Whitworth, Andrew William (2016) *Conservation value, biodiversity value and methods of assessment in regenerating and human disturbed tropical forest.* PhD thesis.

http://theses.gla.ac.uk/7426/

Conservation Value, Biodiversity Value and Methods of Assessment in Regenerating and Human Disturbed Tropical Forest

Andrew William Whitworth

MSc Conservation Biology (distinction) – Manchester Metropolitan University

BSc Zoology (Hons) – The University of Leeds

A thesis submitted in partial fulfilment of the requirements for the degree of doctor of philosophy.

The Institute of Biodiversity, Animal Health and Comparative Medicine,
The College of Medical, Veterinary & Life Sciences,
The University of Glasgow.

Supervisors: Dr Ross MacLeod and Prof. Roger Downie

June 2016

“Uniformity is not nature’s way; diversity is nature’s way.”

Vandana Shiva

Thesis Abstract

Although the value of primary forests for biodiversity conservation is well known, the potential biodiversity and conservation value of regenerating forests remains controversial. Many factors likely contribute to this, including: 1. the variable ages of regenerating forests being studied (often dominated by relatively young regenerating forests); 2. the potential for confounding on-going human disturbance (such as logging and hunting); 3. the relatively low number of multi-taxa studies; 4. the lack of studies that directly compare different historic disturbances within the same location; 5. contrasting patterns from different survey methodologies and the paucity of knowledge on the impacts across different vertical levels of rainforest biodiversity (often due to a lack of suitable methodologies available to assess them). We also know relatively little as to how biodiversity is affected by major current impacts, such as unmarked rainforest roads, which contribute to this degradation of habitat and fragmentation. This thesis explores the potential biodiversity value of regenerating rainforests under the best of scenarios and seeks to understand more about the impact of current human disturbance to biodiversity; data comes from case studies from the Manu and Sumaco Biosphere Reserves in the Western Amazon.

Specifically, I compare overall biodiversity and conservation value of a best case regenerating rainforest site with a selection of well-studied primary forest sites and with predicted species lists for the region; including a focus on species of key conservation concern. I then investigate the biodiversity of the same study site in reference to different types of historic anthropogenic disturbance. Following this I investigate the impacts to biodiversity from an unmarked rainforest road. In order to understand more about the differential effects of habitat disturbance on arboreal diversity I directly assess how patterns of butterfly biodiversity vary between three vertical strata. Although assessments within the canopy have been made for birds, invertebrates and bats, very few studies have successfully targeted arboreal mammals. I therefore investigate the potential of camera traps for inventorying arboreal mammal species in comparison with traditional methodologies. Finally, in order to investigate the possibility that different survey methodologies might identify different biodiversity patterns in habitat disturbance assessments, I investigate whether two different but commonly used survey methodologies used to assess amphibians, indicate the same or different responses of amphibian biodiversity to historic habitat change by people.

The regenerating rainforest study site contained high levels of species richness; both in terms of alpha diversity found in nearby primary forest areas (87% ±3.5) and in terms of predicted primary forest diversity from the region (83% ±6.7). This included 89% (39 out of 44) of the species of high conservation concern predicted for the Manu region. Faunal species richness in once completely cleared regenerating forest was on average 13% (±9.8) lower than historically selectively logged forest. The presence of the small unmarked road significantly altered levels of faunal biodiversity for three taxa, up to and potentially beyond 350m into the forest interior. Most notably, the impact on biodiversity extended to at least 32% of the whole reserve area. The assessment of butterflies across strata showed that different vertical zones within the same rainforest responded differently in areas with different historic human disturbance. A comparison between forest regenerating after selective logging and forest regenerating after complete clearance, showed that there was a 17% greater reduction in canopy species richness in the historically cleared forest compared with the terrestrial community. Comparing arboreal camera traps with traditional ground-based techniques suggests that camera traps are an effective tool for inventorying secretive arboreal rainforest mammal communities and detect a higher number of cryptic species. Finally, the two survey methodologies used to assess amphibian communities identified contrasting biodiversity patterns in a human modified rainforest; one indicated biodiversity differences between forests with different human disturbance histories, whereas the other suggested no differences between forest disturbance types.

Overall, in this thesis I find that the conservation and biodiversity value of regenerating and human disturbed tropical forest can potentially contribute to rainforest biodiversity conservation, particularly in the best of circumstances. I also highlight the importance of utilising appropriate study methodologies that to investigate these three-dimensional habitats, and contribute to the development of methodologies to do so. However, care should be taken when using different survey methodologies, which can provide contrasting biodiversity patterns in response to human disturbance.

Contents

Thesis Organisation

This thesis is presented in the form of six main chapters, each of which has been prepared for publication in the style of a stand-alone manuscript (including introduction and discussion) suitable for submission to scientific journals in the fields of ecology and conservation. Presentation as manuscripts does mean that some information, particularly in methods, is repeated more than once, but this should make each chapter of the thesis more readable without a constant need to refer back to other chapters.

1) Chapter one assesses the overall biodiversity and conservation value of a regenerating rainforest site from the Amazon region of SE Peru. The chapter compares the values of this regenerating study site with the biodiversity found at a selection of well-studied primary forest sites and the predicted species list for the region. In addition it focuses upon key indicator species and species of key conservation concern.

2) The second chapter investigates the biodiversity within a regenerating forest once subjected to different types of historic anthropogenic disturbance. This is achieved through a multi-taxa analysis including data on amphibians, butterflies, birds and mammals.

3) Chapter three assesses the ecological disruption to faunal biodiversity of the presence of a small, unmarked Amazonian road; from the eastern Amazon region of Ecuador. The study is multi-taxa, including an assessment of amphibians, birds and butterflies.

4) Chapter four examines terrestrial and arboreal patterns of biodiversity within a regenerating rainforest, focussing upon butterflies. Measures include species richness, species diversity, community structure and abundance.

5) The fifth chapter examines the efficiency and potential for the use of arboreal camera traps in generating rapid inventories of arboreal rainforest mammals; in comparison with traditional survey techniques. The comparison includes both the effectiveness of data gathering and a financial cost-benefit comparison.

6) This final data chapter examines patterns of amphibian biodiversity, with the aim to directly assess whether different survey methodologies provide the same or contrasting patterns of biodiversity in response to different forms of historic human disturbance; a potentially contributing factor towards the controversy about the biodiversity value of regenerating rainforest.

In addition to these six chapters, the thesis begins with a general introduction on biodiversity and finishes with a short final discussion which brings together the major findings and conclusions of the main chapters and assesses their overall significance; with suggestions for future progression of research in this field.

Appendices that provide supporting materials for the data chapters are all located at the very end of the thesis. In addition to the appendices for each of the main chapters, the appendices contain five additional published or accepted supporting papers on regenerating rainforest biodiversity and survey methods. These demonstrate my ability to produce publication quality research on these subjects. These relate to: 1. the effects of human habitat disturbance on amphibian and reptile communities; 2. trialling bobbin tracking methodologies for amphibians and reptiles, for the first time in a tropical rainforest; 3. the use of bamboo traps as refugia for two poorly known species of rainforest amphibians; 4. the first distribution map and range extension for a species of frog of the genus, *Osteocephalus*; and 5. the first distribution map and range extension of a species of hummingbird. These supporting papers were co-authored, initiated and overseen by myself and their inclusion follows university thesis guidelines. In the appendix I explain what my specific role and involvement was for each paper, explain their relevance to the thesis and provide the full citation for published versions. They are added into this section to separate them from the papers/manuscripts on which I am the first author.

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Acknowledgements

If I were to thank each individual who has contributed in some way to this thesis, in terms of ideas, comments, data collection or personal support, then the thesis would be double the length. As my eyes are hurting and I want to relax with a beer, I have limited this to the following acknowledgements. If I have missed anyone that feels I have forgotten their words of encouragement or time when they found ‘that special frog’, then I am sorry and here is your acknowledgement; ‘Thank you’.

I would like to begin with huge appreciation for the Crees Foundation (www.crees-manu.org) and most especially its visionary director Quinn Meyer for supporting this research as part of Crees’ research programme. Thanks to both Crees and the University of Glasgow for supporting my field work conducted at the Manu Learning Centre (MLC) and other areas of the Manu Biosphere Reserve. I gratefully acknowledge the financial support and encouragement of the TJMF Foundation who provided crucial support for this work through their Amazon Research Programme grant to the University of Glasgow and the Darwin Initiative for financial support of the Sustainable Manu project. I also thank Chicago Herpetological Society, the Louise Hiom Fund and Glasgow Natural History Society for financial support towards herpetological field work equipment. Appreciation is given to Bushnell for providing some free camera traps and a great deal on those which we purchased to gather mammal and bird data for this study. Thanks also to the Universidad Nacional Mayor de San Marcos for providing a base in which to deposit valuable specimens and also to the Ministerio de Agricultura of Peru for providing the permit to conduct research in Peru (Permit provided by the Ministerio de Agricultura of Peru; Permit Number ‘Codigo de Tramite’: 25397; Authorisation Number ‘Autorización No.’ 2904-2012-AG-DGFFS-DGEFFS). A special thanks is also given to the National Park authority of Peru; El Servicio Nacional de Áreas Naturales Protegidas (SERNANP), in particular ex and current heads of the park, Carlos Nieto and Jhon Flores.

A huge thanks to all the Crees Foundation researchers, volunteers and visitors (including Glasgow University Peru Expeditions 2011-2014) who have assisted over the years, developing species lists and conducting field work; without their dedication and support to rainforest conservation and Crees, this research wouldn’t have been possible. Volunteers choose to contribute something special to our planet instead of a typical holiday and I thank them for making that choice to work with us at Crees. I have had the pleasure to see the first batches of Crees interns pass through the MLC over the past few years and develop into budding conservationists, environmentalists and educators for the future; keep conserving!

Thanks to all the staff of the MLC and Crees for providing a comfortable and beautiful place to stay and conduct my research; Alcides, Karla, Darwin and Dino have fed me and everyone at Crees incredibly well (99.9% of the time; we had porridge once or twice); Juvenal and Nelson whom worked hard to uphold the research programme when I first arrived in 2011; a number of maintenance staff and boat drivers, Julian, Carlin, Willy, Yelsin, Tomas and Aurelio, who have always been enthusiastic to help out in any way; housekeeping staff whom help to keep things at the MLC beautiful and clean, Jenni, Maria and Katia; the Crees Cusco office whom deal with a huge amount of logistics, permits and general issues, Juan Carlos, Gladys, Luz Marina, Yulli, Jose Luis, Edwin and Susannah; the Crees community team, Louise, Eduardo, Reynaldo, Tabitha, Esperanza and Jose; the Crees education team who have taken care of and helped to train all of our volunteers and interns, Nicola, Louise, Tabi, Eleanor and Tilman; and finally the Crees London office whom have sent us an ever increasing number of guests, volunteers and interns, Fran, Alex, Cia, Gabrielle, Sarah, Alyssa, Olivia and Matt. Many thanks to Crees’ rainforest journalists Marcus Brent-Smith and Katie Linn who document some of the incredible wildlife we find with beautiful images. I also need to thank a set of very special field research staff which has worked tirelessly to help collect an incredible amount of data for Crees; these people are; (in no particular order) Alex Fowler, Laura Allen, Alice Risely, Lawrence Whittaker, Grant Reekie, Emily Waddell, Alice Brown and Laura Dominie-Braunholtz. It has also been a pleasure to work alongside and train two very special and talented young Peruvian Biologists, Ruthmery Pillco Huarcaya and Jennifer Serrano Rojas, of whom I am both very proud and grateful for their incredible work ethic and passion. Their futures are incredibly bright.

I would like to show a huge appreciation for my supervisors Prof. Roger Downie, who I will always be grateful for providing me with ruthless and rapid edits upon

my drafts; and especially Dr. Ross MacLeod who has been incredibly important to me both professionally and personally. I could not have wished for a better primary supervisor who has always been calming in times of stress and who shares my passion for tropical fieldwork and wildlife. Thanks also to my good friends and colleagues Oliver Burdekin, who helped to set up the database management systems for the Crees Foundation research programme, and Dr Christopher Beirne who has always displayed incredible patience when I needed help with modelling aspects of my analyses. Dan Challender has always provided a healthy level of competition, keeping me to a tight PhD deadline.

Probably the two most instrumental and incredible people in helping me to organise the initial projects and incorporate these within Crees' volunteer and intern programmes were Jaime Villacampa and Jasmine Rowe; without either one we could not have achieved the level of work that we have been capable of at Crees. Not only did they help me professionally but they have also both supported me personally in times of panic and exhaustion (both physical and mental).

My family have been an unbelievable rock of support throughout not only the past four and a half years of this PhD but throughout my life. My brother, Lee, has kindly been able to explain very clearly to friends in the pub that 'I catch frogs for a living', whilst my dad, Ron, has continually displayed an interest each day to see whether I have finished 'that thesis yet'. It was wonderful to have my mum, Sheila, travel all the way to Cusco to meet the Crees team and spend a week in the rainforest at the MLC. Even though she never actually caught a butterfly on her transect, she did carry the clipboard and fill out the data sheet. Their support is never-ending. Finally, I want to dedicate this thesis to the memory of my wonderful big sister Debbie. Like the rest of my family, Debs was incredibly understanding of my work away in the rainforests of South America this past seven years and I have no doubt that had she been well enough, she would have travelled there with me. I have a box full of letters sent to Ecuador and Peru, addressed to 'my special brother'. I will never forget her love for, and belief in me.

Thank you everyone.

General Introduction

What is biodiversity?

The term ‘biodiversity’, a contraction of the phrase ‘biological diversity’, was first created by Walter G. Rosen at “The National Forum on BioDiversity in Washington DC, 1986 (Maclaurin & Sterelny 2008); but was first popularised by Edward Owen Wilson’s book “The Diversity of Life” (1992). As such, both the term and the field of Conservation Biology remain relatively young, especially considering that governments only really began to seriously realise that wild and natural landscapes were at risk of disappearing until after the intensive land-use of the Second World War (Maclaurin & Sterelny 2008). Since then however, global biodiversity change has been referred to as “one of the most pressing environmental issues of our time” (Pereira et al. 2012); and at the 10th Conference of the Convention on Biological Diversity in Nagoya, Japan, 20 Aichi Biodiversity Targets were set for 2011 – 2020, to achieve global biodiversity conservation (CBD 2010; Woodley et al. 2012).

The term biodiversity refers not specifically to the number of given species, but more to the diversity of organisms and array of functions within a biological system or most simply, as “life on Earth” (Pereira et al. 2012). Although referred to as a single property, biodiversity represents a complex variety of processes and physical measures, and as such, conservation biologists have had to create numerous identifiable and measureable surrogates for these complex biological systems. According to Woodley et al. (2012), biodiversity underpins ecosystem functioning and the provision of ecosystem services essential for human survival and well-being. It provides food security, clean air and water and as such, it contributes to livelihoods, human health and economic development (Pereira at al. 2012). This means that biodiversity conservation is an essential component for the achievement of the Millennium Development Goals, including poverty reduction (Woodley et al. 2012).

Outside of conservation, why does biodiversity matter to people?

The importance of biodiversity may seem obvious to a conservation scientist who typically works in the field of conservation due to an affinity for nature, and who regards biodiversity as having key intrinsic values (Blicharska & Grandin 2015; Soulé 2013). However, it is essential to understand more about the benefits beyond biodiversity conservation and view the importance to the planet and to its people (Blicharska & Grandin 2015; CBD 2010). This was clearly reflected within Strategy Goal C of the 20 Aichi Biodiversity Targets, which is as follows: ‘*To enhance the benefits to all from biodiversity and ecosystem service*’ (CBD 2010).

