals between harvesting, and making better local management decisions on thinning of weeds, and burning (Brockerhoff et al. 2008). Large scale suggestions include the placement of different types, sizes, and shapes of plantations in the landscape, placement of plantations around native habitats to function as buffers, as well as making plantation's in fragmented landscapes act as stepping stones between forest fragments (Brockerhoff et al. 2008). Wilcove and Koh (2010), recommended bans on converting forests to oil palm plantations, financial incentives for organic practices, fines for undesirable activities, and promotion of alternate, more sustainable methods of the local resources, a few suggestions to reduce growing negative impacts of oil palm plantations in Southeast Asia. Such information can only be obtained if long-term monitoring plans are in place. With several plantations being in biodiversity hotspots in the tropics, we need to address ways of being able to manage these plantations such that the biodiversity on these unique properties can be supported. In an innovative study from Tanzania, Norfolk et al. (2013) found that floral-dependent insects were found in much higher richness and abundance on the path between tea bushes than on the tea bushes themselves, leading them to suggest that plantation managers should encourage the wild plants that grow in-between tea bushes because they do not compete with tea. In a study comparing cultivated land with hedges and cultivated land without hedges, Nopper et al. (2016) found that corn growing land with hedges in Madagascar had three species of reptiles (*Astrochelys radiata*, *Pyxis arachnoides*, and *Phelsuma breviceps*) that were not present in adjoining forested land and were reported in the first three threat categories of the IUCN red list. With access to guidelines before designing plantations, it

is possible to make these necessary evils as sustainable as possible. The present review shows that it is hard to come up with generalized global suggestions to make plantations sustainable habitats for remnant native flora and fauna. The need of the hour is several local studies in different plantations types, targeting different groups of plants and animals such that the most appropriate long-term suggestions can be implemented in each case.

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Tables

Region and subregions	Total plantation forests ³				
	Area			Change %	
	1990	2000	2005	1990-2000	2000-2005
Africa	12 245	12 865	13 338	0.5	0.7
Asia	46 591	55 665	64 888	1.8	3.1
Europe	22 530	26 588	27 694	1.7	0.8
North and Central America	10 782	17 937	18 844	5.2	1.0
Oceania	2 447	3 491	3 865	3.6	2.1
South America	9 132	11 437	12 189	2.3	1.3
World	103 728	127 984	140 818	2.1	1.9

Table 1.1. Global area of plantation forests based on 1000 acres (FAO 2005)

glm(formula = SP ~ Harvest + SF + Age + BH, family = poisson(log))				
Number of Fisher Scoring iterations: 5				
Coefficients	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	1.4837861	0.1695084	8.753	< 2e-16 ***
Harvest[T.Periodic]	0.3287687	0.1162714	2.828	0.00469 **
SF	0.031625	0.0032394	9.763	< 2e-16 ***
Age	0.0002845	0.001343	0.212	0.83223
BH[T.Y]	-0.0048103	0.121556	-0.04	0.96843

Table 1.2. Generalized Linear Model (GLM) for the number of species in plantations (SP) being described by the type of harvest (Periodic), number of species in the control forest (SF), age of plantation, and whether the plantation is present in a Biodiversity hotspot (BH) or not.

Sno	IUCN Category	Endemic	Name	Eucalyptus	Shola	Tea
1	Critically Endangered	Y	<i>Raorchestes chlorosomma</i>	+	+	-
2	Critically Endangered	Y	<i>Raorchestes munnarensis</i>	-	+	+
3	Critically Endangered	Y	<i>Raorchestes griet</i>	+	+	+
4	Critically Endangered	Y	<i>Rhacophorus pseudomalabaricus</i>	+	-	+
5	Critically Endangered	Y	<i>Raorchestes sushili</i>	-	+	-
6	Endangered	Y	<i>Rhacophorus calcadensis</i>	+	-	+
7	Endangered	Y	<i>Indirana</i> sp	+	+	-
8	Vulnerable	Y	<i>Raorchestes dubois</i>	+	+	+
9	Vulnerable	Y	<i>Duttaphrynus microtympaum</i>	+	-	+
10	Near Threatened	Y	<i>Duttaphrynus parietalis</i>	+	-	+
11	Near Threatened	Y	<i>Raorchestes beddomii</i>	+	+	+
12	Near Threatened	Y	<i>Raorchestes theuerkaufi</i>	-	+	+
13	Near Threatened	Y	<i>Micrixalus</i> sp1	-	+	-
14	Near Threatened	Y	<i>Micrixalus</i> sp2	-	+	-
15	Near Threatened	Y	<i>Uperodon species (cf montana)</i>	-	+	+
16	Least Concern	N	<i>Duttaphrynus melanostictus</i>	+	+	+
17	Data Deficient	Y	<i>Fejervarya</i> sp	+	+	+
18	Data Deficient	Y	<i>Ghatixalus asterops</i>	-	+	+
19	Not Evaluated	Y	<i>Raorchestes uthamani</i>	-	+	-
20	Not Evaluated	Y	<i>Raorchestes jayarami</i>	+	-	+
21	Not Evaluated	Y	<i>Raorchestes kadalarensis</i>	+	+	+
22	Not Evaluated	Y	<i>Nyctibatrachus poocha</i>	-	+	+
23	Not Evaluated	Y	<i>Nyctibatrachus acanthoderms</i>	+	-	-
24	Not Evaluated	Y	<i>Beddomixalus bijui</i>	-	-	+
25	Not Evaluated	Y	<i>Ghatixalus magnus</i>	-	+	-
	Totals	All except 1		14	18	17

Table. 1.3. Checklist of amphibians recorded in different habitats (eucalyptus, sholas, and tea) in tea plantations in Munnar, Kerala, India according to the IUCN threat status (Endemic to Western Ghats; Y=yes, N=no; IUCN 2016).

Figures

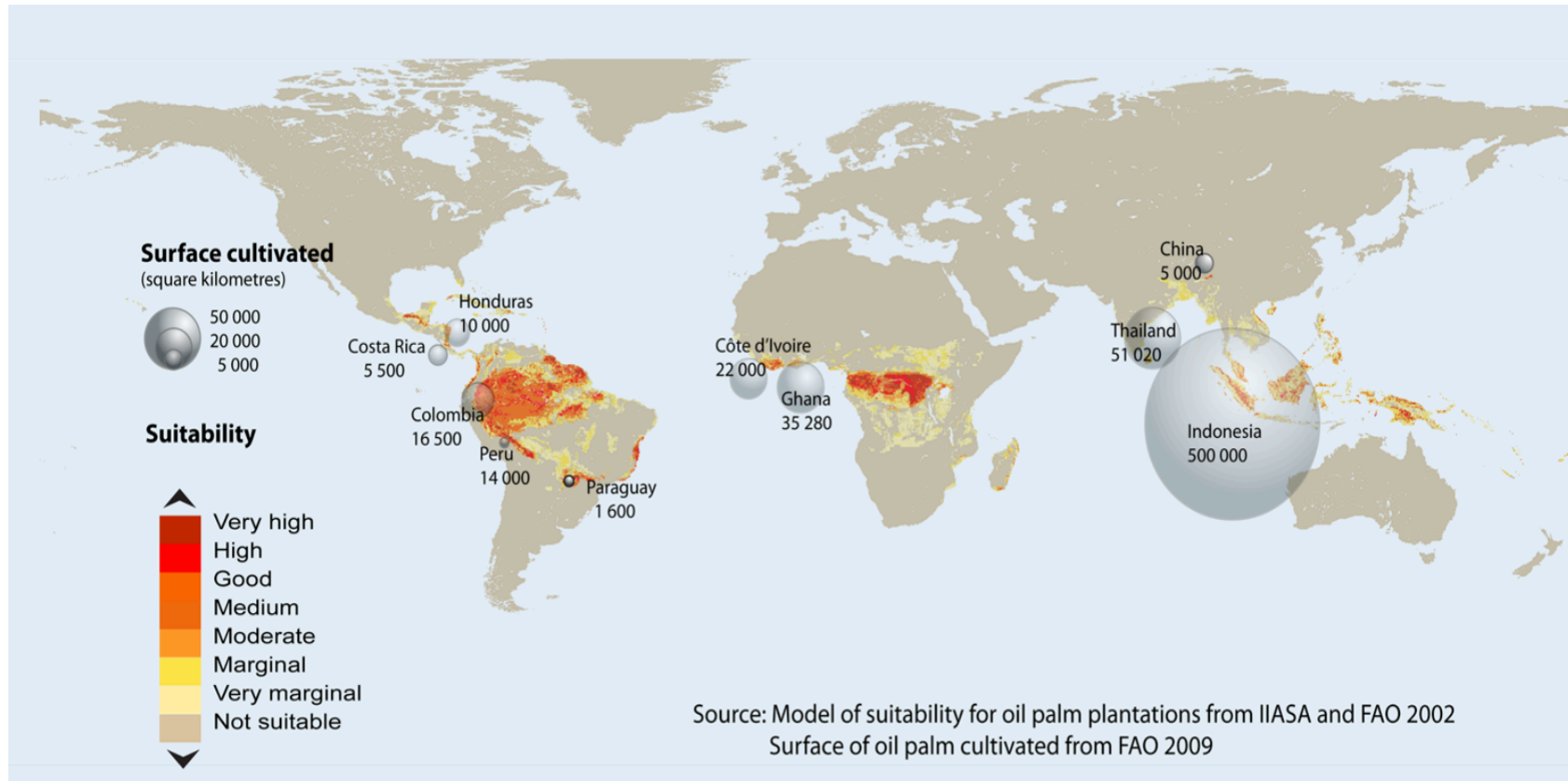


Figure 1.1. Land suitable for palm oil plantations (IIASA, 2002; FAO 2009)



Figure 1.2. Location of field site (white dot) within the Western Ghats and Sri Lanka Biodiversity hotspot in, Munnar, Kerala. (Map from IUCN, 2016)

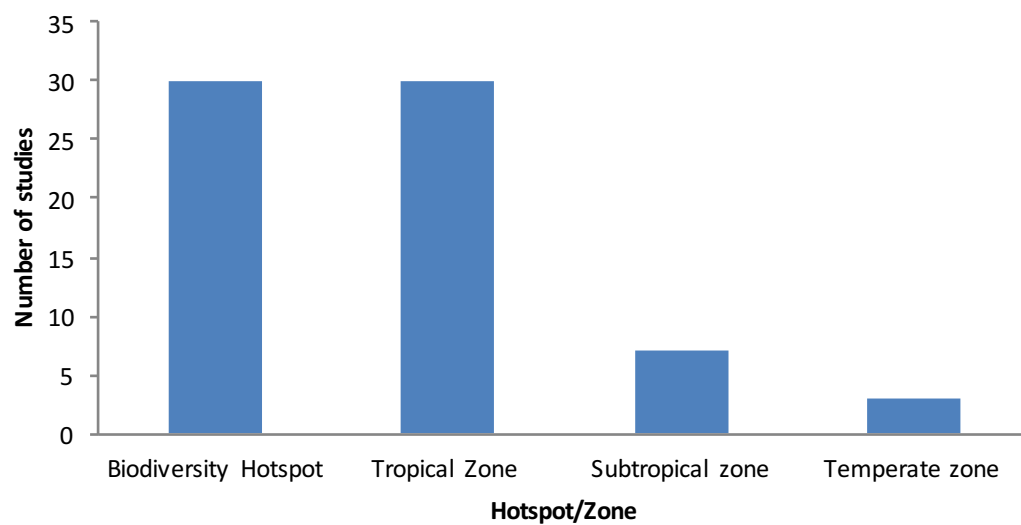
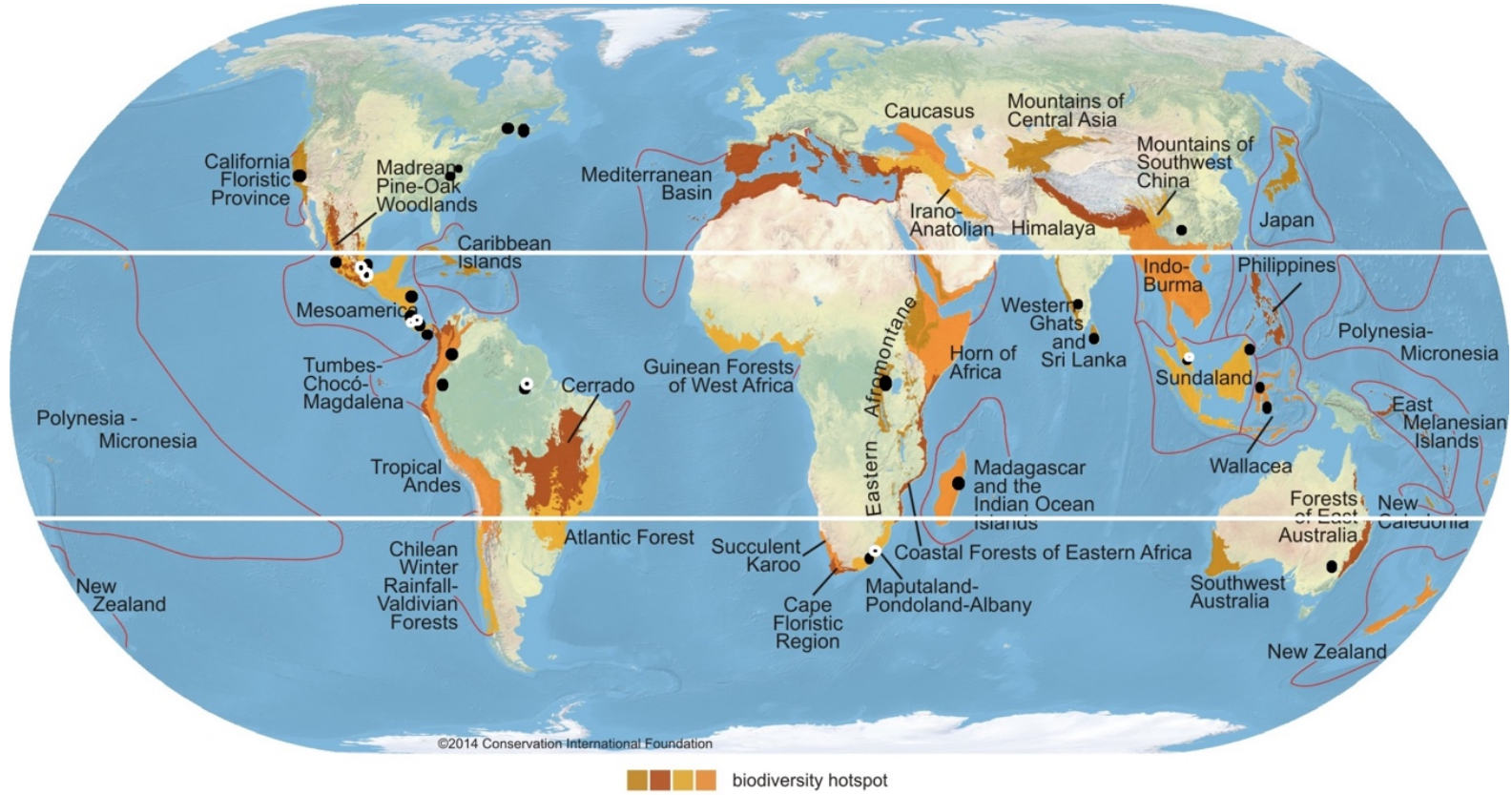


Figure 1.3. Number of studies in each latitudinal zone.



Conservation International (conservation.org) defines 35 biodiversity hotspots — extraordinary places that harbor vast numbers of plant and animal species found nowhere else. All are heavily threatened by habitat loss and degradation, making their conservation crucial to protecting nature for the benefit of all life on Earth.

Figure 1.4. Location of all the 40 study sites (Conservation International Foundation, 2014).

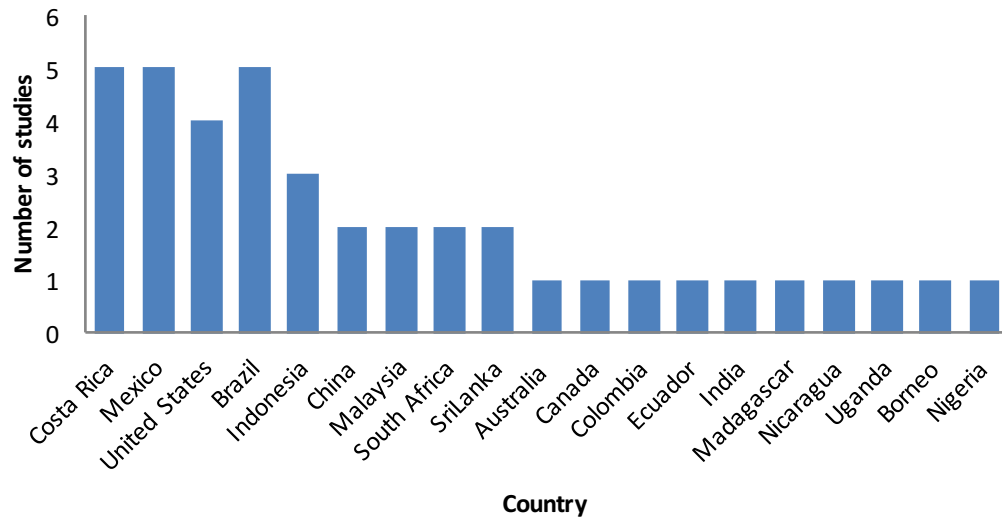


Figure 1.5. Number of studies in different countries of the world (N=40).

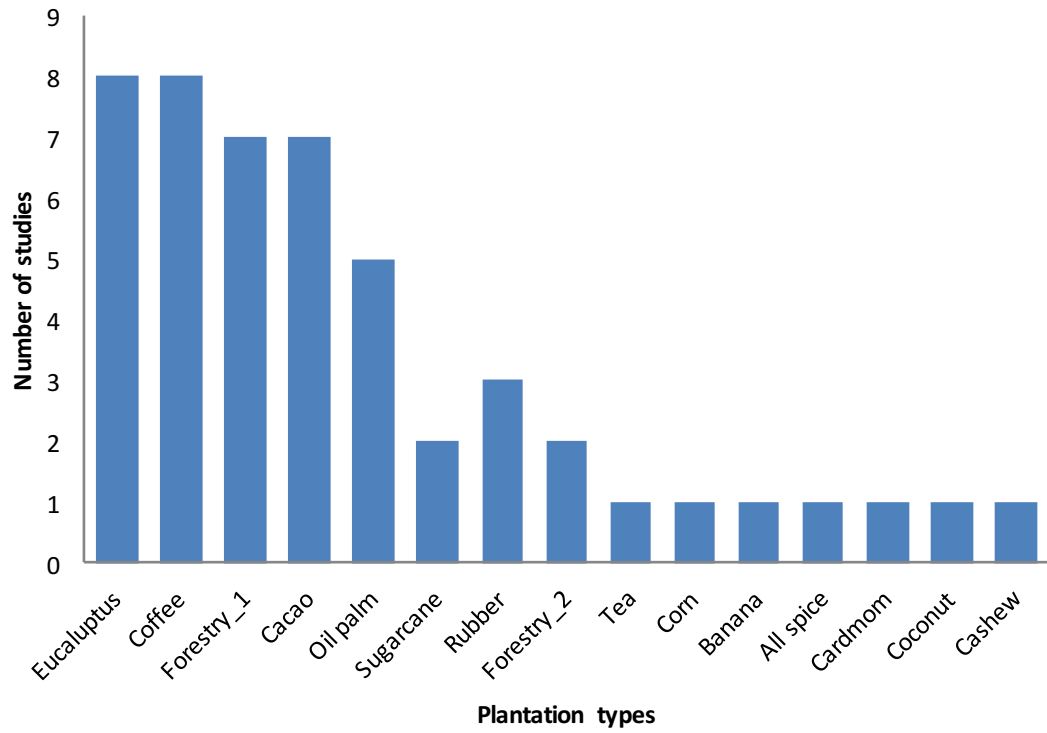


Figure 1.6. Types of plantations with comparisons of amphibians between plantations and primary forests.

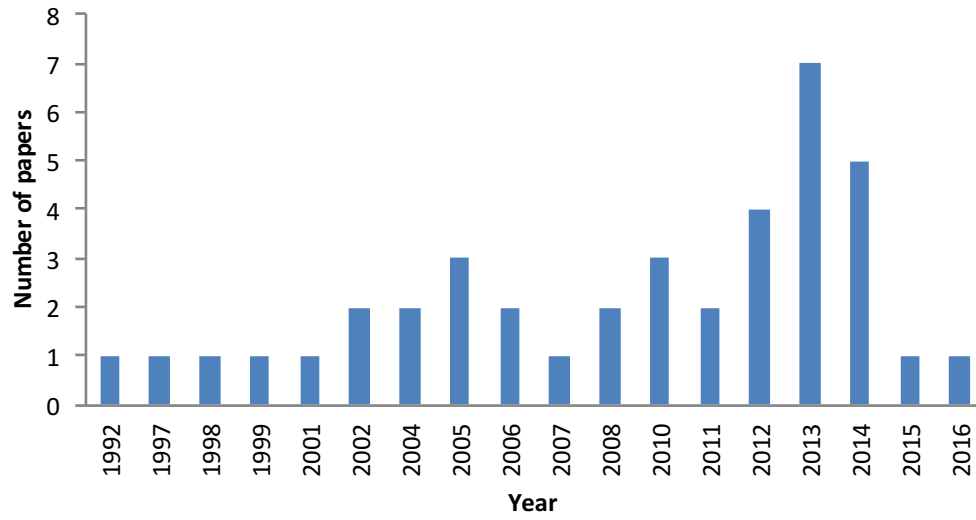


Figure 1.7. Number of papers published by year.

Appendices

Appendix 1.1. Forty papers used for the review in chapter 1.

- Behm, J.E., Yang, X. and Chen, J., 2013. Slipping through the cracks: rubber plantation is unsuitable breeding habitat for frogs in Xishuangbanna, China. *PloS One*. DOI:org/10.1371/journal.pone.0073688.
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Appendix 1.2. Information from 40 studies used for the review in chapter 1 (Y:Yes; N: No; S_P: Number of species in dominant plantation; S_F: Number of species in forest).

Sno	Publication_Year	Initial_clearing_age	Age	Plantation_type	Country	Biodiversityhotspot	Harvesting	S_P	S_F	Difference
1	2014	1980	34	Oil_palm	Colombia	Y	Complete	9	9	0
2	2014	1950	64	Cacao	Costa Rica	Y	Complete	11	13	2
3	2014	1990	24	Eucalyptus, Sugar Cane	South Africa	N	Complete	6	23	17
4	2014	1970	44	Oil_palm	Malaysia	Y	Complete	4	6	2
5	2014	1950	64	Coffee	Costa Rica	Y	Complete	5	22	17
6	2013	2000	13	Cacao	Ecuador	Y	Complete	4	14	10
7	2013	1970	43	Rubber	China	N	Complete	2	5	3
8	2013	1980	33	<i>Pentaclethra macroloba</i> , <i>Virola koschnyi</i> , <i>Vochysia guatemalensis</i>	Costa Rica	Y	Complete	31	66	35
9	2013	1890	123	Coffee	Mexico	Y	Complete	4	4	0
10	2013	1970	43	Oil_palm	Malaysia	Y	Complete	12	9	-3
11	2013	1974	39	Pine	Uganda	N	Complete	7	6	-1
12	2013	1890	123	Coffee	Mexico	Y	Complete	7	8	1
13	2012	1960	52	Eucalyptus, Sugar Cane	South Africa	Y	Complete	10	8	-2
14	2012	1960	52	Oil_palm	Indonesia	Y	Periodic	0	0	0
15	2011	1970	41	Pine	SriLanka	Y	Periodic	7	11	4
16	2012	1950	62	<i>Lophostemon confertus</i> (Brisbane box)	China	Y	Periodic	4	9	5
17	2012	1890	122	Eucalyptus, Tea	SriLanka	Y	Periodic	17	12	-5
18	2011	1960	51	Coffee, Corn	Mexico	Y	Periodic	14	36	22
19	2010	1990	20	Cacao	Indonesia	Y	Periodic	11	18	7
20	2010	1970	40	Eucalyptus	Brazil	N	Periodic	7	8	1
21	2008	1970	38	Eucalyptus	Brazil	N	Periodic	13	17	4
22	2008	1970	38	Coffee	Costa Rica	Y	Periodic	22	22	0
23	2006	1960	46	Eucalyptus	Brazil	N	Periodic	13	17	4
24	2006	1999	7	Allspice	Nicaragua	Y	Periodic	17	29	12
25	2005	1850	155	Coffee, Cardamom	India	Y	Periodic	1	17	16
26	2005	1970	35	Cocunut, Cacao	Indonesia	Y	Periodic	7	12	5
27	2004	1950	54	Coffee	Mexico	Y	Periodic	2	6	4
28	2004	1932	72	Pine	Australia	Y	Periodic	2	7	5
29	2002	1850	152	Eucalyptus, Rice	Madagascar	Y	Periodic	14	15	1
30	2002	1910	92	Eucalyptus	Uganda	Y	Periodic	5	22	17
31	2001	1967	34	Pine	Uganda	Y	Periodic	15	15	0
32	1999	1970	29	Black Spruce	Canada	N	Periodic	6	8	2
33	1998	1970	28	Conifer	Uganda	N	Periodic	6	10	4
34	1997	1940	57	Pine	Uganda	N	Periodic	13	21	8
35	1992	1950	42	Cacao	Costa Rica	Y	Periodic	16	19	3
36	2015	2000	15	Oil palm	Borneo	Y	Periodic	11	19	8
37	2007	1950	57	Cacao	Brazil	Y	Periodic	14	17	3
38	2016	1887	129	Cacao	Nigeria	N	Periodic	19	26	7
39	2005	1880	125	Coffee	Mexico	Y	Periodic	13	16	3
40	2010	1950	60	Rubber	Brazil	Y	Periodic	26	39	13

III. CHAPTER 2

A Comparative Study of Standard Methods for Surveying Anurans in Tea Plantations in the Southern Western Ghats

Abstract

Global amphibian declines have been reported since the late 1980s and some major drivers of these declines are reported to be habitat loss and the emergence of chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis*. While amphibian species face declines globally, several new species of amphibians are being described in many parts of the world, especially in Asia. The Western Ghats and Sri Lanka Biodiversity Hotspot is a zone of high endemism for amphibians, but is also a region that is affected by increasing human population pressures as forestlands are being converted to agricultural areas. Here, I test the efficiency of standard methods of surveying amphibians in active tea plantations in the Indian state of Kerala. Visual encounter surveys, stream transects, and drift fences with funnel traps were tested in three main habitats, tea fields, sholas (remnant forest patches), and eucalyptus forests. A total of 407 individual frogs from 17 species (plus one category for unidentifiable *Raorchestes* juveniles) were encountered from June to November, 2012. Drift fences with funnel traps were discarded after we had four captures from two species after 2016 trap hours (84 trap nights). Stream transects in shola habitats gave the highest species per person effort, followed by Visual Encounter Surveys in eucalyptus patches. The greatest number of individuals per person-effort were obtained in the shola habitat. We recommend a

combination of nocturnal Visual Encounter Surveys and Stream surveys to sample for anurans in the tea plantation landscape of the Southern Western Ghats, India.

Key words: Amphibians, Standard methods, Tea plantations, Western Ghats

Introduction and Background

Global amphibian declines have been the central theme of many conferences and symposia since the phenomenon was described in the late 1980s (Hayes et al. 2010; Lips 1998). Some driving factors of these declines have been identified as fragmentation and loss of habitat, pollution and increase in pesticide use, infection by chytrid fungus and other diseases, dam and water management systems, ultraviolet β radiation, and the introduction of alien species (Alford and Richards 1999; Bickford et al. 2010; Blaustein and Kiesecker 2002; Cushman 2006; Hayes et al. 2010; Nowakowski et al. 2016; Stuart et al. 2004; Whitfield et al. 2007; Whitfield et al. 2013). Scientists are now referring to this period of declines and extinctions as the sixth global mass extinction event, and several direct anthropogenic effects are being compounded by global warming which is difficult to study (Wake and Vredenburg 2008). Rising temperatures in upland habitats are hypothesized to create conditions which are favorable for the growth of chytrid in areas that were historically not conducive for the fungus, especially high mountainous habitats which were historically too cold for *Batrachochytrium dendrobatidis* (Bd) (Pounds et al. 2006). Amphibians, many with a biphasic life history, have permeable moist skin and diverse modes of reproduction (Duellman and Trueb 1986). These animals function as natural biological indicators, and their declines signal possible declines in

local environmental health (Daniels 1991; Lips 1998; McDiarmid 1994). The fungus *Batrachochytrium dendrobatidis* (Bd) has been identified as being responsible for a large number of mass amphibian die-offs in Australia and Central America (Berger et al. 1999; Lips 1998; Pechmann et al. 1991). When faced with such declines, although several short-term studies have been completed, it is imperative to have long-term monitoring projects of amphibians in place to enable sound conservation decisions derived from population trends of local species (Storfer 2003). Papers on amphibian ecology and conservation are on a rise and this is a very healthy sign, however, not all regions of the globe have been studied to the same extent as those in North America and Central America (Ficetola 2015; Whitfield et al. 2007). Several regions of high species endemism are unfortunately the least studied. For example, we have little long-term ecological information regarding amphibians of the Western Ghats in India where scientists are still actively describing new species.

The Western Ghats (WG) are a part of the Malabar biogeographic region (Gadgil 1996; Rodgers and Panwar 1988). The WG form a continuous chain of small to medium-sized tropical mountain ranges running north to south nearly 1600 km, along the west coast of southern India, from 8° to 21°N. The elevation is moderate compared to the Himalayas to the north; the hills generally vary between 600 and 1000m asl elevation. The Western Ghats are hypothesized to have originated as the Indian plate was moving over the volcanic Deccan trap region (Bossuyt and Milinkovitch 2001; Briggs 2003; Karanth 2006; Radhakrishnan 1993). It is believed that the volcanic eruptions of the Deccan trap lead to the extinction of several old world species (Cretaceous–Tertiary extinction) and

played a major role in the evolution of the biota of the Western Ghats (Bossuyt and Milinkovitch 2001). Bossuyt and Milinkovitch (2001) suggest that after the Deccan trap eruptions, the isolation of the Indian plate resulted in major speciation events. Ecological studies of this rich diversity has shown that the amphibian composition can vary greatly between two hills (separated by <100km) in the same mountain range and the number of amphibian species increases as one moves south along the Ghats towards the equator (Daniels 1992).

The isolated hills of the Western Ghats may function as sky islands that support high levels of endemism in the biota. They have been hypothesized to serve as centers of speciation for amphibians, orchids and balsams (Aravind et al. 2007). Sky islands are isolated terrestrial islands that have varying degrees of connectivity (Robin et al. 2011; Warshall 1994). Robin et al. (2011) found that populations of White-Bellied Shortwing birds from different 'sky islands' in the Western Ghats varied in their songs. The populations of the White-bellied Shortwing (*Brachypteryx majoron*) on different mountains tops, as well as, recently isolated populations (as a result of deforestation) showing similar variations in their calls, that suggest that mountains of the Western Ghats act as isolated units allowing for speciation by geographical isolation (Robin et al. 2011). It might be expected that amphibian communities in the Western Ghats may be affected to a much greater extent by the ecosystem connectivity because of their limited dispersal capability. Watling and Donnelly (2006), after reviewing fragmentation studies that used the Equilibrium Theory of Island Biogeography to analyze the effects of fragment size and species richness, found that the effect of isolation was not studied in most cases.

They observed that species on isolated mountain tops seemed to be more severely isolated than species in other patchy habitats, irrespective of the patch size (Watling and Donnelly 2006). Watling et al. (2011) found strong effects of the matrix composition in a meta-analysis on 184 different animal taxa found in 283 fragmented landscapes.

The vegetation of the Western Ghats varies from dry deciduous and deciduous rain forests, to montane and evergreen forests, with short stunted forests also called ‘sholas’ or ‘stunted forests’ and grasslands at high elevations (>1000m; Krishnamurthy 2003; Thomas and Palmer 2007). It might be expected that the amphibian biodiversity of the Western Ghats is influenced by variation in forest types, matrix type, altitude, latitude, the degree of isolation of each shola forest patch on an already isolated sky island and high overall species diversity could also be driven by high beta diversity (Watling and Donnelly 2006; von May 2009). The Western Ghats region in the ‘Western Ghats and Sri Lanka Biodiversity Hotspot,’ is home to over 384 amphibians, approximately 80% of which are endemic (Frost 2016; Mittermeier et al. 2011). Of these amphibian species from India, approximately 56% are found in the Western Ghats and more than 73 new species of amphibians have been described from India in the last 5 years (Figure 2.1; Dinesh et al. 2015a; Frost 2016). The number of new species continues to rise by the month (Figure 2.1; Frost 2016).