At the 14th World Congress on Public Health, February the 14th, 2015 in India, a new report entitled “Connecting Global Priorities: Biodiversity and Human Health” suggested that the protection of the world’s biodiversity, ultimately benefits human health (CBD 2015). The report highlights the links and impact of interactions between biodiversity and the following key factors related to humans; water and air quality, food production and nutrition, microbial diversity, infectious diseases, medicines and intrinsic factors related to mental, physical and cultural well-being. Biodiversity is suggested to improve the resilience of ecosystems, allowing a greater potential to adapt to climatic change and natural disasters. The report investigates strategies and tools for protecting biodiversity and enhancing public health. These involve the best management of ecosystems, the promotion of sustainable biodiversity-friendly lifestyles, public education of health sectors, tackling drivers of environmental change (e.g. deforestation) and the effective monitoring of progress towards these. Ultimately the report calls for biodiversity and human health linkages to be better recognised, valued and reflected in national and biodiversity conservation policy.

An excellent illustration of the relations between biodiversity and the benefits to people can be seen in the reduced levels of Amazonian deforestation between 2001-2012 (falling by 40%), resulting in a 30% decrease in particulate emissions, translating to 1700 fewer human deaths (related to particulate-caused cardiopulmonary disease and lung cancer) from the region, per year (Reddington et al. 2015). Negative impacts of land-use change (e.g. dams and deforestation) to human health have become increasingly clear in the form of increased disease transmissions, such as malaria, schistosomiasis, leishmaniasis, and many others (Myers et al. 2013). Land-use change and biodiversity loss impacts on entire ecosystems and the services they provide. A furthere example is the degradation/loss of coastal mangroves and other coastal barriers, which act as important flood defences against storms; for an estimated one third of human beings, all thought to live within 100km of coastlines and 50m above sea level (Myers et al. 2013). Two direct examples of an association between human health and biodiversity, from the field of disease ecology, were the rising risk of West Nile virus exposure, associated with a decrease in avian biodiversity (Keesing et al. 2010) and higher Lyme disease risks, associated with falling levels of mammal diversity (Suzan et al. 2009). A further example from the Brazilian Amazon, shown through statistical models, connects high levels of biodiversity with reduced malaria transmission rates (Laporta et al. 2013). A number of examples also exist in relation to the importance of diversity for healthy agriculture. Many important food crops need wild animal populations, such as butterflies, bees, bats and birds, to provide essential pollination services (Eilers et al. 2011). Nutritional health has also been linked to a healthy and abundant level of wild meat supplements to human diets, particularly for those peoples living in marginal and rural areas (Golden et al. 2011). As such, as biodiversity suffers, so do many key food supplies for human populations.

How fast is biodiversity being lost, and why?

According to McCallum (2015), the rate of vertebrate extinction since 1500 was 24-85 faster than during the Cretaceous-Paleogene mass extinction (K-Pg); since 1980 this has exploded to 71-297 times greater than the K-Pg event. If species identified by the World Conservation Union (IUCN) as globally threatened went extinct in the near future, then vertebrate extinction levels would reach 8900-18,500 times the levels of the K-Pg period. Ceballos et al. (2015) utilised conservative assumptions of background extinction rates (two mammal extinctions per 10,000 species per 100 years; twice as high as previously used estimates) in order to confirm the claim (Wake & Vredenburg 2008) that the world is entering a sixth mass extinction. Their estimates suggest that vertebrate species loss over the last century was 114 times higher than the background rate, thus verifying the arrival of the Anthropocene era, the sixth mass extinction (Dirzo et al. 2014). Only through intensified conservation efforts and rapid action (Ceballos et al. 2015; McCallum 2015) can this loss of biodiversity and decay of ecosystem services be avoided. This message has again been reflected within the 20 Aichi Biodiversity Targets, through Strategy Goal A, as follows: '*To address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society*'; *and Go*al B: '*To reduce the direct pressures on biodiversity and promote sustainable use*' (CBD 2010).

According to the IUCN Red List (2013), which uses very strict criteria for extinction, and gives very different numbers to estimates based on habitat loss, 27 species were known to go extinct between 1984 and 2004. Of these 27 species, habitat loss and degradation were thought to have played a major role in 13 and these causes (along with invasive exotics and disease) are thought to be playing a greater role in recent extinctions than observed in previous centuries (Pereira et al. 2012). As such, habitat loss and degradation are recognised by many as the major drivers of global biodiversity change (Pereira et al. 2012) and are identified as the major threat to amphibians, birds and mammals (IUCN 2013). However, not all species respond similarly to habitat change; some species can increase in abundance, some species may remain unaffected, whilst others, particularly habitat/niche specialists, will decline or even become locally extinct (Pereira et al. 2012). Three major types of habitat alteration have been defined (Pereira et al. 2012): 1. the conversion of natural habitat to human-dominated landscapes; 2. areas of human disturbance further intensified for human-use; and 3. regenerating landscapes following previous clearance and use by humans. These alterations display a distinct latitudinal pattern. Although tropical forest ecosystems are undergoing the largest amount of natural to human habitat change, intensification and natural recovery are fast increasing. For example, it has been suggested that tropical secondary forests (regenerating forest on abandoned lands) had already replaced one-sixth of all the primary forest areas that were cut down within the 1990s by the early 2000's (FAO 2001); and in many countries, these regenerating areas now exceed the cover of primary forest (Gardner et al. 2007a; Wright 2005).

Why are tropical forests so important for biodiversity? and how fast are they being lost and degraded?

Tropical forests are one of the world's most biodiverse ecosystems and have been a key focus for conservation biologists (Bradshaw et al. 2008; Myers et al. 2000), as they contain a disproportionate contribution of the world's biodiversity; harbouring over half of the world's animals and plants in less than 10% of the land surface (Bradshaw et al. 2008). The species within these environments contain a huge proportion of species important for conservation (de Queiroz et al. 2014), including rare, endemic, and evolutionary distinct species (Steege et al. 2013), and species already threatened due to habitat loss and degradation (Myers et al. 2000). The specific biota and functions associated with tropical forests are distinct in a variety of ways, particularly in relation to evolutionary history, dispersal mechanisms, demography and sensitivity to environmental change (Steege et al. 2013; Stratford & Robinson 2005). It is often, these specific traits which make tropical ecosystems vulnerable to rapid environmental change and ultimately, to extinction (Steege et al. 2013) of both species and services (Stork et al. 2009).

Tropical rainforests not only support themselves, but the processes that take place within them are of global significance, providing many ecosystem services such as clean water and climate regulation (Edwards et al. 2014a). For example, forests can act as a reservoir for species that make a substantial contribution to pollinating crops in neighbouring land (Blanche et al. 2006). The dense vegetation of tropical forests itself acts as a carbon sink, removing atmospheric carbon and storing it in plant tissue, thereby reducing the levels of carbon dioxide in the atmosphere (Pan et al. 2011). This vegetation also plays valuable ecosystem service roles in soil retention, nutrient capture, the prevention of erosion and landslides, preservation of water quality and the maintenance of groundwater stores (Edwards et al. 2014a).

Tropical forests have been exploited by humans for many centuries, but the total area affected by anthropogenic disturbance, such as deforestation, has increased dramatically in the last few decades (Peres et al. 2006; Peres et al. 2010; de Queiroz et al. 2014). Aide et al. (2013), estimate that for Netropical regions between 2001 and 2010, there was a net loss of almost 18 million ha of woody vegetation, resulting from ~ 54 million ha of deforestation and ~36 million ha of reforestation. Deforestation was shown to be highest in South America; with Argentina, Brazil, Paraguay, and Bolivia accounting for 80 percent of the deforestation in all of Latin America. However, global deforestation rates between 1990 and 2005 (despite still being around 13 million ha/year) did show some degree of decline (Chazdon et al. 2009a). Target five of the 20 Aichi biodiversity targets relates to continuing these declines in forest loss and by 2020 the aim is to '*at least half the rate of loss of all natural habitats, including forests*'; and where feasible, should bring these to zero (CBD 2010), whilst also reducing degradation and fragmentation.

What are the greatest threats to tropical forest biodiversity?

Habitat change and degradation are currently the major drivers of global biodiversity change (Pereira et al. 2012). Some of the most common activities resulting in rainforest degradation include: logging, mining, oil exploration, hunting and conversion of land for agriculture (Edwards et al. 2012; de Queiroz et al. 2014). These factors (with the exception of hunting for local subsistence) are all economic driving factors of habitat disturbance; with each activity providing short-term profitable utilisation of natural resources. Instead of sustainable land uses practices through, land is used for large-scale, biodiversity depauperate agriculture, such as soybean and oil palm (Gasparri et al. 2013; Grau & Aide 2008), illegal gold-mining (Swenson et al. 2011), hydro-carbon exploration and utilisation (Finer & Orta-Martínez 2010) and illegal logging enabled through logging concessions (Finer et al. 2014).

Despite protected areas covering 13% of the Earth's terrestrial surface (Le Saout et al. 2013), 13% of the land area in developing countries (Miranda et al. 2014; Miranda et al. 2016) and 16.3% in Peru (de Queiroz et al. 2014), the success of these areas depends heavily on the management of surrounding areas and the inclusion of local peoples in management strategies (Laurance et al. 2012a; Miranda et al. 2014; Oldekop et al. 2015). Laurance et al. (2012a) conducted a systematic and uniquely comprehensive assessment of long-term changes within 60 protected areas spread across the world's major tropical forests, aiming to appraise both the ecological integrity and threats to tropical protected areas on a global scale. They found that the most sensitive guilds in tropical protected areas include apex predators, large non-predatory vertebrates, bats, stream-dwelling amphibians, terrestrial amphibians, lizards and larger reptiles, non-venomous snakes, freshwater fish, large-seeded old-growth trees, epiphytes and ecological specialists. Several other groups were somewhat less vulnerable, including primates, understorey insectivorous birds, large frugivorous birds, raptorial birds, venomous snakes, species that require tree cavities, and migratory species. In addition, five groups increased markedly in abundance in the reserves, including pioneer and generalist trees, lianas and vines, invasive animals, invasive plants and human diseases.

Protecting biodiversity involves more than just safeguarding the reserves themselves (Melo et al. 2013; Miranda et al. 2014). In many instances, the landscapes and habitats surrounding reserves are under imminent threat (de Queiroz et al. 2014). For example, 85% of the reserves assessed suffered declines in surrounding forest cover in the last 20 to 30 years, whereas only 2% gained surrounding forest (Laurance et al. 2012a). Of the potential drivers of declining reserve health, three of the most important involve ecological changes outside reserves (declining forest cover, increasing logging and increasing fires; see Laurance et al. 2012a). The most important drivers within the reserves included declining forest cover and increasing hunting, as well as increasing logging and harvests of non-timber forest products (de Queiroz et al. 2014). Thus, changes both inside and outside of protected areas determine their ecological viability, with forest disruption (deforestation, logging and fires), and overexploitation of wildlife and forest resources (hunting and harvests of non-timber forest products) having the greatest direct negative impacts. Other environmental changes, such as air and water pollution, increases in human population densities and climatic change (changes in total rainfall, ambient temperature, droughts and windstorms) were suggested to be more indirect effects (Laurance et al. 2012a).

Overall, these findings suggest that the fates of tropical protected areas will be determined by environmental changes both within and around them. This indicates that the best strategy for maintaining biodiversity within tropical reserves is to protect them against their major nearby threats, especially habitat degradation; but it is not enough for efforts to focus upon protected areas while ignoring the surrounding landscapes, areas which are often being rapidly deforested, degraded and overhunted (de Queiroz et al. 2014).

Regenerating landscapes and their biodiversity and conservation value

Eighteen countries have now shown an increase in forest cover, owing to afforestation projects and natural regeneration (Chazdon et al. 2009a). However, research in tropical forests has historically focussed mainly on a relatively small number of field sites, most frequently found within protected reserves and most often situated within primary forest habitat (Chazdon et al. 2009a; Gardner et al. 2009; Melo et al. 2013; Peres et al. 2010).

Although the importance of protecting primary rainforest for biodiversity conservation has been well documented (Barlow et al. 2007a; Chazdon et al. 2009b; Anand et al. 2010; Sodhi et al. 2010; Gibson et al. 2011), we know relatively little in comparison of the huge areas of forests already degraded by humans (Gardner 2010). If the focus of current reserve networks is to protect only areas of primary forest and biodiversity outside of these areas is not considered, then we could potentially lose thousands of species and many valuable ecosystem functions and services (Soulé & Sanjayan 1998; Putz et al. 2001). This realisation has led to a growing amount of research into the importance of these human modified forests for biodiversity conservation. This assessment however, has not been a straightforward one.

Human-modified landscapes may hold the potential to provide important refuges for forest biodiversity (Chazdon et al. 2009b) but information on biodiversity recovery from devastating environmental change is often difficult to determine (Gardner et al. 2010). One reason for this is that whilst regenerating, the environment often continues to experience a variety of human impacts such as hunting, agriculture or harvesting of wood. In addition to this, there are many potential reasons for studies to identify contrasting patterns about the conservation value of regenerating areas, including geographic context, study scale, timeframe since disturbance and the tendency to focus on overall species richness patterns (Anand et al. 2010; Barlow at al. 2007a; Chazdon et al. 2009b; DeClerck et al. 2010). This may restrict the ability to clearly measure the ecological recovery of biodiversity following a significant period of regeneration (Chazdon et al. 2009a; Letcher & Chazdon 2009) and constitutes just a few of the possible reasons why there appears to be a lack of agreement on the overall conservation value of tropical forests regenerating after human disturbance, and where conservation focuses should be (Wright 2005). Some authors suggest that the conservation and integration of such areas can sustain significant proportions of biodiversity (Chazdon et al. 2009a; Norris et al. 2010; Tabarelli et al. 2010): for example, “most biodiversity can be retained in tropical forest impacted by logging” (Edwards et al. 2014b); whereas other authors have suggested that regenerating landscapes contain significantly less biodiversity than natural landscapes (Gibson et al. 2011; Mackey et al. 2014) and that even low levels of selective logging and habitat clearance can “halve the levels of species richness” (Burivalova et al. 2014).

However, it is increasingly clear that if secondary areas are not recognised as valuable habitat in current conservation efforts, and regenerating forest protection remains low, then there is a dangerous risk of conversion to agricultural land of known poor biodiversity value, such as oil palm monoculture (Gillespie et al. 2012). Chazdon et al. (2009b) recommend that older, more species-rich regenerating forests, in close proximity to protected areas should be of the highest conservation priority, whilst an investment in younger areas could prove essential in the longer-term. Without knowing clearly the potential value of these older regenerating forests it might become increasingly difficult to protect younger areas of secondary forests, potentially compromising the future of secondary forest cover and the possibility to create biological corridors and buffer zones from such areas (Tabarelli et al. 2010). By preserving a balanced age structure of secondary forests, we may be able to maximise the conservation potential for old growth associated species, increasing richness values at the landscape level (Chazdon et al. 2009b).

This thesis therefore focuses on three areas where further research could potentially help improve understanding and knowledge of conservation value, biodiversity value and methods of assessment in regenerating and human disturbed tropical forest. These are: 1) an improved understanding of the potential of regenerating rainforest biodiversity, 2) information on the effects of different types of human disturbance to biodiversity and 3) the need to develop and understand the methods available to study rainforest biodiversity and responses to human disturbance. In the following sections I discuss some of the key areas of research surrounding chapters and appendices presented within this thesis. In doing so I suggest the importance of research needed to further understand tropical forest biodiversity and the response of biodiversity to habitat change in reference to each of these topics.