The endemism in the ‘Western Ghats and Sri Lanka Biodiversity Hotspot’ has been typically attributed to the subcontinent’s geographical history of long-term isolation (Aravind et al. 2007; Bocxlaer et al. 2009; Dutta et al. 2004; Gunawardene et al. 2007).

Phylogenetic studies also provide evidence of “back dispersal” of a single lineage of tree frogs from the island of Sri Lanka back to mainland India (Bossuyt et al. 2004). Of all the biodiversity hotspots in the world, the Western Ghats and Sri Lanka hotspot ranks the highest in human population density, with the Philippines, Caribbean, and Madagascar hotspots following the Indian site (Cincotta et al. 2000). The high anthropogenic pressure and the alarming rate of habitat loss in India are reason enough to monitor and to protect the biodiversity of this region (Gunawardene et al. 2007). Over 25.6% loss of forest cover has been reported in the states of Karnataka, Kerala, and Tamil Nadu from 1973 to 1995 (Gunawardene et al. 2007). However, in case of amphibians even with all these dramatic threats to the taxon, the rate of discovery of new species remains an increasing function because of the recent focus on amphibian research in India (Dinesh et al. 2015a; Frost 2016). Our knowledge regarding the distribution patterns, abundances and ecological interactions of these newly described species is still lacking (Aravind et al. 2007). Very few long-term community-level studies of amphibians have been conducted in India, and the present study focused on identifying the best standard surveying method for amphibians in the tea plantation landscape of the southern Western Ghats in Kerala, to identify methods used be in a comparative study of sites across the tea plantation landscape.

Replacing natural vegetation with crops has been a part of regular landscape dynamics ever since humans ended their nomadic lifestyle and started to farm in 9000 BC (Gignoux et al. 2011). Global deforestation rates are around 13 million hectares per year and most of this is concentrated in the tropics (Bremer and Farley 2010). Simultaneously, the

annual rate of change of planted forests in the world has increased by 2.09% in the past 10 years, up from 1.88% in the decade before (FAO 2011a). Of late however, over 48% of the global population lives in urban settlements, with several countries like the US and the UK, having long surpassed this statistic (Miller 2005). A lack of connection with the natural environment, because of life in urban settlements may drive a lack of empathy towards conservation (Miller 2005). With increasing human population size comes an increase in the demand for resources like food, timber, oil, and coffee and these demands are likely to increase in the future. Conversion of natural habitats to plantations cannot be prevented but ideally this conversion should inflict the least possible damage to the natural environment (Bali et al. 2007; Gardner et al. 2009; Wanger et al. 2010). Urbanization reduces unique habitats worldwide and encourages common species to thrive in altered conditions (Miller 2005).

Amphibians occupy intermediate positions in food webs because they act as both predator and prey. They primarily consume arthropods that in the brown food webs and they are consumed by larger animals, thus forming a connecting link in the food web (Reider et al. 2013). Gardner et al. (2007b) conducted studies on the effect of primary, secondary, and plantation forests on herpetofaunal diversity in the Brazilian Amazon and the results suggest that secondary forests might be able to provide habitats that can sustain a highly diverse community hosting up to two-thirds the amphibian diversity in the primary forests. There are several studies on amphibians in plantations; some showing an increase in species richness with fragmentation (Tocher et al. 1997) and others showing decreases (Gardner et al. 2007a; Hanlin et al. 2000; Vallan 2000); only

two of these studies were conducted in India (Daniels 2003; Pawar et al. 2004). In his short study (six days in the wet season and four days in the dry season) Daniels (2003) reported 13 species of amphibians from a single tea estate; which include both seasonal and explosive breeding anurans (see Table. 2.2). The fact that such a short study revealed the presence of such a diverse anuran fauna suggests that the plantation landscape in the region provides some habitat for frogs. Ryan et al. (2002) in an attempt to monitor the herpetofauna of a managed ecosystem found that different sampling methods identified different species. Ryan et al. (2002) demonstrated that the study of amphibians and reptiles in plantations or other agro-ecosystems, requires pilot studies to choose the most appropriate sampling techniques. The results from Ryan et al. (2002) were a part of the motivation for the present study. In a recent study derived from 14 different global climate models (including data for 5527 species of amphibians), areas with the highest rates of amphibian decline, overlapped with regions most strongly affected by climate and land use changes (Hof et al. 2011). The fact that land-use change was more significant than effects of disease shows that habitat modification still remains the prime driver of amphibian decline. Apart from the direct effects on diversity, plantations also result in the fragmentation of the landscape, which affects the connectivity of populations (Becker et al. 2007). In case of vertebrates that cannot escape disturbed areas because of the limited capacity for dispersal, it is important to understand that disruptions, such as fragmented habitats can have strong effects on gene flow of species. In a detailed study trying to explain population gene flow patterns by landscape changes over time, Nowakowski et al. (2015) found that the genetic variation in the leaf-litter species *Craugastor bransfordii* was strongly explained by the prevalent landscape structure at the

time of the study, while the genetic variation in another leaf-litter amphibian, *Oophaga pumilio*, was explained partially by a combination of isolation and old barriers. The realized permeability of similar habitats was different in each of these species and the authors placed emphasis on this as a factor that makes gene flow very species specific (Nowakowski et al. 2015). In another study, Becker et al. (2007) observed that species with aquatic larval stages seemed most affected by fragmentation.

While keeping track of species, presence/absence data are just one type of data.

Information on the abundances of the species using methods that are comparable are extremely important to be able to track the health of populations over time (Campbell and Christman 1982; Hayek 1994; Mengak and Guynn 1987; Rodda et al. 2001; Stoffer 2003). Each standard method has several assumptions and it is important to ensure that the underlying assumptions are met, or addressed while interpreting the data (Heyer et al. 1994). There are several standard methods described for sampling amphibians that are developed by studies in certain parts of the world do not function with the same efficiency globally (Storfer 2003). Other methods can be successful implemented in different places, for example, Pearman (1997) in Ecuador, Doan (2003) in Peru and Donnelly et al. (2005) in Guyana.

The availability of resources such as capital, human power, terrain, and the species in question, all affect the success rates of the methods used (Doan 2003; Storfer 2003; Donnelly et al. 2005). A 2001 study conducted in Brazil comparing the Large Plot Method (LPM) versus the Small Plot Leaf Removal (SPLR) method, found that the

former method underestimated the diversity of the region though the SPLR method is known to be a 'quasi destructive' method (Rocha et al. 2001). The need to understand the short-comings of a method is well exhibited by Bridges and Dorcas (2000), when the usual post midnight to dawn calls of the species *Rana sphenocephala* during the month of July were noticed. The call of the species was completely missed when using standard methods that were stopped at midnight (Bridges and Dorcas 2000). Donnelly et al. (2001) were unable to use the pit fall traps with a drift fence method for several months because the elevated water table pushed the buckets out of the ground during El Niño flooding. In a study comparing random quadrats and adaptive cluster sampling in the Southern Western Ghats, Vasudevan et al. (2001) found that the latter gave density estimates which were almost three times of that using the random quadrat method. In certain areas a combination of methods need to be used to find species that occupy very different niches, since they cannot all be encountered by using a single method (Ryan et al. 2002). Some methods are more efficient than others, and depending on the habitat in question and the terrain of the study site (Doan 2003). When a mix of transects and pitfall traps were used to study amphibians in Mississippi, it was seen that no single method represented the diversity of the region as well as all methods combined (Fogarty and Jones 2003). However, the pit fall trap captures were predated upon by raccoons, which may have lead to an underestimation of the diversity in some sites (Fogarty and Jones 2003).

In India, Vasudevan et al. (2006) reported a high turnover of amphibian species in the Anamalai hills of the southern Western Ghats, with about 40 species each in neighboring hill ranges separated by >2 degrees of longitude, while the total regional species richness

of the landscape was 130 species. Naniwadekar and Vasudevan (2007) reported that intense transect surveys and Visual Encounter Survey's accounted only for 49% and 83% of the species documented in the study site in Kalakad Mundanthurai Tiger Reserve, Tamil Nadu, India. They also reported that the alpha diversity increased with elevation and that there were more than two peaks of diversity, when considering gamma level diversity. Sung et al. (2011) in a study in Hong Kong used cover-boards, pitfall traps and transects to survey herptofauna and found that transects were better for a quick diversity count while combinations of methods gave the largest number of animals. It is important to find the best methods for an area to maximize the use of available resources. Sampling methods must provide data that answers questions effectively. If an accurate species list is the goal then all the methods including opportunistic observations, must be employed. Ideally one has a large number of methods with high returns for investment in costs of supplies and and human resources

The present study tests the efficiency of established standard methods in a tea plantation landscape in the southern Western Ghats of India. In this chapter I describe the amphibian community in three different habitats, shola forests, eucalyptus forests, and tea, within a large tea plantation landscape using Visual encounter surveys (VES), stream transects (ST), and drift fences with funnel traps (DF). The site mimics a classic terrestrial island-matrix fragmented landscape (Gardner et al. 2007b; Watling and Donnelly 2006). I predict the native shola patches in the area will have the highest richness and abundance of anurans species. I also predict that the VES method will be the most efficient method, as it is a popular method used successfully across different

habitats globally. Since tea cultivation is important across India and across the world, my study provides important background information regarding sampling amphibians in this large agro-ecosystem, from a site in a biodiversity hotspot.

Study Area

This study was conducted near the town of Munnar, in the state of Kerala in the Southern Western Ghats of India. Kerala is an important producer of tea in South India accounting for 8.5% of the total production of India. Munnar falls under the Devikulam Forest Division (Figure 2.2). The word ‘Munnar’, translates into three rivers; Kadalar, Nallathanni Aar, and Kallar. The Munnar mountain range in the Munnar Forest Division has over 106 km² of reserve forest, most of which is interspersed among tea plantations. The elevation varies from 600m asl to 2695m asl which is on Anamudi (Elephant Peak), the highest peak in peninsular India. Though present in the tropical zone, the climate in this area is moderated by altitude. Munnar receives rainfall both during the Southwest and the Northeast monsoons. The Southwest monsoons account for over 80% of the annual precipitation and occur during June and July, and the rest of the precipitation is received during Northeast monsoon which from October to November (Figure 2.3). The minimum temperature in the last three years was 0°C and the maximum temperature in the last three years was 29°C (Figure 2.4). The humidity in these areas is high year around, usually above 80%. The area supports wet evergreen forest, semi-evergreen forest, moist deciduous forest, dry deciduous forest, broad-leaved hill forest, montane wet temperate forest, and grasslands according to the revised classification (Champion and Seth 1968; Thomas and Palmer 2007) . I conducted surveys in the Kanan Devan Hills

Tea Plantation Company Private Limited (henceforth KDHP; 10° N and 77° E) located in the Idukki district, of Kerala, in the Southern Western Ghats. Tea plantations in this area were originally established in 1877, after John Daniel Munro leased the land from the Poonjar Chief without any time stipulation (KDHP Management Plan 2002) and have been actively harvested ever since. The Kanan Devan Hills Tea Plantation Cooperation Private Limited was established in 2005 and is a 238 Km² holding, 98 Km² of which is under tea cultivation (Figure 2.3). The plantation holdings include 27 tea estates under the same management plan. The other major habitat types, in addition to tea are eucalyptus patches and remnant patches of shola forest. The plantation shares borders with the Eravikulam National Park (established in 1978), a 100km² tract of undulating highlands, including Anamudi, that was originally a part of the tea plantation (KDHP Management Plan 2002). The company donated the land to the Government of India to create a park to protect the critically endangered Nilgiri Tahr, an endemic mountain goat (KDHP Management Plan 2002; Thomas and Palmer, 2007). Other tea plantations in the area are owned by American Direct Tea Trading Cooperation Limited, Thalayar Tea, The Malayalam Plantation Limited, and some private plantation holdings.

The KDHP plantation was chosen, because it was the single largest land holding in the region. In tea plantations, tea bushes are maintained at a height of approximately 1.5m to facilitate hand picking and they are planted 50cm from each other in rows. My study was conducted in the Kannaimallai and Perriavarai plantations approximately 7 km East of Munnar (Figure 2.2.). Drift fences made of plastic sheeting (8m long) with wire funnel traps, nocturnal visual encounter surveys, and stream transects were tested at sites located

at elevations between 1500 and 2000 m asl to avoid elevation effects. The southern montane temperate forests (shola forests) interspersed within tea fields were one of the focal habitats of my study. These natural forests usually grow in shallow mountain valleys that are protected from harsh climatic events. The trees are seldom taller than 10m and they are stunted forests. The families of trees represented includes Lauraceae, Rubiaceae, Symplocaceae, Myrtaceae, Myrsinaceae, and Oleaceae (Bunyan et al. 2012; Champion and Seth 1968). Sholas forests are usually found above 1500m asl. It is mostly forests of this type along with grasslands between the shola that were cleared to establish tea plantations over 130 years ago. There are two schools of thought regarding the presence of these high elevation grasslands: 1) they are the climax ecosystem in the area and 2) they are a pre-climax ecosystem that is limited by fire and frost (Bunyan et al. 2012; Thomas and Palmer 2007). At elevations above 1800m, most of the land that is not covered by rock faces is covered by grasslands. Almost 80 Km² of this area has been converted into fuel wood stands of eucalyptus which are harvested every seven years. The most common tree used for fuel is eucalyptus which in addition to being used to dry tea during its processing is also used as fire wood by the tea picking community.

Methods

This study was focused on anurans. Standard animals handling protocols were approved by Florida International University's Institutional Animal Care and Use Committee (IACUC-12-004 #A3096-01). Four standard methods (Diurnal and Nocturnal Visual Encounter Surveys, drift fences with funnel traps, and VES along stream transects) were

used to sample anurans and build a species list for the tea estate. For all the methods, except the funnel traps, animals were captured by hand or by small aquarium net to ensure that the animal was exposed to minimal stress. Upon capture, frogs were identified to species or morphospecies, measured (snout-to-vent length in mm) with a plastic field calipers (SPI Plastic Dial Caliper Model 31-415-3), weighed by placing the animal in a clean plastic bag and weighing it with a Pesola scale (Pesola® LightLine Spring Scales, 10g and 30g) to the nearest mg and released at the middle of the transect (Crump and Scott 1994). Any species encountered off transects were added to the species list to make it complete, but these opportunistic data were not used in any statistical comparisons. Detection probability was maintained uniformly by having the same two observers work during the period of the study and alternating the order among transects. Spatial autocorrelation was avoided by sampling habitats which were not contiguous with each other and by using different streams. The experimental design ensured that transects in each habitat type (tea, shola, eucalyptus, and streams) were sampled in a single night to avoid bias of anuran detection because of differences among nights. Data from nights during which sampling was interrupted because of heavy rain or dangerous animal encounters (elephants and gaurs) were discarded from the analysis. Four replicates were established for each of the habitats and each of these were at least a km apart (Figure. 2.5). Data were collected during the monsoons from June to November, 2012.

1.1 and 1.2 Nocturnal and Diurnal Visual Encounter Surveys (VES): These are standard methods used for sampling amphibians and reptiles which are found on the ground and on bushes and trees. I set up 25m long transects in all the three habitat types (sholas, tea and eucalyptus). The transects were marked at every 5m with flagging tape. The VES

method involved two people walking along the transect and searching carefully for frogs, 1m on either side of the transect line and up to 2m above the ground for 15 minutes (Crump and Scott 1994). The transects were conducted during the day (09:00hrs-12:00hrs) and the night (19:00hrs-00:00hrs using Black Diamond Icon Headlamp). The start of each transect within a particular habitat was at least 5m away from a different habitat type.

1.3 Stream sampling with Nocturnal VES: Stream transects were set up in the habitats for 25m in both tea and shola habitats. The transect were at marked every 5m using flagging tape on vegetation in the riparian zone. The VES method involves two people walking in the middle of the stream searching carefully for frogs, 1m on either side of the transect line and up to 2m above the ground for 15mins (Crump and Scott 1994). The transects were sampled between 09:00 hrs-12:00hrs and 19:00hrs-00:00hrs using headlamps for nocturnal surveys. Each stream was independent.

1.4 Drift Fence with Funnel Traps: This method involves placement of 8m long plastic barrier fences with funnel traps on each end to capture amphibians moving through the habitat. The method is free of investigator bias (detection is equivalent for all captures) and generates a catch-per-unit-effort value for analysis of the number of captures per trap hour (Corn 1994). In some cases, drift fences are the best method for surveying sites and capturing secretive amphibians (Corn 1994). When animals contact the plastic fence they can turn completely around and walk away but often animals walk along the fence and enter the funnel trap at the end of the fence. The fence made from three plastic sheets, 8m long and three funnel traps were placed on each end (Corn 1994). Four replicates of drift fences with funnel traps (Figure 2.6) were deployed in the three different habitats and

trapping was conducted for seven consecutive nights, which is a total of 84 trap nights. The effort to set up these traps was high and it took eight person hours (two people working for four hours) to set up each array and as well as, hiking up twice a day regularly to the traps to check for captures was also quite high.

Analysis and Results

The lowest temperature recorded in the area in 2012 was 1°C during a few days in February and the highest temperature recorded was during the month of May and was 28°C. The area is reported to have a few days of frost during the month of January or February. The Southwest monsoons arrived on the 3 June as predicted by the Meteorological Department of India. The average of minimum and maximum temperatures for the study site is plotted in Figure. 2.3. The area received 3859.02 mm of rainfall in the year when the study was conducted. The rainfall received in 2012 lies between the usual average (add year here) of rainfall received annually in the area. Of this 2848.86 mm was received in the months of June, July, and August during the South West Monsoon, in 2012. Therefore, 73.8% of the annual rainfall was received in just three months (Figure 2.4).

A total of 407 individual frogs from 17 species. Since I could not differentiate among juvenile *Raorchestes* species all of them were put in one category and were not included in species richness measures. were encountered during the months of June to November using the standard methods (Table 2.2). The maximum number of individuals were sampled in the month of July, followed by June, and September (Figure 2.7). Seven

species encountered during the study are in the first three threat categories of the IUCN red list, and five are considered to be data deficient (IUCN 2016). Of these, four species are critically endangered, one is vulnerable, and the other two were reported as near threatened (IUCN 2016). Species from three genera, *Fejervarya*, *Micrixalus*, and *Indirana* are categorized together as *Fejervarya* sp., *Micrixalus* sp., and *Indirana* sp., respectively, as the individuals could not be differentiated with confidence in the field. The genus *Micrixalus* was recently revised and 14 new species described (Biju et al. 2014). Eight new species have been described from Kerala which are not easily distinguishable in the field. The species were described on the basis of molecular differences (Biju et al. 2014). Nair et al. (2012) revised the genus *Indirana* and found the presence of many cryptic species that await description. Dinesh et al. (2015b) revised the genus *Fejervarya* and call for caution in identifying species of this genus in the field because of uncertainties associated with collection of types and of the locations of historical collections. These cryptic species suggest that my list is conservative for these two plantations and is likely to include several more species. I generated Coleman curves for the data set to estimate the number of species using each the standard method in each habitat (Figure 2.8). The number of species in each of the transects sampled was averaged after 100 randomizations to remove the difference associated with the sampling order and the variation in the number of individuals collected per sample. The analysis was conducted using the community ecology vegan package in the software R version 3.3.0 (Oksanen et al. 2015).

1.1 Diurnal Visual Encounter Surveys: After seven days of diurnal VESs, this method was discontinued because I had zero detections.

1.2 Nocturnal Visual Encounter Surveys: A total of 68 nocturnal VESs were conducted in each of the three habitat types from June to November 2012. During the VES sampling a total of 102 hours was spent in all the three habitats (Table. 2.4). A total of ten species were encountered in the shola forests and of these two species were found exclusively in the shola forest transects (Table. 2.2 and Figure 2.10). The species *Rhacophorus pseudomalabaricus* and *Ghatixalus magnus* both arboreal species, which depend on streams for reproduction, were found only in shola stream transect. Eight species were detected in tea bushes with a single bush frog species, *Raorchestes jayarami* being exclusive to the tea habitat. Seven amphibian species were encountered in eucalyptus forests and two species, frogs from the genus *Fejervarya* and *Uperodon cf. montanus* were found only along transects in eucalyptus (Table. 2.2).

1.3 Stream sampling with Nocturnal VES: A total of 98 transects each were conducted in tea (49) and shola (49) streams from June to November, 2012. Nine species of amphibians were encountered in the shola stream transects and seven species were encountered in the tea stream transects, with three amphibian species, *Raorchestes chlorosomma*, *R. munnarensis*, and *R. dubois* found exclusively in shola stream transects and frogs in the genus *Fejervarya* were found exclusively in tea stream transects (Table. 2.2 and Figure 2.10). The total number of individuals sampled using the stream transects was 173 (Table. 2.3).

1.4 Drift Fence with Funnel Traps: Four replicates of drift fences with funnel traps were deployed in the three different habitats and trapping was conducted for seven consecutive nights from July 27 to August 4, 2012 (4x3x7=84 trap nights) for 2016 trap-hours. A total of four individual frogs from two species (*Duttaphrynus melanostictus* and *Indirana* species) were trapped, two each in tea and eucalyptus habitats and none in shola forest. These two species are terrestrial and are wide spread in India with *D. melanostictus* being one of the most widely distributed species in the country.

I used the community ecology packages `vegan` from the software package R (Version.3.3.0) to calculate a Bray Curtis dissimilarity matrix of the total data from each transect (Oksanen et al. 2015). A Meta non-Metric Dimensional Scaling plot (meta nMDS) was constructed and best fit polygons were constructed around sites using the standard sampling method used, as well as, the habitat type (Figure. 2.10 and Figure 2.11). The composition of species obtained using visual encounter surveys were more similar to each other than the species obtained from stream transects. The anuran species community from transects in the tea, sholas, and eucalyptus habitats showed statistically significant differences (Figure 2.11).

Discussion

The Visual Encounter Survey method deployed at night worked most efficiently in the tea estates at my study site in Munnar, Kerala. The species found only in the tea field

transects, *Rarochestes jayarami*, was described in 2010 from five male specimens collected in forest undergrowth in Valparai, Tamil Nadu (Biju and Bossuyt 2009). Bush frogs have direct development, which means that they lay their eggs outside of water unlike anurans with a generalized amphibian life cycle (Biju and Bossuyt 2009; Duellman and Trueb 1986). The tea bushes seem to be providing *R. jayarami* a hospitable microhabitats (Biju and Bossuyt 2009). *Rarochestes jayarami* was also commonly seen inside the houses of tea plantation workers, suggesting that it is tolerant of human-caused disturbance.

Rhacophorus pseudomalabaricus and *Ghatixalus magnus*, both rhacophorids found only from the shola forests transects, are species that are usually found in forests trees but move down to vegetation over-hanging streams during the breeding season (Abraham et al. 2015; Vasudevan and Dutta 2000; Harpalani et al. 2015). *Rhacophorus pseudomalabaricus* was spotted in large numbers around low over-hanging vegetation and eucalyptus trees surrounding a marshy area in the tea plantation during the months of June and July (Harpalani et al. 2015) but not in the transects that were randomly selected for the study. *Ghatixalus magnus* was only described in 2015 and we do not have much information about the life history of this species (Abraham et al. 2015). Although I encountered two adults, I did not witness any breeding behavior or observe tadpoles. The three *Fejervarya* species and *Uperodon cf. montana* were found only in the eucalyptus patches and are terrestrial frogs in the tea plantation. Frogs from the genus *Fejervarya* were often spotted in marshy areas that were scattered across the Kanniamallai estate. *Uperodon cf. montana* was only spotted in a eucalyptus forest transect, but it was

observed in other locations off the transects in tea fields. In one instance, over 30 individuals were spotted on the bases of pruned tea bushes in a tea field. Frost (2016) reports that this taxon is a complex of morphologically similar species. Little is known about the breeding behavior of *Uperodon cf. montana*. Though Krishna and Bosch (2007) report the closely related species *Uperodon montana* males, were seen calling from tree holes, I have seen instances of males calling while floating in puddles of water on access paths in the plantations. Sandeep Das (personal communication) also observed the same male calling behavior as well as egg laying by female *Uperodon cf. montana* in puddles along access ways in cardamom plantations in Tamil Nadu. While the Nocturnal Visual Encounter Survey and the Stream transects gave the best catch-per-effort, the funnel traps with drift fence methods were not successful in this landscape (Table. 2.3). The highest diversity per person-hour was obtained using stream transects in the Shola forest, followed by nocturnal VES in eucalyptus forests (Table. 2.3). The highest number of individuals per person-hour was also obtained using the stream transect method in the shola forest, followed by the VES in shola forests (Table. 2.3). The slight overlap of the species composition shown in the nMDS plot during visual encounter surveys and stream transects was because of the species *Duttaphrynus melanostictus*, and juvenile *Raorchestes* species which were encountered using both methods (Figure 2.10). We recommend that the Stream Transect method and the nocturnal Visual Encounter Survey methods are the most efficient methods for standard surveys of anurans in the tea plantation landscape of the Southern Western Ghats. More specific methods around breeding swamps are likely to target other species as well (Scott and Woodward 1994). My difference in community composition in close by transects provides some support for

the idea that the high species diversity of the Western Ghats is explained by high beta diversity. Taxonomy and field identification is a challenge for species complexes, but there are now baseline data for two plantations in Munnar during the monsoon season (June-November).

Urbanization reduces unique habitats worldwide and encourages common, less sensitive flora and fauna to thrive (Miller 2005). Sometimes different methods applied in the same region provide different estimates of richness and abundance, hence a combination of methods is better or at least the least expensive method can be applied (Rocha et al. 2001). In some cases, the knowledge of the most efficient method for amphibian surveying can help in situations where rapid assessments of diversity are required (Vonesh et al. 2009). Studies comparing standard methods provide information on the time, money, and other logistics that are required for a particular method. Roelants et al. (2007) calculated the background rate of extinction of amphibians as 200-2700 times faster than the norm of 0.2926 events per lineage per million years using the number of extinctions in the past 26 years. The predicted rates of extinction are reason enough to encourage more studies of amphibian diversity, life history, behavior and all other areas possible.

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Tables

Serial No	English name	Species name	IUCN	Endemism	Eucalyptus	Shola	Tea
I. ORDER ANURA							
1. Family BUFONIDAE (toads)							
1	Common Indian Toad	<i>Duttaphrynus melanostictus</i>	Least Concern	India	Present	Present	Present
2. Family DICROGLOSSIDAE (fork tongued frogs)							
2	Fejervarya sp	-	-	Western Ghats	Present	Present	Present
3. Family MICRIXALIDAE (dancing frogs)							
3	Micrixalus sp	-	-	Kerala	Present	Present	Present
4	Jerdon's Balloon Frog	<i>Uperodon cf montanus</i>	Near Threatened	Kerala	Present	Absent	Absent
4. Family NYCTIBATRACHIDAE (night frogs)							
5	Nyctibatrachus	<i>Nyctibatrachus poocha</i>	Not Evaluated	Kerala	Absent	Present	Present
5. Family RANIXALIDAE (leaping frogs)							
6	Indirana sp	-	-	Western Ghats	Present	Present	Present
6. Family RHACOPHORODAE (tree frogs)							
7	Ghat Tree Frog	<i>Ghatixalus asterops</i>	Data Defficient	Kerala	Absent	Present	Present
8	Beddome's Bush Frog	<i>Raorchestes beddomii</i>	Near Threatened	Kerala	Present	Absent	Present
9	Green Eyed Bush Frog	<i>Raorchestes chlorosomma</i>	Critically Endangered	Kerala	Absent	Present	Absent
10	Kodaikanal Bush Frog	<i>Raorchestes dubois</i>	Vulnerable	Western Ghats	Present	Present	Present
11	Griet Bush Frog	<i>Raorchestes griet</i>	Critically Endangered	Kerala	Present	Absent	Present
12	Anil's Bush Frog	<i>Raorchestes anili</i>	Least Concern	Western Ghats	Present	Present	Present
13	Jayaram's Bush Frog	<i>Raorchestes jayarami</i>	Not Evaluated	Kerala	Absent	Absent	Present
14	Kadalar Bush Frog	<i>Raorchestes kadalarensis</i>	Not Evaluated	Kerala	Present	Absent	Absent
15	Munnar Bush Frog	<i>Raorchestes munnarensis</i>	Critically Endangered	Kerala	Present	Present	Present
16	Malabar False Tree Frog	<i>Rhacophorus pseudomalabaricus</i>	Critically Endangered	Western Ghats	Absent	Present	Absent
17	Giant Tree Frog	<i>Ghatixalus magnus</i>	Not Evaluated	Kerala	Absent	Present	Absent
18	Juvenile <i>Raorchestes</i>	-	-	India	Present	Present	Present
Totals					12	13	13

Table 2.1. Complete list of species encountered from standard sampling methods from June to November, 2012.

Transect type	Shola			Tea			Eucalyptus	
	VES number	ST number	FT days	VES	ST	FT days	VES	FT days
No of Transects/days of trapping	68	49	28	68	49	28	68	28
Person hours/trap-hours	34	24.5	672	34	24.5	672	34	672
No of Species	10	9	0	8	7	2	12	2
Total number of individuals	131	106	0	62	67	2	37	2
Species/person-hours *100	29.4	77.6	0	23.5	28.6	0.3	35.3	0.3
Individuals/person hours	3.853	4.327	0	1.824	2.735	0.003	1.088	0.003

Table 2.2. Number of species, number of individual amphibians encountered, catch person-hour or trap-hour both by habitat, and method of sampling. (VES: Visual Encounter Survey; ST: Stream Transects; FT: Funnel Traps)

Figures

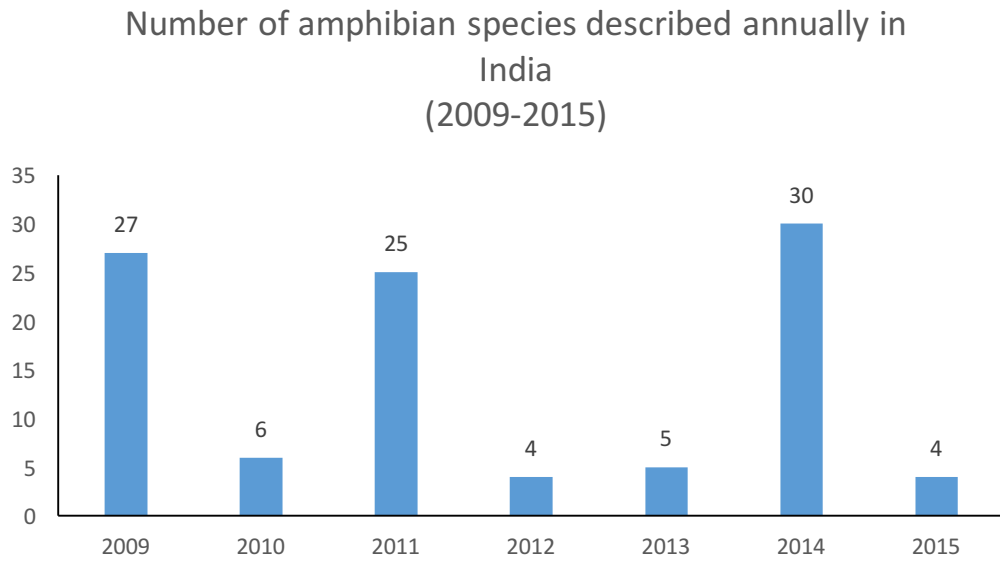


Figure 2.1. Number of amphibian species described in India from 2009 to 2015 (Frost 2016).

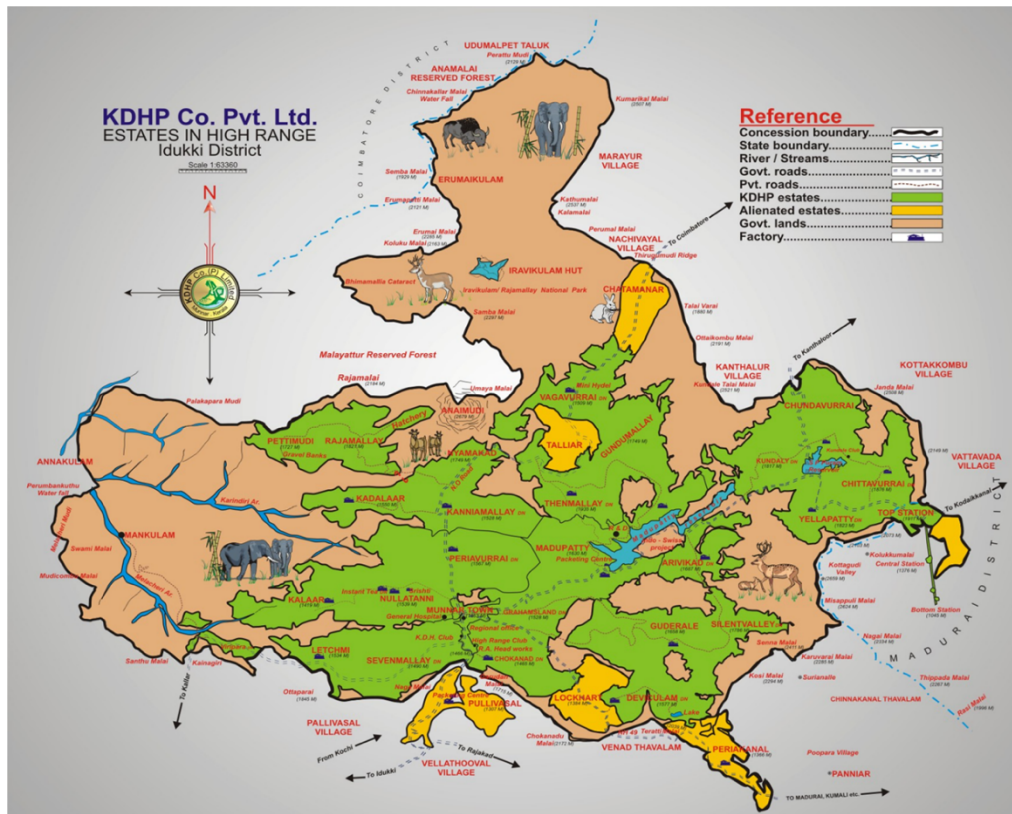


Figure 2.2. Map of KDHP Co Lt. (Kanan Devan Hills Private Cooperation Limited 2016).

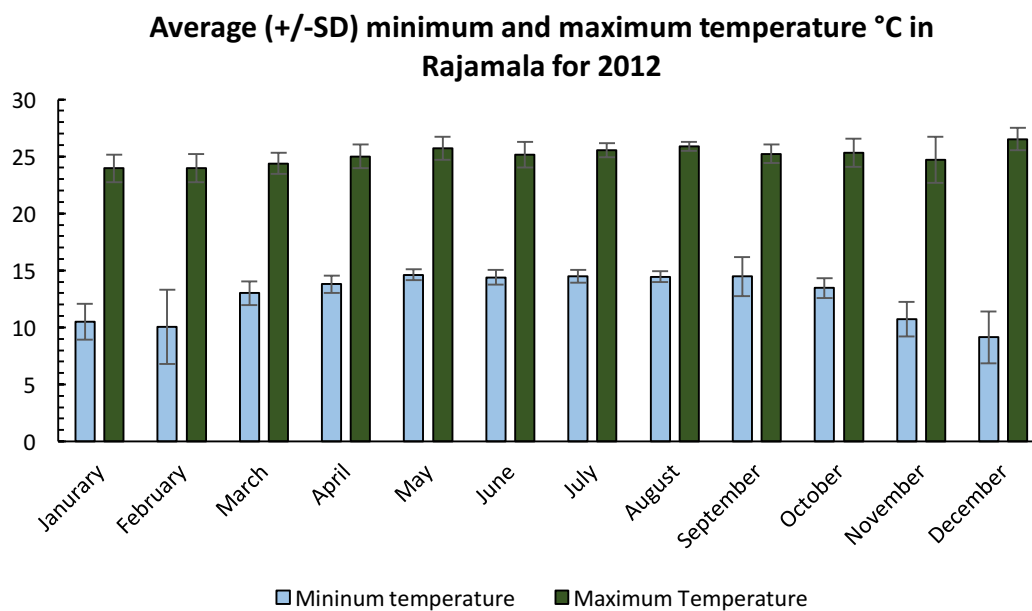


Figure 2.3. Monthly averages of minimum and maximum temperatures for year 2012 in Rajamala tea estate, Munnar, India.

**Average Rainfall+SD(mm) and Relative humidity+SD(%)
for Rajamala in 2012**

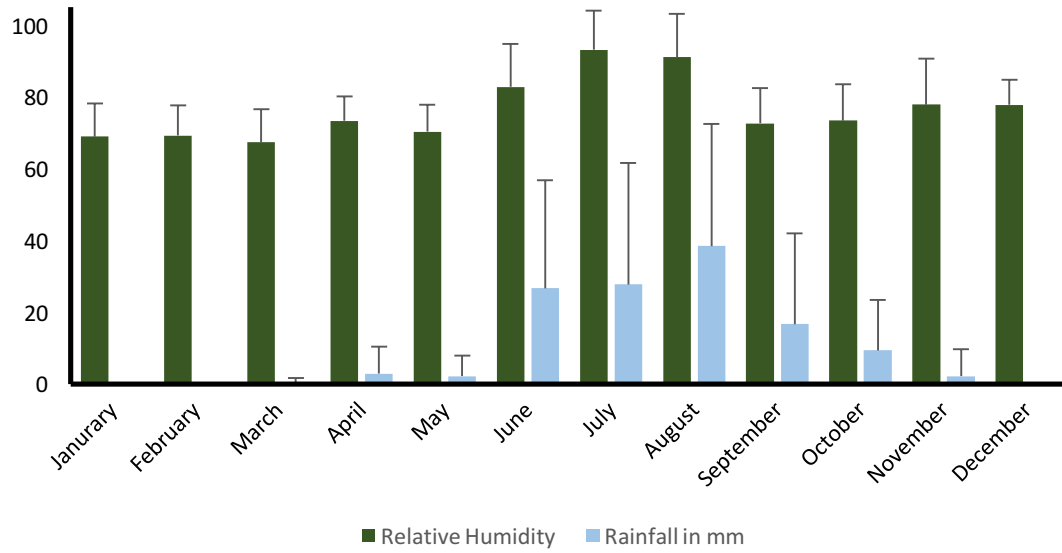


Figure 2.4. Monthly averages of Rainfall and humidity for year 2012 in Rajamala tea estate, Munnar, India.

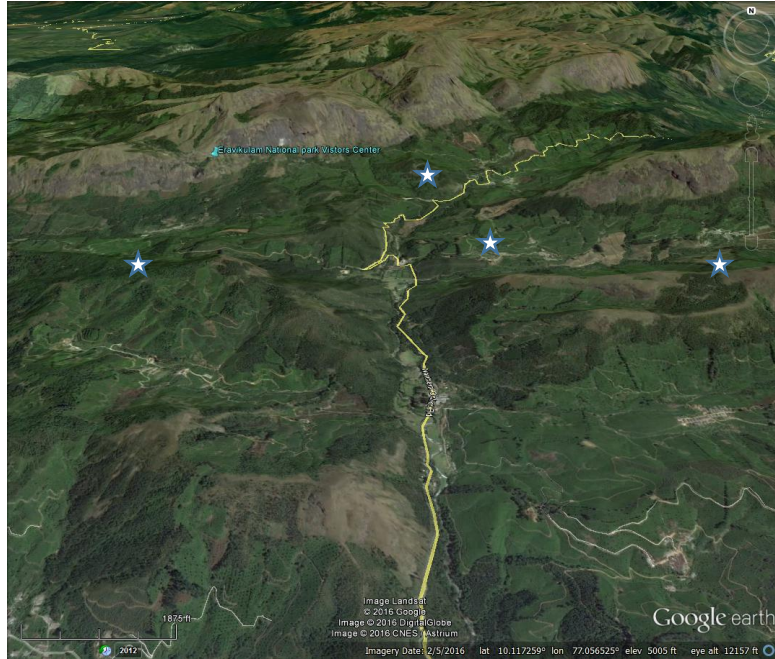


Figure 2.5. Map of Kannaimallai and Periarrai estates with transect locations (Shola, tea, eucalyptus, and a stream) were setup.



Figure 2.6. Drift fence with funnel trap image (Photos: Lilly Eluvathingal).

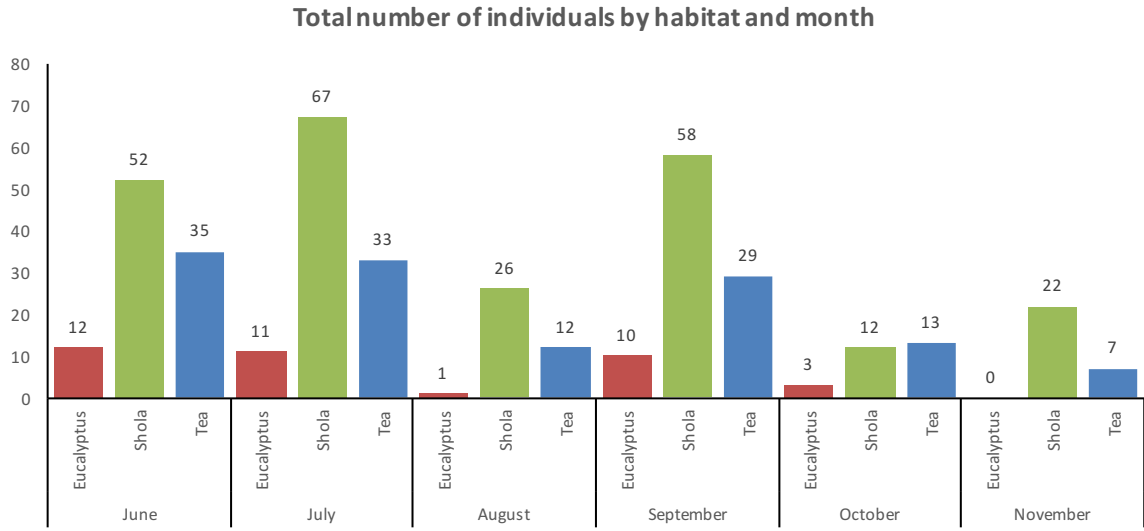


Figure 2.7. Number of individuals found in each of the habitats monthly from June to November, 2012. (Green: Shola; Red: Eucalyptus; Blue: Tea).

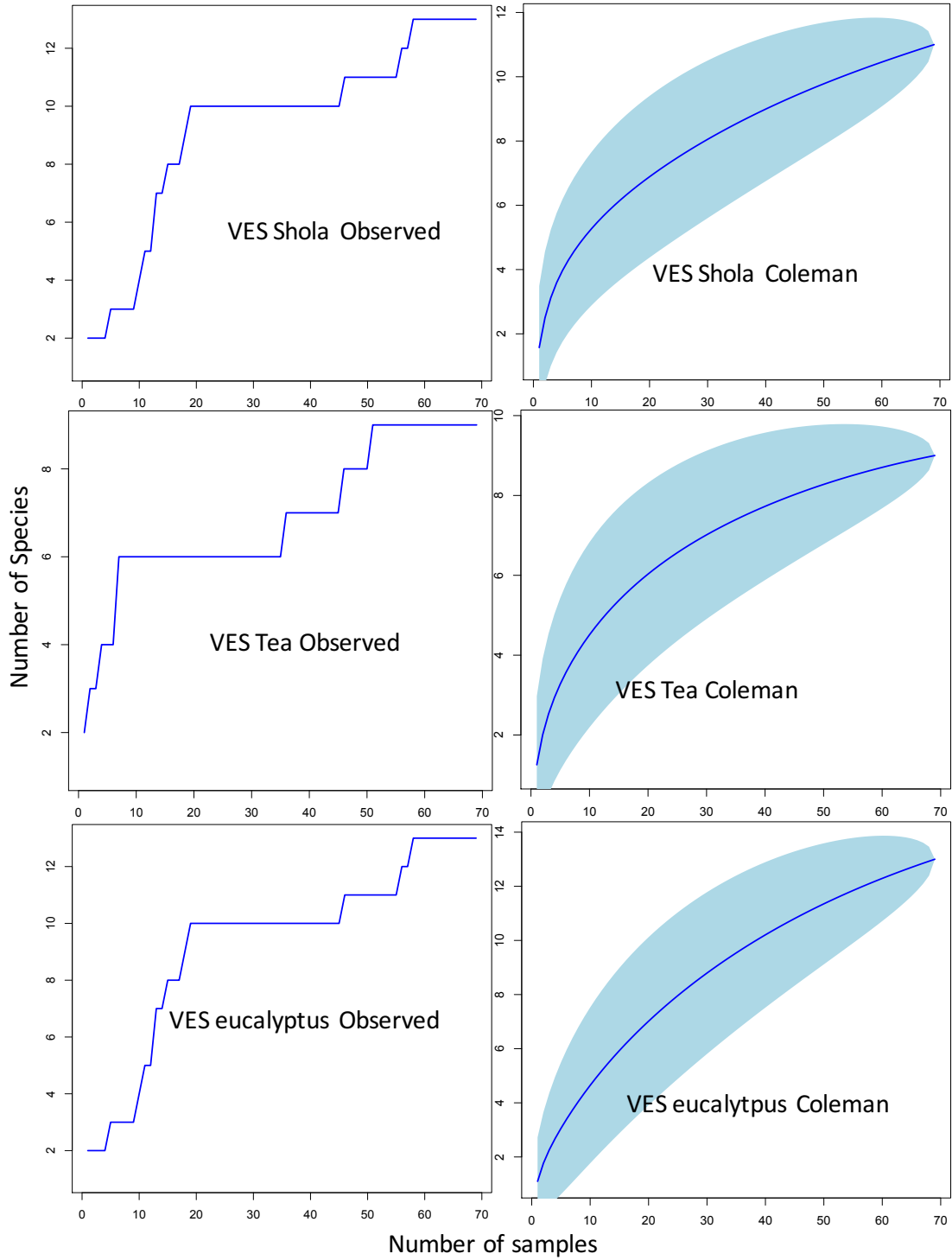


Figure 2.8. Observed and Coleman's species accumulation curves for 2012 data from Munnar. Samples using using visual encounter surveys and stream transects in each of the three habitats (Shola, tea, and eucalyptus).

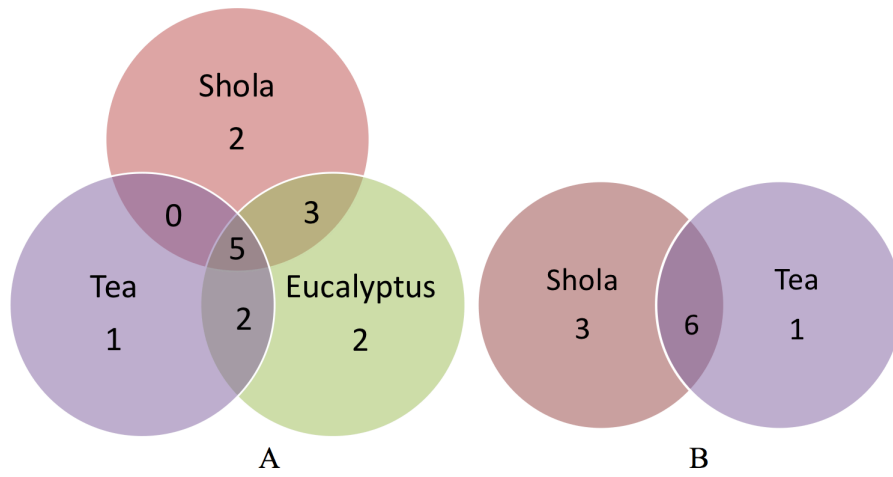


Figure 2.9. Number of exclusive species and number of overlapping species in different habitats types using Visual Encounter Surveys (A) and stream transects (B).

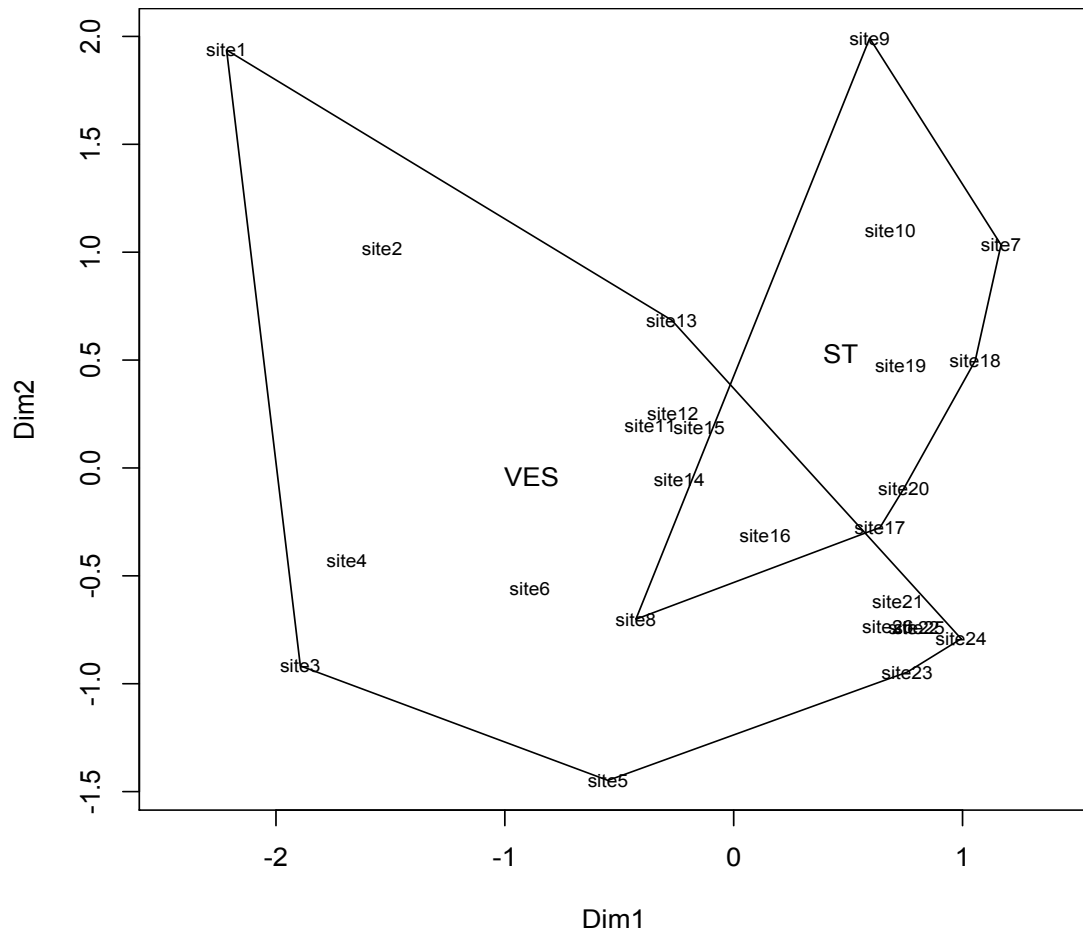


Figure 2.10. Meta nMDS plot of pooled transect data with polygons drawn around transects using the same standard methods: Visual Encounter Surveys and Stream Transects (VES and ST; Stress: 0.2214079).

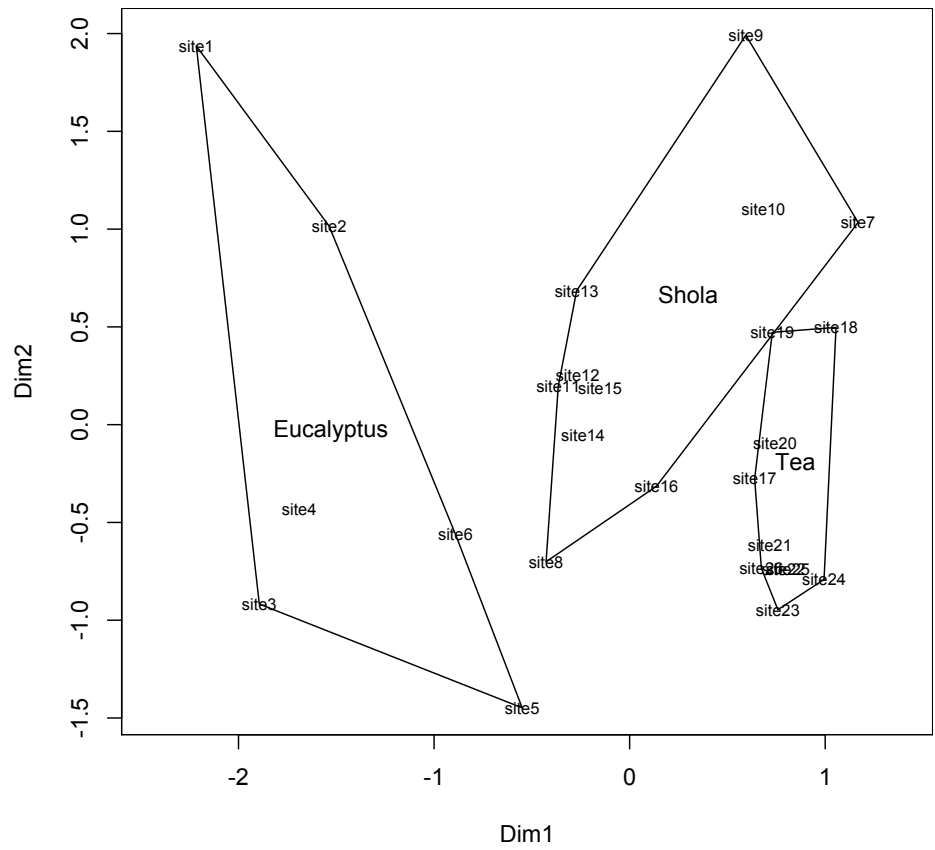


Figure 2.11. Meta nMDS plot of pooled transect data with polygons drawn around transects in conducted in the same habitats (Tea, Shola, and Eucalyptus; Stress: 0.2214079).

IV. CHAPTER 3

Patterns of Anuran Community Structure in a Tea Plantation Landscape

Abstract

Global amphibian declines have been reported since the late 1980s and some of the major drivers of these declines are reported to be habitat loss and the emergence of infectious diseases like chytrid. While amphibians are declining globally, new species of amphibians continue to be described from countries like India. It is critical to study amphibian species diversity in poorly studied regions of the world before we lose the habitat that supports such species. The Western Ghats and Sri Lanka Biodiversity Hotspot is a zone of high endemism for amphibians, and is a region that is being affected by increasing human population pressure. We hypothesized that the anuran community composition would differ across four sites in the tea plantations in Munnar, India, as well as, across three habitat types in the landscape. A total of 648 visual encounter surveys and 216 stream surveys were conducted between April to December, 2013 and May to December, 2014. In 2013, 724 individuals were encountered on the VES transects; in 2014, 632 individuals were encountered using VES transects. In case of stream transects, 536 and 600, individuals were encountered in 2013 and 2014, respectively. There was no difference in community structure between 2013 and 2014, and the data from the two years were analyzed together to describe how the anuran assemblages varied across three main habitats in the tea plantation setting: tea, eucalyptus, and remnant patches of shola forests. A total of 14 species were encountered in both years. I used multivariate statistical methods to determine how anuran communities were organized in the area.

Anuran communities were similar at two of the sites, Madupetty, and Silent Valley. The community of anurans in stream transects differed slightly from the anuran community in upland transects with three species driving the difference. The present study creates baseline data of anuran communities in the tea plantation landscape near Munnar in the southern Western Ghats.

Key words

anuran, diversity, eucalyptus, habitat, plantation, shola, tea

Introduction

Amphibian declines have been reported by scientists all over the world since the late 1980s and some driving factors of these declines have been identified as loss and fragmentation of habitat, pollution and increase in pesticide use, chytrid fungus infection and other diseases, ill-planned dam and water management systems, ultraviolet- β radiation, and the introduction of alien species (Alford and Richards 1999; Bickford et al. 2010; Blaustein and Kiesecker 2002; Cushman 2006; Grant et al. 2016; Whitfield et al. 2016). The modification of the earth's surface by humans has contributed to severe biodiversity loss around the globe (Fischer and Lindenmayer 2007; Vitousek et al. 1997). According to the FAO (2011), approximately 15 million ha of forests are destroyed every year, and a major part of the deforestation occurs in the tropics where the richest biodiversity is located. According to the International Union for Conservation of Nature and Natural Resources (IUCN) over 41% of global amphibian diversity is threatened, and

this percentage does not account for species that are data deficient or those that have not been assessed for their threat status (Hoffmann et al. 2010). Of the many predicted drivers of amphibian declines, the fungus *Batrachochytrium dendrobatidis* (*Bd*) and its salamander counterpart *Batrachochytrium salamandrivorans* (*Bsal*) have been identified as being responsible for a large number of mass amphibian die-offs in Australia, and Central America, and northern Europe (Berger et al. 1999; Kolby and Daszak 2016; Martel et al. 2013; Pechmann et al. 1991; Whitfield et al. 2016). Species of amphibians vary in their susceptibility to *Bd* and the fungus varies in virulence, and in conjunction with warming global temperatures, the stressor will affect species differently (Rollins-Smith, 2016).

Even though amphibian declines are occurring globally, there are several regions that are not represented by detailed studies. The anurans of the Western Ghats in southern India, for example, have not been subject to ecological study. In the last 30 years there has been a spike in the number of anuran species described from the Western Ghats Biodiversity Hotspot (Frost 2016). The Western Ghats is an area which has conservation need as it faces increasing anthropological stresses from from a growing population. The area is home to many amphibian species, and several are critically endangered and locally endemic (Bossuyt et al. 2004; Myers et al. 2000; Figure 3.1). To qualify as a “biodiversity hotspot”, a region must meet two strict criteria: (1) it must contain at least 1,500 species of endemic vascular plants (> 0.5 percent of the world’s total) and (2) the region must have lost at least 70 percent of its original habitat because of some external pressure (Conservation International 2012). The Western Ghats region in the Western

Ghats and Sri Lanka Biodiversity Hotspot (area 18 in Figure 3.1), is home to 198 amphibians, 80% of which are endemic (Frost 2016; Table. 3.1). High endemism in the Western Ghats-Sri Lanka region has been attributed typically to the geographic history of long-term isolation of the mountain chain (Bossuyt et al. 2004; Gunawardene et al. 2007; Van Bocxlaer et al. 2009).

The Western Ghats are a part of the Malabar biogeographic region and form a continuous chain of small to medium-sized mountain ranges running nearly 1600 km along the western coast of Southern India, from 8 to 21° N latitude (Rodgers and Panwar 1988; Figure 3.2). The mountains of the Western Ghats generally vary between 600 and 1000 m. However, there are several mountain peaks that extend between 1000-2600 m between 8 and 13° N and 18 and 19° N (Daniels 1992). The only break in the mountain range occurs in the Palghat Gap (Figure 3.2). The Palghat Gap (~10°35'N and 10°50'N) is a 35km wide, low mountain pass (Bossuyt 2002; Figure 3.2). The annual 0.44% deforestation rate in the Western Ghats is a strong motivation to study amphibian community structures in these regions (Lo Seen et al. 2010). In the case of the amphibians of the Western Ghats, in spite of these anthropogenic threats, the rate of discovery of new species is still an increasing function (Figure 3.3). Previous studies show that the amphibian diversity can vary greatly between hills (separated by <100km) in the same mountain range (Daniels 1992). Thus, the isolated hill ranges of the Western Ghats act as spatially isolated islands supporting high levels of endemism (Bossuyt et al. 2004; Krishnamurthy 2003). A large phylogenetic study using individuals of the Bush Frog genus, *Raorchestes*, by Vijaykumar et al. (2016) showed that geographical isolation

and events of exchange of genes across the Palghat gap, climate-driven isolation because of a Quaternary glaciation event, and divergence of species along the elevation gradients of the Western Ghats are all responsible for high species richness of the genus. The vegetation of the Western Ghats varies from dry deciduous and deciduous rain forests, to montane and evergreen forests, with short stunted forests also called 'sholas' and grasslands at high elevations (Rodgers and Panwar 1988). Very little is known about the effects of habitat differences on community structure of amphibians in India. It can be expected that the amphibian fauna of the Western Ghats is influenced by the variation in forest types, habitat diversity, altitude, latitude, and that overall diversity is in part dictated by high beta diversity across habitats (von May 2009).

Replacing natural vegetation with the cultivation of crops has been occurring since humans adopted a sedentary lifestyle. The modification of the earth's surface by humans has contributed to severe biodiversity loss around the globe (Fischer and Lindenmayer 2007; Vitousek et al. 1997). According to the FAO (2011), approximately 15 million ha of forests are destroyed every year, and a major part of the deforestation occurs in the tropics where the richest biodiversity is located. New plantation areas are being established at the rate 4.5 million ha per year and occurs primarily in Asia and South America (FAO 2011). With an increasing population size comes an increase in the demand for resources, food, timber, oil, coffee, and other resources. These demands are not going to disappear or decrease in the future. In light of the increases in land area being converted to plantations, it becomes extremely important to understand the effect that plantations have on native fauna and flora (Fischer and Lindenmayer 2007; Gardner

et al. 2007a, 2007b). Conversion of naturally forested land into monocultures has an effect on the quality of the habitat that is available for the native flora and fauna (Fischer and Lindenmayer 2007; Vitousek et al. 1997). Some of the major concerns that arise as a result of reduction in forest cover are habitat loss and fragmentation, which most people view as the same (Fahrig 2003). Habitat loss deals with reduction in total habitat size and fragmentation with the problem of small patches (Fahrig 2003). The formation of small patches leads to many effects driven by factors that change at the edges of the patches and vary according to the connectivity of the patches themselves. The effects of the matrix surrounding the habitat patch must also be considered (Watling et al. 2011 and Nowakowski et al. 2016) There are several detailed reviews that look at the effects of fragmentation and edge effects (Ewers and Didham 2006; Saunder et al. 1991).

Plantations require heavy inputs of agro-chemicals like pesticides and fertilizers, which affect the region of the plantation directly and neighboring forests indirectly through runoff (Chen et al. 2009). Constant agro-chemical modifications of the soil can change the characteristics of the land holding such that it cannot recover even if native forest species are planted again (Chen et al. 2009). In contrast, the addition of nitrogen (through fertilizer application) increased the species evenness but did not cause a change in beetle diversity in tea plantations (Chen et al. 2009). The coverage of secondary forests has increased in the tropics (Wright 2005). In addition to acreage covered by plantations, other anthropogenic changes accompany development to support farming like roads and access routes which affects the connectivity of remnant forest patches. Loss of natural habitats to plantations cannot be prevented however it is possible to try and reduce

damage to the natural environment (Bali et al. 2007; Gardner et al 2007b; Wanger et al. 2010). It is important to study biodiversity within plantations to determine if they harbor diversity, or if they are just “green deserts” (Bremer and Farley 2010). Some studies show that the extent of damage by plantations depends on the type of ecosystem that was cleared to make way for the plantation (Bremer and Farley 2010; Cassano et al. 2009; Gardner et al. 2007b). Gardner et al. (2007b) conducted studies on the effect of primary, secondary, and plantation forests on herpetofaunal diversity in the Brazilian Amazon and the results suggest that secondary forests had approximately 66% of the natural amphibian community from the landscape, while plantations supported only five generalist amphibian species.

Large plots of lands in the mid and high elevations of the southern Western Ghats have been under cultivation for plantation crops like tea, coffee, rubber, and other spices and cash crops since the late 1870s. Herein, I describe the anuran community in a landscape dominated by tea plantations naturally interspersed with small patches of forests creating a classic fragmented landscape (Gardner et al. 2007a; Watling and Donnelly 2006).

Watling et al. (2011) found that the quality of the matrix has a large impact on species diversity because some matrix types cannot be crossed. Tea is a tree which grows in the tropics and sub tropics, and all commercial tea is produced from a single species, *Camellia sinensis* (Shalleck, 1972). Tea plants are regularly sprayed with chemicals to keep fungi and other diseases at bay (Table. 3.2). The European Union (EU) regulations require that the maximum limit of chemicals sprayed not exceed those in Table 3.2 and that spraying be restricted to dry months of the year (Table. 3.2; Daniels 2003; European

Union MRL, 2015). Personal observations and prior research showed that restricted spraying is not always practiced on the ground, because the reality of tea production is different from theoretical tea production (Daniels, 1991; Daniels, 2003). In the only study (six days in the wet season and four days in the dry season) of amphibians in tea, Daniels (2003) reported 13 species of amphibians from a single tea estate. The species list included both prolonged and explosive-breeding taxa (Table. 3.3). In a preliminary study, Hegde (2012) reported 20 species of frogs in an Arecanut plantation in northern Karnataka, India. The fact that such short studies revealed the presence of anurans suggests that plantation landscapes provide habitat for frogs.