Roads a major cause of degradation to tropical forests

It is well known that most forms of tropical forest exploitation are facilitated by the introduction of roads, an increasingly common feature of landscapes all over the world (Caro et al. 2014; Fraser 2014; Laurance & Balmford 2013). Considering that at least 25 million kilometres of new roads were anticipated globally by 2015 (Laurance et al. 2014a), the degradation of forests following these roads could be dramatic. Roads not only cause mortality through vehicle collisions (Bissonette & Rosa 2009) but also influence the spread of disease (Hahn et al. 2014), increase the risk of fires (Fraser 2014) and act as dispersal barriers limiting the movements of individuals within and between populations (Bisonette & Rosa 2009; Goosem 2007). However, in addition to these direct disturbances, roads likely cause unknown and more subtle effects upon biodiversity (Peres et al. 2006).

The western Amazon had until recently remained largely intact, but growing pressure on the exploitation of resources suggests that these regions will likely become highly fragmented (Finer et al. 2008; Laurance et al. 2014b; Oliveira et al. 2007). According to Brandào Jr. and Souza Jr. (2006), the average growth rate for unofficial roads in the Amazon region approximately doubled in ten years; from 9.85 km/10,000km^2 per year (between 1990-1995) to 19.25 km/10,000km^2 per year (between 1996-2001) and the Brazilian road network grew by almost 17,000km per year between 2004 and 2007 (Ahmed et al. 2013). There is currently little information about the impacts of such unmarked rainforest roads, despite knowing that nearly 95% of all deforestation within the Amazon occurs within 5.5km of roads or 1km of rivers (Barber et al. 2014).

Understanding the canopy

A key part of tropical forest spatial complexity is the vertical stratification of biodiversity, with widely differing communities often found in the canopy compared to terrestrial levels (DeVries et al. 1997; Dumbrell & Hill 2005; Maguire et al. 2014; Tregidgo et al. 2010). Although less well studied, understanding vertical differences may be as important for understanding biodiversity patterns as more traditional assessments along the horizontal gradient (de Faria Lopes et al. 2014). In fact, it has been suggested that habitat alteration due to anthropogenic impacts may cause an even greater disruption to arboreal than to terrestrial biodiversity (Dumbrell & Hill 2005; Francis 1994; Klimes et al. 2012; Kurten et al. 2015; Tregidgo et al. 2010; Walther 2002). This indicates that sampling within a single vertical stratum could lead to under or over estimation of true overall levels of biodiversity within rainforest habitats, and therefore bias judgements about the relative conservation value of different areas (Dumbrell & Hill 2005; Klimes et al. 2012; Ribeiro et al. 2015; Tregidgo et al. 2010). As such, improving our understanding of arboreal rainforest biodiversity more generally is important, as many species serve as charismatic flagship species for conservation (Kays & Allison 2001), are important dispersers of fruits and seeds (Kurten et al. 2015; Vieira & Izar 1999), act as key rainforest pollinators (Ganesh & Devy 2000) and are essential ecosystem engineers (Chapman et al. 2013).

The importance of understanding the results of different survey methodologies

As we have seen, determining how well tropical forest biodiversity can recover following human disturbance is complex (Gardner et al. 2010), especially as studies from different locations often produce contrasting results. One causal factor often overlooked is the potential for alternative survey methodologies to indicate different results on the response of biodiversity to habitat change (Barlow et al. 2007b). In the case of butterflies for example, line transect studies carried out in a number of locations have suggested that butterfly biodiversity does not show a significant degree of difference between human disturbed and primary forest (Devy & Davidar 2001; Kudavidanage et al. 2012; Posa & Sodhi 2006). In contrast, studies using traps undertaken at other sites suggest that butterfly biodiversity does show a significant degree of difference between human disturbed and primary forest (Dumbrell & Hill 2005; Ribeiro & Freitas 2012; Ribeiro et al. 2015). Understanding more about how these contrasting patterns might relate to differences due to survey methodologies can therefore help to improve our ability to assess the true value of regenerating tropical forests and better understand the response of specific communities. Otherwise, assessments of a specific community may under or overestimate the potential biodiversity value for such forests, especially if the results from single surveys are over generalised (Barlow et al. 2007b).

Thesis study areas

The western Amazon is a huge area encompassing the Amazonian rainforest of Bolivia, Colombia, Ecuador, Peru, and the furthest reaches of western Brazil. Periodical floods in the banks of upper western Amazonian rivers create complex successional vegetation gradients which favour habitat heterogeneity. These processes generate a mosaic of forest types which largely explains the extraordinary biodiversity of western Amazonia along with significant altitudinal gradients ranging from high up in the Andes right down into lowland Amazonian rainforests (Gascon et al. 2000; Salvador et al. 2011). It is characterized by extraordinary species richness across taxa, and large tracts of road-less and relatively intact humid tropical forest (Bass et al. 2010), and is considered as one of the world’s last high-biodiversity wild areas. A recent global analysis of biodiversity risks from fossil fuel extraction found that the western Amazon is under particular threat (Butt et al. 2013). Laurance et al. (2014a) in a recent global analysis on road building stated that much of the western Amazon is a road-free area and should remain so.

One of the study areas within this thesis is the Manu Biosphere Reserve, a UNESCO and IUCN World Heritage Site designated to protect the globally important Amazon rainforest in and around Manu National Park, SE Peru. As part of western Amazonia, an area which holds the highest levels of biodiversity in the Amazon (Salvador et al. 2011), the Manu Biosphere Reserve consists of a network of core protected areas surrounded by areas designated as a cultural or buffer zone due to high human impacts; including extensive logging or clearance for subsistence/commercial agriculture. The core area of the Manu National Park contains over 1.5 million ha of mainly primary tropical forest.

The second study area lies within the lowland western Amazon region of eastern Ecuador, located within the cultural zone of the Sumaco Biosphere Reserve; situated between Yasuni (>1 million ha created in 1979) and Sumaco (>206,000ha created in 1994) National Parks, large swathes of protected and largely intact primary forest. The areas of cultural use inbetween consist of predominantly primary lowland rainforest, as well as abandoned and active plantations, small abandoned and active pasturelands, fragmented forest patches and a growing network of roads.

The importance but lack of multi-taxa research

Most assessments of biodiversity patterns in response to habitat loss and degradation in tropical forests are single taxon orientated (Anand et al. 2010; Gardner et al. 2008), likely as a result of limited funds within biodiversity and conservation research (Lawton et al. 1998; Gardner et al. 2008). Biologists therefore often need to look for the most cost effective solution. One approach to avoid the cost of intensive multi-taxa sampling is to focus on a single indicator group or taxon (Gardner et al. 2008; Anand et al. 2010). However, this could lead to erroneous conclusions about the overall biodiversity responses and research has shown that a multi-taxon approach is essential for monitoring changes in biodiversity (Edwards et al. 2014c; Lawton et al. 1998). However, within site multi-taxa research is often lacking from assessments of tropical biodiversity in response to habitat change, as was highlighted over ten years ago by Dunn (2004a). Dunn (2004a) reviewed 34 studies and found just two that provided multi-taxa information. However, since this study, multi-taxa research has been carried out in a number of studies from tropical forest regions (Louzada et al. 2010; Fonseca et al. 2009; Pardini et al. 2009; Silveira et al. 2015). Louzada et al. (2010) studied nine different taxa, Pardini et al. (2009) seven taxa and Fonseca et al. (2009) studied 13 taxa, all within the Brazilian Atlantic forest. Silveira et al. (2015) studied four taxa from the Brazilian Amazon. Despite this, there more recently there has been shown a bias in many regions to focus upon certain taxa and many studies still focus upon a single taxon. For example, Taylor & Goldingay (2010) found a taxonomic bias within research assessing the impacts of roads towards mammals (53% of studies), with just 10% focussing on birds, 8% on amphibians and only 20% including multi- taxa data.

Survey groups within this study

This thesis therefore measured the biodiversity of five key taxonomic groups (amphibians, birds, butterflies, medium-large mammals and reptiles), chosen because they are of well-known conservation importance and provide numerous ecosystem functions (Lawton et al. 1998). Other groups were considered but not selected for various reasons. Small ground-living mammals were not chosen due to difficulties in identification in the field, bats due to the necessity of specialist methods and equipment that was not available and other invertebrates due to the lack of easily accessible taxonomic identification for many Amazon species, which would have made it difficult to compare results with other sites. Details for each specific taxon studied are provided below:

Amphibians

A key taxonomic group frequently utilised to study the impacts of habitat disturbance in tropical forests is amphibians, chosen due to their high conservation importance (31% of evaluated species are threatened with extinction; IUCN 2013), and because they are key components within their ecosystems (Ficetola et al. 2014; Hocking & Babbitt 2014). Specifically, more than 70% of the world's amphibian species are thought to be in decline (Hayes et al. 2010). Amphibians display a high level of sensitivity to disturbance due to low mobility, limited dispersal capacity and narrow ecological requirements (Lawler et al. 2010). Habitat change is therefore likely to affect amphibians more severely than other vertebrate groups (Ficetola et al. 2014), especially as small changes in vegetation structure can create significant alterations to amphibian communities (Cortés-Gómez et al. 2013). As a result, habitat destruction and fragmentation are among the leading causes of the global threat to amphibians (Catennazi & von May 2014; Eigenbrod et al. 2008), especially in tropical regions where levels of diversity are highest (Ficetola et al. 2015).

Birds

Rainforest birds, especially understorey guilds, have been found to be sensitive to environmental changes and habitat fragmentation (Banks-Leite et al. 2010). They are therefore likely to be a useful group for understanding how biodiversity may respond to rainforest regeneration. Nocturnal birds in particular have received very little attention within tropical forests due to the challenge in successfully surveying them (Goyette et al. 2011), but many species hold a potentially disproportionate ecological importance at the top of the food chain (Sberze et al. 2010).

Butterflies

Butterflies are key components within their ecosystems and are effective in detecting ecological change due to their sensitivity to forest disturbance (Bonebrake et al. 2010; Hamer et al. 2003), particularly through association with specific food plants (Horner-Devine et al. 2003). Butterfly biodiversity assessments are therefore well suited to assess changes in biodiversity due to anthropogenic habitat disturbance.

Medium-to-large mammals

Medium–to-large mammals were chosen because they serve as charismatic flagship species for conservation (Kays & Allison 2001), while remaining relatively understudied within tropical forests (Salvador et al. 2011). For the purpose of this thesis, medium-to-large mammals represent those species which can be accurately and relatively easily identified from camera trap footage (the smallest species generally the size of a green acouchy or tamarin monkey); as such, smaller rodents and bats are not included. Many of these species play a key role in forest ecosystems, as important dispersers of fruits and seeds (Kurten et al. 2015; Vieira & Izar 1999), are essential ecosystem engineers (Chapman et al. 2013) and are involved in prey population control by helping to maintain assemblages of other faunal groups (Beck et al. 2010; Cassano et al. 2012)

Reptiles

Reptiles are a vastly understudied group both globally and within Amazonia, despite acting as important meso-predators within many ecosystems. Close to 21% of evaluated reptile species are threatened with extinction (IUCN 2013). However, these threats remain underestimated due to the lack of basic ecological knowledge of reptiles (Böhm et al. 2013); this can be seen from the 18.6% of reptiles that are classified as data deficient (IUCN 2013). This is further emphasised by the small proportion of reptiles that have been evaluated by the IUCN; just 41.5% of known species compared with almost all known species of birds and mammals and 91% of amphibians.

Chapter 1 - How much potential biodiversity and conservation value can a regenerating rainforest provide? A site-level, comparative species list approach to infer the value of local reserves for conservation.

Authors and contributions

Andrew Whitworth[1,2*], Roger Downie[2], Rudolf von May[3], Jaime Villacampa[1] and Ross MacLeod[1,2]

[1] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.

[3] Museum of Vertebrate Zoology, University of California, Valley Life Sciences Building, Berkeley, US.

Designed the study: AW RM

Collected the data: AW RvM JV

Performed the analysis: AW

Contributed to writing of the manuscript: AW RD JV RvM RM

Abstract

The majority of the world's tropical forests have had their structure and underlying functions disrupted by human impacts but the potential biodiversity and conservation value of regenerating forests is still debated. Reviews suggest that on average, regenerating tropical forests hold 57% (±2.6%) of primary forest species richness; creating some doubt about whether there remains a viable second chance to conserve biodiversity through rainforest regeneration. Average values however, may underestimate the potential benefit to biodiversity and conservation because they contain many studies of short-term regeneration and studies with on-going human disturbance. We suggest that the true potential biodiversity and conservation value of regenerating could be best be assessed in the absence of such factors and present a multi-taxa case study of faunal biodiversity in regenerating tropical forest in lowland Amazonia rainforest. In addition we suggest that a species list assessment approach for local reserves could be utilised as a cost-effective solution, with the aim to highlight the value of local reserves for biodiversity conservation. Our results show that biodiversity of this regenerating site case-study was higher than might have been expected, reaching 87% (±3.5%) of primary forest alpha diversity and an average of 83% (±6.7) of species predicted to have occurred in the region before disturbance. Further, the regenerating forest held 37 species of special conservation concern, representing 88% of species of highest conservation importance predicted to exist in primary forest from the region. We conclude that this specific regenerating rainforest has high biodiversity and conservation value and suggest that our approach can be a cost-effective solution to highlight the value of this reserve (and others) to attract future researchers and visitors, with the aim to provide support for its future protection. Whilst preserving primary forest is essential, our results suggest that effective conservation management of regenerating lands can aim to return high levels of biodiversity to heavily disturbed tropical forest ecosystems.

Introduction

Many of the world's tropical forests have had their structure and underlying functions disrupted by human impacts (Gardner 2012). The Global Forest Resources Assessment (FAO 2010) classifies just 36% of global forest cover as primary and shows that despite forest regeneration contributing to considerable progress being made towards reversing the overall trend of global forest loss in recent years, most net forest loss still occurs in tropical regions with tropical countries showing a net loss of 12.3 million hectares per year (FAO 2012). According to Wright (2010) this includes approximately 64,000 km^2 of tropical forest per year being deforested, with approximately 21,500 km^2 per year of natural forests regenerating on abandoned land. As a result there is a pressing need to understand the future biodiversity and conservation value of areas following tropical rainforest regeneration (Dent & Wright 2009; Kinnaird et al. 2003; Peres et al. 2006). Within this study we use the term regenerating rainforest to refer to once pristine or primary tropical forests that have undergone significant human impacts (including clear felling, heavy logging resulting in partial clearance or substantial levels of selective logging) and have then regenerated to a state where a closed canopy has been re-established (Chazdon 2014). Regenerating forests can represent a number of types of original land use and modification and as such, have been shown to display different values for biodiversity conservation; such as logged regenerating forests, secondary regenerating areas, once cleared regenerating forest and abandoned agricultural regenerating lands (Burivalova et al. 2014; Chazdon 2014; Edwards et al. 2014b; Gibson et al 2011). Despite such ecosystems representing the majority of remaining tropical forest, the potential of such human-modified landscapes to provide important habitat for the conservation of rainforest biodiversity is contentious. Whilst some authors propose that the key conservation priority is to protect primary forest (Barlow et al. 2007a; Gibson et al. 2011; Sodhi et al. 2010), others suggest that regenerating and secondary forests will become increasingly important as human populations in tropical countries increase and primary forest is converted to agricultural land and later abandoned to regenerate (Anand et al. 2010; Chazdon et al. 2009b; Durães et al. 2013; Irwin et al. 2010; Letcher & Chazdon 2009; Norris et al. 2010; Tabarelli et al. 2010).