My project was designed to determine how the community composition, abundance, and habitat use of the anurans in tea plantations varied among distinct sites and years, in the state of Kerala in the southern Western Ghats of India. I used nocturnal VES method in three upland habitat components of the tea landscape: Shola forest patches, eucalyptus patches, tea fields, and along steams. Upland permanent transects (25m long) were used during the two years in four sites. Each habitat type had three transects ($3 \times 3 \times 4 = 36$ upland transects). The stream transects were 50m long and there were three independent streams at each site. These four sites were picked because of the presence of weather stations that were already in operation and maintained by the tea plantation management. The sampling was conducted from April to December, 2013 and May to December, 2014, in each of the sites where I sampled, because the climatic patterns in the region are predictable. I hypothesized that the anuran communities would be similar between the two years (2013 and 2014). I expected that sites closest to each other would have more

similar communities of anurans than sites that are further apart. All the sites are separated by less than 111km apart and according to Daniels (1992), these sites should have similar composition. Given that so little is known about the frogs in the region, I hypothesize that the anuran species composition and abundance will vary across the sites and that species richness will be highest in the sholas. I predict that the abundance and richness of individuals will be greater during the Southwest monsoons compared to the Northeast monsoons. I will compare the data obtained from the weather stations at each estate group. Because tea is consumed in India and around the world, and its popularity continues to increase, my study provides important background information regarding anuran diversity patterns in an actively managed agro-ecosystem nested within a biodiversity hotspot and could serve as a basis for future study of the biodiversity of the plantation ecosystem.

Study Area

I conducted the surveys in two years (2013 and 2014) in the Kanan Devan Hills Tea Plantation Company Private Limited (henceforth KDHP; 10° N and 77° E; Figure 3.4 and Figure 3.5) located in Munnar, in Idukki District, Kerala, in the southern Western Ghats. Kerala accounts for 8.5% of the total tea production of India (Tea Board of India, 2016). Munnar, located in the Devikulam Forest Division, is a large tea producing area in Kerala (Figure 3.4). Munnar is located in the Anamallai mountain range in the Munnar Forest Division. More than 106 km² of reserve forest is interspersed in a matrix of tea fields, eucalyptus patches, and communities of tea plantation workers. The landscape includes

undulating hills and ridges between 900-2600m asl, that run in the northeast to the northwest direction. Though present in the tropical belt, the climate in Munnar is temperate because of elevation. Munnar receives 80% of the annual rainfall in June and July from the Southwest monsoon and the remaining 20% during the Northeast monsoons from October to late November (Thomas and Palmer, 2007; Figure 3.7). The minimum temperature in 2013-2014 was 0°C and the maximum temperature was 29°C (Figure 3.8-3.11). The relative humidity during the monsoon season is over 80%. The Idukki district, of Kerala, supports wet evergreen, semi-evergreen, moist deciduous, dry deciduous, broad-leaved hill forest, montane wet temperate forest and grasslands (Champion and Seth 1968; Thomas and Palmer 2007).

Tea plantations were established in 1877, after John Daniel Munro leased the land from the Poonjar Chief without any time stipulation (KDHP Management Plan 2002). The Kanan Devan Hills Tea Plantation Cooperation Private Limited was established in 2005 and is a 238 km² holding, 98 km² of which is under tea cultivation (Figure 3.4). Almost 80 km² of this area has been converted into fuel wood (eucalyptus) stands which are harvested in cycles of seven years. Eucalyptus is used for drying tea as well as, by members of the local community as a source of fuel for cooking. The 27 tea estates are now aggregated into seven estate groups for ease of management. The other major habitat types in this area in addition to tea and eucalyptus, are remnant patches of shola forest, and the lands included in the Eravikulam National Park (Thomas and Palmer, 2007). The other plantations in the area are owned by American Direct Tea Trading Co. Ltd, Thalayar Tea, The Malayalam Plantation Ltd, and some private planters.

Tea bushes are maintained as shrubs (~1.5m tall), and are planted half a meter apart (Shalleck, 1972). The remnant shola forests patches usually grow in steep mountainous valleys that are protected from the wind. Shola trees are shorter than 10m, and form patches of stunted forest. The shola forests include trees from the families, Lauraceae, Rubiaceae, Symplocaceae, Myrtaceae, Myrsinaceae and Oleaceae (Bunyan et al. 2012; Champion and Seth 1968). The forest patches are usually seen above 1500m in elevation. The branches of these trees are covered in mosses and epiphytes supported by the high relative humidity (Thomas and Palmer, 2007).

The sholas and grasslands of the high elevations in Munnar were cleared to establish the tea plantations. At elevations above 1800m most of the land not covered by rock faces is covered with grass. My study was conducted in the three main habitat types (tea, shola, and eucalyptus) replicated in four different tea plantations sites to compare the diversity and abundance of anurans assemblages.

Methods

Sampling was conducted between May and November, of 2013 and between May and December, of 2014. Four sites were selected for the surveys. Site 1: Vaguvarrai, Site 2: Rajamallay and Pettimudi, Site 3: Madupetty and Thenmala, and Site 4: Silent Valley (Figure 3.5). These sites support small remnant patches of forests (sholas), and weather stations were located in each site. Each weather station collected daily precipitation,

humidity, and temperature data. In each of the sites, three replicate transects were established ($3 \times 3 \times 4 = 36$ total upland transects). Each transect was situated in a belt between 1500 to 2000m, to avoid potential effects of elevation on the anuran community because of the high turnover associated with the elevation described for the region (Daniels 1992). In each estate group, three shola patches which were accessible and of sizes less than 3 hectares were surveyed and selected for the transects. These shola patches were surrounded by a matrix of tea, eucalyptus, or a mix of both.

Nocturnal time-constrained Visual Encounter Surveys (VES) were used in the three upland habitats. For the VES, I set up 25m transects in each of the habitat types. The transects were 25m long, because the habitat is too heterogeneous to accommodate standard 50m transects. Transects were started at least 5 m away from the trail or the edge of the habitat to avoid trail effects (von May and Donnelly 2009). Nocturnal sampling was conducted from 1900hrs to 2300hrs by two observers using head lamps (Doan 2003; Donnelly et al. 2005). The same two observers conducted sampling both years. Observer effect was minimized by randomization of the order of the observer who started the transect and the order in which the transects were sampled each night. In each estate, all three habitat types and the stream transects were sampled on the same night (between 19:30hrs and 23:00hrs) to avoid temporal effects on frog encounters. On nights when sampling was interrupted by heavy rain or wildlife encounters (e.g., elephant or gaur encounters), sampling was discontinued and then repeated the next day. All individuals located within 1m of either side of the transect and up to 2m in height were captured and put into zip lock bags (new zip lock bags were used at each transect). The

distance of measurement of the transect was maintained consistently by using plastic poles which were marked at the 1m length. At the end of the transect, the captured individuals were identified, measured and photographed and then released in the middle of the transect in each case. Animals were captured by hand or by small aquarium net to ensure that the animal was exposed to minimal stress. Standard animals handling protocols were approved by Florida International University's Institutional Animal Care and Use Committee (IACUC: 13-034-CR01-200222). Upon capture frogs were identified to species or morphospecies, measured (snout-to-vent length in mm) with a plastic field calipers (SPI Plastic Dial Caliper Model 31-415-3), weighed by placing the animal in a clean plastic bag and measuring mass with a Pesola scale (Pesola® LightLine Spring Scales, 10g and 30g) to the nearest mg and then released (Crump and Scott. 1994). On average, an individual was handled for 2min. Three 50m long stream transects (3*4=12 transects of 50m each) were established within shola patches in each of the estate groups. The stream transects were 50m long, as it was possible to find accessible stretches that met all the criteria. Environmental variables (precipitation, relative humidity and temperature) were obtained from permanent weather stations maintained by the tea estate management and are described graphically herein.

Analysis

Meteorological data obtained from the permanent weather stations were summarized by month). I use the community ecology package *vegan* from the open source software package R version 3.3.0 to calculate a Bray Curtis dissimilarity matrix of the data from transects conducted in each year (Oksanen et al. 2015). To test for similarity of anuran

community structure I used non-metric Multidimensional Scaling (nMDS) plots to visualize the patterns of community structure of the data. The software program R was used to generate the Meta-nMDS plots which produce the most stable result from 20 nMDS iterations using the package *vegan* (Oksanen et al. 2015). I used the analysis of similarity (ANOSIM) to test the statistical significance of clustering seen in the Meta-nMDS plots.

A Meta-nonMetric Dimensional Scaling plot (meta nMDS) was constructed and minimum convex polygons were constructed around sites from the two years. To test the hypothesis that the assemblages did not differ between 2013 and 2014, I ran an analysis of similarity (ANOSIM) between the data obtained in each year. The data show no difference between years, so I used the community ecology package *vegan* from the open source software package R version 3.3.0 to calculate a Bray Curtis dissimilarity matrix of all the data from transects conducted in both years (Oksanen et al. 2015) to test my hypotheses. A Meta-nonMetric Dimensional Scaling plot (meta nMDS) was constructed and minimum convex polygons were constructed around sites using the estate groups, to test the hypothesis that there was no difference in community structure among the sites. I used a ANOSIM analysis to see if there was a significant effect of site on community structure. Similarity ellipses were also constructed around the transect type and the three habitat types. I also used ANOSIM to find out if the communities differed in their use of habitat types. The number of species encountered each month, in both the years of sampling, were summarized to look for patterns in phenology of the species encountered. For both VES and stream transects, the number of species encountered per unit time

taken for sampling each transect was used to calculate person effort in each habitat type to show how the encounter rate varied between habitats. The same calculations were done for each site to see if there was difference in the encounter rates of species and average number of individual depending on the site being sampled.

Results

I sampled 324 upland Visual Encounter Surveys transects, and 108 stream transects each year. A total of 648 upland transects and 216 stream transects were completed, such that each transect was sampled nine times each year. In 2013, 724 individuals were encountered on the VES upland transects. In 2014, 632 individuals were encountered along upland VES transects. In the case of stream transects, 536 frogs were encountered in 2013, and I encountered 600 individuals in 2014. I used the total species abundance data for each transect to run ordination plots. The ANOSIM showed no effect of year on the frog community ($R^2=0.01781$; $p<0.1138$), and data from 2013 and 2014 were analyzed together (Figure 3.6).

All four sites received the highest average rainfall in the month of June in the year 2013. In 2014, the average rainfall across the sites was highest in July (Figure 3.7). The rainfall at Rajamallai and Pettimudi was greatest, while the Silent Valley was the driest site in both years. *Nyctibatrachus poocha* was not encountered in the upland transects in 2013, while *Duttaphrynus microtympanus* was not encountered in the upland transects in 2014. Thus, in each year 13 species were encountered in the upland transects. In the stream

transects, only one species, *Duttaphrynus microtympanus*, encountered in 2013 was not seen in 2014. However, I only encountered a total of 10 individuals *D. microtympanus* in 2013. Fourteen species were encountered during the two years (Table. 3.4). Species from three genera, *Fejervarya*, *Micrixalus*, and *Indirana*, were categorized as genus sp. because individuals could not be differentiated with confidence in the field. They are grouped together as a single taxon in all my analyses (Biju et al. 2014; Dinesh et al. 2015; Nair et al. 2012). Each of these genera has undergone major taxonomic revisions using molecular data (Biju et al. 2014; Dinesh et al. 2015; Nair et al. 2012). The authors of these papers have called on the community to take caution in identifying species in the field because of uncertainties associated with collection of the types, and of the locations of historical sites. These cryptic species suggest that my list of species for the Kanan Devan Tea Estates is conservative and is likely to include several more species.

Of the 14 species we encountered, six are in the first three threat categories of the IUCN red list (IUCN, 2016; Table. 3.5). Two species (*Raorchestes munnarensis* and *Rhacophorus pseudomalabaricus*) are critically endangered, two (*Raorchestes dubois* and *Duttaphrynus microtympanum*) are listed as vulnerable, and another two (*Raorchestes beddomii* and *Duttaphrynus parietalis*) are reported as near threatened (IUCN 2016). I found two species of anurans, *Raorchestes kadalarensis* and *Duttaphrynus microtympanus*, exclusive to the Rajamalla and Pettimudi site, however, only 20 individuals of *Raorchestes kadalarensis* and 10 individuals of *Duttaphrynus microtympanus* were encountered during the two-year sampling period. The critically endangered frog *Rhacophorus pseudomalabaricus* was found only in one shola upland

transect at Silent Valley. The Madupetty and Thenmala site had the largest number of species, with 14 species across the four habitats (shola, tea, eucalyptus, and streams) combined. The Vaguvarrai site with the highest elevation (bordering 2000m) had the lowest species diversity (nine species). The average number of species encountered at each site, per habitat type, was highest in the shola VES transects and shola stream transects (Table. 3.6 and Table. 3.7).

In the 2013 season the most common species in the upland transects in all the months was *Raorchestes dubois* while the juvenile *Raorchestes* peaked in October (Figure 3.12). In 2014, the same pattern was seen in the upland transects (Figure 3.14). Along stream transects, *Ghatixalus asterops* peaked in August and November in 2013, while in 2014, the species abundance peaks are in July and October (Figure 3.13 and 3.15). The largest number of *Micrixalus* individuals were encountered in May and October in 2013, and August and November in 2014 (Figure 3.13 and 3.15). The juveniles of genus *Rarochestes* peak in July and October in 2013, and in August and November in 2014 (Figure 3.12 and 3.15).

The meta nMDS plot shows slight overlap in community structure between the Madupetty and Thenmala site, with the Rajamallay and Pettimudi site (Figure 3.16). The lack of visual overlap in the data even with six common species is because the raw data were entered such that all site data was grouped (Figure 3.16). The Madupetty and Thenmala site, and the Rajamallay and Pettimudi site are separated by the greatest distance. The community composition between Vaguvarrai and Silent Valley sites seem

to be more similar to each other than with any of the other estate groups (Figure 3.16). As is expected, we see very little similarity in the species encountered in the upland transects and stream transects (Figure 3.17). Six species (*Ghatixalus asterops*, *Raorchestes dubois*, *Indirana* sp., *Micrixalus* sp., *R. beddomii*, and *Duttaphrynus melanostictus*) as well as, the *Raorchestes* sp. juvenile category common to all the three upland habitat types (tea, shola, and eucalyptus; Figure 3.18).

Raorchestes jayarami was found exclusively on transects located in tea in all sites except at Vaguvarrai, suggesting that it might be near its upper distribution limit of 2000m (Biju and Bossuyt 2009). In the VES surveys across the sites, *R. jayarami* was found exclusively on tea bushes which suggest that *R. jayarami* has developed an ability to survive in the altered agro-ecosystem. The Jayaram's Bush Frog, was the species that the local tea plantation inhabitants were most familiar with, though it is likely that they assumed both *R. jayarami* and *R. beddomii* were the same species (Biju and Bossuyt 2009). Two toads (*Duttaphrynus parietalis* and *Duttaphrynus microtympanum*) and species of the genus *Fejervarya* were found exclusively in the eucalyptus habitat during my studies. The terrestrial habitat of these species may allow them to survive in the altered eucalyptus habitat (Dinesh et al. 2015; IUCN 2016). Though listed as Near Threatened, the IUCN red list mentions that *Duttaphrynus parietalis* may be found in degraded habitats. I confirm its presence in the tea landscape in Munnar. I also encountered *Duttaphrynus microtympanum*, in the tea plantations, which is known to be species associated with the shola (IUCN 2016). The similarity ellipse of the sites sampled in 2014 was a subset of those from 2013 (Figure 3.11). The composition of species

obtained using visual encounter surveys in uplands were more similar to each other than the species obtained from sampling stream transects. The tea, sholas, and eucalyptus habitats clustered together showing similar groups of species in each of the habitats across the four sites that I sampled (Figure 3.18). The ANOSIM results show that the community structure differs across sites ($R^2=0.47126$ $p<0.002$), and habitats ($R^2=0.15388$ $p<0.002$).

Discussion

I report difference in species composition across the four sites in the tea plantations landscape in Munnar, as well as across the three habitat types (shola, eucalyptus, and tea). According to the International Union for Conservation of Nature (IUCN), one-third of the world's amphibian species are under the threat of extinction and we lack data about the conservation status for approximately 30% of them. In the light of these declines, there is a need to study amphibian community structure in biodiversity hotspots with high amphibian endemism. The Western Ghats have been subjected to great disturbances because of humans (Krishnamurthy 2003). Deforestation, pesticides, and invasive species are some of the major threats to diversity (Krishnamurthy 2003). Data on anuran community structure in plantations, as well as, other landscapes are required to develop and implement successful conservation strategies in such landscapes in the future (Sutherland et al. 2012).

I report the presence of a total of 14 species of anurans from four tea estate groups from the southern Western Ghats of India. Of the 14 species, seven were common to all four

sites. Of these seven common species, three were found in shola stream transects. *Ghatixalus asterops*, *Micrixlausa* sp., and *Nyctybatrachus poocha* were predominantly found in stream transects in all the sites because of their strong dependence on water for breeding (Biju, et al. 2008; Biju et al. 2014). Across the KDHP landscape, *Ghatixalus asterops* and *Micrixlausa* sp. were the most abundant species found in stream transects. Individuals of *Ghatixalus asterops* were encountered repeatedly along the same stream transects and sometimes on exactly the same perch (even though all individuals were released in the middle of transects) suggesting that these species may maintain site fidelity. Future studies are required to understand the social behavior of *G. asterops*.

The community structure did not vary between 2013 and 2014, which is somewhat interesting given that 2013 was reported to be a drought year (Figure 3.7). All four sites received higher average rainfall in June than July in year 2013, while in 2014, the average rainfall across all the sites was the highest in July (Figure 3.6) and there was high variance around the monthly precipitation at each site in the landscape. The early heavy rainfall in June 2013 (monsoon) did not affect the patterns observed in 2014 (Figure 3.6).

The list of species from the region now provides a baseline for anuran community structure in this area and ideally will play a part in long term studies looking at trends of the anuran communities at the four sites in the landscape over time. In my pilot study in 2012, I encountered 17 species of anurans (all juveniles *Raorchestes* sp. were lumped) using standard amphibian surveying methods (species not encountered in 2013-2014:

Uperodon cf montanus, *Raorchestes chlorosomma*, *R. anili*, and *Ghatixalus magnus*) and another seven species (species absent in 2013-2014: *Rhacophorus calacadensis*, *Raorchestes sushili*, *R. theuerkaufi*, *R. uthamani*, *Micrixalus* sp., *Beddomixalus bijui*, and *Nyctibatrachus acanthoderms*) were encountered opportunistically in 2012 in the Kannaimallai and Periarvarai Estates. These species were not encountered using standard methods in the four sites that were sampled during 2013-2014, neither were they encountered opportunistically in the four sites. We believe that Kannaimalli and Periarvarai Estates that were sampled during 2012 support a high species diversity compared to the other sites sampled in 2013 and 2014. This is probably because Kannaimalli and Periarvarai estates are close to the Eravikulam National Park and fall in the elevational belt 1400-2000m asl.

According to Vijaykumar et al. (2016), there is an increased species richness of *Raorchestes* species between 1400m and 1800m near the Anamallai hills. Of the ten species not encountered in the four sites during 2013 and 2014, five species belong to genus *Raorchestes*. Another species *Beddomixalus bijui* was described from its type location, which is only 13 miles away from Kanniamallai estate. Compared to data reported in Daniels (2001), the Western Ghats now support 198 species of amphibians and 80% of them are endemic, as most of the newly described are endemic to the mountain range (Frost 2016). Though the 14 species I sampled, is not extremely diverse as might be expected (see CHAPTER 2), the seven rhacophorid species found in the estates are endemic to Munnar (Vijaykumar et al. 2016; IUCN 2016). The species of *Raorchestes* are known to be direct developers, and I observed several instances of amplexus and egg

laying away from water (Biju and Bossuyt 2009; Biju et al. 2010). The bush frogs, *Rarchestes jayarami*, *R. kadalarensis*, and *R. munnarensis* were never encountered in stream transects, however, some recently metamorphosed individuals were encountered along streams. It is likely that these offspring were either of *R. dubois* or *R. beddomii*, whose adults were encountered along the vegetation in the stream transects. The five species from the genus *Raorchestes* encountered in the present study: *Raorchestes munnarensis*, *R. kadalarensis*, *R. dubois*, *R. beddomii*, and *R. jayarami*, are unique to the Anamalai Hills, and are only reported above 1400m asl (Vijaykumar et al. 2016).

The type specimen of *R. munnarensis* was collected adjacent to tea bushes on the route to Munnar, and in the present study I report that the species was found at all the sites except for Vaguvarrai (Biju and Bossuyt 2009). *Rarchestes jayarami*, *R. kadalarensis*, and *R. munnarensis* were not encountered at Vaguvarrai which includes areas with elevations between 1900m and 2000m asl. *Raorchestes kadalarensis*, was described in 2011 on the basis on two specimens collected near the Kadalar Estate, which shares boundaries with the Rajamallai and Pettimudi sites of the present study (Zachariah et al. 2011). I encountered 20 individuals of *R. kadalarensis*, across habitats, sites, and years. All of the individuals were found along an upland shola transect in the Rajamallai and Pettimudi site. The species was opportunistically observed in the Kannaimallai Estate which also shares boundaries with the Kadalar Estate. Since it is yet to be assessed by the IUCN, we recommend that its distribution in four abutting tea Estates (Kadalar, Kannaimallai, Rajamallai, and Pettimudi) be taken into consideration (IUCN 2016). In the present study, I encountered individuals of *R. beddomii* in all the upland habitats, and in all the

four sites; however, only six individuals were encountered in the Vaguvarrai site, suggesting it was near the upper limit of its distributional range. The species is closely related to the critically endangered species *R. resplendens* that is reported only inside the Eravikulam National Park (Biju et al. 2010). In its description, *R. jayarami* was reported to be collected from a shola forest in Valparai region of the Anamallai hills (Biju and Bossuyt 2009; Vijaykumar et al. 2016) I report its presence on tea bushes in plantations in the Munnar region of the Anamallai hills. *Raorchestes jayarami*, was abundant on tea bushes at all sites except at Vaguvarrai. The frogs in the type series of *R. dubois* were collected from shrubs (1m high) and one of the paratypes was collected from the Eravikulam National Park. In the present study, *R. dubois* was the most abundant species across all the habitats, including Vaguvarrai (Biju and Bossuyt 2009). The juveniles of genus *Raorchestes* peak in July and October in 2013, and in August and November in 2014 suggesting a bimodal pattern in the egg laying of the adults, matching the two monsoon seasons (southwest in June and northeast in October). *Ghatixalus asterops*, a tree frog described in 2008 is still reported as data deficient by the IUCN redlist (Biju et al. 2008; IUCN, 2016). The present study shows that it is a locally abundant species, found perched on low vegetation along the sides of, as well as, perched on overhanging vegetation in streams across the four sites I surveyed. Even though the critically endangered frog *Rhacophorus pseudomalabaricus* was found only in one shola upland transect of Silent Valley, different life history stages of the species have been opportunistically observed in the Kadalar and Kannaimallai Estates in Munnar (Harpalani et al. 2015; IUCN 2016). Specimens of *Nyctybatrachus poocha* were collected from the Munnar area from small hill streams and the present study found the species to be

abundant only in stream transects, across all the four sites (Biju et al. 2011). The individuals were found in the streams or on rocks and grass along the banks of the streams. The species is difficult to handle. On several occasions we noticed egg clutches laid in rocky crevices, just above the water line.

There are studies on novel difference breeding behavior associated with other species in the *Nyctybatrachus* genus, including mud packing to prevent desiccation of egg masses and the dorsal straddle, and dorsal release of sperm onto the female (Gururaja et al. 2014; Willaert et al. 2016). Though egg clutches were observed, we did not observe breeding in the species. The species of the Torrent Frog genus *Micrixalus* were observed abundantly only in stream transects across the four sites, with the highest abundance in the Silent Valley. Across the landscape, we found individuals of this species along the banks of the streams or in the streams which is the usual habitat of the species (Biju et al. 2014). We also encountered individuals from *Micrixalus* from the Kannaimallai Estate, in the 2012 field season. The behavior of foot flagging is reported from individuals of *Micrixalus saxicola* and the behavior was observed in individuals of the genus *Micrixalus*, on occasion during my study (Preininger et al. 2013). There was no information on the morphology of the tadpoles from the genus *Micrixalus*, until Senevirathne et al. (2016) described the tadpoles of *Micrixalus herrei*. The tadpoles of the genus are fossorial until the late stages before metamorphosis (Senevirathne et al. 2016). On one occasion, I encountered a tadpole from the genus *Micrixalus*, in a stream from the Madupetty Estate. Individuals from the genera *Fejervarya* and *Indirana*, were encountered in low numbers along the upland shola transects and streams transects in the current study. During both

the field seasons, a local marsh which received runoff from tea fields, in the Kannaimallai Estate appeared to have several calling individuals, as well as, aquatic tadpoles of species from the genus *Fejervarya*. Individuals from both *Fejervarya* and *Indirana* were encountered in the Rajamallai and Pettimudi site, while we only found individuals of *Indirana* sp. at the Vaguvarrai site and the Madupetty-Thenmala site. I only found *Fejervarya* sp. at the Silent Valley site. Veeranagoudar et al. (2009) report the semi-aquatic nature of tadpoles of the *Indirana* genus. We opportunistically observed tadpoles on moist rock faces in the Vaguvarrai site, during the present study. Additional work is required on trying to carefully identify species using morphological traits, especially from the recently revised genera, *Fejervarya*, *Micrixalus*, and *Indirana* (Biju et al. 2014; Dinesh et al. 2015; Nair et al. 2012). Species specific studies on the reproductive biology of the amphibians in this landscape need to be conducted.

In his study 10-day study in Valparai, Daniels (2003) reported 13 species of amphibians from a single tea estate and this number compares with the number of species encountered in at each site in my study (Md_Th: 10 species; Ra_Pt: 14 species; SV: 11 species; Vg: 8 species) and juvenile *Raorchestes* sp. which were found at all sites. It is important to remember that the list I compiled is conservative because of the presence of several cryptic species. Wordley et al. (2015), found eight out of ten species of bats in a tea plantation dominated landscape in state of Tamil Nadu in southern India. In the light of such studies reporting the presence of species in tea plantations and the anuran species richness and community structure reported in this study from an actively managed tea plantation, both in the same biodiversity hotspot, we are reminded of the role agro-

ecosystems can play in supporting the native biodiversity of an area. Grant et al. (2016) reported a diverse number of drivers for declining amphibians in North America and recommended that conservation plans needed to be tailored to each locality in question, even though amphibian declines are reported on a global scale. With all the information existing on declines of amphibians all over the globe, it is now time to conduct and learn from species specific targeted interventions (Sutherland et al. 2012). With the exponentially increasing human population, the pressures on existing agricultural and plantation lands are only going to keep increasing (Nielsen 2016). Trying to increase awareness among the stake holders managing these land holding can possibly lead to more sustainable long term measures which allow certain subsets of native species to take refuge in microhabitats provided by these systems.

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Tables

Group	Total Species	Endemic Species	% Endemism
Angiosperms	4000	1500	38
Butterflies	330	37	11
Fishes	218	116	53
Amphibians	121	94	78
Reptiles	156	97	62
Birds	508	19	4
Mammals	120	14	12

Table. 3.1. Endemism in the Western Ghats (Daniels 2001).

Group	Name and dosage	Maximum Residue Level (MRL mg Kg⁻¹)
Organochlorine compounds		
	Dicofol 18.5 EC (1000ml/ha)	20.0
	Endosul fan 35 EC (1000ml/ha)	30.0
Organophosphorous compounds		
	Ethion 50 EC (750ml/ha)	2.0
	Quinalphos 25 EC (750ml/ha)	0.1
	Chloropyrifos 20 EC (1000ml/ha)	0.1
Synthetic Pyrethroids		
	Deltamethrin 2.8 EC (500ml/ha)	5.0
Weedicides		
	Paraquat 24% (1500-2250ml/ha)	0.1
	Glyphosate (1750ml/ha)	0.1
Fungicides		
	Copper oxychloride (210-350g/ha)	Not available

Table 3.2. Pesticides commonly used on tea and the maximum residues levels of pesticides permitted by the European Union Regulation (European Union MRL, 2015).

Sno	Species Name	Current Name	Breeding Comments
1	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i>	Explosive breeding
2	<i>Philautus variabilis</i>	<i>Raorchestes variabilis</i>	Calling observed
3	<i>Rhacophorus pseudomalabaricus</i>	<i>Rhacophorus pseudomalabaricus</i>	None observed
4	<i>Euphlyctis cyanophlyctis</i>	<i>Euphlyctis cyanophlyctis</i>	Calling observed
5	<i>Indirana beddomii</i>	<i>Indirana beddomii</i>	None observed
6	<i>Nyctibatrachus beddomii</i>	<i>Nyctibatrachus beddomii</i>	Calling observed
7	<i>Nyctibatrachus</i> species	<i>Nyctibatrachus</i> species	Calling observed
8	<i>Rana temporalis</i>	<i>Indosylvirana temporalis</i>	None observed
9	<i>Rana aurantiaca</i>	<i>Indosylvirana aurantiaca</i>	Calling observed
10	<i>Limnonectes bervipalmata</i>	<i>Fejervarya bervipalmata</i>	Breeding and all stages observed
11	<i>Limnonectes keralensis</i>	<i>Fejervarya keralensis</i>	Adults and juveniles seen
12	<i>Limnonectes limnocharis</i>	<i>Fejervarya limnocharis</i>	Juveniles and metamorphs seen
13	<i>Limnonectes nilagirica</i>	<i>Fejervarya nilagirica</i>	Confused with F . <i>limnocharis</i>

Table. 3.3. All species observed by Daniels in a tea plantation along with updated names (Daniels 2003; Frost 2016).

	English name	Species name	IUCN	Endemism
I. ORDER ANURA				
1. Family BUFONIDAE (toads)				
1	Common Indian Toad	<i>Duttaphrynus melanostictus</i>	LC	
2	Small-eared Toad	<i>Duttaphrynus microtymphanum</i>	VU	WG
3	Ridged Toad	<i>Duttaphrynus parietalis</i>	NT	WG
2. Family DICROGLOSSIDAE (fork tongued frogs)				
4	Fejervarya	<i>Fejervarya</i> sp		
3. Family MICRIXALIDAE (dancing frogs)				
5	<i>Micrixalus</i>	<i>Micrixalus</i> sp	NE	KL
4. Family NYCTIBATRACHIDAE (night frogs)				
6	<i>Nyctibatrachus</i>	<i>Nyctibatrachus poocha</i>	NE	KL
5. Family RANIXALIDAE (leaping frogs)				
7	<i>Indirana</i>	<i>Indirana</i> sp		
6. Family RHACOPHORODAE (tree frogs)				
8	Ghat Tree Frog	<i>Ghatixalus asterops</i>	DD	WG
9	Beddome's Bush Frog	<i>Raorchestes beddomii</i>	NT	WG
10	Kodaikanal Bush Frog	<i>Raorchestes dubois</i>	VU	WG
11	Jayaram's Bush Frog	<i>Raorchestes jayarami</i>	NE	WG
12	Kadalar Bush Frog	<i>Raorchestes kadalarensis</i>	NE	KL
13	Munnar Bush Frog	<i>Raorchestes munnarensis</i>	CR	WG
14	Malabar False Tree Frog	<i>Rhacophorus pseudomalabaricus</i>	CR	WG

Table. 3.4. All species (minus juvenile *Raorchestes* sp) encountered in this study from all four estate groups (LC: Least Concern; VU: Vulnerable; NE: Not Evaluated; DD: Data Deficient; NT: Near Threatened; CR: Critically Endangered; KL: Kerala; WG: Western Ghats).