Chazdon et al. (2009b) reviewed 51 studies that evaluated the potential of tropical secondary forests for biodiversity conservation and found that the average proportion of primary forest animal species richness found in regenerating forest in the tropics was 57% (±2.6) of the primary forest species. When considering just the 12 studies with data for forests with at least 20 years of regeneration, the average value of primary forest species found was 66% (±5.4). Barlow et al. (2010) found similar levels and showed that regenerating secondary forests (14-19 years old) within Brazilian Atlantic forest areas held on average 59% of the primary forest species richness (including data from vertebrates, invertebrate and plant groups). Individual studies have shown that regenerating forest can hold higher levels of biodiversity, with Edwards et al. (2011) for example, finding that twice logged forests in Asia sustained levels of 75% of primary forest species, in a study of birds and dung beetles. Despite the notable biodiversity losses indicated by the average values reported above, it has been suggested that regenerating forests could still possess the potential to sustain future levels of biodiversity comparable to those of primary forest habitats (Letcher & Chazdon 2009). Particularly when we take into account the premise that biodiversity will continue to increase over time as forests continue to regenerate (Chazdon et al. 2009b; Wright 2010).

Gardner et al. (2010) suggest that the current lack of agreement on the conservation and biodiversity value of regenerating forests arises largely because information on how well tropical forest biodiversity can recover from such devastating environmental change is often difficult to determine and interpret. We agree with this view and highlight four key reasons that may play a role in the lack of agreement on the response of tropical forest biodiversity to regeneration and therefore on where conservation efforts should be focused (Wright 2005). First, many regenerating tropical forests studied continue to experience human impacts so that studies have measured both the impact of the original disturbance and the impact of any ongoing disturbance (for example hunting or extraction of timber) that is limiting recovery. Second, in many studies although time scales are appropriate for understanding impacts of recent land use on biodiversity, the time scale of assessment following disturbance is often premature, if the aim is to assess the value regenerating rainforest may have for conservation. The types of forest evaluated in the majority of reviews (Anand et al. 2010; Barlow et al. 2007a; Chazdon et al. 2009b; Gibson et al.

2011; Irwin et al. 2010; Letcher & Chazdon 2009; Norris et al. 2010; Sodhi et al. 2010; Tabarelli et al. 2010) are often relatively young areas of regenerating forest (<15 years) that are therefore limited in their ability to measure the potential value secondary areas might have, following a significant period of regeneration (Chazdon et al. 2009b) Third, a key point of criticism suggested by some of the review papers is that there is a bias towards examining changes in overall species richness patterns and that overall richness alone may not be the best measure of biodiversity value from a conservation perspective (e.g. Peres et al. 2006). Anand et al. (2010) suggest a potential dominance of species of low conservation concern in species richness measures and show that two communities can have altered community structures but display similar richness values. One possible way to tackle this issue is to look specifically at species of key conservation concern or groups that have been highlighted as key indicators as opposed to simply analysing estimated overall richness values of communities (Lawler et al. 2003; Pearman 1997), which provide little indication of which conservation targets are present and which absent. Fourth, the vast majority of studies only investigate the response of a single taxon to regeneration and so may only provide a limited insight into the general patterns occurring within a wider biodiversity context.

One possible reason to explain why many assessments of biodiversity are often single taxon orientated (Anand et al. 2010; Gardner et al. 2008), may relate to the limited funds available within biodiversity and conservation research (Lawton et al. 1998; Gardner et al. 2008). Biologists therefore often need to look for the most cost effective solution. This is a challenge generally for many local and private reserves which are often located on marginal abandoned lands which are in some form of regeneration, and are often located in close proximity to areas of primary forest (Sloan et al. 2015). But how can local reserves put into context their own biodiversity if they don’t have resources in terms of funds, access or time to survey in comparative primary forest (Lawton et al. 1998; Gardner et al. 2008)? One potential solution might be to utilise previous knowledge and information from the region more generally and from previously well-studied sites, so that local reserve values can be placed into relative context. Understanding biodiversity and conservation value is key at a number of levels; global, national, regional and local (CBD 2010). Average values may be important at a global, national or even regional level, but assessments are also essential to local reserves in understanding biodiversity and conservation value to attract potential visitors, or in developing payment services systems related to landscape preservation (Phelps et al. 2012; Sommerville et al. 2012).

In this study we aimed to assess the potential conservation value of a privately-owned regenerating tropical rainforest in one of the world's most biodiverse and important conservation areas while controlling for the above difficulties. Our case study focuses on a regenerating study site within the Manu Biosphere Reserve, a UNESCO World Heritage Site designated to protect the globally important Amazon rainforest in and around Manu National Park, SE Peru. Within this area we specifically chose a site that had been effectively protected from confounding on-going human disturbances and that had a long time for regeneration since the initial disturbance (>30 years). We focused on species richness values so that the results could be compared with the majority of studies that had measured biodiversity value in this way but we also assessed specific groups of indicator species and species of conservation concern to allow us to put these species richness values in the context of conservation value. Finally, we looked at multiple taxa to allow us to test the generality of any observed patterns. In this way we aimed to better understand the actual value of such regenerating forest for conservation in Manu but also to use this as a case study to assess the potential value regenerating forest might have as a conservation tool more generally. We believe this is the first multi-taxa study to focus on assessing potential conservation value of regenerating rainforest in the Amazon in the absence of the key potentially confounding factors of young regenerating forest age and on-going human disturbance. In this case study we aimed to answer three key questions to help contribute towards understanding the potential biodiversity and conservation value of regenerating tropical rainforest more generally: 1. How does the observed species richness of the regenerating rainforest study site (alpha diversity) compare with measured alpha diversity of nearby primary forest locations?; 2. How does the observed species richness (alpha diversity) of the regenerating rainforest study site compare with predicted primary species richness of geographically and ecologically similar habitat prior to disturbance?; and 3. How do the numbers of key indicator species and species of special conservation concern (globally threatened and near-threatened species) compare between the regenerating rainforest study site and those predicted to have existed before human disturbance?

Methods

Choice of study site

The regenerating rainforest area selected for this study was chosen on the basis that it had a known human disturbance history and was located in a well understood geographic context, in close proximity to a large protected area network. The study was carried out at the Manu Learning Centre (MLC) research station owned and operated by conservation NGO the Crees Foundation, in the Peruvian Amazon (71°23'28"W 12°47'21"S; Figure 1). The study site lies within the cultural buffer zone of the Manu biosphere reserve and consisted of ~800ha of regenerating lowland tropical forest accessed by a 20km trail system and covering an altitude range of 450-740 m asl. The forest had historically experienced various types of major human disturbance such as selective logging (ceased mid 1980's; ~332ha), partial clearance for small scale (largely subsistence) agriculture (ceased ~1980; ~183ha) and complete clearance by felling and conversion to large scale agriculture (ceased ~1970; ~293ha). This was determined by two of the authors visiting the site to visually inspect it, with further confirmation by consulting local guides whom had expert local knowledge related to historic land-use of the study site. Both approaches identified consistent points that were marked as the boundaries of the different disturbance histories so that the respective areas of disturbance could be calculated. The study site was directly bordered by areas of small-scale agricultural land and areas of current logging practices but had been strictly protected from hunting and other negative human impacts since 2002. Beyond the study site to the west lies the core area of the Manu National Park; over 1.5 million ha of protected tropical forest. To the east of the reserve lies the second largest protected area in the biosphere reserve, the Amarakaeri Communal Reserve (a 402,335 ha forest reserve, created in 2002). By the end of this study the site had been regenerating for 30-50 years, so that the whole area was once again covered by closed canopy tropical forest and had been strictly protected from ongoing human disturbance for >10 years. Ferraz et al. (2014) have recently proposed an ecosystem condition scoring system for tropical forest that provides an objective way of categorising sites for their ecosystem services potential. This system provides a simple methodological framework to evaluate the contribution of past landscape dynamics and present landscape structure, which is applied to score sites based upon the following features of past landscape dynamics and present landscape structure: forest age (used as a surrogate for forest integrity, as old-growth forests have better vegetation structure), proximity to nearby forest (a surrogate of local habitat connectivity, as more connected forest patches allow better biological and physical flows in the landscape), the proportion of surrounding area covered by forest (to represent interior-edge, as interior forest is less exposed to disturbances like fire, strong winds, drought and biological invasions) and the size of the forest patch (forest contiguity, as larger forest patches harbor higher numbers of species and provide better ecological conditions for their long-term perpetuation). The study calculates these based on surrounding quadrants and classifies each metric into four levels according to its level of contribution for ecosystem services provisioning. If we roughly apply this system to the regenerating study site in our study (with >30 years of regeneration time, in close proximity to large swathes of primary protected areas less than 10km distance to both the east and the west and consists of an area over 800ha in size), it would likley fall within the highest categories (a score of >11). It therefore provides an ideal opportunity to investigate the potential biodiversity and conservation value of what we will term a best case scenario for regenerating rainforest without the confounding effects of on-going human disturbance. This allows us to examine the true value that a regenerating rainforest can have under successful conservation management, designed to take advantage of and create the best of circumstances.

Choice of study groups

This study measured the biodiversity of four key taxonomic groups (amphibians, birds, medium-large mammals and reptiles), chosen because they are of well-known conservation importance, have been identified in some cases as key indicators and most importantly, had been well-studied locally at both the regenerating forest study site and primary forests sites in the Manu region.

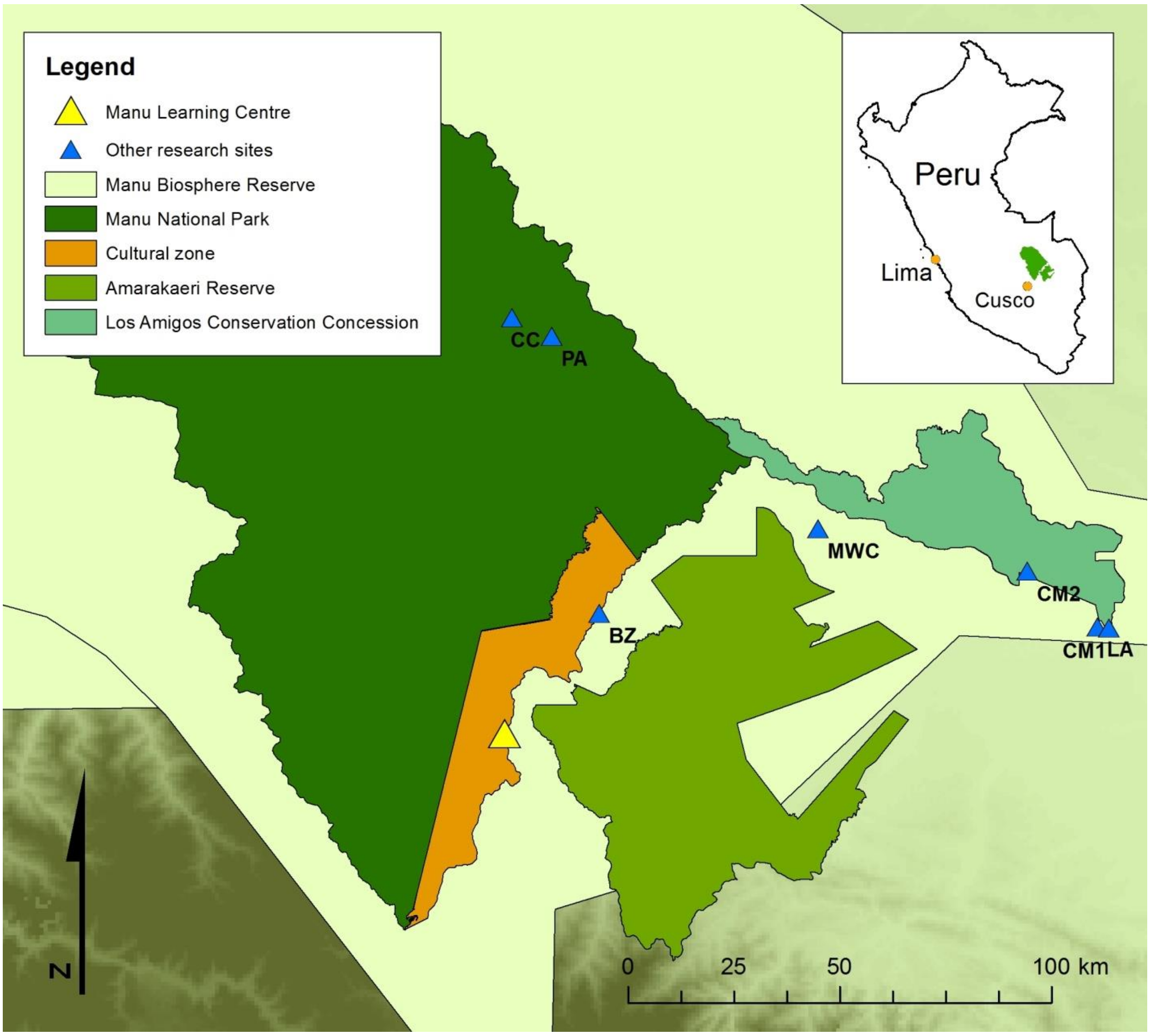


Figure 2. Protected areas in SE, Peru and the relative location of field sites mentioned within the study area and context map of Peru. Site acronyms: LA = Los Amigos, CM1 = Centro de Monitoreo 1, CM2 = Centro de Monitoreo 2, CC = Cocha Cashu, PA = Pakitza, MWC = Manu Wildlife Centre, BZ = Bonanaza and MLC = Manu Learning Centre. Shape files gathered from The World Database on Protected Areas.

Specifically, more than 70% of the world's amphibian species are thought to be in decline (Hayes et al. 2010) and given that habitat destruction and fragmentation are among the leading causes of this global decline (Cushman 2006; Eigenbrod et al. 2008; Schlaepfer & Gavin 2001) it is important to understand how amphibians respond to regenerating landscapes. Birds, in particular understorey guilds, have been found to be sensitive to environmental changes and habitat fragmentation within neotropical rainforests (Banks-Leite et al. 2010) so are likely to be another useful group for understanding how biodiversity may respond to rainforest regeneration. Medium-large mammals remain relatively understudied within tropical forests (Salvador et al. 2011) and play a key role in forest ecosystems, directly through seed dispersal (Beck et al. 2010) and prey population control or indirectly by helping to maintain assemblages of other faunal groups (Beck et al. 2010; Cassano et al. 2012). Reptiles are a vastly understudied group both globally and within Amazonia, despite acting as important meso-predators within many ecosystems (Böhm et al. 2013).