Serial No	English name	Species name	IUCN	Endemism	Eucalyptus	Shola	Tea
I. ORDER ANURA							
1. Family BUFONIDAE (toads)							
1	Common Indian Toad	<i>Duttaphrynus melanostictus</i>	Least Concern	India	Present	Present	Present
2	Small-eared Toad	<i>Duttaphrynus microtympphanum</i>	Vulnerable	India	Present	Absent	Absent
3	Ridged Toad	<i>Duttaphrynus parietalis</i>	Not Threatened	India	Present	Absent	Absent
2. Family DICROGLOSSIDAE (fork tongued frogs)							
4	<i>Fejervarya</i>	<i>Fejervarya</i> sp	-	Western Ghats	Present	Absent	Absent
3. Family MICRIXALIDAE (dancing frogs)							
5	<i>Micrixalus</i>	<i>Micrixalus</i> sp	-	Kerala	Present	Present	Present
4. Family NYCTIBATRACHIDAE (night frogs)							
6	<i>Nyctibatrachus</i>	<i>Nyctibatrachus poocha</i>	Not Evaluated	Kerala	Absent	Absent	Present
5. Family RANIXALIDAE (leaping frogs)							
7	<i>Indirana</i>	<i>Indirana</i> sp	-	Western Ghats	Present	Present	Present
6. Family RHACOPHORODAE (tree frogs)							
8	Ghat Tree Frog	<i>Ghatixalus asterops</i>	Data Deficient	Kerala	Present	Present	Present
9	Beddome's Bush Frog	<i>Raorchestes beddomii</i>	Near Threatened	Kerala	Present	Present	Present
10	Kodaikanal Bush Frog	<i>Raorchestes dubois</i>	Vulnerable	Western Ghats	Present	Present	Present
11	Kadalar Bush Frog	<i>Raorchestes kadalarensis</i>	Not Evaluated	Kerala	Absent	Present	Absent
12	Jayaram's Bush Frog	<i>Raorchestes jayarami</i>	Not Evaluated	Kerala	Absent	Absent	Present
13	Munnar Bush Frog	<i>Raorchestes munnarensis</i>	Critically Endangered	Kerala	Absent	Present	Present
14	Malabar False Tree Frog	<i>Rhacophorus pseudomalabaricus</i>	Critically Endangered	Western Ghats	Absent	Present	Absent
Totals					9(3)	9(2)	9(2)

Table. 3.5. All species encountered in this study using Visual Encounter Surveys (VES) from all four estate groups with information on presence and absence in each habitat and number of exclusive species in each habitat in parenthesis.

Sites		Total of species per habitat		Average no of individuals per habitat		Species/person-hour	Avg Individuals/person-hour
		2013	2014	2013	2014		
Md_Tm	Shola	6	6	3.15±0.42	1.85±0.22	0.22	0.51±0.08
	Tea	7	7	1.48±0.15	0.85±0.08	0.26	0.23±0.03
	Eucalyptus	6	7	1.30±0.27	1.19±0.21	0.24	0.22±0.05
Ra_Pt	Shola	8	8	2.78±0.22	2.78±0.25	0.30	0.43±0.11
	Tea	4	4	1.11±0.19	1.48±0.23	0.15	0.19±0.06
	Eucalyptus	6	5	2.19±0.28	1.11±0.17	0.20	0.35±0.05
SV	Shola	6	6	4.07±0.56	3.30±0.40	0.22	0.66±0.14
	Tea	4	6	1.33±0.17	1.67±0.18	0.19	0.21±0.07
	Eucalyptus	3	3	0.89±0.14	0.93±0.14	0.11	0.15±0.04
Vg	Shola	5	5	2.96±0.50	2.07±0.35	0.19	0.49±0.09
	Tea	2	6	0.48±0.09	3.70±0.54	0.15	0.08±0.16
	Eucalyptus	4	4	3.22±0.69	2.48±0.50	0.15	0.56±0.11

Table. 3.6. Summary of average number of species and average number of individual encountered at each site and habitat type using Visual Encounter Surveys (Calculated for each site (3 replicates*15 minutes*repeated 18 times*2 observers)/60=27 hours per habitat).

Sites	Total No of species		Average No of Individuals in streams		Species/person-hour	Individuals/person-hour
	2013	2014	2013	2014		
Md Tm	4	7	2.48±0.36	4.52±0.48	0.20	0.13±0.02
Ra Pt	8	6	5.93±0.88	6.33±1.11	0.26	0.23±0.04
SV	7	7	7.37±1.01	7.78±1.03	0.26	0.28±0.04
Vg	5	5	4.07±0.73	3.59±0.62	0.19	0.14±0.02

Table. 3.7. Summary of average number of species and average individual counts from each estate group for shola stream transects (Calculated for each estate (3 replicates*30 minutes*repeated 18 times*2 observers)/60=54 hours per habitat).

Figures

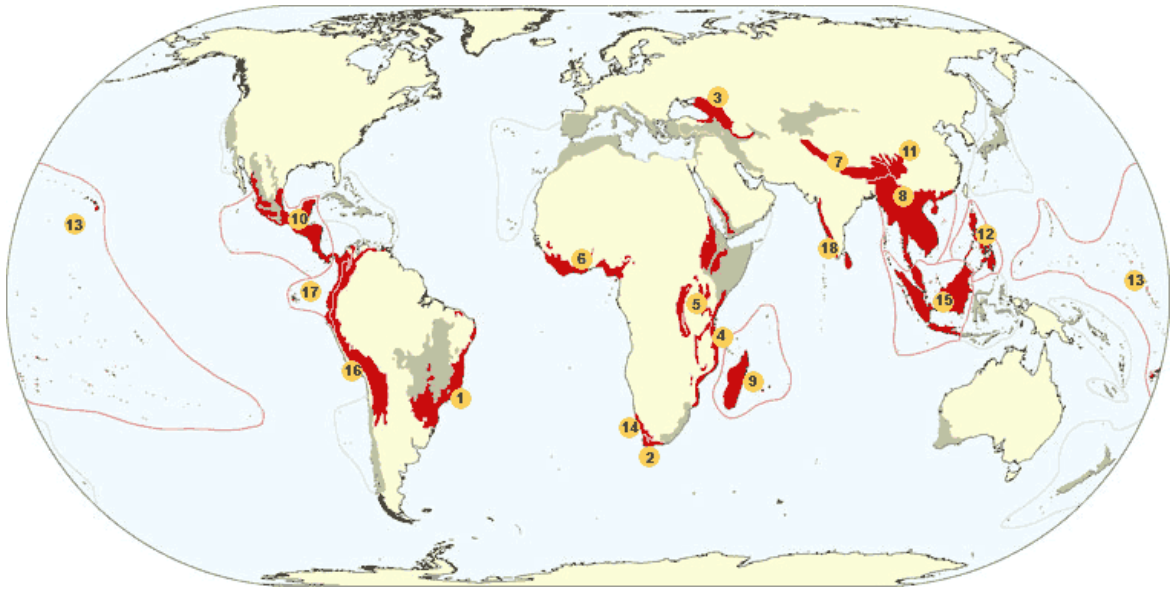


Figure 3.1. The 25 biodiversity hotspots of the world (Conservation International, 2012).



Figure 3.2. The Western Ghats and Sri Lanka Biodiversity Hotspot with a white dot marking the study area which is South of the the Palghat gap (white dash; Map from IUCN, 2016).

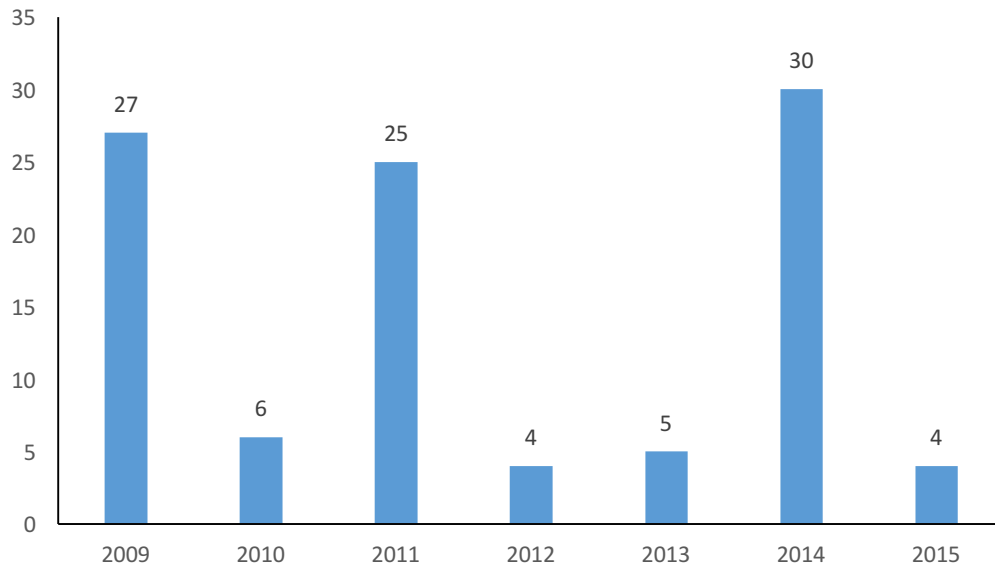


Figure 3.3. Number of amphibian species described in India from 2009 to 2015 (Frost, 2016).

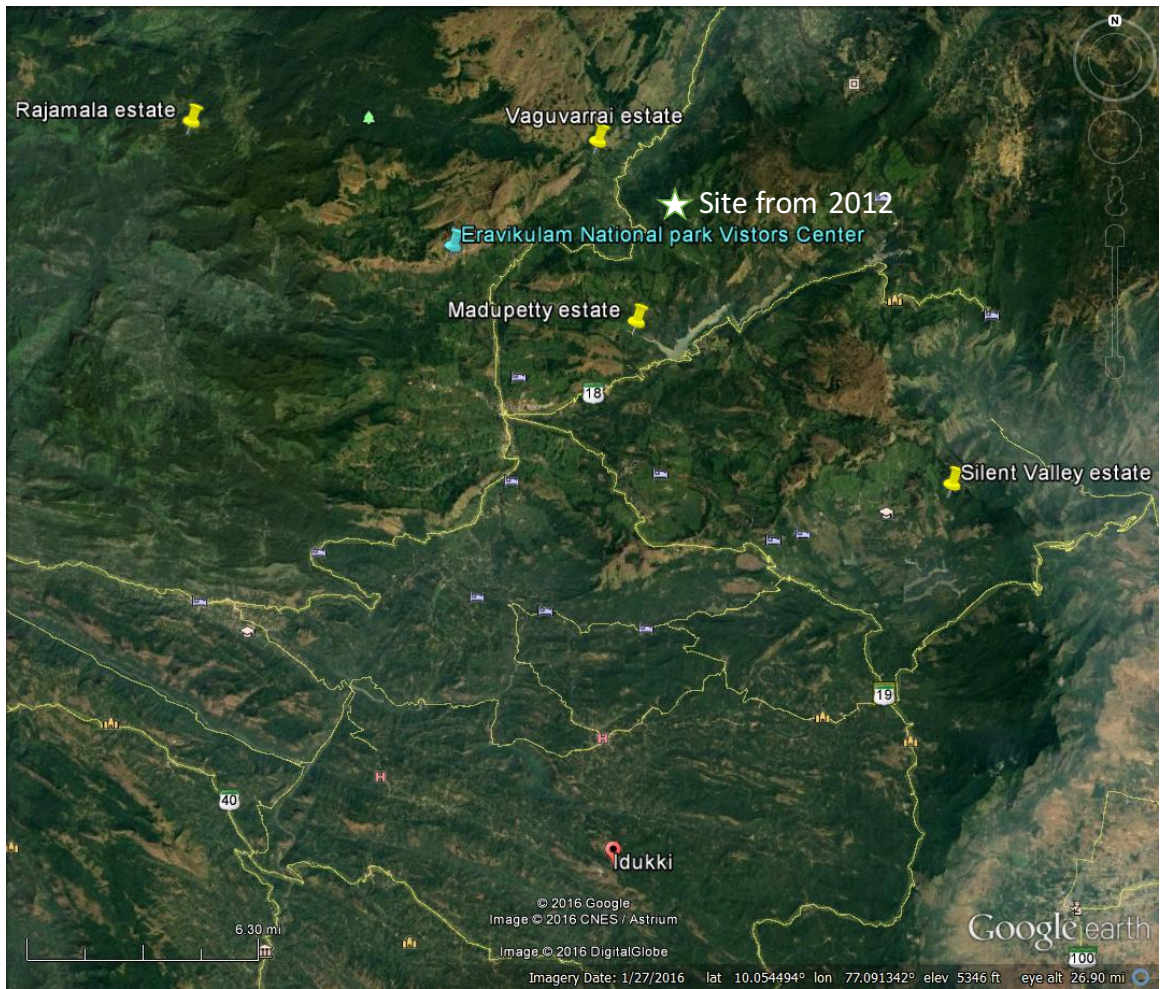


Figure 3.5. KDHP map with the four estate groups (1. Vaguvarrai; 2. Rajamallay and Pettimudi; 3. Madupetty and Thenmala; 4. Silent Valley) marked (Google maps, 2016).

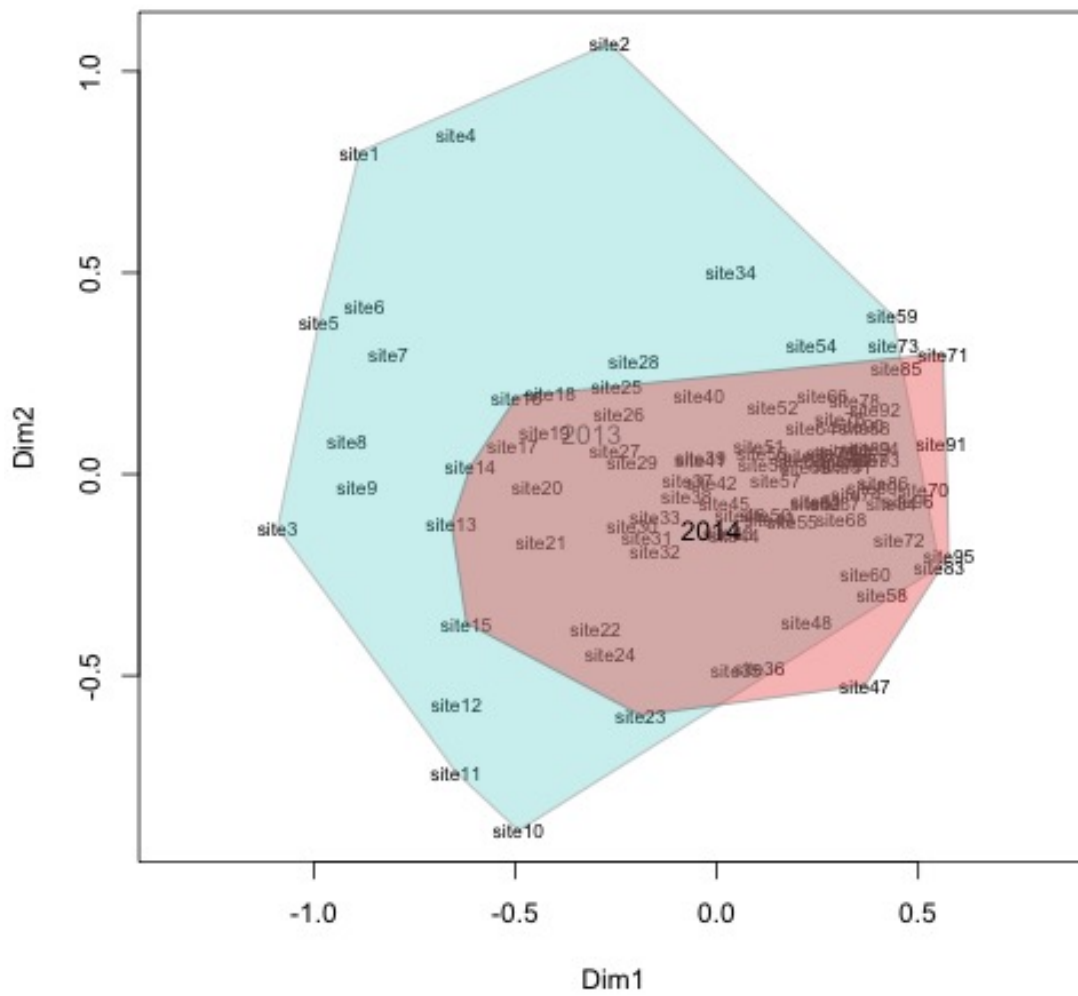


Figure 3.6. MetanMDS plot of similarity with ellipses drawn around samples from each year, 2013 and 2014 (Stress: 0.2510247).

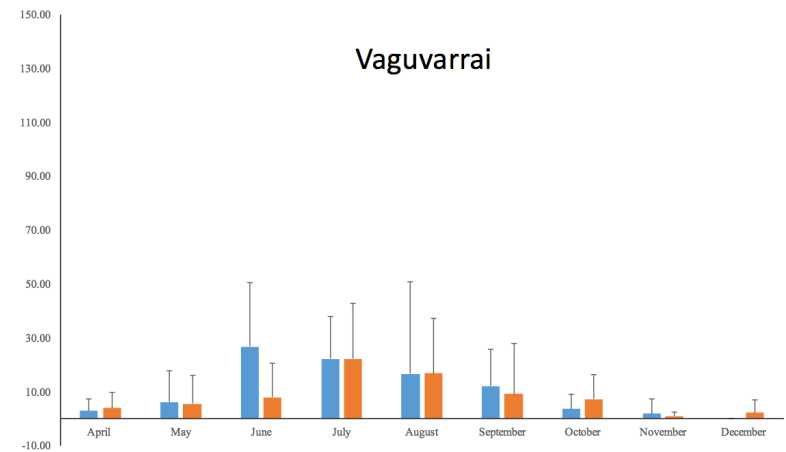
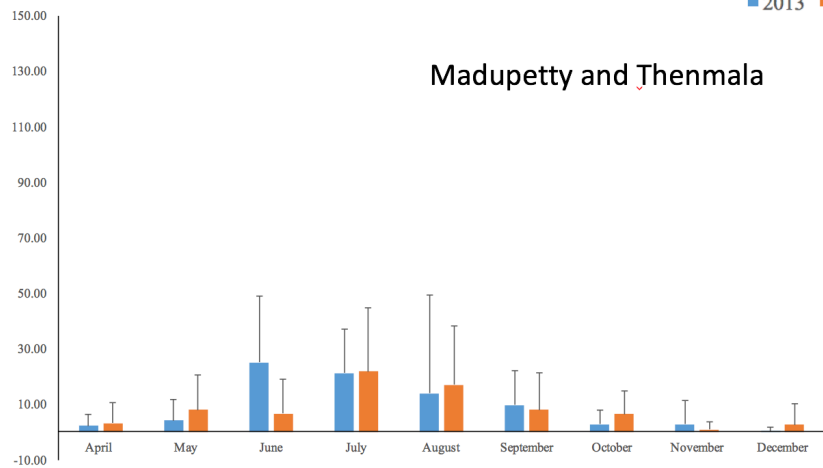
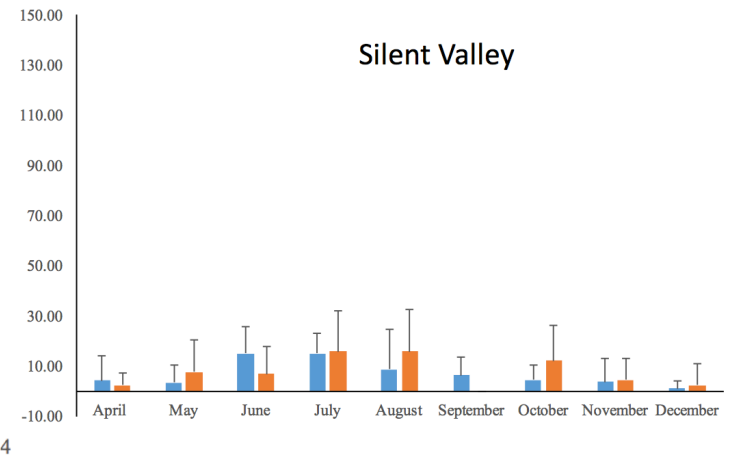
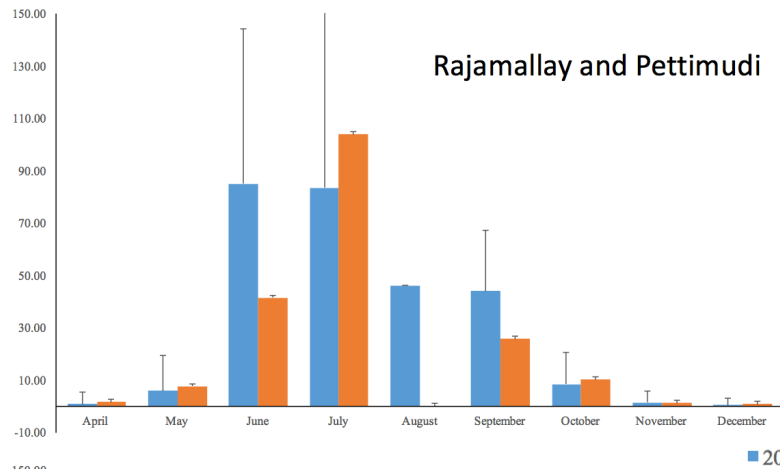


Figure 3.7. Average monthly Rainfall in mm(+1Standard Deviation) for years 2013 and 2014 in Rajamallay and Pettimudi, Silent Vally, Madupetty and Thenmala, and Vaguvarrai sites, Munnar.

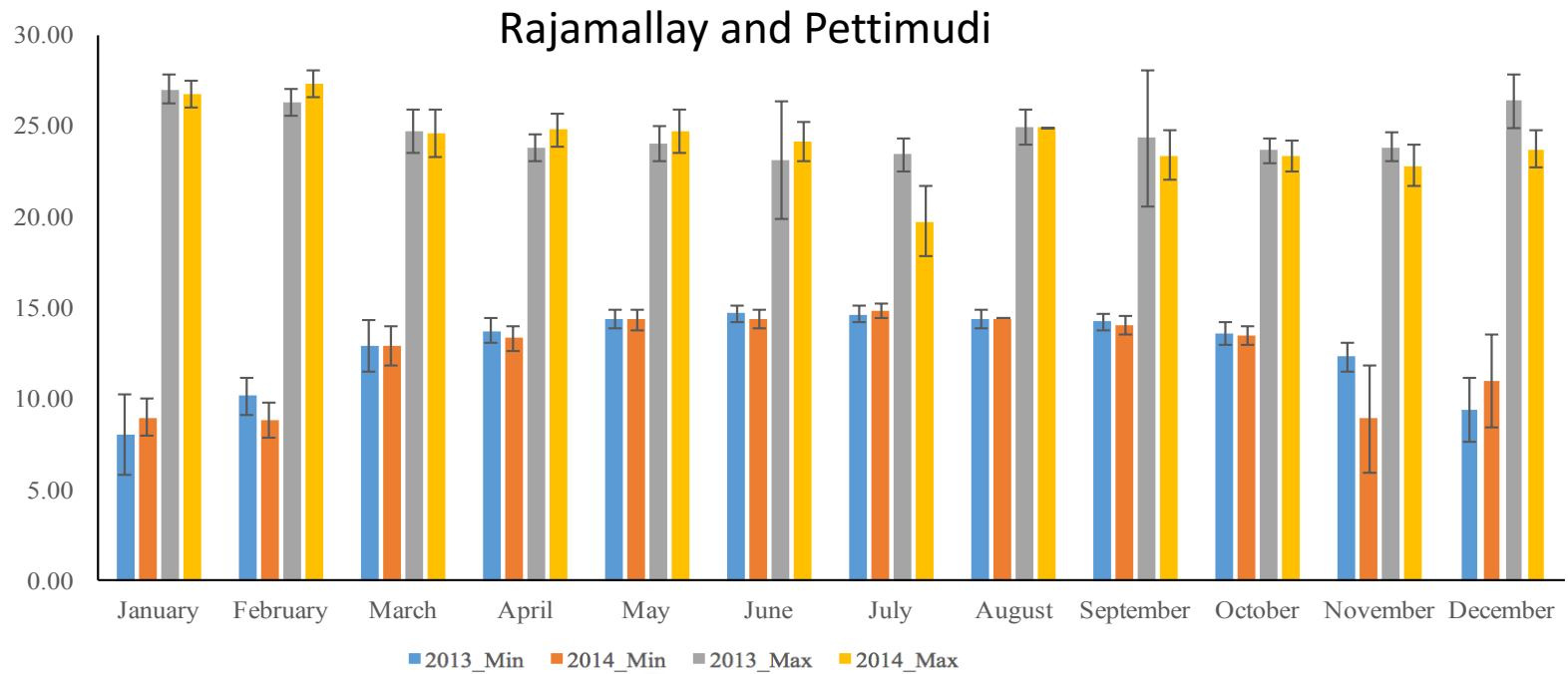


Figure 3.8. Average (\pm Standard Deviation) monthly minimum and maximum temperature in degrees Celcius for years 2013 and 2014 in the Rajamallay and Pettimud site, Munnar.

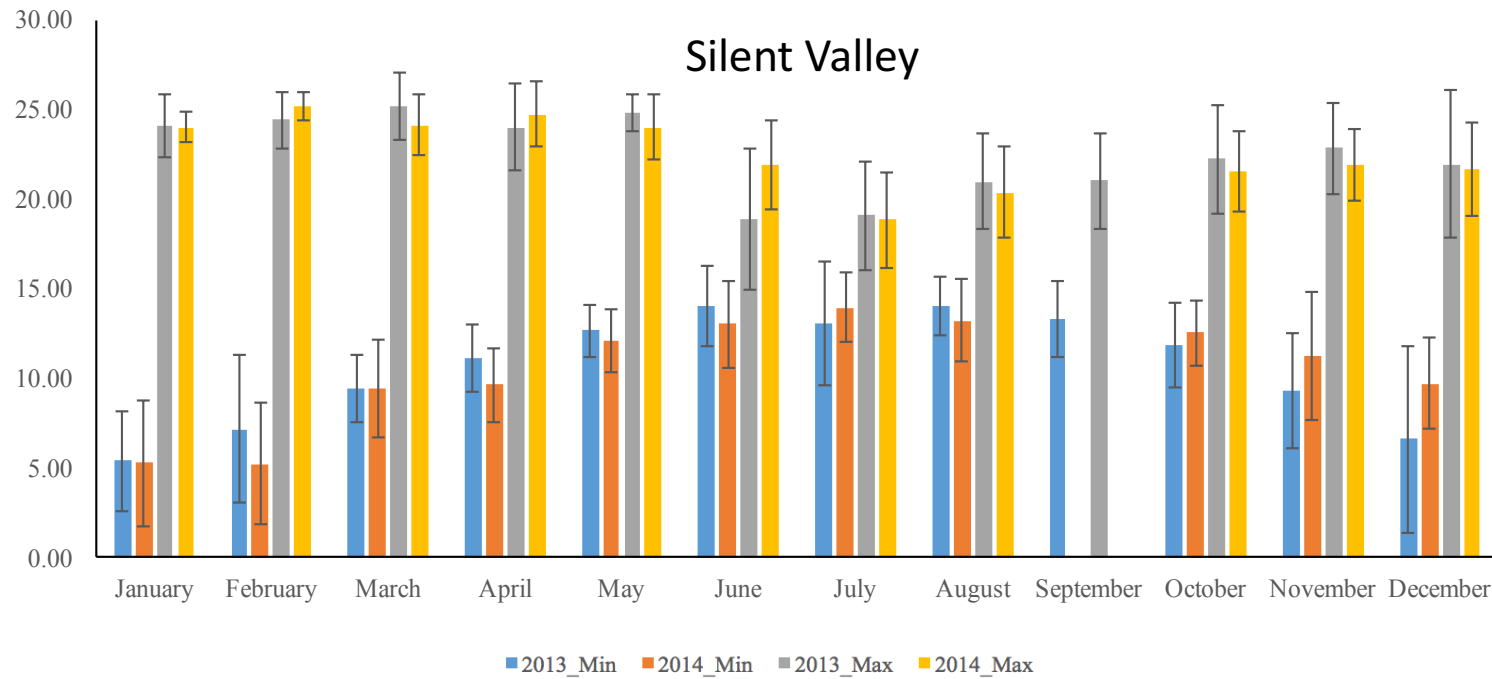


Figure 3.9. Average (\pm Standard Deviation) monthly minimum and maximum temperature in degrees Celcius for years 2013 and 2014 in the Silent Vally site, Munnar.

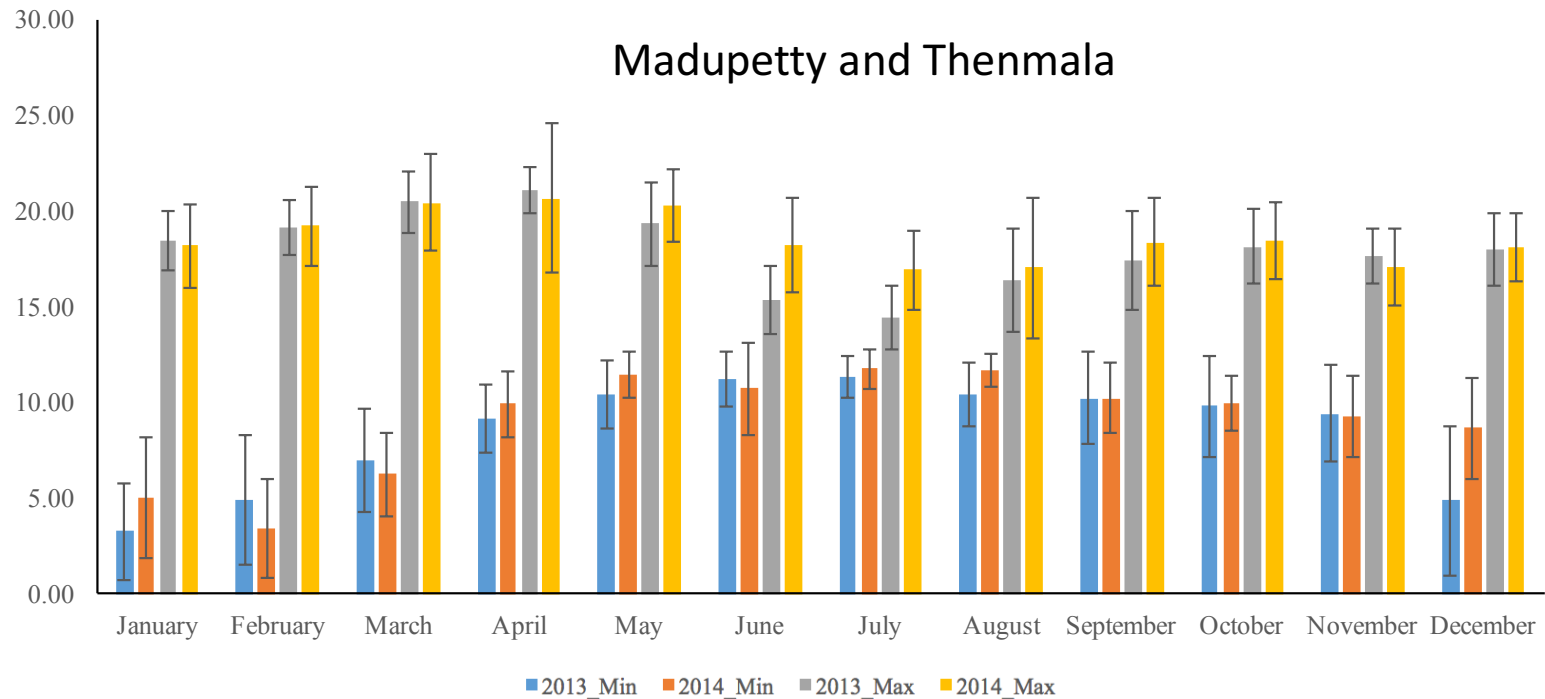


Figure 3.10. Average (\pm Standard Deviation) monthly minimum and maximum temperature in Celcius for years 2013 and 2014 in the Madupetty and Thenmala site, Munnar.

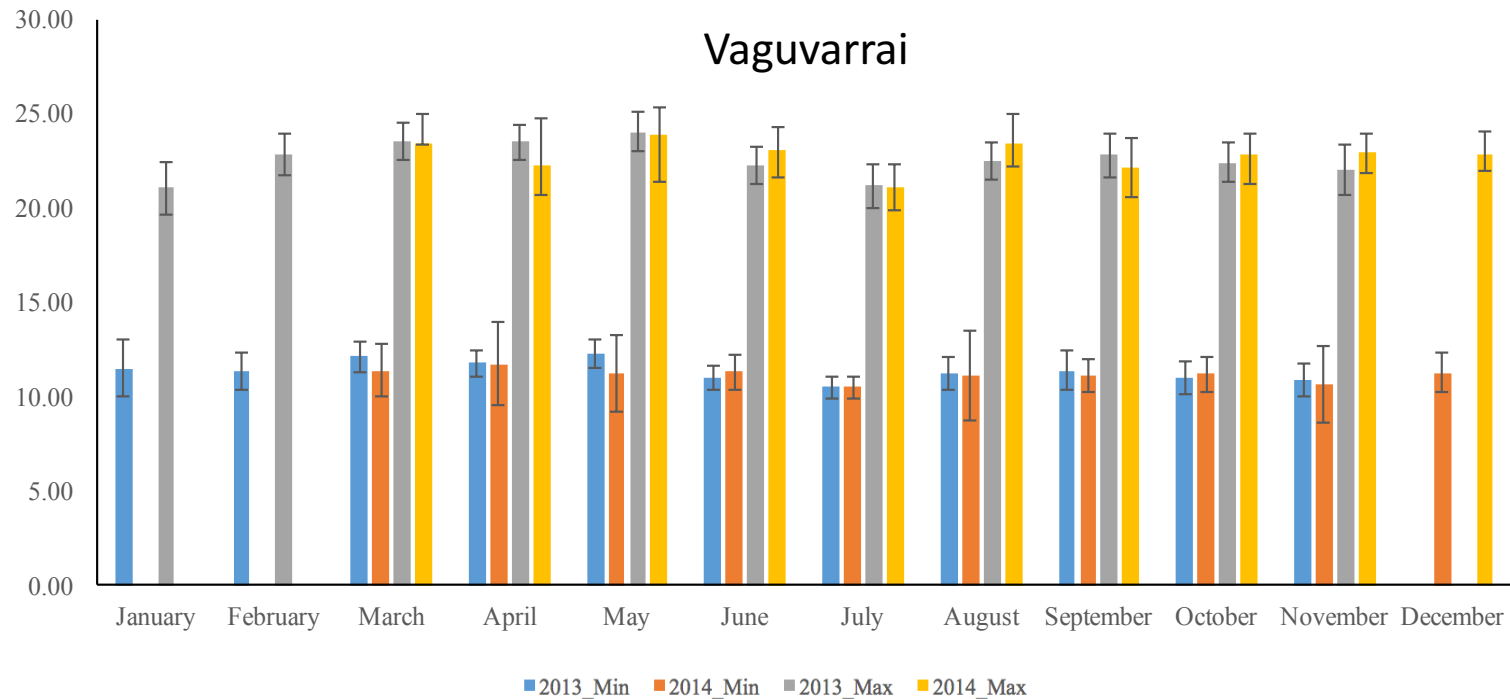


Figure 3.11 Average (\pm Standard Deviation) monthly minimum and maximum temperature in Celcius for years 2013 and 2014 in the Vaguvarrai site, Munnar

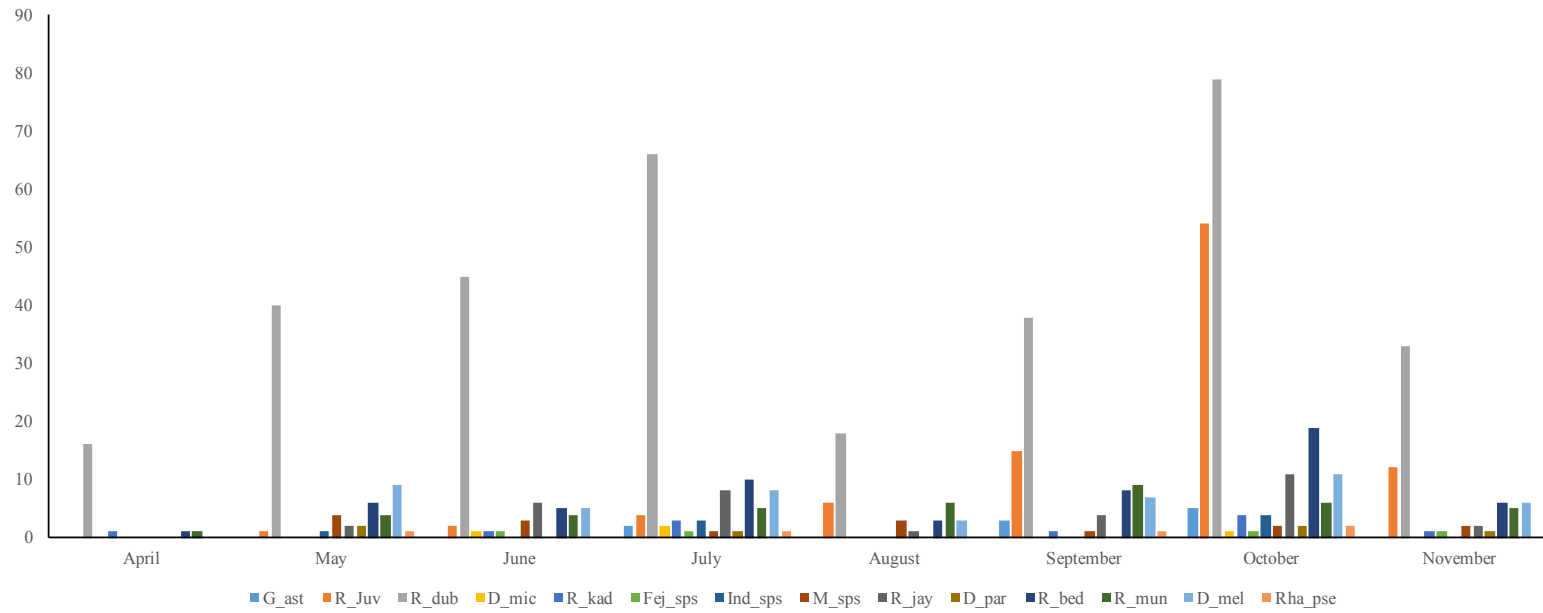


Figure 3.12. Species encountered per month in 2013 in the upland transects.

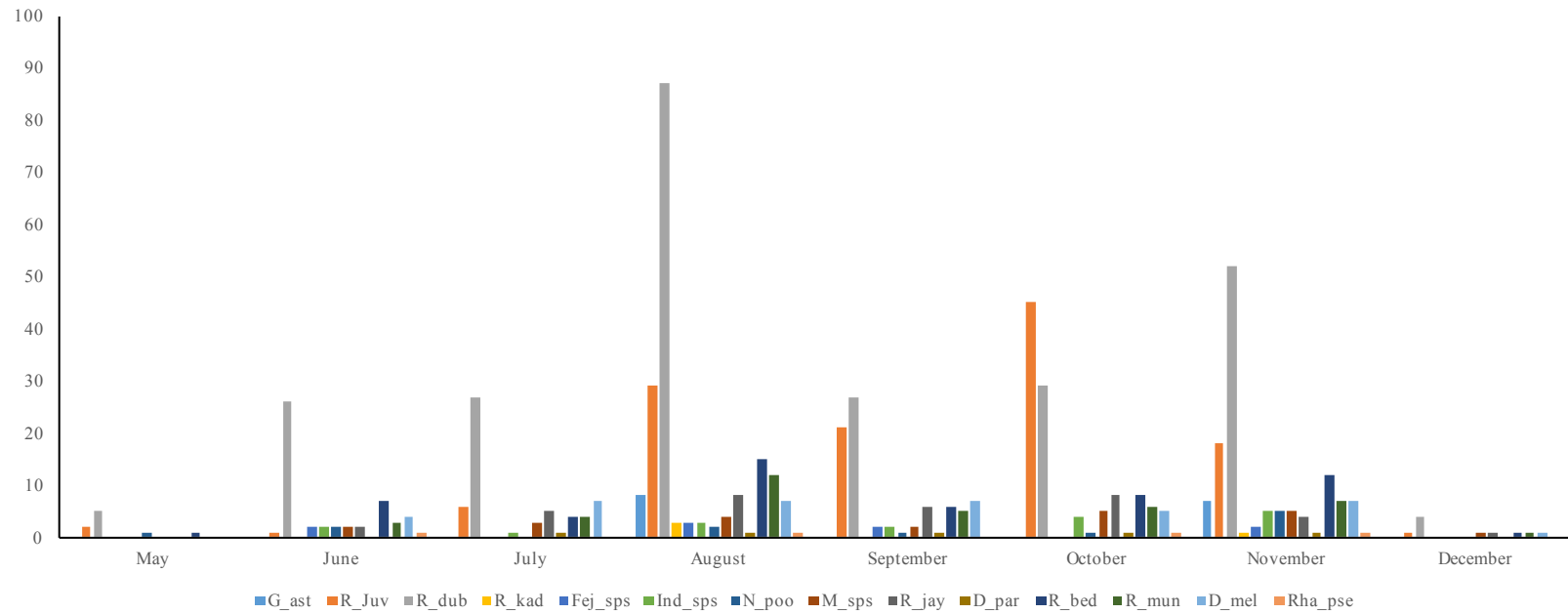


Figure 3.13. Species encountered per month in 2014 in the upland transects.

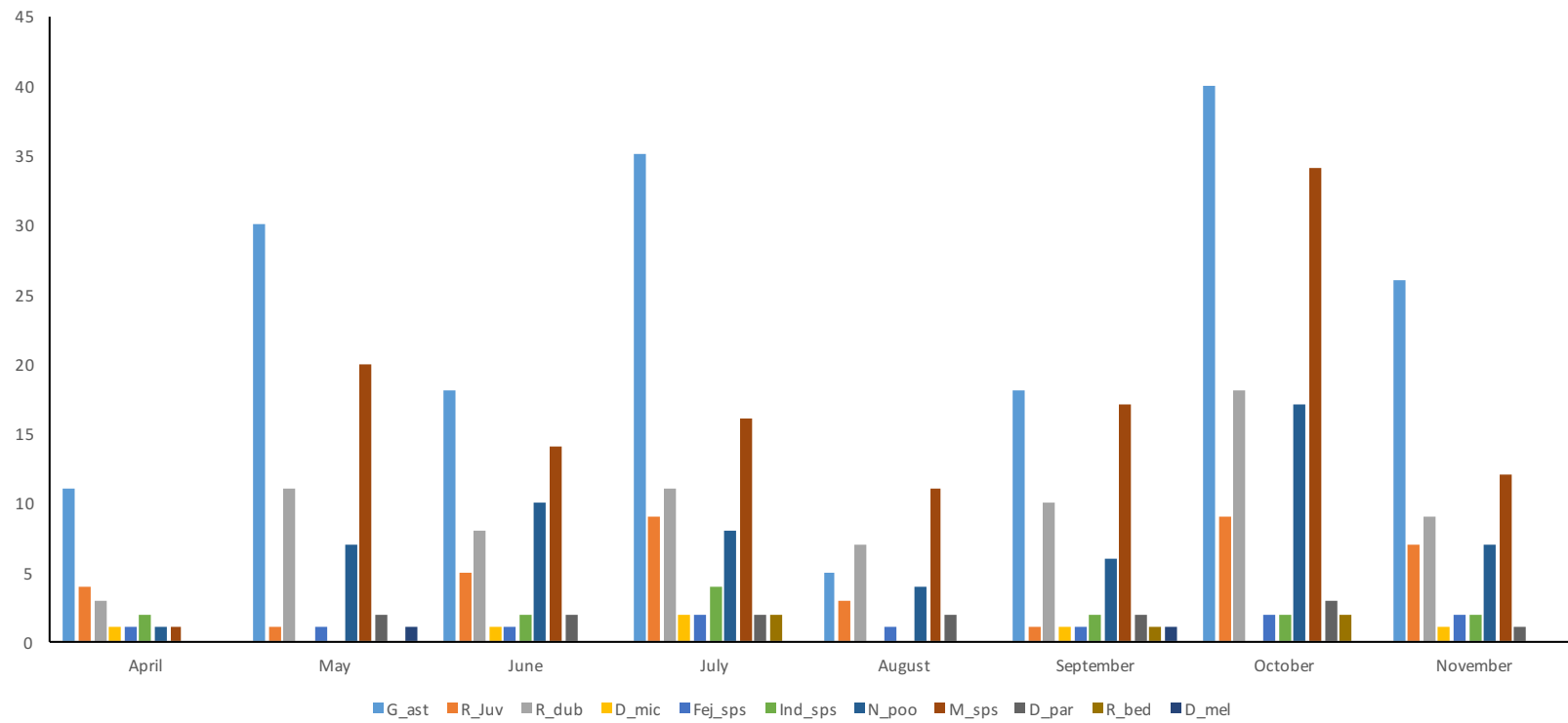


Figure 3.14. Species encountered per month in 2013 in stream transects.

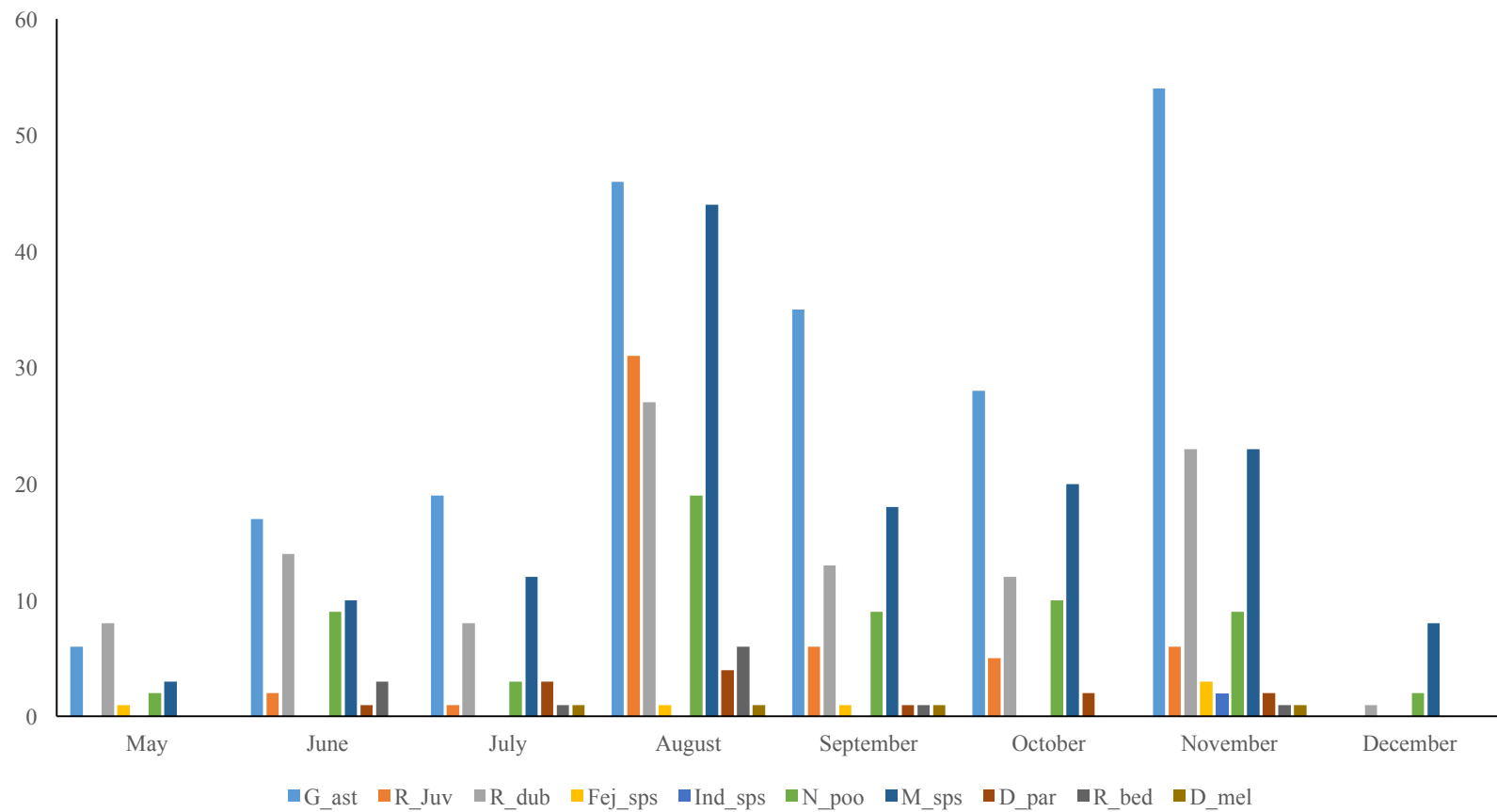


Figure 3.15. Species encountered per month in 2013 in stream transects.

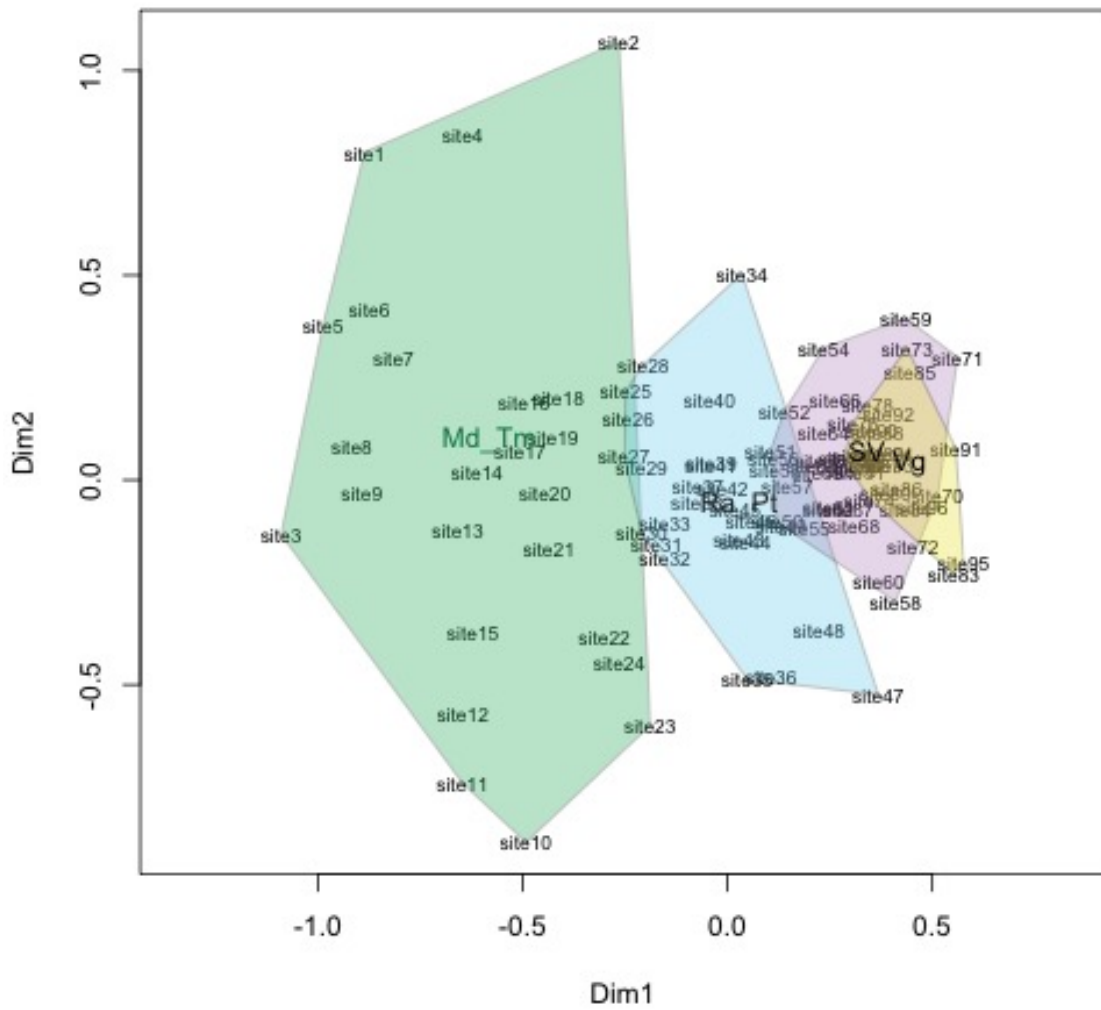


Figure 3.16. MetanMDS plot of similarity with ellipses drawn around the four different sites (Stress: 0.2510247).

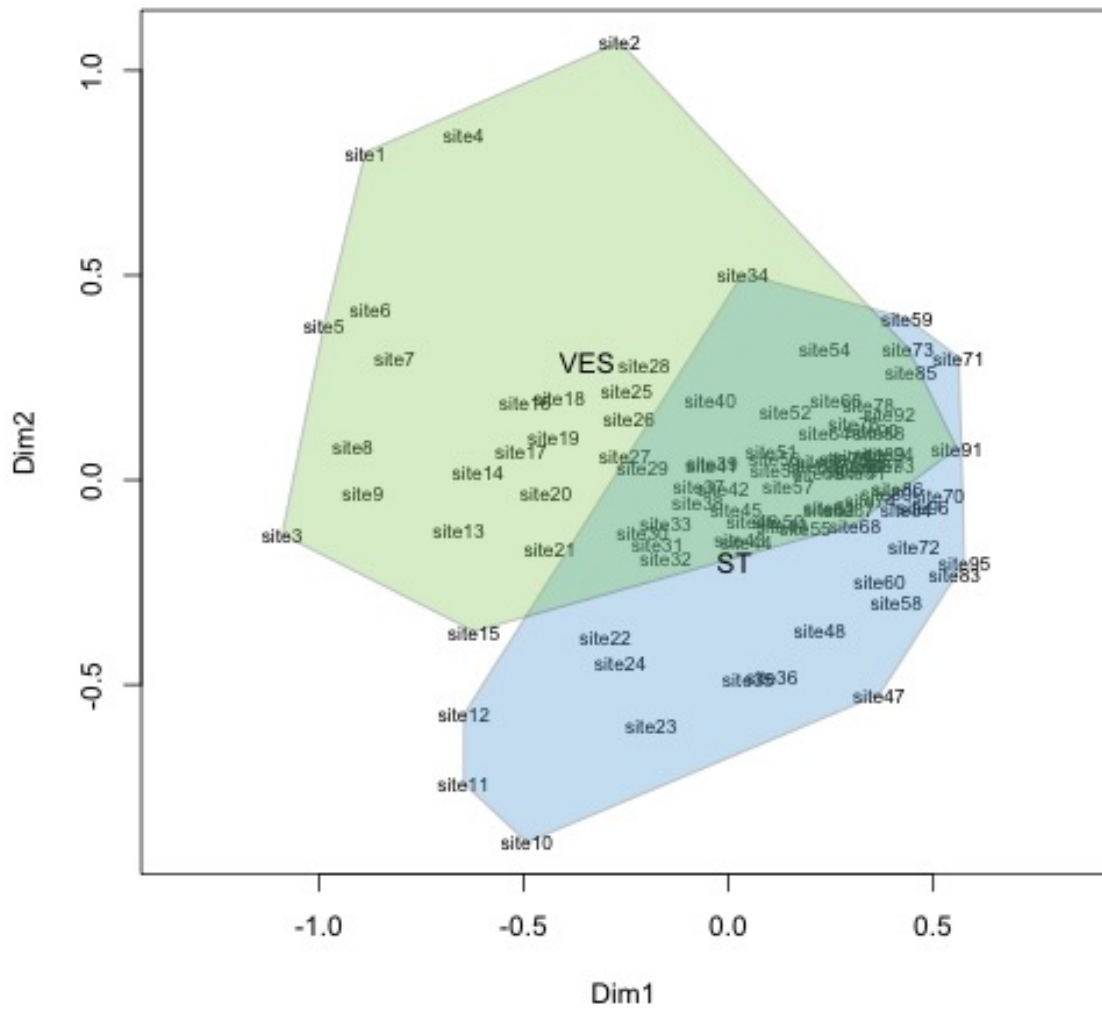


Figure 3.17. MetanMDS plot of similarity with ellipses drawn around the sites using either VES (light green) or stream transects (light blue; Stress: 0.2510247).

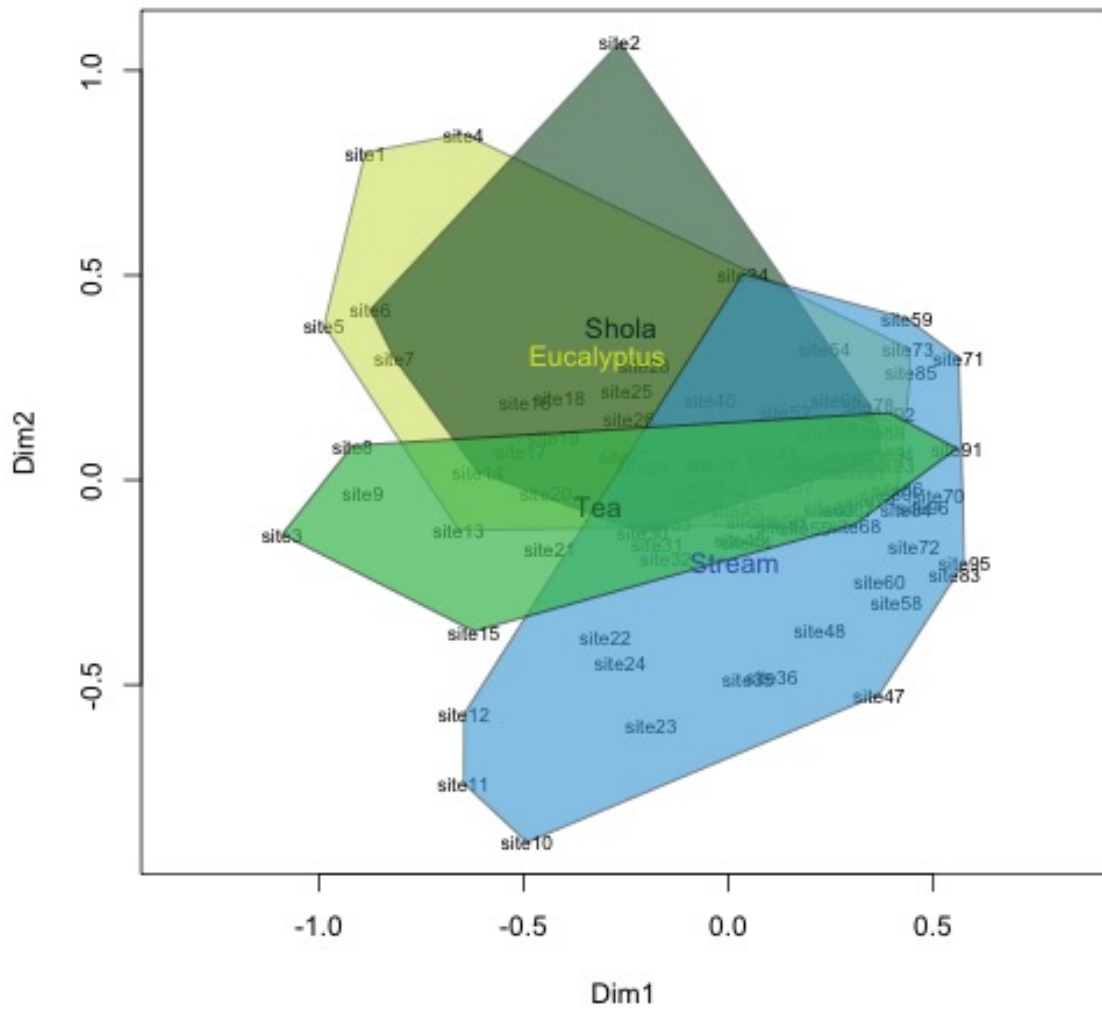


Figure 3.18. MetanMDS plot of similarity with ellipses drawn around sites from similar upland habitats, shola (dark green), tea (light green), eucalyptus (yellow), and around sites from stream transects (light blue; Stress: 0.2510247).

V. CHAPTER 4

Testing for Chytrid Infection in Tea Plantations in the Southern Western Ghats, India

Abstract

With the recent declines in amphibian populations attributed to infectious diseases, it is imperative to monitor for the presence of disease causing fungi like *Batrachochytrium dendrobatidis* (*Bd*) in areas that do not show obvious mass declines of amphibians. In the present study, I swabbed anurans encountered in tea plantations in the southern Western Ghats. The site is located in the Sri Lanka and Western Ghats Biodiversity hotspot. I swabbed 216 individuals of 17 species, including four critically endangered species, between 2012 and 2015. Even though six samples showed amplification in simple PCR, none of them were positive for *Bd* in triplicate quantitative PCR experiments. Even though we report the absence of the Asian strain of *Bd* in Munnar, Kerala, using the PCR method, it is important to continue monitoring for the presence of chytrid and other infectious diseases. My study was conducted in a managed tea plantation that has regular application of fertilizers and fungicides. My data provide baseline information for the region. Future sampling and continued monitoring of frogs in this hotspot will help us manage amphibian populations in agricultural systems.

Keywords

amphibians, *Batrachochytrium dendrobatidis*, chytrid, swab, tea plantations, Western Ghats

Introduction

Mass extinctions have been periodic events on Earth, however each of the past five mass extinction events have taken place over very long periods of time as opposed to the current sixth mass extinction event (James et al. 2015; Wake and Vredenburg 2008). The current extinction rates are higher than any extinction event rate since 1980 and the extinction rates of amphibians are 136-2707 times greater than the background amphibian extinction rate (McCallum 2007). The IUCN World Conservation Union Global Amphibian Assessment (GAA) has shown that approximately 2,468 amphibian species (~43.2%) are experiencing some form of population decrease, and approximately 7.5% are critically endangered which exceeds the numbers for endangered birds or mammals (Stuart et al. 2004). The amphibian decline phenomenon refers to the global-scale enigmatic reduction in populations of several species of amphibians in North America, Central America, Europe, Australia, and other locations (Araújo et al. 2006; Berger et al. 2016, Collins and Storfer 2003; Daszak et al. 1999; Laurence et al. 1996, Lips 1999; Lötters et al. 2009; Pounds et al. 2006; Skerratt, et al., 2007). Since the early 1980s, scientists documented declines of amphibians in different parts of the world without any obvious cause (Alford and Richards 1999; Baringa 1990; Berger et al. 2016; Blaustein et

al. 1994). During the first World Congress of Herpetology in 1989, scientists reported declines and the need to examine these supposed declines was discussed by herpetologists around the world (Baringa 1990; Lips 1998).

Initially all the drivers that were considered responsible for these declines included habitat change, pollution, deforestation, UV radiation, alien species, over-exploitation, and emerging infectious diseases (Alford and Richards 1999; Becker et al. 2015; Collins and Storfer 2003; Daszak et al. 1999). Extensive studies suggested that emerging infectious diseases, particularly chytridiomycosis, was responsible for many of these declines (Berger et al. 2016; Berger et al. 1998; Laurance et al. 1996; Lips 1998). Some of the tell-tale signs recorded were that these declines occurred suddenly, over a short period of time (as few as a few months), only adults seemed to be affected, no significant changes in environmental characteristics were noted over the period of the decline, stream-dwelling frogs seemed most severely affected, and mass mortalities were observed in some sites (Berger et al. 1999a; Laurance et al. 1966; Lips, 1999). The first scientific papers tying these declines to the fungus *Batrachochytrium dendrobatidis* were published in 1998 and 1999 (Berger et al. 1998; Berger et al. 1999a; Longcore et al. 1999). Berger et al. (1998) found no signs of fungal spores on specimens of frogs from museums in both Central America and Australia before the declines. Some claimed that amphibian populations were highly dynamic and that assuming that the declines were just a result of incomplete sampling without several continuous years of data was premature (Blaustein et al. 1994; Pechmann et al. 1991). Several scientists have since conducted

extensive studies and associated *Bd* with reduced amphibian abundances in Central America, and suggested that these results can be extended to other regions of the world showing similar declines (Berger et al. 1998; Lips et al. 2006; Lips et al. 2008). Lips et al. (2008) modeled the movement of *Bd* from the upper reaches of Central America down, through Costa Rica and Panama, and were able to document the spatiotemporal movement of the fungus. They failed to find support for the climate-linked epidemic hypothesis of Pounds et al. (2006) that suggests that climate change was making amphibians more vulnerable to *Bd* (Pounds et al. 2006). Rohr and Raffel (2010) found that factors like climate change, and habitat loss might have increased the severity of *Bd* attacks on amphibians. Rohr and Raffel (2010) also showed how over 80% of amphibian declines could be explained by variables directly pertaining to temperature variations and El Niño dependent variables, and are correlated to global *Bd* related declines. Thus, showing that global climate change makes amphibians more susceptible to infectious diseases.