Comparing observed richness (alpha diversity) in the regenerating forest and primary forest

Many studies comparing biodiversity rely on calculations of estimated species richness often based on relatively short periods (a few months) of intensive, standardised survey at individual sites (Magurran 2004; Magurran 2013). Biodiversity estimators have many advantages but they can only produce an overall figure that estimates the number of species present and cannot provide information on which individual species of conservation importance are likely to make up the overall species richness figure. In this study we wanted to assess the conservation value that regenerating forest may have in comparison to primary forest and this meant we needed to be able to compare actual biodiversity in terms of the alpha diversity recorded in regenerating forest with that of primary forest. Species richness estimators also require at least some standardised information on the frequency at which each individual species has been observed (e.g. whether a species has been seen once, twice or more etc). In reviewing the data available for potential primary forest comparison sites in the Manu area it became clear that although many sites have richness data in the form of overall lists of species observed there was little standardisation between different primary forest sites in the abundance data available thus making the calculation of comparable species richness estimates impractical. For this study we therefore selected observed species richness as the most appropriate measure for allowing biodiversity of regenerating and primary rainforest to be compared. Additionally, the majority of previous studies assessing the importance of regenerating forest for biodiversity have based comparisons on species richness so it was desirable to do the same to make the results of our study comparable to the majority of previous work. Using observed values will provide a conservative estimate as this is likely to be an underestimate of the true value such a biodiverse region, despite such intensive survey efforts over a number of years.

In order to assess the alpha diversity of the regenerating forest study site, we used a combination of intensive field work surveys and long term data that had been recorded at the site since its creation as a protected area in 2002. The long term data consisted of relatively comprehensive lists of birds and medium to large mammals but depauperate lists of amphibians and reptiles, partly due to the bias and interest of previous researchers and visitors towards birds and mammals but also due to the fact that visitors often walk diurnally as opposed to nocturnally, when many amphibians and reptiles are active. More intensive survey data were collected between August 2011 and February 2013 in order to maximise the data coverage and detect as many species as possible that exist within the regenerating forest study site. Birds and mammals were surveyed through early morning line transects (Salvador et al. 2011; Bibby 2000) and for birds mist nets were also used (Blake &Loiselle 2001). Camera traps were used for medium to large mammals and game birds (Munari et al. 2011) and amphibians and reptiles were surveyed using nocturnal visual encounter transects (Beirne et al. 2013; Doan 2003), pitfall traps (Beirne et al. 2013) and leaf litter plots (Doan 2003; Heyer et al. 1994).

Survey effort during the intensive phase was: for mist netting 3180 net hours (10m long x 3m high), providing 1143 captures; 227 early morning bird/mammal transect hours by teams of two observers (covering 213.72km along 19.83km of trail – 11 transects comprising 118 transect surveys); 4860 camera trap days from 10 survey sites; 754 observer hours of nocturnal herpetological transects; 2060 pitfall array days and 30 leaf litter plots ($5m^2$). Uncertain or doubtful records (from the less intensive long term data collection phase) were excluded from the overall site species lists unless species presence could be confirmed during the intensive data collection phase. Incidental records outside of systematic survey times were added to each of the overall species lists. The result was a species list for each taxonomic group that recorded all species detected in the regenerating tropical forest study site over a 10 year period. This provided the data for assessing observed species richness in regenerating tropical forest.

In order to provide data on primary forest biodiversity to which we could compare the data from our regenerating forest site we reviewed published inventories and identified all tropical forest survey sites within 100km of the regenerating forest study site that had detailed species lists and significant amounts of research conducted at them (see Appendix 1A). As with the regenerating forest site the data from each comparison site was generated from a similar combination of long term biodiversity recording supplemented by periods of intensive survey effort during more focused studies. In general the primary forest sites had longer spans of data collection by more scientists and so were judged likely to have species lists that were at least as comprehensive as the regenerating forest sites (see Appendix 1A for a detailed description of comparative sites and respective survey efforts). Use of observed species richness (rather than estimated species richness, which was not possible due to insufficient published data on the frequency of species records at the comparison primary sites) should therefore provide a conservative test of the relative biodiversity and conservation value of this regenerating forest compared to nearby primary forest sites.

Predicting overall richness of the study sites regional area prior to disturbance

Although the primary forest comparison sites are the best studied sites available in Manu, we considered the possibility that observed species richness based on the species lists available for the primary forest comparison sites might not be exactly comparable to the regenerating site due to local variation in elevation, habitat types and the fact that some species are likely to remain undetected at each site despite comprehensive monitoring efforts. We therefore also used a second method, to provide alternative primary forest species lists, for comparison to the regenerating site. To do this we used existing ecological and distributional information on each individual species to assess whether species known from the general Manu area were likely to have existed in the regenerating forest area prior to any human impact (i.e. we estimated species lists of primary forest habitat with conditions similar to the regenerating forest study site). We started with the conservative assumption that current records from the regenerating forest indicated that the species was likely present in the area before any anthropogenic effects occurred. This assumption seemed reasonable as most generalists found following human disturbance will usually found prior to these events because natural disturbance creates opportunities and species that had re-colonized the regenerating site from nearby source populations were likely to have once existed before disturbance. This allowed our results to act as conservative estimates of original biodiversity at the regenerating site. To the list of the regenerating forest records we added all species that were known from the general Manu area based on the most authoritative species lists available for each taxonomic group (the sources of these lists are described in Appendix 1B). We then edited the primary forest lists to omit species that occurred outside of the altitudinal range of the regenerating study site and, with regards to the birds in particular, species strictly associated with large water bodies (e.g. oxbow lakes), as this particular habitat type was absent from the regenerating site (to do this we used available ecological information from books and online resources; AmphibiaWeb 2013, Schulenberg et al. 2010, The Reptile Database 2013 and information from IUCN 2013). This meant that the focus would remain specifically upon forest associated species situated within an expected altitudinal range. The result was a predicted primary forest species list for each taxonomic group that included all species predicted, based on ecology and range information, to have occurred in the regenerating forest area, before human disturbance; i.e. we aimed to estimate species lists for primary rainforest habitat around where the study site would have been.

The following steps were followed to produce the primary forest comparison lists. Information from the International Union for Conservation of Nature (IUCN) Red List was obtained for each species with known distributions covering the Manu area to provide distributional data on all species (including species of least conservation concern) in the target taxonomic groups (IUCN 2013). Altitudinal ranges were noted (Schulenberg et al. 2010) along with information available from the range maps provided by IUCN. These range maps were used to assess whether the species distributions (a) were included directly within the range of the regenerating forest site and therefore automatically included, or (b) were within 50km of the site or within 100km of the site depending on taxonomic group (assessed using the IUCN rangemap viewer and Google Earth). The distance of 50km was chosen for amphibians, as amphbians are generally more range restricted than the more vagile birds and mammals. If an amphibian species was within 50km of range then we assumed that it could have been present providing no significant physical barriers (such as major rivers or mountain ranges) were present between the known distribution and regenerating site. For mammals and birds the cut off figure was 100km due to their general potential to move over greater distances but again special physical features such as rivers were considered for each case, using the ecological information resources mentioned previously. Resources, outlined below, were then used to aid decisions in any cases that were difficult to determine from this initial information. The predicted bird lists were confirmed with the aid of information in Schulenberg et al. (2010) which is the most authoritative and up to date source on bird distributions in Peru. The amphibians were confirmed by information from AmphibiaWeb (2013), one of the most up to date resources for amphibians globally and the reptiles from The Reptile Database (2013), another updated global online database. This part of the analysis aimed at predicting the historic presence of individual species in the regenerating rainforest study area prior to disturbance. The method generally produced clear outcomes for species in the majority of study groups. However, for the reptiles due to lack of available information we felt that species presence in the generated list was fairly arbitrary and more dependent on the quality (or lack) of data available for each species rather than reflecting whether the true distribution included the regenerating study site. We therefore chose not to use this approach for reptiles.

Choice of species of special conservation concern / indicator groups

In order to look beyond overall species richness levels to assess the conservation value of the regenerating forest study site, we assessed in more detail the occurrence of specific well known indicator groups for good habitat quality and species of special conservation concern (defined for this study as globally threatened or near-threatened species, as categorised on the IUCN Red List of threatened species, IUCN 2013). This avoided the potential problem of simply observing regenerating forest species richness values that might be elevated through the inclusion of generalist or common species. This allowed us to see if regenerating forest richness levels were dominated by generalists or might contain comparable conservation importance to primary forest if considering only indicator species and species of conservation importance. Since previous research has found that at-risk species can perform well as indicators themselves (Lawler et al. 2003) and as no more specific list of indicator species was available, we focused on this group for mammals. For birds the indicator species used were the indicators of good quality habitat for Southern Amazonian lowland tropical forest, as described by Stotz et al. (1996; see Appendix 1C). Amphibian indicators of good quality habitat were assessed by focussing on the family Craugastoridae, containing the genus *Pristimantis* (previously *Eleutherodactylus*), a key indicator group identified by Pearman (1997). Leaf-litter and understorey dwelling herpetofauna lend themselves well to biological conservation studies as they are abundant in neotropical forests and are relatively easy to sample (Peaman 1997; Kati et al. 2004; Oldekop et al. 2012; see Appendix 1D). No suitable list of indicator species of good habitat quality for reptiles has yet been proposed so reptiles were excluded from this section of the analysis.

Results

Observed richness in the regenerating forest and comparable primary forest sites (alpha diversity)

We recorded high levels of biodiversity in the regenerating rainforest study site, with a total of 570 species detected in the four target groups. These observed totals included 60 amphibian species, 406 bird species (species associated with forest habitat and excluding those associated with open water bodies), 38 medium-large mammal species (this list excludes bats and small ground mammals) and 66 reptile species (see Appendices 1E-H for full species lists). When compared with alpha diversity of nearby primary forest sites, we found that alpha diversity of the regenerating forest site was 81% for amphibians, 84% for birds, 80% for medium-large mammals and 100% for reptiles (an average of 87% ±3.5; Table 1).

Table 1. Species richness in the regenerating tropical forest (alpha diversity) site compared to primary forest sites. Primary forest site abbreviations; CC = Cocha Cashu, LA = Los Amigos, MWC = Manu Wildlife Centre, PA = Pakitza. Values in brackets represent the percentage of the species present at the regenerating forest site (MLC).

Taxa	**Primary forest sites used to determine average alpha diversity from primary forest**					***Alpha diversity at the MLC***
	CC	***CC-PA***	***LA***	***MWC***	***PA***	
Amphibians	78 (77)	-	82 (73)	-	63 (95)	60
Birds	454 (89)	-	499 (81)	501 (81)	-	406
Mammals	-	47 (81)	48 (79)	-	-	38
Reptiles	64 (103)	-	75 (88)	-	60 (110)	66

Three sites, Cocha Cashu, Los Amigos and Pakitza had more developed amphibian and reptile lists compared to the 60 and 66 species, respectively, of the regenerating site (Figure 2). The most detailed medium to large mammal inventories from primary sites close to the regenerating rainforest site, Cocha Cashu-Pakitza and Los Amigos, contain 47 and 48 species respectively, compared to the 38 found in the regenerating forest. When we look at the forest associated bird inventories from primary sites close to the regenerating site these have: Cocha Cashu (454 species), Manu Wildlife Centre (501 species) and Los Amigos (499 species), compared to the 406 species of the regenerating site.

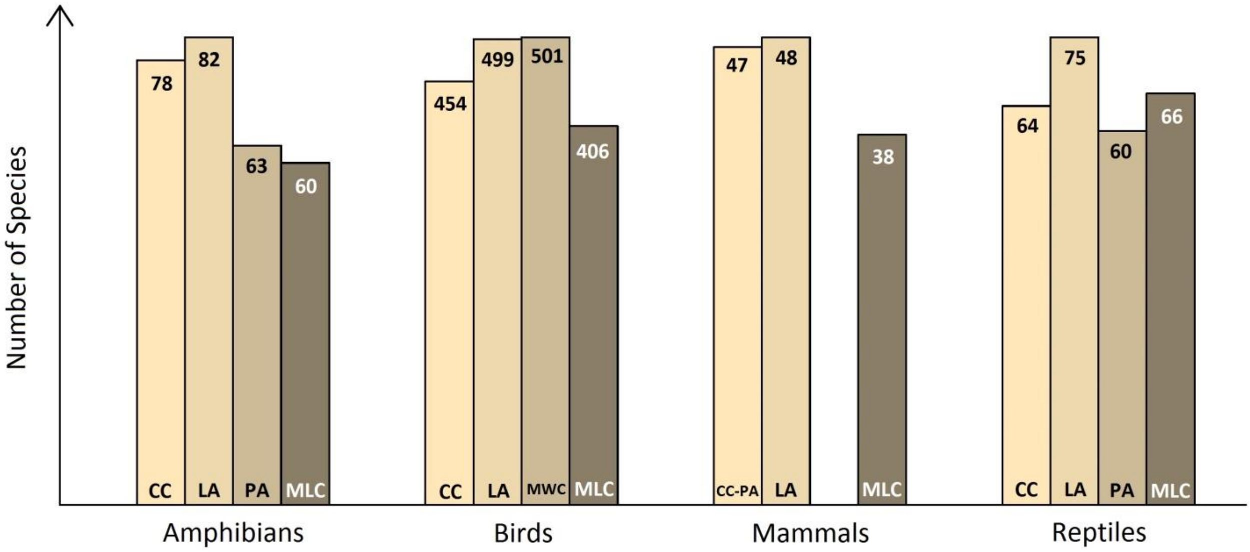


Figure 2. Species richness in the regenerating tropical forest site (MLC) compared to primary forest sites. Primary forest site abbreviations; CC = Cocha Cashu, LA = Los Amigos, MWC = Manu Wildlife Centre, PA = Pakitza.

Comparing alpha diversity of regenerating forest to inferred species lists of the region

When inferred species lists were estimated for the regenerating forest area, 74 amphibian, 563 bird and 40 mammal species were predicted to have existed prior to human disturbance (Table 2). There was insufficient distribution data available for reptiles to make a reliable prediction for this group. If the three taxa had been sampled to completion at the MLC in this study, this would suggest the regenerating forest site contains 81% of amphibians, 72% of birds and 95% of medium-large mammals that would have once existed in primary forest before human disturbance (Table 2). Overall this represented an average value of 83% (±6.7) of species from inferred species lists from the region detected within the regenerating forest site.

Table 2. Number of species in the regenerating forest of the MLC compared to inferred species lists for the region and observed primary forest sites. Primary forest site abbreviations; LA = Los Amigos, CC = Cocha Cashu, PA = Pakitza, MWC = Manu Wildlife Centre.

Taxa	Alpha diversity at the MLC (as % of inferred regional species lists)			Inferred regional species lists			Average species richness from primary forest sites	Species richness of MLC as a % of species richness at primary forest sites	Primary forest sites used to determine average primary forest species richness
	Total	*Indicators*	*Conservation concern*	*Total*	*Indicators*	*Conservation concern*			
Amphibians	60 (81)	13 (72)	-	74	18	-	74	81	LA, CC, PA
Birds	406 (72)	30 (86)	27 (87)	563	35	31	485	84	LA, MWC, CC
Mammals	38 (95)	-	10 (91)	40	-	11	48	80	LA, CC-PA
Reptiles	66	-	-	-	-	-	66	100	LA, CC, PA

Comparing key species of conservation concern / indicator groups from the regenerating forest with primary forest

Overall, an average of 84% (±4.1) of indicator species and species of conservation concern predicted for the region were detected within the regenerating forest site. Thirteen medium-large mammal species of special conservation concern (threatened and near-threatened species) are known from the Manu region (Appendix 1I). Based on range data and ecology it was predicted that 11 of these species could have existed before human disturbance at the regenerating forest study site (Table 2) and of these, 10 species (91%) were recorded in regenerating forest by this study. The only species not recorded was the pacarana (*Dinomys branickii*). Therefore, we found that the regenerating forest held almost all mammal species of high conservation concern that had the potential to have existed in the study area. Of the 36 bird species of special conservation concern in the Manu region, we predicted based upon range data and ecological information that 31 species could have existed in the regenerating forest prior to disturbance. Of these, 27 species (87%) were recorded within the regenerating forest site (Appendix 1J). The scarlet-shouldered parrotlet (*Touit huetii*), the green-thighed parrot (*Pionites leucogaster*), the cerulean warbler (*Dendroica cerulea*) and the black-and-white tanager (*Conothraupis speculigera*) were the four threatened/near-threatened species not recorded. In total, 37 bird and mammal species of special conservation concern (threatened and near-threatened species) were found during this study in the regenerating forest and this represented 88% of the 42 species predicted to be present in primary forest in the area before human disturbance.