Co-infections of *Bd* with ranavirus in four species from Costa Rica were reported by Whitfield et al. (2013) and the same co-infections have been reported by Warne et al. (2016) from five species in Peru. However, neither paper provides support for the facilitation of one infection by the other. On the other hand, mesocosm studies by Becker et al. (2015) show that frogs from pristine temperate forests show higher *Bd* loads than those from disturbed tropical forests when inoculated with standardized *Bd* zoospores and suggested that deforestation or habitat loss might support faster *Bd* growth because of

the added stress of habitat alteration. Becker et al. (2015) also reported that previous exposure to the fungus reduced the spore load on the naïve frogs than on frogs with prior exposure to *Bd*.

After almost three decades of research, scientists are still concerned about the consequences of chytrid because it is a generalist fungus, that puts over 41% of the worlds amphibians at risk (James et al. 2015; Monastersky 2014; Skerratt et al. 2007; Swei et al. 2011). *Batrachochytrium dendrobatidis* is also reported from non-anuran amphibians from Africa, Japan, and Mexico (Frias-Alvarez et al. 2008; Goka et al. 2009; Gower et al. 2013; Lips and Donnelly 2005). The first conclusive demonstration of non-amphibian hosts of *Bd* were crayfish from Louisiana and Colorado; the study also demonstrated that healthy frogs were infected when exposed to water with infected crayfish (McMahon et al. 2013). Exposure of crayfish to different concentrations of *Bd* inoculated water caused increased rates of molting and then mortality in crayfish (McMahon et al. 2013). These observations need to be taken into consideration during conservation reintroduction plans. A study conducted at the La Selva Biological Station by Whitfield et al. (2007) showed declines in both reptiles and amphibians over a span of 35 years. *Batrachochytrium dendrobatidis* had only been reported to affect amphibians from mid-elevation sites and reptile declines were not expected. Whitfield et al. (2007), suggested that changes in leaf litter, along with increasing temperatures might explain the declines they observed. In a later study at the same site, Whitfield et al. (2012) detected *Bd* spores from anuran frog skin swabs taken in the coolest months of the year.

Scientists are still debating about whether to call *Bd* a novel pathogen or whether the declines resulted from some abnormal change in an environmental variable that made amphibians more susceptible to the fungus (Skerratt et al. 2007). Another species of chytrid, *Batrachochytrium salamandrivorans* (Bsal), found only on urodeles was found during sampling in 2010 in Europe. *Batrachochytrium dendrobatidis* and Bsal are the only two species in the chytrid family of fungus that are known to affect vertebrates (Berger et al. 2016; Martel et al. 2012; Martel et al. 2013). *Batrachochytrium salamandrivorans* has a slightly lower optimal temperature range (10°C to 15° C) than *Bd* (Martel et al. 2012). The new species of *Batrachochytrium* has been reported as the factor causing salamander population declines in the Netherlands (Martel et al. 2013).

Though several studies pin the declines of amphibians solely on *Bd* or *Bsal*, scientists now believe that synergistic effects with disease have accelerated this phenomenon of global declines (Berger et al. 2016; Lips 1998; Pounds et al. 2006; Sodhi et al. 2008; Whitfield et al. 2007). Sodhi et al. (2008) used multi-model Bayesian inferential statistics on information compiled for 5,717 species of amphibians and found that models including a combination of climatic seasonality, habitat degradation, and human effects seemed to predict population trends better any single driver. James et al. (2015) suggests that instead of looking at *Bd* hotspots, it might be time to shift our attention to *Bd* coldspots and try and understand the factors that prevent amphibians from being

susceptible to the fungus in these areas and also predict which areas in the Americas are more susceptible to *Bd* and should be monitored closely.

Swabs from ten species of amphibians between 2010 and 2013 from the Seychelles Islands report no *Bd* present in representatives of the Order Anura and the Order Gymnophiona (Labisko 2015). Olsen et al. (2013) predicted that the Seychelles would be affected by *Bd* soon (Olsen et al. 2013). Olsen et al. (2013) calculated areas that were likely to be *Bd* positive using a database with over 5000 samples (Figure 4.1). Olsen et al. (2013) also showed that the range of occurrence of *Bd* on amphibians has not reached an equilibrium infection status as there are still new locations where *Bd* is being reported from every year showing an extremely heterogeneous spatial spread of positive results globally. The Olsen et al. (2013) study also predicts the odds of chytrid being present in hylids in Asia as very high (Figure 4.3; Swei 2011). Hence, scientists in Asia are still testing new sites for the presence of this fungus and are closely monitoring old sites for the possible chance of novel chytrid fungus introductions (Figure 4.4; Swei et al. 2011).

Longcore et al. (1999) first cultured a pure line of the amphibian fungus *Batrachochytrium dendrobatidis* (henceforth referred to as *Bd*) from a captive frog. The amphibian chytrid species (Phylum Chytridiomycota, Class Chytridiomycetes, Order Chytridiales) is a new species and was isolated from a Blue Poison Dart Frog (*Dendrobates tinctorius*) that was housed in the National Zoological Park, Washington DC. A close fungus to *Bd*, *Homolaphlyctis polyrhiza* gen. et sp. nov., was recently

described and its infection status has not yet been established (Longcore et al. 2012) suggesting that *Bd* is just one of the many members of the Chytridiales that could be infectious in nature. In the lab, cultures of *Bsal* differed from those of *Bd* by the formation of germ tubes (Martel et al. 2013). Both these fungi from the order Chytridiales are aquatic and have flagellated zoospores used for transport (Berger et al. 1999a).

Most studies associating chytrid with amphibian declines have shown that the declines have taken place in species that are characterized by an aquatic larval stages (Figure 4.2) which is where they come in contact with the zoospores (Lips 1998; Collins 2013). Since anuran larvae lack a keratinized epidermis the tadpoles are not generally affected by the fungus except for their mouthparts which are keratinized (Berger et al. 1998; Berger et al. 1999b; McDiarmid and Altig 1999; Rachowicz and Vredenburg 2004). The dental ridges of the tadpoles are affected to different extents depending on the temperature and the extent of infection, and infected tadpoles are reservoirs and they all metamorphosed into infected adults (Rachowicz and Vredenburg 2004). However, it is uncertain if the fungus is lethal to larval stages or if the infections reduce larval feeding efficiency. Kobly et al. (2015a) reported that 76.1% of the times a swab from a leaf where a *Bd* positive frog was previously perching resulted in a *Bd* positive result. Hence, the transfer of *Bd* from the aquatic habitat to the terrestrial habitat could occur from recent metamorphs of species with aquatic larval stages and then infect terrestrial direct developing species, for example, species in the genus *Craugastor* (Bielby et al. 2008; Kobly et al. 2015a; Lötters et al. 2009). The presence of chytrid spores in rain drops in recent studies in Cusco, Peru

suggests that strong winds and rain could help in the spread of *Bd* spores (Kolby et al. 2015b). High altitude and small geographic range are reported by Bielby et al. (2008) as the factors driving the relation between *Bd* positive, rapidly declining species and other *Bd* positive species. Nowakowski et al. (2016), proposed and successfully tested the idea that as mismatch in thermal tolerances of anuran hosts and *Bd* increased, the rate of *Bd* infection decreased. The zoospores of *Bd* mature and grow on the keratinized epidermis of amphibians and they might be able to survive as saprophytes on non-amphibian keratin (Longcore et al. 1999). Early studies showed that the colonies of *Bd* grew best at temperatures between 4°C and 25°C, with the pH between 4 and 8 (Piotrowski et al. 2003). Stevenson et al. (2013) reported that different isolates of *Bd* from Australia showed different thermal tolerances (up to 28°C) suggesting local adaptations in the isolates varied with local ambient temperatures. Recent work in Central America and Spain shows that the *Bd* load of individual amphibians is tightly connected with temperature (Berger et al. 2016; Fernández-Beaskoetxea et al. 2015; Whitfield 2011). The swabs taken from frogs in warm seasons had lower *Bd* loads than from frogs in cool areas and loads increased in the cool season when a favorable temperature range for *Bd* was obtained. Since the *Bd* fungus cannot sustain spore integrity at temperatures above 30°C, when monitoring for *Bd*, seasonality of the sampling needs to be taken into consideration (Johnson et al. 2003). Whitfield et al. (2012) show that there is a strong temporal affect on the *Bd* spore load on amphibians and the highest spore counts were obtained during the cold seasons at La Selva, Costa Rica which allow for the growth of temperature sensitive *Bd* spores. Experimental studies showed that holding populations of infected amphibians at 37°C caused the individuals to be cleared of the infections within

16 hours (Woodhams et al. 2003). Zoospores of *Bd* were found to survive in water from a lake without a host for up to six weeks, for up to four weeks in tap water, and up to five weeks in deionized water (Johnson and Speare 2003). Researchers in Australia have reported a behavioral response to chytrid in which frogs were basking on rocks which killed the fungus (Danskin et al. 2011; Puschendorf et al. 2011; Rowley and Alford 2013).

Work by Chestnut (2014) showed that five different fungicides in varying concentrations inhibited the growth of *Bd* in culture temporarily, and that *Bd* started recovering from the effects of fungicide addition after 7 days and was equal to the *Bd* load in the control samples within 21 days. Chestnut (2014) also observed a difference in the growth rates of individuals over time under different treatments. *Batrachochytrium dendrobatidis* affects frog species differently; some merely play the role of carriers whereas others are killed by the fungus (Briggs et al. 2010; Lips 1999; Lips et al. 2006). The spores of *Bd* do not die when the host dies, but persist on the skin of dead amphibians and these spores remain dormant until another host is obtained (Longcore et al. 1999). The presence of *Bd* can be detected in two different ways: by histological examination of the skin or by genetic methods using Polymerase Chain Reaction (PCR) amplification. The environmental DNA (eDNA) from water or soil can also be used to detect the presence of *Bd* in an area (Kolby et al. 2015a). The molecular method, though more expensive (\$13 per sample) than histological sectioning, is preferred, because PCR quickly quantifies the *Bd* load, i.e., the number of spores on a frog (Boyle et al. 2004; Kriger et al. 2006a). Quantification of

Bd zoospores can be done accurately using the histological sectioning method which are 2.46 times less expensive than the swabbing but it requires killing of the adult frog, as shown by a direct comparison of costs by Speare et al. (2005). However, counting individual spores on histological slides to assess *Bd* load is a cumbersome process. A study comparing these two techniques shows that the Taqman PCR procedure is much more efficient at detecting *Bd* and it is more ethical as it only involves swabbing the skin of frogs as opposed to harvesting the entire individual (Kriger et al. 2006a).

Recent studies by Farrer et al. (2011) have shown that there are three distinct lineages of *Bd*: BdGPL (found globally), BdCAPE (from South Africa), and the BdCH (from Switzerland). The BdGPL lineage seems to be recent and its emergence occurred at the same time that declines of amphibians were reported in North and Central America, Europe, Australia, and elsewhere (Farrer et al. 2011). The BdGPL lineage seems to be hyper-virulent and bears all the signs of a recombinant lineage because of short sequence length overlaps with several other strains (Farrer et al. 2011). The chance of such recombination occurring between such widely spread strains was probably assisted by the international trade in amphibians and reptiles (Farrer et al. 2011; Laurence et al. 1996). A study from South Africa predicts the regions that have an increased likelihood of *Bd*, determined on only positive samples from all over South Africa, which is important from a monitoring perspective, even though there are no clinical symptoms in any of these populations currently (Tarrant et al. 2013). Scientists believe that trade in amphibians especially, *Xenopus laevis*, a frog used for pregnancy testing, and *Lithobates*

catesbeianus (used as food) might have resulted in the recombination of the harmless or non-virulent strains of *Bd*, to result in the formation of the virulent killer fungus (Mazzoni et al. 2003; Parker et al. 2002; Weldon et al. 2004). Some researchers suggest that the difference in susceptibility to *Bd* is tied to innate immunity to *Bd* in certain species, whereas another school of thought states that the immunity to *Bd* is acquired because amphibians are known to have a cocktail of peptides or other compounds stored in their glands that are known to have antibiotic like synergistic effects on fungi and bacteria (Richmond et al. 2009; Rollins-Smith and Conlon 2005; Woodhams et al. 2006; Woodhams et al. 2007). Tadpoles with *r* and *g* haplotypes are more resistant to *Bd* than others, and resistance is determined by the presence of particular Major Histocompatibility Complexes (MHC) (Barribeau et al. 2008, McMahon et al. 2014). *Anaxyrus quercicus*, *Osteopilus septentrionalis*, and *Litoria booroolongensis* were shown to actively avoid *Bd* inoculated areas, as well as show a reduced infection rate when exposed to dead cultures of *Bd* before being inoculated with live *Bd*, showing the development of active immunity to the fungus (McMahon et al. 2014). In a detailed study on *Bd* infected frogs with symptoms, infected frogs without symptoms and uninfected control frogs of the species *Lithobates yavapaiensis*, Savage et al. (2016) reported an increase in the blood neutrophil to lymphocyte ratio in the *Bd* infected frogs, which is similar to a response shown during infection or stress. A decrease in the growth rate of infected individuals compared to the control group was also reported, even though the food intake was uniform in all groups (Savage et al. 2016). Savage et al. (2016) suggest that infected individuals might show prolonged tolerance to *Bd*, to buy enough time for their body's adaptive immune response to start functioning. An earlier study by Savage

and Zamudio (2011) suggested that frogs with the Major Histocompatibility Complex Q (MHC Q) and frogs which are MHC heterozygotes in general appear to show significantly reduced risk of death when exposed to *Bd*. Bataille et al. (2015) reported that the MHC binding pockets of three MHC-II antigen binding pockets (four, six, and nine) in 17 species of amphibians that survived *Bd* infections showed similar amino-acid binding conformations (Bataille et al. 2015), suggesting a similar global fingerprint for *Bd* resistant anurans; the presence of specific antigen binding pockets remains to be tested in additional *Bd* resistant species. Woodhams et al. (2007) showed that the survival rates after ex-situ infection of four different species (*Litoria caerulea*, *Litoria chloris*, *Mixophyes fasciolatus*, and *Limnodynastes tasmaniensis*) of Australian amphibians were different (*Lit. caerulea* and *M. fasciolatus* suffering 95% mortality) and that the species (*Lit. chloris* and *Lim. tasmaniensis*) with the highest resistance to mortality showed the presence of skin peptides secretions in larger quantities than the others. Melzer and Bishop (2010) found that four native species of leiopelmatid frogs in New Zealand were more resistant to *Bd* and produced more skin secretions than two naturalized *Litoria* species. *Leiopelma archeyi*, which was reported to have an 88% decline in five years was found to produce the largest quantity of peptide skin secretions (Melzer and Bishop 2010). When three different strains of *Bd* were used to infect frogs in a study, the individuals of all species died, however the time to mortality was very different for each of the strains showing that each strain of the fungus had a different intensity of infection (Berger et al. 2005). Therefore, the success of treatment of the infected populations can depend on the initial susceptibility of the species in question.

Harris et al. (2006), isolated several bacteria from 10 genera, from the skin of two amphibian species that showed *Bd* inhibition in ex-situ tests. Scientists showed that the addition of the anti-fungal bacterium found on amphibian skin, *Janthinobacterium lividum*, to amphibians infected with chytrid reduced the mortality associated with the infection (Harris et al. 2009). Bataille et al. (2016) showed that the microbiome of captive versus wild caught species of amphibians differed, as did the diversity between the dorsal and ventral communities in both groups. The difference in microbial communities could drive the presence of different peptide complexes on amphibians (Bataille et al. 2016). A study from Panama reported five frog species sharing a larger number Operational Taxonomic Units (OTU) of microbes between their body swabs and perch swabs, than having unique OTU's either from their body swabs or from their perch swabs (Rebollar et al. 2016). Rebollar et al. (2016) also reported that individuals of *Craugastor fitzingeri* found in *Bd* naïve places had a higher microbial diversity than individuals from places which had endemic *Bd* populations, suggesting that historical microbial data from amphibians may have played a role in the biogeography of the infection patterns of *Bd*. Lubick (2015), reported clearing a wild population of toads of chytrid on the Spanish island of Mallorca. Scientists are still trying to understand the links between the innate and acquired immunities that seem to cause different infection patterns of *Bd* in different species (Richmond et al. 2009).

An in-vitro study by Voyles et al. (2014) showed an increase in rates of zoospore formation and general population growth rates in two lineages of *Bd* that were diluted 10 and 50 times, respectively. Though there was no significant difference in the infection patterns of the two cultures, it is still important to consider differences in growth rates and spore formation, when storing *Bd* in culture (Voyles et al. 2014) and as well monitoring its evolution in the wild. Thus the global amphibian decline phenomenon has brought together a community of biologists, all trying to understand the drivers of these declines and a discover a way to reverse these declines.

Though sampling for *Bd* has been conducted on a large scale globally, Asia has been underrepresented in sampling for the presence of chytrid (Swei et al. 2011). Une et al. (2008) reported the first case of *Bd* in Asia, from a consignment of 45 frogs, with *Bd* reported from 9 of 18 species (27 out of 45 individuals) in a private pet collection. Following this finding, Goka et al. (2009) conducted sampling in Japan of native and exotic amphibian species, both in captive and wild condition including 265 from pet shops (3 native species and 15 non-native species), 294 (17 native species and 58 non-native species) bred in-situ and 2103 (47 native species and two non-native species) collected from the field, and they found positives for *Bd* in *Ceratophrys cornuta*, *Ceratophrys ornate*, *Lepidobatrachus laevis*, *Lithobates catesbeianus*, *Xenopus laevis*, *Afrixalus fornasinii*, *Ambystoma* sp., *Laurasiarana ornativentris*, and *Andrias japonicus*. For both captive and wild amphibians, 26 different haplotypes of the ITS1-5.8S-ITS2 region of *B. dendrobatidis* were found, including three specific to the Japanese giant salamander (*Andrias japonicas*). The species used in pet trade (*Lithobates catesbeianus*)

and for Biology classroom education (*Xenopus laevis*) were the two hosts with the largest number of *Bd* haplotypes (Goka et al. 2009), and since the incidence in the wild species was low, the authors suggest a major role by these two introduced species in the spread of *Bd*. Yang et al. (2009) reported the first observation of *Bd* from wild amphibian populations of three species (*Bufo gargarizans*, *Hyla japonica*, and *Lithobates catesbianus*) in South Korea. Again, as in the case of Japan, *Lithobates catesbianus*, an introduced free-ranging species, had most of the *Bd* positive samples. However, none of the swabbed individuals tested showed outward signs of chytridiomycosis which suggested that this exotic species was not susceptible to the strain of *Bd* in Korea. Kadekaru et al. (2015) showed that 20 out of 21 tadpoles of *Lithobates catesbianus* with abnormal mouth lesions tested positive for *Bd*. Chytrid was first reported from Indonesia in four amphibian species in the early part of the 21st century (*Rhacophorus javanus*, *Chalcorana chalconota*, *Leptobrachium hasseltii*, and *Limnonectes microdiscus*) by Kusrini et al. (2008). Three of these were found in streams and the fourth species was found near a stream. The tadpoles of *Leptobrachium hasseltii*, with thin keratinous dental ridges were found in the streams from which the adults were swabbed (Kusrini et al. 2008). In 2012, individuals of *Bombina orientalis*, *Cynops orientalis*, *Paramesotriton hongkongensis*, and *Xenopus laevis* obtained from import stock in Hong Kong, were shown to have low *Bd* loads (Kolby et al. 2014). The authors also reported low *Bd* loads from water samples taken from containers transporting these amphibians, raising concerns about spreading *Bd* infection through the improper disposal of water by the pet trade industry (Kolby et al. 2014). Since Hong Kong is a large center for the amphibian pet-trade industry, reports of *Bd* from captive amphibians here, is a matter of global

concern. Bai et al. (2010) first reported *Bd* from mainland China from four species (*Lithobates catesbeianus*, *Laurasiarana chaochiaoensis*, *Babina pleuraden*, and *Odorrana andersonii*). Later, Bai et al. (2012) reported the presence of 30 haplotypes of *Bd* from China in both wild caught and captive species, with *Lithobates catesbeianus* showing the highest prevalence of *Bd*. *Laurasiarana dybowskii*, which is intensively hunted for human consumption was reported to be *Bd* free by Wei et al. (2010).

The presence of unique *Bd* lineages to Asia, as suggested by Swee et al. (2011) is also supported by the study by Bai et al. (2012) with the identification of a haplotype that is unique to the Chinese endemic, *Babina pleuraden*. Several other studies have since confirmed the presence of *Bd* in China (Zhu et al. 2014; Zhu et al. 2016). Vörös et al. (2012), reported the first record of *Bd* from Thailand from a single individual of the species *Ingerophrynus parvus* (n=6), that did not show any external signs of infection. Savage et al. (2011), reported low *Bd* loads from four families (Megophryidae, Microhylidae, Ranidae, and Rhacophoridae) of amphibians from Malaysia with no external symptoms of chytridiomycosis, again suggesting that there might be a native strain of *Bd* in Asia. Fong et al. (2015) detected the presence of *Bd* spores in skin sections from formalin fixed species of amphibians from the 1910-1929 (Korean Peninsula). Yumi et al. (2010) also reported the detection of *Bd* spores in skin sections from amphibian samples from 1902 to 1937 in Japan. The detection of *Bd* spores in amphibian specimens when no reports of chytridiomycosis, supports the hypothesis of the presence of native non-infective, *Bd* haplotypes in Asia. Zhu et al. (2014) reported the observation

of low *Bd* spore counts from six out of 67 museum specimens between 1930-1939. A large sample of 3363 amphibians (Anura=3315; Caudata=48) from 15 Asian countries, reported an overall *Bd* prevalence rate of 2.35% with positive samples (greater than 1 genomic equivalent of *Bd*) from Indonesia, Kyrgyzstan, Laos, Malaysia, Philippines, and South Korea (Swei et al. 2011). None of the samples from the Order Caudata were positive for *Bd* but they were not tested for the presence of *Bsal* (Swei et al. 2011). Labisko et al. (2015), reported the absence of chytrid in samples from the Orders Anura, and Gymnophiona from 291 individuals from the Seychelles Islands. Bataille et al. (2013) reported that 11 out of 17 South Korean species had a *Bd* prevalence between 12.5% and 48.0%. The exotic species *Lithobates catesbeianus* had a prevalence of 24.7% (Bataille et al. 2013). On the basis of multi-locus genotyping, three of the 50 known haplotypes of *Bd* from South Korea were unique and different from the globally emerging recombinant lineage ('*Bd*-GPL'), as well as, from the Brazilian *Bd* Lineage (Bataille et al. 2013).

The presence of such large variations in the *Bd* haplotypes suggests that there have been several complex interactions between the native *Bd* lineages, and the globally ubiquitous *Bd*-GPL strain (Bataille et al. 2013). *Batrachochytrium dendrobatidis* was also reported from 19 out of 105 sample from lakes across Turkey with the positive samples from four amphibian species (*Pelophylax ridibundus*, *Hyla orientalis*, *Pseudepidalea variabilis*, and *Pelophylax caralitanus*) found in seven water bodies (Erismis et al. 2014). Although Bataille et al. (2013) reported a higher prevalence of *Bd* than Swei et al. (2011), the

Bataille group did not notice any outward signs of infections in the individuals sampled from South Korea and suggested that that particular haplotype of *Bd* might be endemic to the country. *Batrachochytrium dendrobatidis* has not been detected from the low-lying plains of northwest Borneo by Kaiser and Grafe (2011) or from the swabs from the United Arab Emirates (UAE) in both captive and wild samples (Soorae et al. 2012). Absence of *Bd* in Borneo and UAE may be the result of unfavorable high temperatures for the survival of *Bd*. No positives were reported in a study of *Bd* in Vietnamese salamanders, however, experimental ex-situ inoculations led to infection and death with three weeks, suggesting that this species is very susceptible to *Bd* infections (Thien et al. 2013). These studies did not test for the presence of *Bsal*.

The first studies of *Bd* in India reported that there was no *Bd* in histological samples of seven wide spread and commonly found amphibian species (*Duttaphrynus melanostictus*, *D. stomaticus*, *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, *Fejervarya limnocharis*, *Polypedates maculatus* and *Uperodon systomus*) from the state of Gujarat, however, no information on sample size was reported and only the tissue histology method was used (George et al. 2008). *Duttaphrynus melanostictus*, *D. stomaticus*, *Polypedates maculatus*, and *Uperodon systomus* are mostly terrestrial and arboreal species, while *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, and *Fejervarya limnocharis* are aquatic. The histological method does not quantify the spore load. The absence of *Bd* in Gujarat could be explained because the average maximum temperature in Gujarat is above the maximum temperature at which *Bd* is viable (30°C) for 10 months

of the year. Nair et al. (2011) reported one positive sample of the Asian *Bd* strain in one frog (*Indirana brachytarsus*: Family Ranixalidae) out of 142 samples from eight species from the Western Ghats in India. The authors report that the chances of a false positive are low since the positive was tested by two independent laboratories and suggest that such low rates of positives are not unique to the Western Ghats. Dahanukar et al. (2013) report the presence of an Asian strain of *Bd* from three species, *Nyctibatrachus humayuni*, *Indirana leithii* and *Raorchestes bombayensis* found in the northern Western Ghats on the basis of eight positive samples. Dahanukar et al. (2013) used diagnostic nested PCR, quantitative PCR, DNA sequencing, and histopathology. They reported external clinical symptoms in one of three species (*Nyctibatrachus humayuni*) suggesting that the Asian strain may not be lethal to at least two species (Dahanukar et al. 2013). Studies by Molur et al. (2015) report the presence of the generic Asian *Bd* strain in eight individual samples from eight different species from a total of 497 samples representing 22 different genera. They tested for *Bd* with nested PCR and the positive samples were tested in two independent laboratories.

Since the number of *Bd* monitoring studies in India are few and mostly from protected areas, I wanted to check for the presence of *Bd* in tea plantations in southern Kerala. I swabbed anurans using standard methods, in tea plantations in Munnar, India, to determine if *Bd* was present in this region of the southern Western Ghats. Since, there have been no mass die-offs of anurans reported from the Western Ghats, we predicted that all of our samples would either test negative for *Bd* or if any of them test positive

for *Bd*, we predicted that the spore load will be minimal. The study site is located in the Western Ghats and Sri Lanka Biodiversity Hotspot. Sampling was done for four years we ensured that sampling was done during months with lower temperatures (November and December). For the current study, swabs from 216 anurans were collected from four tea estates near Munnar, India from November, 2012 to December, 2015.

Study Area

The swabbing was conducted in the Kanan Devan Hills Tea Plantation Company Private Limited (10° N and 77° E) located in the Idukki district, of Kerala, in the southern Western Ghats. The company owns 240 Km² of land, and 100 Km² of which is under tea cultivation (Figure 4.5). The landscape has undulating hills and ridges that run from the northeast to the southwest direction. The altitude varies from 600 m above sea level (asl) to 2695 m asl. The highest point in the park (2695m), Anamudi (Elephant Peak), is the highest peak in peninsular India. Though located in the tropical belt, the climate in this area is temperate because of its altitude. Munnar receives rainfall both during the Southwest and the Northeast monsoons. The Southwest monsoon accounts for over 80 percent of the annual precipitation and occurs during June and July, and the rest of the precipitation is received during the Northeast monsoon which extends from October to November (Figure 4.6). The minimum temperature in the study period was 0°C, and the maximum temperature was 29°C. The humidity in these areas is high year around, usually above 80%. The area has wet evergreen, semi evergreen, moist deciduous, dry deciduous,

broad-leaved hill forest, montane wet temperate forest and grasslands according to the revised classification by Champion and Seth (1968).

Methods

Field Protocol for Swabbing Frogs

Time constrained and opportunistic surveying were used to find the anurans for swabbing in five estates, Kanniamallay, SilentValley, Madupatty, Rajamallay, and Vagavurrai (Figure 4.5). Swabs (IACUC-13-034-CR01 and IACUC-15-041) were taken from all the species that were encountered and individuals were swabbed using standard methods to obtain the material required for detection of the Bd fungus. Swabbing was conducted between 19:00hrs and 23:00hrs. Sampling was done in all available habitats in the tea plantations (tea, eucalyptus, shola forests, streams, along roads, as well as around human settlements). Each individual was handled with a new pair of sterile gloves and the standardized technique by Kriger et al. (2006a) was used to swab animals. For each sample, a new cotton swab was run firmly first along the dorsal surface, then along one lateral surface, then along the ventral surface, on the ventral surface of inguinal region and then along webbing of one of the legs of the frog (Kriger et al. 2006a). Each swipe was repeated five times on each of the body surfaces specified. No cross contamination between samples occurred. Each sterile swab was then placed in an individually labeled vial for analysis. The snout-vent length and weight for each of the swabbed individuals was measured using Vernier calipers (0.05mm least count) and a spring balance (0.5g least count), respectively. The condition of the skin of the frog was noted. The plastic

waste generated during the swabbing process (gloves and covers of the swabs) was carried away from the site and disposed of elsewhere. The samples were stored in a freezer (-4°C) until they were transported to the Laboratory for Conservation of Endangered Species (LaCONES), in Hyderabad, where the samples were analyzed.

Laboratory protocol for swab processing

The DNA extraction was done by adding 300µL of Extraction Buffer (Table 4.1) into a 2mL micro-tube. The tips of the swabs were cut so that they entered the tubes. Three tungsten carbide beads (Qiagen) (3mm) were added to each tube and bead beating was conducted for 150 seconds using a Qiagen tissue lyser. The tubes were centrifuged and then swabs were removed from the tube following further centrifugation (Goka et al. 2009). The supernatant was pipetted into new Eppendorf tubes and 20 µl of crude DNA was taken and diluted with 180 µl of deionized water (Goka et al. 2009). This diluted DNA was used as template for PCR.

The Polymerase Chain Reaction was performed in a 10ul reaction volume by using EURx master mix (Table. 4.2) containing 1ul of template, 5 picoMolar of each forward and reverse *Bd* specific ITS1-5.8S-ITS2 gene primers *Bd* a (5'- CAG TGT GCC ATA TGT CAC G-3') and *Bd* b (5'- CAT GGT TCA TAT CTG TCC AG - 3') and nuclease free water. The PCR results were visualized on a 2% agarose gel. Qualitative PCR was performed using the *Bd* specific primer, ITS1-5.8S-ITS2 gene primers *Bd* a (5'- CAG TGT GCC ATA TGT CAC G-3') and *Bd* b (5'- CAT GGT TCA TAT CTG TCC AG -

3') and the Taqman MGB probe designed by Boyle et al. (2004). Single sample qPCR was performed first (Kriger et al. 2006a). Samples showing amplification were then repeated in triplicate qPCR reaction. Samples were considered positive for *Bd* only when more than one out of the three replicates showed amplification. Qualitative PCR for *Bd* was done by using the protocol outlined by Boyle et al. (2004) and later modified by Kriger et al. (2006b).

Results

I obtained 216 samples from 17 species of anurans known to occur in the region. The individuals were swabbed between 2012 and 2015 (Table. 4.3). These include the critically endangered species *Raorchestes griet*, *Raorchestes munnarensis*, *Rarocheustes chlorosomma*, and *Rhacophorus pseudomalabaricus* (Table. 4.4). The threat status allocated by the IUCN Red-list for eight of these species was either data deficient or not evaluated. Eight of the 17 species were from the family Rhacophoridae represented by the genera *Raorchestes* (six species), *Rhacophorus* (one species), and *Ghatixalus* (one species). *Ghatixalus asterops* was the most represented species with 31 samples, this species was encountered perched on vegetation in slow flowing streams (Table. 4.5). In the 216 samples, only six samples showed amplification with simple PCR, and on further processing with pPCR in tri-replicate, none of the samples showed a positive result. Since all the samples showed up as negative, the prevalence of *Bd* in the Munnar region of the southern Western Ghats is reported to be zero. Throughout the period of the study the visual appearances of the frogs were noted and abnormalities or skin wounds were not

observed. The lowest temperature recorded when swabbing was 8°C and the highest temperature recorded was 26°C (Figure 4.7) which coincides with temperatures favorable for *Bd* growth. The largest number of samples are from the month of December and the fewest from the month of April (Figure 4.8) this is because swabbing was not uniformly distributed across the months and effort was taken to swab during December, to test for the presence of *Bd* in winter.

Discussion

My study showed that over a period of four years *Bd* was not present in 17 species of anurans near Munnar in the Western Ghats. The species I sampled included aquatic and terrestrial species, as well as several direct developing species from the genus *Raorchestes*. The daily minimum temperatures in Munnar are lower than those in the plains by 10°C, however, the highest temperature during mid-day exceeds the optimal range for *Bd*, throughout the majority of the year, except for a few days in late December.

A study conducted in Nevada, showed that two species of amphibians had *Bd* spores with load levels which can be damaging even when the day time temperatures were as high as 32°C (Forrest et al. 2015). The authors suggest that even though the air temperature, is high the amphibians never reach such temperatures because of their nocturnal habits and aquatic habitat (Forrest et al. 2015). Because temperature has a major influence on growth and survival of the *Bd* fungus, the temperature at our study sites might be one of

the explanations for why Munnar has not suffered a mass die-off of amphibians. Daniels (2003) suggested that a possible explanation for the absence of any visual skin deformities of anurans in the tea plantations, in his study could be the regular application of fungicides which are directed at Blister Blight, caused by a fungus that damages tea production (Daniels 2003). Munnar in the Western Ghats, a biodiversity hotspot, is a site where several new amphibian species are waiting to be described. Although there are reports of a few positives *Bd* samples, there have been no mass die-offs of amphibians reported from any part of the country (Dahanukar et al. 2013; Molur et al. 2015). These studies suggest that the Asian *Bd* strain may not be as lethal as other strains. The Asian strain may not be as virulent as other strains, or the lack of infection could be because *Bd* is temperature limited in most parts of India as the daily temperature regularly exceeds 24°C. Even though the general trend of declines because of *Bd* are not seen in the Indian subcontinent it is critical to monitor frogs to assess their infection status.

Since fungi like *Bd* are reported to be extremely dynamic (Olsen et al. 2013; Swei et al. 2011), strains that currently infect anurans but show no symptoms of visible infection in amphibians may mutate into ones that are more infective than the strains already found in the community or infective strains of *Bd* may be introduced into a naïve community of amphibians causing mass die-offs. Swei et al. (2011), suggest that the reasons for low *Bd* prevalence in Asia are 1) *Bd* is not yet established 2) *Bd* and the anurans in Asia have co-existed and hence, it does not cause a threat to the amphibians here and 3) *Bd* is inhibited by some biotic or abiotic factor that scientists are yet to understand (Figure 4.4). Goka et

al. (2009) report the detection of *Bd* in old formalin fixed specimens of *A. japonicas* from 1901, suggesting that the *Bd* haplotypes found in Japan are probably native to Japan and have existed with amphibians in Japan from several hundred years, explaining why there have been no massive die-offs reported from the country. The Japanese pattern is very different from the studies of amphibian from the New World and Australia (Berger et al. 1998.). Several of the *Bd* positive individuals in Asia have haplotypes that are mixes between the globally emergent *Bd*-GPL strain and other native strains suggesting that the pet trade industry may play a role in the global spread of *Bd*. In addition, to the presence of several haplotypes of *Bd*, the new threat of *Batrachochytrium salamandrivorans*, a fungus found in European salamanders (Martel et al. 2013) gives us reason to be concerned about the spread of these fungi globally. In January of 2016 the US Fish and Wildlife Services added 20 genera of salamanders to their list of injurious wildlife species, as a preemptive measure to prevent the spread of *Bsal* to the United States. The synergistic action of humans, climate change, habitat loss, and fragmentation are difficult to quantify though a few studies have shown that humans have added to the pressures on amphibians (Venesky et al. 2014). A review of *Bd* cases in South America showed a steady decline in the lag times between the first detection of *Bd* and mass declines of amphibians at a site (Philips and Puschendorf 2013) suggesting that *Bd* may become increasingly virulent with time. With a life-cycle of approximately 61 days, it is likely that *Bd* is undergoing mutations each generation, whether or not these mutations in the fungus are directly correlated to increased rates of amphibian population declines remains to be seen (Philips and Puschendorf 2013).

Scheele et al. (2014b) put forth a detailed management plan to tackle *Bd* spread and mitigation. The plan included suggestions for prevention, treatment, and increasing resilience in case of *Bd* infections, both long-term and short-term. While some of management ideas are harder to implement globally than others (translocation to *Bd* free areas), where some suggestions such as prevention of infection in captive population by thermal manipulation are more feasible options. Whitfield et al. (2016) put forward 11 suggestions for the future management of chytrid in Central America and these recommendations are applicable globally; further research in amphibian life history and disease, researching interactions of amphibian stressors, *Bd* reduction plans, identification and protection of rare species, starting long-term research, establishing captive seed populations, and most importantly developing a viable long term conservation policy. Though these are directed for Central America, they would work well in other parts of the world.

In the last two years, more than one group of scientists have agreed that it might be more important to study *Bd* coldspots and understand what makes some species more resistant to *Bd* than others, than to study *Bd* hotspots (James et al. 2015; Mendelson 2011). These coldspots could be areas of high prevalence of *Bd* but low negative impacts on native populations which makes the study of the innate and acquired immunity of the amphibians in these areas important from a conservation perspective (James et al. 2015). The recolonization of the Australian species *Litoria verreauxii verreauxii*, in old habitats and those rich with wooden debris, 16 years after *Bd* caused declines is a sign that there is

hope that *Bd* affected species may recover, albeit at different rates (Scheele et al. 2014b). The emergence of generalist fungi and their spread over the decades warrants the constant monitoring for the presence of novel infections in amphibians around the world (James et al. 2015). Alroy (2015) reported that approximately 200 species (3.1%) of amphibians have gone extinct since the 1970s and that we might lose another 6.9% of our amphibian diversity in the next century if we continue at the same extinction rate. With herpetologists focusing on how to reverse the current trend of global amphibian declines, it is imperative to try and formulate several strategies that attach the primary driver of these declines (Scheele et al. 2014a). Fundamental to these strategies is to test and monitor sites that do not show mass amphibian declines yet, ex-situ sites where species can exist without exposure to *Bd*, and gradual increasing of immunity of native populations to *Bd* strains (James et al. 2015; Scheele et al. 2014b). In times of global concern because of declining amphibian populations it is especially important to check and keep monitoring areas for the presence of infectious diseases on a regular basis. With empirical information we should continue to monitor known populations. It is only by a combination of several methods that we may be able to revert the current trends of amphibian declines.

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Tables

0.1M Tris-Cl (pH8.0)	1ml
0.5M EDTA (pH8.0)	2ml
Non-idet P-40	50uL
1M NaCl	100uL
Mili Q water	6.85m

Total	10ml

Table. 4.1. Composition of Extraction buffer.

Master mix	5 ul
Forward primer (10 pmole/ul)	0.5ul
Reverse primer (10 pmole/ul)	0.5ul
Mili Q water/ nuclease free H ₂ O	3 ul
DNA	1 ul

Total	10ul

Table. 4.2. Composition of PCR mix

Sn/Year	Species Name	Tea	Shola	Eucalyptus	Other
1	<i>Duttaphrynus melanostictus</i>	Yes	No	Yes	road
2	<i>Duttaphrynus parietalis</i>	No	No	Yes	Roadside, Village housing
3	<i>Fejervarya brevipalmata</i>	No	No	No	Swamp
4	<i>Fejervarya</i> sps	No	No	Yes	Swamp
5	<i>Ghatixalus asterops</i>	Yes	No	No	Stream vegetation
6	<i>Indirana</i> sps	No	Yes	No	Stream
7	<i>Micrixalus adonis</i>	No	Yes	No	Steam
8	<i>Micrixalus fuscus</i>	No	Yes	No	Steam
9	<i>Nyctibatrachus poocha</i>	Yes	No	No	Steam
10	<i>Ramanella cf montana</i>	Yes	Yes	No	-
11	<i>Raorchestes dubois</i>	Yes	Yes	Yes	-
12	<i>Raorchestes beddomii</i>	Yes	Yes	Yes	-
13	<i>Raorchestes chlorosomma</i>	Yes	Yes	No	-
14	<i>Raorchestes griet</i>	Yes	Yes	No	Roadside
15	<i>Rarocheates jayarami</i>	Yes	No	No	Roadside
16	<i>Raorchestes munnarensis</i>	Yes	Yes	No	Roadside
17	<i>Rhacophorus pseudomalabaricus</i>	No	Yes	No	Stream vegetation

Table. 4.3. Habitats where frogs were swabbed during the years 2012-2015 in Munnar, Kerala

Sn/Year	Species Name	2012	2013	2014	2015	Total
1	<i>Duttaphrynus melanostictus</i>	0	4	0	0	4
2	<i>Duttaphrynus parietalis</i>	0	1	0	0	1
3	<i>Fejervarya brevipalmata</i>	1	0	0	0	1
4	<i>Fejervarya</i> sps	0	8	10	4	22
5	<i>Ghatixalus asterops</i>	7	3	10	11	31
6	<i>Indirana</i> sps	0	1	2	1	4
7	<i>Micrixalus adonis</i>	0	0	0	3	3
8	<i>Micrixalus fuscus</i>	3	7	3	0	13
9	<i>Nyctibatrachus poocha</i>	1	4	4	9	18
10	<i>Ramanella cf montana</i>	0	5	0	0	5
11	<i>Raorchestes dubois</i>	0	5	4	2	11
12	<i>Raorchestes beddomii</i>	3	1	3	18	25
13	<i>Raorchestes chlorosomma</i>	9	5	0	1	15
14	<i>Raorchestes griet</i>	0	0	3	0	3
15	<i>Rarocheches jayarami</i>	0	17	12	0	29
16	<i>Raorchestes munnarensis</i>	6	5	8	9	28
17	<i>Rhacophorus pseudomalabaricus</i>	0	0	3	0	3
Total		30	66	62	58	216

Table. 4.4. List and number of species swabbed during the years 2012-2015 in Munnar, Kerala.

SN/YEAR	SPECIES NAME	IUCN RED LIST STATUS
1	<i>Raorchestes chlorosomma</i>	Critically Endangered
2	<i>Raorchestes griet</i>	Critically Endangered
3	<i>Rhacophorus pseudomalabaricus</i>	Critically Endangered
4	<i>Raorchestes munnarensis</i>	Critically Endangered
5	<i>Fejervarya brevipalmata</i>	Data Deficient
6	<i>Fejervarya</i> spp	Data Deficient
7	<i>Ghatixalus asterops</i>	Data Deficient
8	<i>Indirana</i> spp	Data Deficient
9	<i>Duttaphrynus melanostictus</i>	Least Concern
10	<i>Duttaphrynus paritelis</i>	Near Threatened
11	<i>Micrixalus fuscus</i>	Near Threatened
12	<i>Raorchestes beddomii</i>	Near Threatened
13	<i>Micrixalus adonis</i>	Not Evaluated
14	<i>Nyctibatrachus poocha</i>	Not Evaluated
15	<i>Ramanella cf montana</i>	Not Evaluated
16	<i>Rarocheastes jayarami</i>	Not Evaluated
17	<i>Raorchestes dubois</i>	Vulnerable

Table. 4.5. IUCN Red List categories all the species tested for Bd between 2012-2015 (IUCN Red List, 2015).

Figures

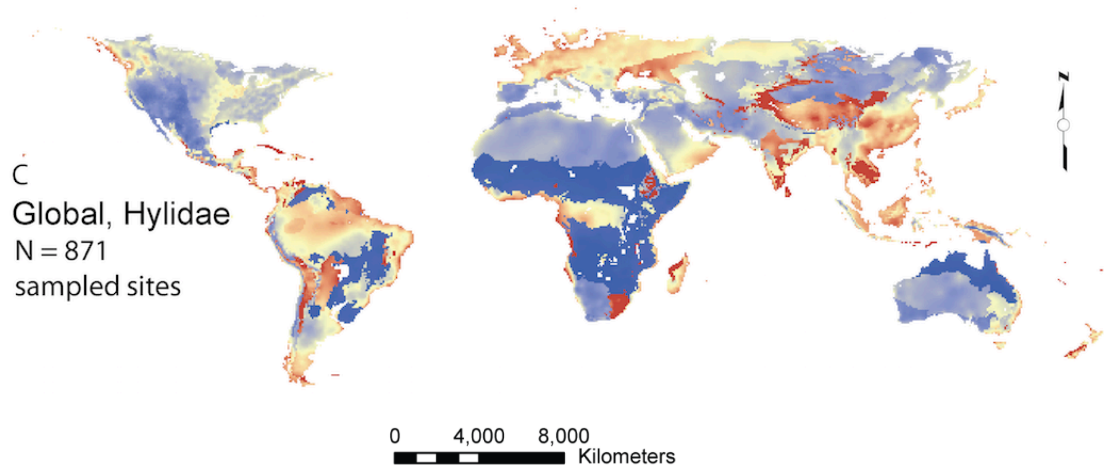


Figure 4.1. Probability of chytrid infection for Hylid amphibians globally from extrapolated maps (Olsen et al. 2013)

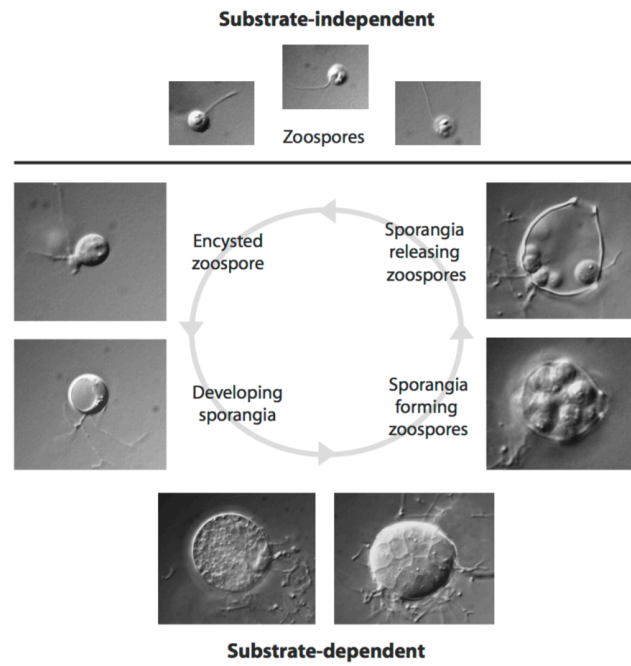


Figure 4.2. Life-cycle of Chytrid showing both aquatic (substrate independent) and terrestrial phases (substrate dependent) (Rosenblum et al. 2008).

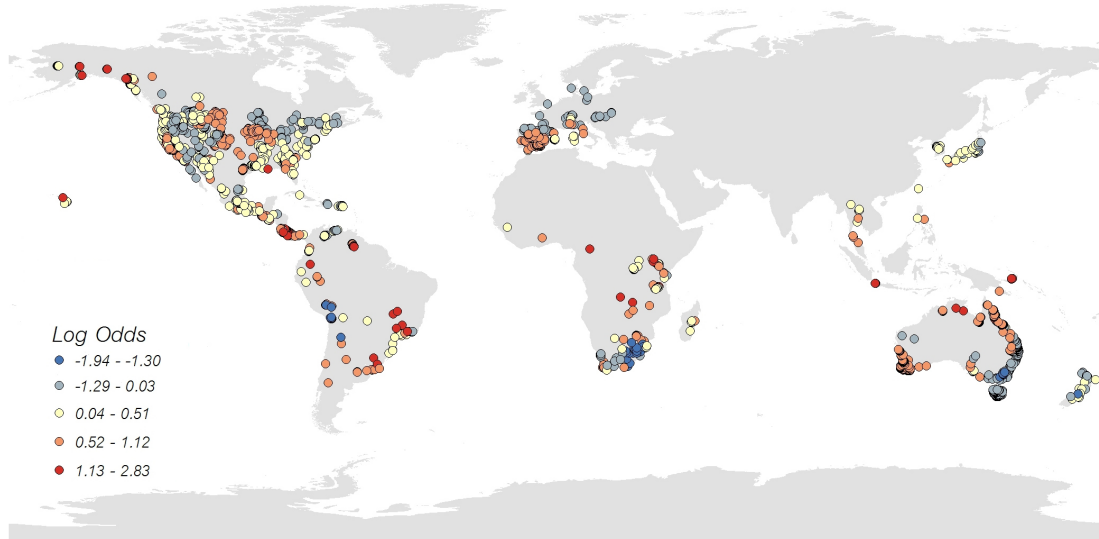


Figure 4.3. Global odds of chytrid detection in amphibians (Olsen et al. 2013).

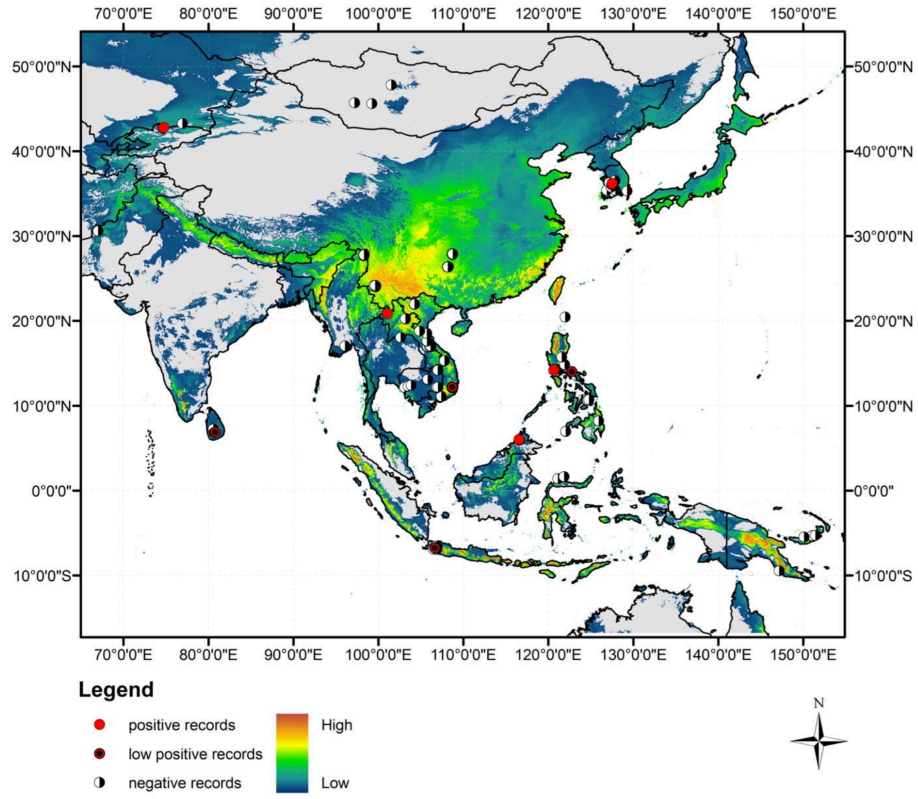


Figure 4.4. Map of predicted and observed *Batrachytrium dendrobatidis* detection in Asian amphibians (Swei et al. 2011).

**Average Rainfall+SD(mm) and Relative humidity+SD(%)
for Rajamala in 2012**

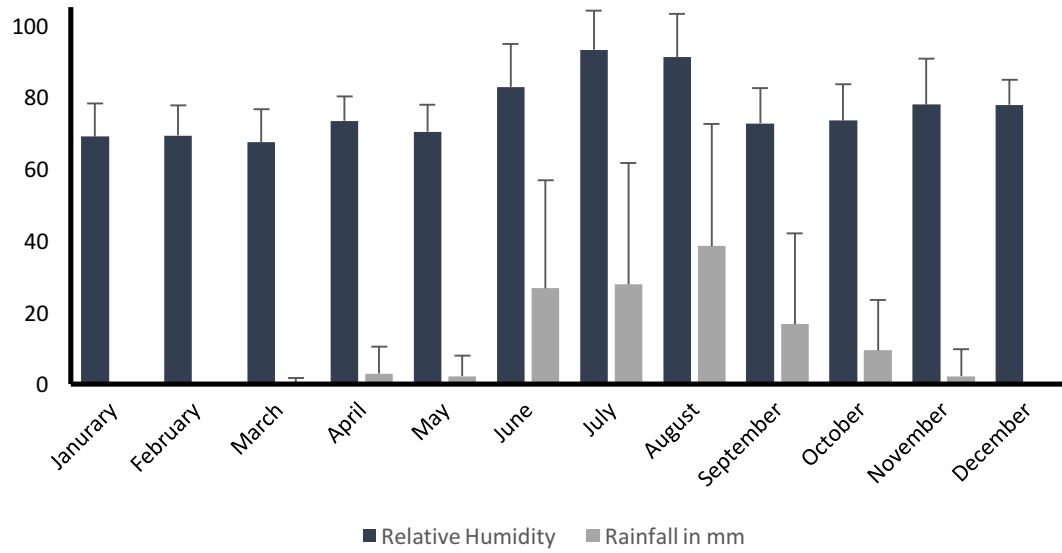


Figure 4.6. Monthly averages of Rainfall and humidity for year 2012 in Rajamala tea estate, Munnar, India.

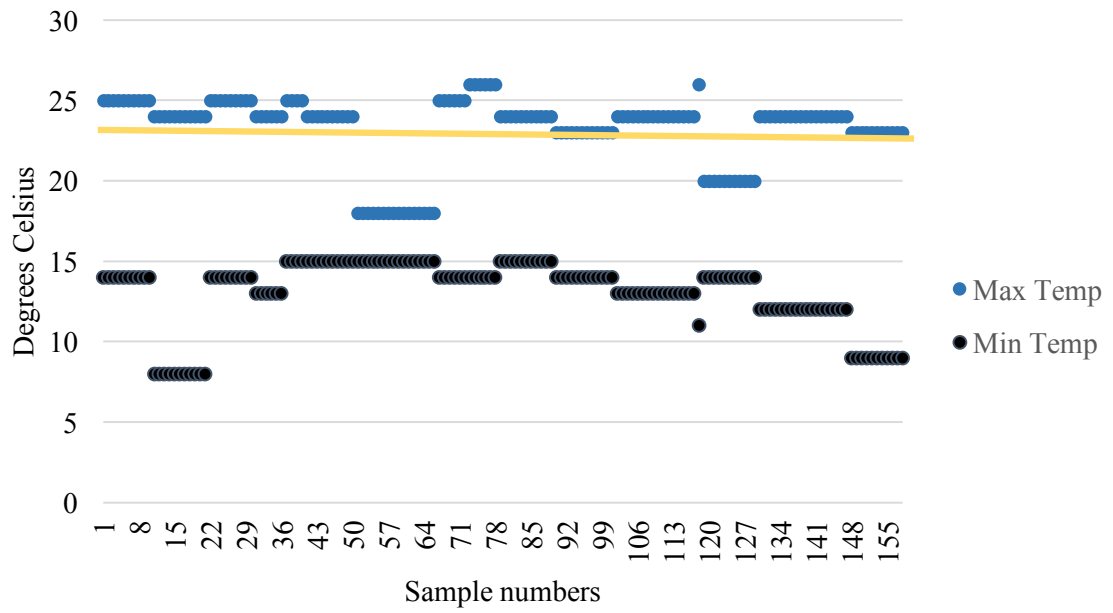


Figure 4.7. Maximum and minimum temperatures on days when samples were collected along with a line to represent the highest temperature that Bd spores can withstand.

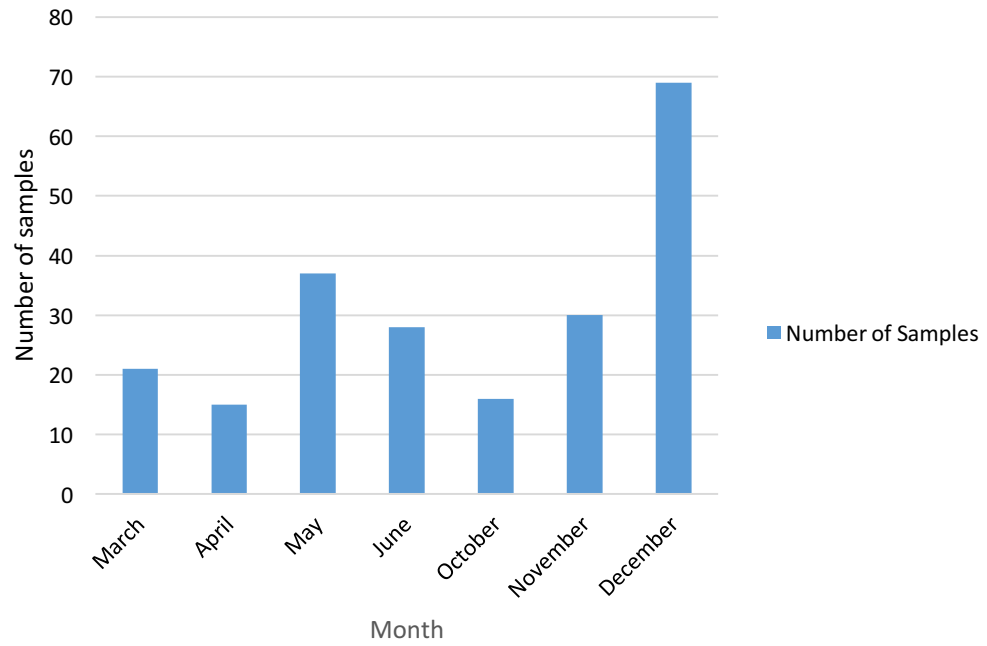


Figure 4.8. Number of swabs collected per month between 2012 and 2015.

VI. CONCLUSION

The tea plantation landscape in south India is dominated by tea fields, eucalyptus patches, and small remnant patches of shola forests. My goal was to test determine if there was a difference in the amphibian communities in different locations across the tea landscape or whether the amphibian communities were better explained by the dominant habitat patches repeated across the landscape. Another goal for this project, was to generate information on the anuran communities in tea plantations in the Munnar area. Ecological studies of anurans are in their infancy in India and my study focused on dominant feature of altered tropical habitats at mid-elevations. I was also interested about the status of *Batrachochytrium dendrobatidis* (*Bd*), a strong driver of global anuran declines, in this landscape.

Chapter One is a review of all papers comparing amphibian diversity in plantations to amphibian diversity in a natural forest habitat nearby, and a case study of amphibian species richness from four estates in the tea plantation landscape in southern India. From each paper obtained, I extracted information about the age of the plantation, the type of plantation, the type of harvesting employed (complete or periodic), whether it was in a Biodiversity hotspot or not, the number of species in the dominant plantation type, the number of species in the paired forest habitat, and which latitudinal zone the study was conducted in. I found none of these factors affected anuran diversity, except for the type of harvesting, where the plantations which were harvested periodically, like coffee, tea, and cacao had higher amphibian species richness than those that practiced complete harvesting. Using three standard amphibian sampling methods and opportunistic

encounters during 2012, at elevations between 1500m and 2000m asl in the Munnar district of Kerala, India, I report about the presence of 25 species of anurans. Of these 25 species, nine are from the first three categories of the IUCN Red list. Another nine anuran species were either data deficient or not evaluated. Also, 24 of these species are endemic to Western Ghats and several species from the *Rarochestes* genus are endemic to the Munnar region of the Western Ghats. The Western Ghats is known to have highly endemic species with narrow ranges of distribution, therefore the presence of 25 species in an old tea plantation landscape, suggests that these old tea plantations in the area are providing some refuge to these species.

In Chapter two, I rated the efficiencies of different standard methods of surveying amphibians in three different habitats (tea, shola forests, eucalyptus forests). I used nocturnal and diurnal, upland Visual Encounter Surveys (VES), Stream Transects (ST), and funnel traps with drift fences. Pit falls traps were discouraged by the plantation managers and hence, were not used. Funnel traps with drift fences are not recommended, as I only encountered four individuals after 64 trap nights in each habitat. Diurnal VESs were discarded after 0 amphibians were encountered. Stream transects in shola forests gave the highest species per person effort, followed by Visual Encounter Surveys in eucalyptus patches. The greatest number of individuals per person-effort was also obtained in the shola habitat. I recommend a combination of nocturnal Visual Encounter Surveys and Stream transects for long-term sampling of anurans in the tea plantation landscape of the Southern Western Ghats, India.

Using the methods tested in chapter 2, I surveyed amphibian diversity and composition across four sites in the tea plantation landscape of Munnar, India, during the monsoon seasons of two years: 2013 and 2014. There was no significant difference in community structure between 2013 and 2014, and data for the two years was combined in all subsequent analyses. A total of 14 species were encountered in both years. Species from the genera *Fejervarya*, *Micrixalus*, and *Indirana*, as well as, juvenile *Raorchestes* individuals, were grouped together as they could not be confidently identified in the field and therefore, the species list for the site is conservative because of presence of cryptic species. Of these 14 species, six were in the first three IUCN red list threat statuses and another three were not yet evaluated. I used multivariate statistical methods to determine how anuran communities were organized in the area. There was a difference in the community structure at the four sites that was driven by the presence of exclusive species at some sites. The critically endangered species, *Rhacophorus pseudomalabaricus*, was encountered only in the Silent Valley site. The species *Raorchestes kadalarensis* and *Duttaphrynus microtympanus* were found in low numbers, only in the Rajamalla and Pettimudi site. Transect type (upland Visual Encounter Surveys or Stream Transects) were factors that drove some of the anuran community species similarity. The species *Ghatixalus asterops*, *Micrixlausa* sp., and *Nyctybatrachus poocha* were predominantly found in stream transects at all the four sites across the landscape.

Between 2012 and 2015, I swabbed 216 individuals of 17 species including four critically endangered species to test for the presence of the global and Asian strain of the fungus *Batrachochytrium dendrobatidis* (*Bd*). Even though six samples showed

amplification in simple PCR, none of them were positive for *Bd* in triplicate quantitative PCR experiments. Even though we report the absence of the Global and Asian strains of *Bd* in Munnar, Kerala, using the PCR method, it is important to continue monitoring for the presence of chytrid and other infectious diseases. We are in the process of testing the samples with nested PCRs to see if there are any weak signals of the presence of different strains of *Bd* fungus that were missed during our initial molecular work.

My research is the first long term study of anurans conducted in a managed tea plantation landscape of Munnar, India and one of the few ecological studies of amphibians in India. My works lays a baseline for any future anuran community work in the area. I found that because of the variation in studies looking at diversity of amphibians in different plantations around the world, the data did not support any one driver of amphibian diversity in plantations and it is likely that each study outcome was unique because of differences in plantation type, location, age, and management. However, since there were amphibians present in each of the plantations, even though the diversity was poor compared to native forests, plantations should be looked at areas that provide refuge to a subset of the native amphibian pool in an area. We need to increase the number of studies conducted in plantations, and provide the information gathered to the stakeholders who can implement recommendations that may help these resident species. Future sampling and continued monitoring of frogs in plantations will help us manage amphibian populations in agricultural systems. The ideal method for long term sampling in the tea plantation landscape in this area is using stream transects in the small remnant shola forest patches. However, there was one species (*Raorchestes jayarami*) that was found exclusively in Visual Encounter Surveys in tea fields, and hence my recommendation is

to study all the habitats available in the landscape. Though this is still a conservative list because of the presence of several cryptic species, it does provide a baseline for future studies in the area. Continued information on the presence, abundance, habitat preferences, community structure, and health of anuran in agricultural systems all over the world can help us better manage these systems.

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Publications and Presentations

- Eluvathingal, M. L., Shanbhag, B. A., and Saidapur, S. K. (2009). Association preference and mechanism of kin recognition in tadpoles of the toad *Bufo melanostictus*. *Journal of Biosciences*. Vol 34, 3: 435-444.
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Endangered Anamalai gliding frog *Rhacophorus pseudomalabaricus* in the Cardamom Hills of Western Ghats, India. *Herpetological Bulletin*, 133, 1-6.

‘Association preference and mechanism of kin recognition in tadpoles of the toad *Bufo melanostictus*’. 7-12 July, 2010. American Society of Ichthyologists and Herpetologists. Joint Meeting of Ichthyologists and Herpetologists. Contributed Paper. Providence, Rhode Island, USA.

‘A Global Analysis of Factors Driving Amphibian Diversity in Plantations’. 15-19 July, 2015. American Society of Ichthyologists and Herpetologists. Joint Meeting of Ichthyologists and Herpetologists. Contributed Paper. Reno, Nevada, USA.

‘Conservation Activities in Tropical Plantations: A Case Study of Tea plantations from the Southern Western Ghats’. 7-12 August, 2016. Invited Symposium. Ecological Society of America Annual Meeting. Fort Lauderdale, Florida, USA.