Of 40 lowland tropical forest indicator bird species (for high quality habitat) known from the region, 35 were predicted based on range data and ecological information to have existed before human disturbance at the regenerating forest site (Appendix 1C). Of these 35 predicted, 30 species (86%) were recorded at the regenerating forest site. The Amazonian barred woodcreeper (*Dendrocolaptes certhia*), the ruddy spinetail (*Synallaxis rutilans*), the banded antbird (*Dichrozona cincta*), the striated antthrush (*Chamaeza nobilis*) and the red-crowned ant-tanager (*Habia rubica*) were the five indicator birds not yet recorded.

Of the 23 species of Craugastoridae (indicator amphibians) found within the lower Manu region, we predict that 18 could have existed before human disturbance at the regenerating forest site (Appendix 1D). Thirteen (72%) of these species were recorded at the regenerating study site. *Oreobates cruralis, Pristimantis diadematus, P. mendax, P. ventrimarmoratus* and *Strabomantis sulcatus* were the five species not recorded at the regenerating site (Table 2.).

Discussion

In this study we report what we believe is the first multi-taxa case study study to focus specifically on assessing potential conservation value of a regenerating rainforest study in the Amazon, in the absence of the key potentially confounding factors of young regenerating forest age and on-going human disturbance. We detected an average species richness value of 87% (±3.5) of alpha diversity found in primary forest areas and an average value of 83% (±6.7) of the inferred species lists from the region. This included 88% (37 out of 42) of the species of highest conservation concern. Our assessment of the actual biodiversity and conservation value of this regenerating tropical forest therefore suggests that high levels of vertebrate species richness could be found in areas of regenerating forest; particularly forests which score highly on the ecosystem condition scoring system as proposed by Ferraz et al. (2014). The results have additionally shown that the high levels of biodiversity found within a regenerating forest study site need not simply be dominated by generalist species but can contain high levels of key indicator species and species of conservation concern.

We suggest our results for the alpha diversity of the regenerating forest of the MLC reserve are likely to be conservative estimates of the biodiversity value of regenerating forest. Since, despite utilising an array of survey methods and techniques and even with a significant amount of effort, it would be unlikely within such a diverse and species rich landscape to have detected the presence of all species, so our comparisons identify only the minimum biodiversity value of the regenerating forest. For example, five of the mammals not found at the regenerating site but found at comparison primary sites are primates, at least one of which (the pygmy marmoset, *Cebuella pygmaea*), is restricted to lower elevations and likely absent due to physical geographic barriers (e.g. the Manu river) rather than the site’s regenerating status. The giant river otter (*Pteronura brasiliensis*) is a species restricted to low lying areas with a presence of oxbow lakes, a feature not found along the Alto Madre de Dios river (location of the regenerating forest site) but a common feature along the Manu and Madre de Dios rivers (primary forest comparison site locations). Also, the two mammals predicted to have once existed that remain undetected so far are particularly cryptic species; the silky pygmy anteater *(Cyclopes didactylus)* and the pacarana *(Dinomys branickii).* Both are secretive and inconspicuous species that are easily overlooked and evade detection. It is quite plausible that they do exist at the MLC but have thus far not been recorded. Similarly, considering our indicator amphibian group, there was a presence of three species found at the regenerating site that could not be identified (*Pristimantis* sp1, sp2 and sp3). It is quite possible that they are variations of *P. diadematus*, *P. ockendeni* and *P.*

ventrimarmoratus, or even new species to science, which would again suggest that our results are conservative. Finally, two of the four threatened/near-threatened bird species not recorded at the regenerating site are migratory species and are only potentially present at the site for part of the year, so may have been overlooked. The cerulean warbler is a patchily distributed species, absent from many primary forest areas and the regenerating study site resides at the lower edge of its altitudinal range so may be absent for reasons not linked to the area's status as regenerating forest. As a result the 87% occurrence rate of bird species of conservation concern is again, likely a conservative estimate of species of conservation interest.

Our results from this best case scenario are higher and more promising for future biodiversity conservation than average value approaches of assessing regenerating rainforest biodiversity (Figure 3). This suggests that a large proportion of the original, primary forest vertebrate species richness might be well conserved in regenerating rainforest cases that score highly under the ecosystem condition scoring system (Ferraz et al. 2014). Especially considering the context of the study site location, situated between two large protected areas of largely primary tropical forest; a factor shown to be one of the most important related to high levels of biodiversity (Anand et al. 2008; Ricketts et al. 2001). Recent reviews have shown average values of regenerating rainforest species richness to be 59% (Barlow et al. 2010), 57% (Chazdon et al. 2009b) and 68% (Chazdon et al. 2009b) of primary forest biodiversity. These figures provide valuable information on current biodiversity levels at a wide variety of regenerating forest sites following various types of original disturbance; however, the majority of the studies that contribute to these lower average values have confounding effects of on-going disturbances and a short regeneration period. These effects are likely to have depressed average biodiversity levels and as such, may be less useful in assessing the potential future biodiversity and conservation value of rainforest regeneration under the best of circumstances. We suggest that the type of best case scenario approach adopted here should be a focus for further research using a replicated study design, in order to increase our understanding and awareness of the potential of regenerating study sites that score highly under the ecosystem condition scoring system (Ferraz et al. 2014), in the absence of on-going human impacts.

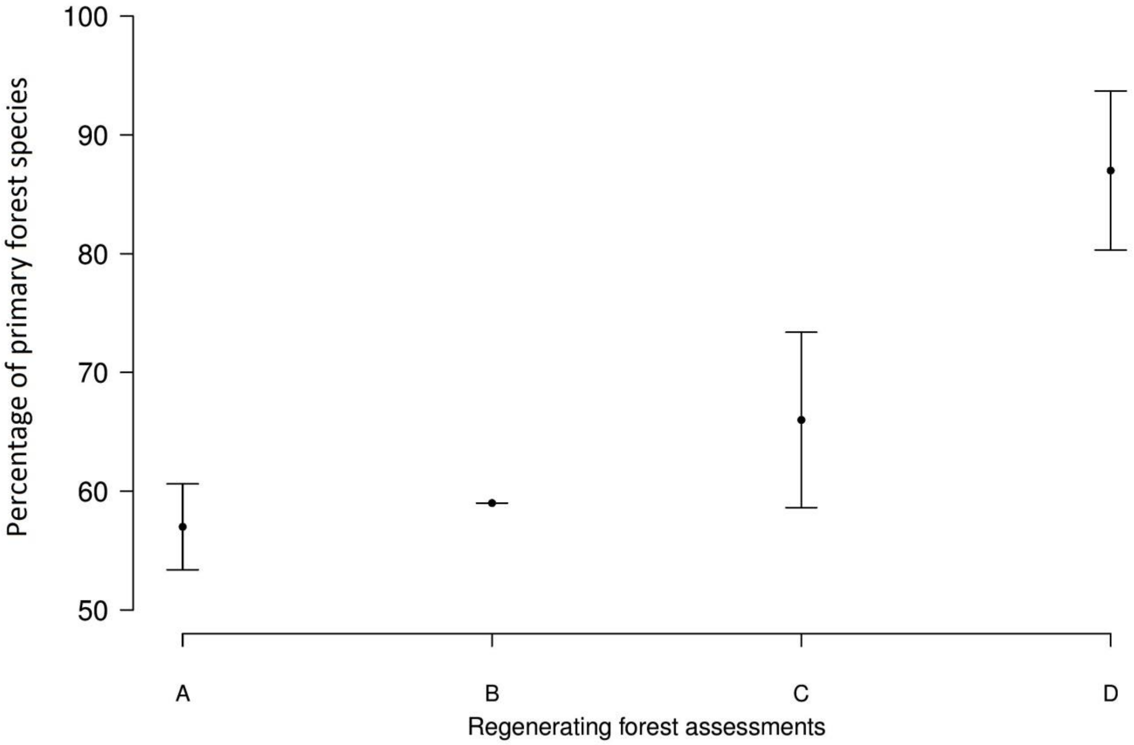


Figure 3. The overall percentage of primary forest species detected within regenerating forests from two previous papers (A-C) compared to the values presented within this study (D). Where appropriate data is available, 84% confidence intervals are included. The assessments included are: A = Chazdon et al. (2009b), containing data from invertebrates and vertebrates from regenerating forests 1-100 years; B = Barlow et al. (2010), containing data from invertebrates, vertebrates from regenerating forests aged 14-19 years (no confidence intervals are provided as only this average value was provided within the manuscript cited); C = Chazdon et al. (2009b), containing data from invertebrates and vertebrates from regenerating forests aged 21-100 years; and D = data on amphibians, birds, mammals and reptiles from regenerating forest aged 30-50 years, the local reserve assessed within this study.

More generally, our findings are consistent with the work of Dunn (2004b) who found that richness could be predicted to resemble that of primary forest at roughly 20-40 years of regeneration after abandonment, for ants and birds. We also found this pattern for birds and show that similar high levels may be true for other taxa including amphibians, medium to large mammals and reptiles. Our results are also consistent with the patterns observed by Dent and Wright (2009) who reviewed 65 studies that compared the level of similarity between primary and regenerating areas and found that similarity was higher with increasing age of the regenerating areas and when they were contiguous to primary forest. Information on the situation of the study site and the types of forest surrounding are two factors that have been proved vital in the pace of recovery and composition of regenerating areas (DeClerck et al. 2010), along with other important factors including fragment size, the distance to contiguous primary forest patches and historic scale of disturbance (Daily et al. 2003; Fahrig 2001; Horner-Devine et al. 2003). However, on-going habitat disruption, hunting and forest exploitation for example, have been suggested to be the strongest predictors of declining reserve health and a failure to halt further degradation could increase the likelihood of serious biodiversity loss (Laurance et al. 2012a).

Caveats and limitations

The use of inferred species lists is only feasible for relatively well studied groups and at the very least basic distributional and altitudinal information is needed. For birds and mammals this was relatively straightforward, for amphibians slightly more complex but for the reptiles this proved unfeasible. Böhm et al. 2013 emphasise the need to discover more about the status, distribution and ecology of reptiles, particularly within tropical forest regions. This method of predicting species presence, however, did provide suitable results to comparing the regenerating site richness values with inferred species lists for primary forest in the area and has potential as a useful and cost-effective way of assessing biodiversity value of regenerating forest for sites when information from nearby primary forest sites is unavailable. In addition, choosing sites for relative comparison must be approached with care. Assessments should attempt to ensure that comparative sites are chosen with at least similar levels of survey effort and conducted over a similar spatial scale in order to have reliable assessments. If the survey efforts or spatial scales are greatly imbalanced then so likely will be the comparrisons in species richness patterns. In this study we were careful to select sites that had received extensive survey effort and were also carried out over a relatively similar small spatial scale (see Appendix 1A).

In this study, as is common in previous efforts at assessing conservation value of regenerating forest, species richness levels are used for comparative analysis. It would have been desirable to use detailed information on abundance and community structure but this level of detail was not available in comparable forms from many of the primary control study sites. Dunn (2004b) found that community composition specifically can take longer than species richness to resemble the original state found within primary areas. Nevertheless, we were still able to show that regenerating forest holds high levels of key conservation species and species recognised as indicators of good quality habitat. Richness levels within this study are therefore not simply dominated by generalist species or species associated with disturbed habitats, as has often been suggested (Anand et al. 2010; DeClerck et al. 2010; Dent & Wright 2009; Peres et al. 2006).

It should be noted that although biodiversity assessments are often based upon estimates of species richness, such estimates often use one or a small number of survey techniques that might only target specific subsets of a community over a relatively short timeframe. This is certainly useful where rapid assessments are necessary and survey effort can be controlled for in detail (Gardner et al. 2008; Lawton et al.1998), but this does not allow for a detailed representation of the overall biodiversity at the sites, as provided by well-developed species inventories from intensively surveyed field sites over a number of years.

Not only does this provide a more complete and detailed representation of biodiversity but this can also be a very cost-effective form of assessing sites that have already been well surveyed by conservation researchers (Gardner et al. 2008; Lawton et al.1998). This is an especially attractive potential tool for small private reserves/landowners/native communities, who might wish to better understand the value of their own land for biodiversity and conservation (Torres-Sovero et al. 2012). This could provide a potentially attractive tool in which to draw in visitors to their land, and provide the opportunity for alternative more sustainable forms of income, than logging and deforestation activities via agriculture (Hunt et al. 2015). Ecotourism has often proved to be a sustainable and viable option in many circumstances, but being able to promote the value of the land and the wildlife it contains is a key factor related to attracting visitors (Broadbent et al. 2012).

Finally, we acknowledge that the high level of connectedness and situation of the regenerating study site within this case study, likely influences the high values of biodiversity recorded. It must be noted that if the study site was less-well connected and situated farther from primary forest, then values would likely be much lower. The high level of connectedness likely also means that some species recorded within the study may be transient, and not necessarily use the site permenantly, or even exist in a less well connected region. However, our site is fairly typical of abandoned lands in the Manu Biosphere

Reserve, in that large tracts of primary habitat can be found relatively close, and to date, disturbances have been restricted to a relatively small area.

Implications for conservation

In conclusion, we suggest that the type of case study conducted here, that focuses on best case scenarios for regenerating rainforest and avoids confounding effects of ongoing disturbance and short regeneration times, could provide a clearer way of assessing the potential biodiversity and conservation value of regenerating tropical forests in the best of cases. Such an approach would avoid the problems of relying on average values across many studies, which if the aim is to assess long term conservation value of well protected rainforest regeneration, are likely to be confounded by on-going disturbance, maybe in isolation from contiguous primary forest and have shorter regeneration periods.Our results also provide a potential cost-effective solution for sites that may have limited access to funds (Gardner et al. 2008; Lawton et al.1998), in utilising data from sites that have already been well surveyed by conservation researchers, in order to give context the conservation and bidoversity value of such reserves. Although our results highlight a high potential of “best case scenario” regenerating areas to conserve biodiversity, they also show that primary rainforest does hold higher levels of biodiversity and remains of the utmost importance in safeguarding the future persistence of species potentially not found within regenerating forests. Nonetheless, we suggest that effective protection and management of regenerating rainforest could offer a second chance to conserve and support species biodiversity and wildlife of high conservation value. Given that the majority of the world’s remaining tropical forests are in various states of regeneration following human disturbance or destruction, and are often located in close proximity to primary forest (Sloan et al. 2015), our results are encouraging for regenerating areas, in particular those scoring highly under the ecosystem condition scoring system (Ferraz et al. 2014) and emphasise the potential value of regenerating rainforest areas to buffer the pressure of deforestation and habitat alteration to remaining primary forests.

Chapter 2 - Long lasting impressions: After decades of regeneration, rainforest biodiversity remains differentially affected following varied human disturbances; but has high potential conservation value.

Authors and contributions

Andrew Whitworth[1,2*], Roger Downie[2], Jaime Villacampa[1], Laura Dominie Braunholtz[1,2] and Ross MacLeod[1,2]

[1] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.

Designed the study: AW RM LDB

Collected the data: AW LDB JV

Performed the analysis: AW LDB

Contributed to writing of the manuscript: AW RD LDB JV RM

Abstract

Despite large-scale human disturbance of the world's tropical forests followed at times by substantial regeneration, there remains a lack of agreement on the potential conservation value of regenerating forest. Recent reviews have suggested contradictory conclusions, with contrasting interpretations from "most biodiversity can be retained in tropical forest impacted by logging", to suggestions that even relatively low levels of selective logging and habitat clearance "halves the levels of species richness". Here we report the findings of a natural experiment that enabled the first direct (within-site) multi-taxa comparison of faunal biodiversity between areas of a regenerating rainforest site previously subjected to different levels of human disturbance and in the absence of the potentially confounding effects of on-going human disturbance. Even after a long (30 year) recovery period, biodiversity levels (or community composition) of amphibians, butterflies, understorey birds and nocturnal birds were found to be lower (or distinct) in regenerating forest that had historically been completely cleared, compared to regenerating forest that had been selectively logged; in contrast mammals showed no obvious difference. Across the taxonomic groups, species richness in once completely cleared forest was on average only ~13% (±9.8) lower than in the historically selectively logged forest. Community structure and abundances also displayed differences related to historic human disturbance type. Amphibians, butterflies and nocturnal birds displayed differences in overall community diversity patterns, whilst diurnal birds and mammals only displayed distinct differences when considering indicator species and specific feeding guilds. While we agree that preventing further impacts on the world's remaining primary tropical forests is imperative, our findings suggest that even historically highly degraded regenerating tropical forests could, if managed for conservation, provide important resources for conserving tropical forest biodiversity.

Introduction

Large-scale disturbance of primary tropical forests (FAO 2010; Gilroy et al. 2014) has resulted in an increasing conservation and scientific interest in the potential for regenerating landscapes to contribute to the preservation of biodiversity and species of high conservation value (Chazdon et al. 2009b; Edwards & Laurance 2013; Melo et al. 2013). The findings of many studies have indicated that protecting primary forests should be a key priority (Mackey et al. 2014) where the goal is to maintain the highest levels of global tropical biodiversity (Barlow et al. 2007a; Gardner et al. 2007a; Gibson et al. 2011; Sodhi et al. 2010). Yet a growing number of studies, whilst acknowledging the irreplaceable diversity of primary tropical forest, suggest there may also be opportunities to maintain high biodiversity value forests in human modified and now regenerating tropical landscapes. This has led towards increasing calls for greater conservation efforts to be made to protect and manage regenerating tropical forest (Anand et al. 2010; Chazdon et al. 2009a; Irwin et al. 2010; Laurance & Edwards 2014; Letcher & Chazdon 2009; Norden et al. 2009; Norris et al. 2010; Tabarelli et al. 2010). Such regenerating tropical forest includes forests recovering from a broad range of human disturbances, from selective logging to complete clearance that has been followed by the regrowth of secondary forest.

Despite the growing awareness of the potential value of regenerating tropical forest, recent reviews have suggested largely contradictory conclusions on actual biodiversity value of such forest. Even when focusing on regeneration after less than complete forest clearance some conclude that “most biodiversity can be retained in tropical forest impacted by logging” (Edwards et al. 2014a; 2014b), where others suggest that even relatively low levels of selective logging and habitat clearance can “halve the levels of species richness” across the majority of taxonomic groups (Burivalova et al. 2014). Gibson et al. (2011) suggest that forests that had been selectively logged showed limited ecological disruption and biodiversity loss and concluded that such forests could contribute significantly towards tropical biodiversity conservation. However, in the same study, Gibson et al. (2011) suggest that other areas of regenerating forest that had regrown following greater levels of clearance for agricultural uses had limited potential for preserving global biodiversity. A similar review by Putz et al. (2012) concluded that between 85-100% of mammal, bird, invertebrate and plant species found in un-logged forests remained after selective logging. In contrast, Burivalova et al. (2014) conducted a meta-analysis on data from 48 tropical studies and concluded that as selective logging intensity increased, amphibian, invertebrate and mammal species richness was heavily reduced. Amphibian and mammal species richness were even shown to fall below 50% of primary forest levels under some logging scenarios. The contradictory nature of these findings and the literature on which they are based indicate why there is little consensus on the true value of regenerating forests for biodiversity conservation.

It has been suggested that this lack of agreement may be driven by regenerating tropical forests varying enormously in their capacity to support biodiversity, which is known to relate to the initial type of historic disturbance, the timeframe since disturbance, continuing confounding impacts, the taxa or group being studied and the scale at which the study is conducted (Daily et al. 2003; DeClerck et al. 2010; Fahrig 2001; Horner-Devine et al. 2003; Letcher & Chazdon 2009). Chazdon et al. (2009a) found a range of 33–86% of primary forest species being recorded between studies within regenerating forests. However, many of these were based upon assessments of young regenerating forest or sites still undergoing human impacts (Anand et al. 2010; Barlow et al. 2007a; Dent & Wright 2009; Gibson et al. 2011; Norris et al. 2010). These on-going impacts, through hunting or logging, have the potential to impact biodiversity and therefore limit its recovery (Burivalova et al. 2014; Roldán & Simonetti 2001; Urquiza-Haas et al. 2011). This has been suggested to restrict the ability to clearly measure the ecological recovery of biodiversity following a significant period of regeneration (Chazdon et al. 2009a; Letcher & Chazdon 2009).

Broad-scale reviews can be beneficial in accumulating information for a number of taxonomic groups across multi-regional scales, but essential within site information often remains sparse (Bowen et al. 2007). In the case of such complex human-ecological scenarios, it has been suggested that the compilation of comparable data sets from multiple spatial and temporal scales will be difficult and perhaps unlikely to generate significant insights regarding the drivers of biodiversity change in modified systems (Gardner et al. 2009). As a result, we suggest here, that rather than relying mostly on the conflicting results of large scale reviews to try and assess potential biodiversity value of regenerating rainforest, it could be useful to also explore within site variation of biodiversity in areas that are effectively natural experiments. That is to say, identify small-scale sites where a key forest disturbance factor has varied within the site, while other potentially confounding factors are controlled for by being similar across the site.

Where studies have been carried out to investigate within-site variation, there is often a tendency for information or circumstances to be lacking that would be useful in order to compare directly between different disturbance histories. This was highlighted by Dunn (2004a), who showed that just two of 34 studies assessed more than a single type of forest disturbance within a site. The low frequency of direct comparisons is a concern considering that previous research has indicated disturbance history to be the most important factor driving species richness levels (Ross et al. 2002). The limited number of studies that have directly compared the effects of different disturbance types on tropical forests have suggested a negative correlation by which heavier levels of disturbance (such as complete clearance) lead to more impoverished biodiversity and increased ecological disruption. Bowen et al. (2007) reviewed 68 studies to investigate faunal recovery in regenerating forests from a global perspective; just two of which were found to directly compare faunal communities following different forms of forest clearance. Dent and Wright (2009) reviewed 65 studies across 114 regenerating forest sites and emphasised the importance of understanding different types of disturbance history by categorising forests into four different prior land-uses, but again, the studies are dominated by between-site comparisons rather than using direct within-site comparisons that control for other potential between-site differences.

Also evident in this review by Dent and Wright (2009) is that a large proportion of the study sites are young regenerating forest; with 65 sites (57%) of an age <21 years since abandonment. This is true for many review studies (Anand et al. 2010; Barlow et al. 2007a; Chazdon et al. 2009b; Gibson et al. 2011; Irwin et al. 2010; Letcher and Chazdon 2009; Norris et al. 2010; Sodhi et al. 2010; Tabarelli et al. 2010) where the types of regenerating forest evaluated are often relatively young (< 15 years). Although short time scales are useful for understanding the impacts to biodiversity of recent land uses, if the aim is to assess the future value regenerating rainforest may have for conservation, then older regenerating forest would be preferable for such an assessment (Chazdon et al. 2009b).

The goal of this study was therefore to investigate a natural within-site experiment and for the first time at such a scale, to assess for multiple taxa how differences in historic human disturbance might influence current biodiversity of regenerating rainforest, in the absence of the potentially confounding effects of young age of regeneration and on-going human disturbance. We did this by assessing within-site variation of species richness, diversity, abundance, community structure, similarity and composition of four taxonomic groups at a regenerating forest site. We also investigate the response of species of conservation concern, indicator species of primary habitat (as identified in Chapter one of this thesis) and specific feeding guilds within groups. The site had been subjected to three different types of disturbance, had over 30 years of time for regeneration and had no on-going disturbance from hunting or logging. This study sought to answer three key questions: 1) To what extent do differences in type of historic disturbance still affect current levels of biodiversity in an older regenerating forest? 2) Do different taxonomic groups (and species of special conservation concern and indicators of primary habitat) show the same response patterns to different levels of historic disturbance in an older regenerating forest? And, 3) Do different feeding guilds of birds, butterflies and mammals show the same response patterns to different levels of historic disturbance in an older regenerating forest? This,with the aim of better understanding the potential biodiversity value different types of regenerating tropical forest might have if they are protected and managed for conservation, rather than experiencing on-going human impacts that might be limiting recovery of biodiversity.

Methods

Choice of study site

The regenerating rainforest area selected for this study was chosen on the basis that it had distinct known human disturbance histories within a small area, so that areas with different histories could be compared without potential confounding effects from variables that might differ over larger spatial scales. It was located in a well-defined geographic context in close proximity to a large protected area network providing source populations for recolonizing the forest as it regenerated. The study was carried out at the Manu Learning Centre (MLC) research station owned and operated by conservation NGO the Crees Foundation, in the Peruvian Amazon (71°23'28"W 12°47'21"S). The MLC is situated within the Manu Biosphere Reserve, a UNESCO World Heritage Site designated to protect the globally important Amazon rainforest in and around Manu National Park, SE Peru (Figure 1). The biosphere reserve consists of a network of core protected areas surrounded by areas designated as cultural buffer zones due to historically high human impact, including extensive logging or clearance for subsistence agriculture. The study was situated within one of these cultural buffer zones and consisted of ~800ha of regenerating lowland tropical forest accessed by a 20km trail system and covering an altitude range of 450-740m asl.

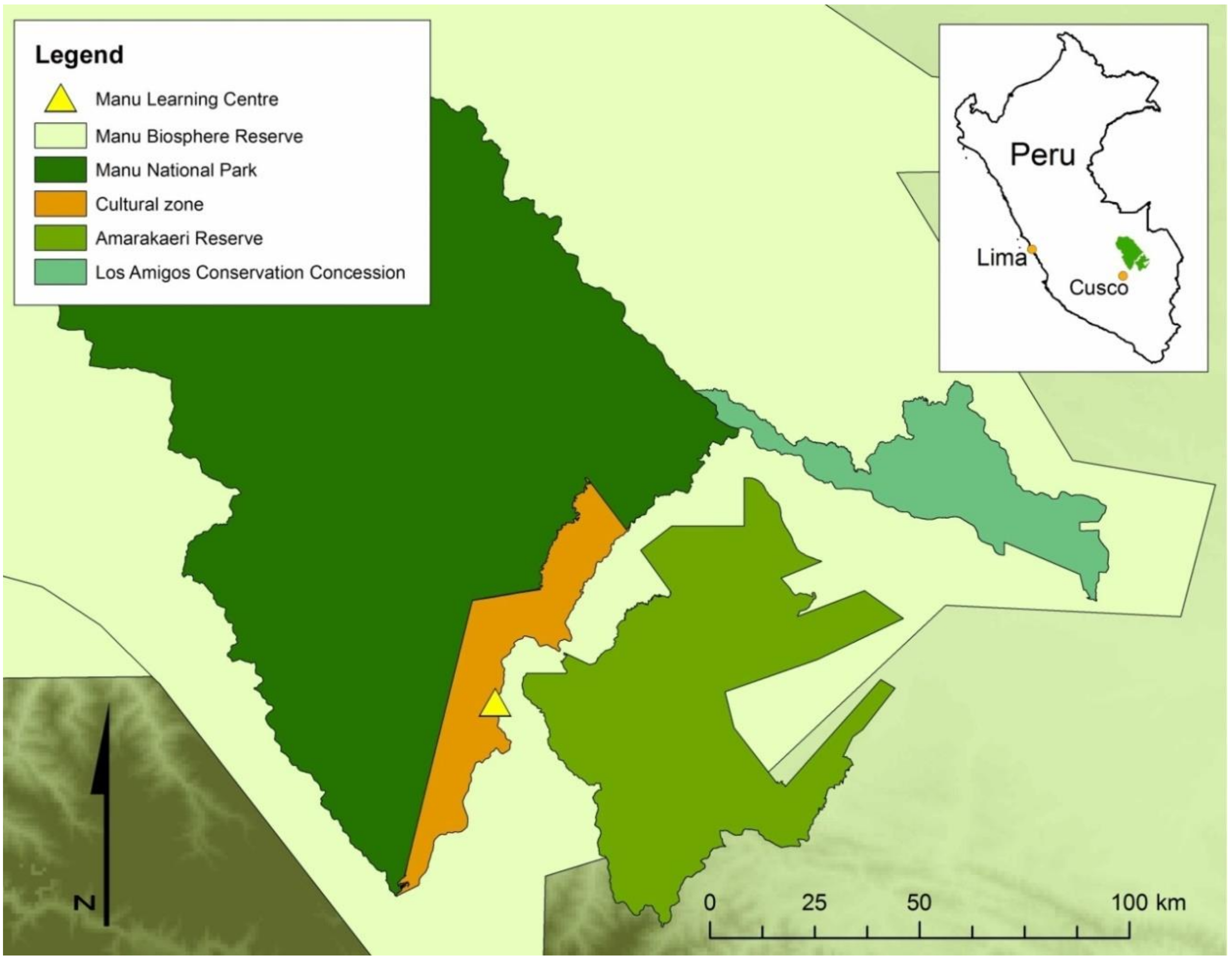


Figure 1. Context of the study site in Manu Biosphere Reserve, southeast Peru.

Key features of the study site for this research were: 1. the known history of different anthropogenic disturbance types (see habitat classification methods for further details); which included selective logging (identified herein with the acronym SLR – selectively logged and now regenerating; ~332ha) and complete clearance due to conversion to intensive agriculture for coffee and cacao (acronym CCR – completely cleared and now regenerating; ~293ha). There was a mixed area between the two disturbance types that once consisted of a mosaic of completely cleared and selectively logged areas through partial clearance for agriculture, which is now regenerating (acronym MXD; 183ha); 2. the absence of current confounding effects of human disturbance. The site is directly bordered by areas of agricultural land and areas of current logging practices but has been strictly protected from hunting and other human impacts since 2002 by the presence of the MLC research station, allowing on-going human disturbance to be excluded as a causal effect; 3. the site has had a long period for regeneration since disturbance events. Disturbances to the site occurred during the 1960s-1980s and the study site has had at least 30 years of regeneration time so at the time of the study the whole area was covered by closed canopy forest.

Habitat classification

Initially the boundaries between habitats with different disturbance histories were identified by two of the authors visiting the site to visually inspect it. This allowed distinct points of transition between the two distinct forest types to be identified based on subjective observation of forest structure, with confirmation by consulting local guides who had expert local knowledge related to the specific historic land-use of the study site. The guides were asked to point out areas of different historic land use and indicate from memory where transition between areas of different disturbance types had been. Both approaches identified consistent points that were marked as the boundaries of the different disturbance histories. To confirm if these identified boundaries related to current differences in forest structure, vegetation data relating to 12 different measures of forest structure were collected across the study site. This included 571 survey points (each 10m in diameter), both along and off trails, evenly distributed over the whole study area. The following variables measured were: upper and mid canopy height, base height of the canopy (m) calculated using clinometers; upper and mid canopy coverage, % estimate conducted by two trained observers (to the nearest 5%); leaf-litter depth (to the nearest 0.5cm); the largest tree diameter at breast height (DBH) within a five metre radius from the survey centre point; the number of trees within a five metre radius from the centre point with a DBH >5cm; shrub layer and herb density through the detection of visible markers from a set distance; and finally, herb coverage, bare-ground cover and coarse woody debris coverage were estimated using a modified Braun-Blanquet scale (see Beirne et al. 2013; Hurst & Allen 2007).

In order to ground truth the initial determination of habitat demarcations set by the authors and guides, a factor analysis (Ansell et al. 2011) was performed using Minitab v.14.12 (Appendix 2B). Factor scores were sorted and rotated with a Quartimax rotation in order to provide the most logical representation of the data visually, and the factor scores for each vegetation site were stored and then mapped using a kriging technique in ArcMap v9.3.1 (Gómez et al. 2012). The shape file boundaries separating habitats of different disturbance histories determined by the authors were overlaid onto the kriging map for visual assessment of the suitability of the border placement (Appendix 2C). In order to verify the statistical difference of factor scores between assigned habitat areas, an analysis of variance analysis (ANOVA), using Minitab v.14.12 was carried out.

To assess the floristic composition of each disturbance are we summarised Gentry data from an unpublished undergraduate project, assessing the feeding habitats of Peruvian woolly monkeys (Pillco Huarcaya 2014). In each disturbance area a 0.1 –ha Gentry plot was carried out, where each 0.1 ha sample represents the sum of ten 2 x 50m subplots. All plants with a steam diameter at breast height (1.37 m) of 10 cm or more were included in the samples (for a detailed methodology see Phillips & Miller 2002).

Choice of faunal study groups

This study measured the biodiversity of four key taxonomic groups (amphibians, birds, butterflies and medium-to-large terrestrial mammals), chosen because they are of well-known conservation importance and provide numerous ecosystem functions. Specifically, more than 70% of the world's amphibian species are thought to be in decline (Hayes et al. 2010) and habitat destruction and fragmentation are among the leading causes of this global decline (Eigenbrod et al. 2008a). Utilising three different survey methods, we chose to look at three potentially distinct bird communities; the overall diurnal community, understorey specialists and nocturnal birds. We use these different methods because bird communities, in particular under-storey guilds, have been found to be sensitive to environmental changes and habitat fragmentation (Banks-Leite et al. 2010) and have been found to respond differently to habitat disturbance. Nocturnal birds have received very little attention within tropical forests due to the challenge in successfully surveying them (Goyette et al. 2011), but many species hold a potentially disproportionate ecological importance at the top of the food chain (Sberze et al. 2010). Butterflies have been shown to be effective in detecting ecological change due to their sensitivity to forest disturbance (Hamer et al. 2003) and their association with specific food plants (Horner-Devine et al. 2003). Medium-to-large mammals were chosen because they remain relatively understudied within tropical forests (Salvador et al. 2011) and play a key role in forest ecosystems, directly through seed dispersal and prey population control or indirectly by helping to maintain assemblages of other faunal groups (Beck et al. 2010; Cassano et al. 2012).

Study approach

The study was planned to assess how biodiversity was distributed across different human-disturbed rainforest areas following a long period of regeneration. Initiation of a human designed experiment waiting at least 30 years before investigating any impacts wasn’t a practical option, so instead a natural experiment was investigated (Hurlbert 1984) at a regenerating rainforest study site where historic human disturbance had varied across a small area (~800 ha). Studying within site differences in biodiversity distribution across this small spatial scale allowed us to avoid confounding effects of large scale drivers of spatial auto-correlation, such as climatic or geographic differences. With an absence of any significant geographic barriers (e.g. large rivers or mountains) within the site and a small spatial scale there were no barriers to hinder species dispersing across the site, so we predicted that in the absence of any effects of differences in historic disturbance, biodiversity would be distributed randomly across the site. If human disturbance history differentially impacted biodiversity, we predicted that we would find differences between areas once subjected to different forms of disturbance.

Survey methodologies summary (see Appendix 2C for a detailed outline of methods and Figure 2 for specific sampling locations.)

Survey sites for all groups had similar although not identical levels of survey effort due to weather and other logistical constraints. These differences resulted because, due to logistic constraints, some survey sites were first installed during the 2012 field season and additional sites were installed in the 2013 field season. This meant that some transects were more intensively surveyed than others but since this was true for all of the disturbance types, would therefore not be expected to influence the patterns identified. We account for this within the analysis by creating extrapolated accumulation curves to represent equal numbers of detections and verify that patterns in the observed data are congruent with these projections (see Biodiversity analysis section).

Amphibians were surveyed nocturnally through visual encounter surveys (Beirne et al. 2013) at 12 locations within the study area (within each location five 100x4m transects were surveyed repeatedly to build up a picture of the community present); four locations were present within each disturbance history type (SLR, CCR and MXD). Each study location was a minimum of 200m apart to ensure spatial independence of sites (see Ribeiro-Junior et al. 2008). Survey effort accumulated to ~454 observer hours and each set of transects was repeated 7-10 times. Morning line transects were conducted to survey the overall diurnal bird community. In total 15 survey locations (each consisting of a 400m long transect) were walked throughout the study area (5 located in CCR, 5 in MXD and 5 in SLR) to monitor all bird species both visually and by call (Bibby et al. 2000). Each transect was walked between nine and 12 times to build up a record of the bird community around each transect location. Overall, 157 transect surveys were carried out over the course of the study; 52 within CCR, 54 within MXD and 51 within SLR. Understorey birds were surveyed across 9 mist net locations (three locations per disturbance history area).

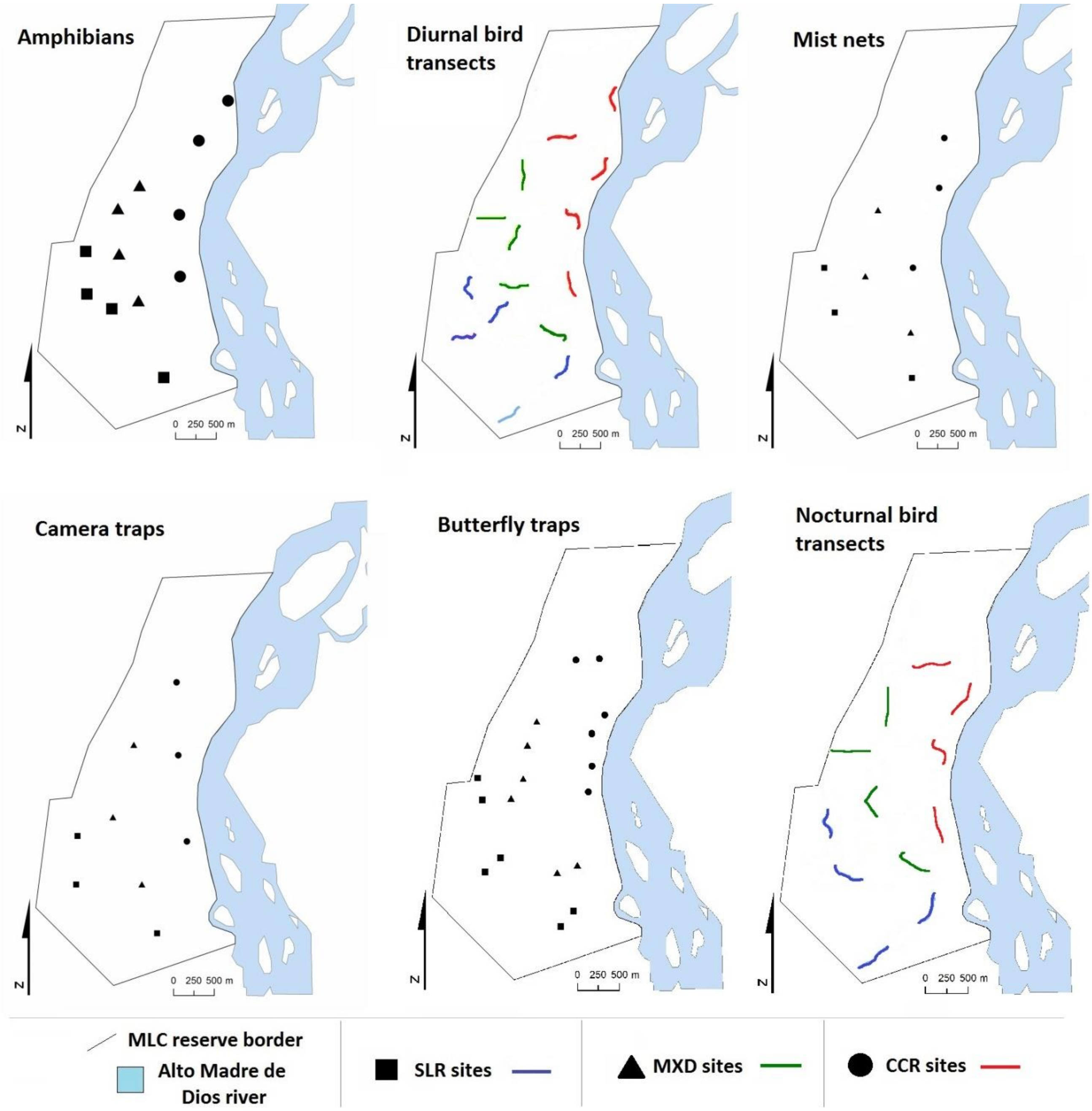


Figure 2. The situation of sampling sites within the study area for each study **group.**

The total net hours of the study accumulated to 3428 net hours; 1055 within CCR (42 mornings), 1264 within MXD (46 mornings) and 1109 within SLR habitat (38 mornings). Nocturnal birds were surveyed along 12, 500m length line transects (4 located in CCR, 4 in MXD and 4 in SLR), with each transect walked six times and detections made both audibly and visually were made ninety degrees to the transect line to avoid overlapping survey areas. All bird sample locations were a minimum of 500m apart to ensure spatial independence of sites (see Hamer et al. 2015).Overall 72 transect surveys were collected over the course of the study, 24 within CCR, 24 within MXD and 24 within SLR. Butterflies were surveyed across 18 locations, six in each of the three disturbance types using Van Someren-Rydon traps (Hughes et al. 1998). Each of the 18 locations was subjected to the use of two bait types (rotten banana and rotten fish), accumulating to 30 days of trapping (6 trapping sessions per site; 450 trap days overall, 150 in CCR, 150 in MXD and 150 in SLR). All survey locations were situated a minimum distance of 200m apart to ensure sampling independence (see Barlow et al. 2007a; Lewsi 2001). Mammals were surveyed across nine camera trap locations (three in each disturbance area) accumulating to 4228 camera trap days (1400 in SLR, 1456 in MXD and 1372 in CCR). Sites were spaced between 0.75-1.5km apart to ensure there were no major gaps in the sampled areas (Sharma et al. 2010). Terrestrial medium-to-large mammals were the target group for the analysis, defined as any mammal large enough to be identified reliably from a camera trap image (e.g. larger than acouchy), therefore excluding arboreal species, small rodents and most aquatic species (see Tobler et al. 2008).

Biodiversity analysis

Overall study groups

In order to investigate differences in biodiversity distributions of faunal groups between disturbance types we assessed a number of frequently used biodiversity metrics; species richness, species diversity, abundance and community evenness, beta similarity and composition (Bruton et al. 2013; Hu et al. 2013). We additionally investigated the biodiversity patterns of species of key conservation concern and indicators of primary forest habitat (as identified within Chapter one of this thesis); and the patterns of specific feeding guilds within four of our study groups.

To assess observed species richness levels and the extent to which our effort had detected as many species as are likely to be found within each disturbance type, we created rarefaction curves for each taxonomic group using the Rich package (Rossi 2011) and plotted using program R (R Core Team 2012). Where the sampling effort detected fewer individuals in one area, we used Estimate S (Colwell 2013) to extrapolate the lower lying curve towards an equal number of individuals for a clearer comparison of richness levels (Colwell et al. 2012). Three estimators of species richness were calculated for all survey groups (Gotelli & Colwell 2011). The three estimators; Jack 1, Chao 2 and Mmmeans, have previously been shown to provide effective estimates for birds, butterflies or mammals (Fermon 2005; Herzog et al. 2002; Hughes et al. 2002; Ramesh et al. 2010; Tobler et al. 2008), while the most effective estimators for amphibians remain unresolved (Veith et al. 2004). To determine a comparable average value of estimated species richness for each survey method across all three habitat types the average of the above three estimators was calculated for each group across each habitat (where an estimator performed eratically, this value was omitted from the calculation of the average value, but still presented in brackets). The 84% confidence intervals for the average estimated species richness were then calculated for each group in CCR and SLR habitats as when comparing two confidence intervals no overlap at this level indicates a statistically significant difference at p=<0.05 (Altman & Bland 2011; Gotelli & Colwell 2011; MacGregor-Fors & Payton 2013).

Species diversity was defined as the Shannon diversity index (Seshadri 2014; Trimble & van Aarde 2014). Repeating the analyses using Fisher's Alpha, Simpson and Shannon Exponential diversity indices did not change the pattern of results significantly and therefore are not presented. All richness and diversity estimators were calculated in Estimate S (Colwell 2013) and plotted using program R (R Core Team 2012), once again with 84% confidence intervals.

As this was a natural experiment and not human designed, it was not possible to intersperse independent sampling locations as a simple way to demonstrate treatment replication (in addition to the sampling replication already described) therefore analytical approaches were used to confirm independence of sampling locations. It has been highlighted that many tropical forest studies investigating effects of human disturbance on biodiversity due to logging have the potential for pseudo-replication due to spatial auto-correlation (Ramage et al. 2013; in agreement with Hurlbert 1984, Heffner et al. 1996). In this context, Ramage et al. (2013) suggest that whilst interspersion is a desired goal where human designed experiments are practical, other approaches such as natural experiments still provide useful scientific evidence if causes of spatial variation, other than the “treatment” effect, are investigated and controlled for as necessary. As such, pseudo-replication only occurs if the results are over generalised (Ramage et al. 2013). Therefore following Ramage’s (2013) recommendations we included additional control variables in our analysis, utilised spatial statistics to confirm the absence of spatial auto-correlation and finally, considered the likelihood of alternative inferences from our results.

In order to investigate if differences in observed sample species richness, sample level abundance and sample level Shannon diversity were significantly different between disturbance areas, a series of Generalized Linear Mixed Models (GLMM’s; with either a poisson distrubition, or where overdispersion was detected as a result of zero-inflation, a negative binomial distribution, both using a log link function) were carried out. To account for repeat measures across sampling locations, sampling location identification was included as a random effect. Mist-netting hours for each survey session were included as an offset for mistnetting data and bait type was included as a fixed factor within butterfly models. Having excluded potential large scale causes of spatial auto-correlation by focussing on a small scale study area over which large scale factors would not vary, we also considered if there were any consistent local scale differences between sampling locations. As a result of a general trend for altitude to gently increase south to north (range between survey sites; 478-711m asl) and distance from the river to increase east to west (range between survey sites; 51-1631m), ‘altitude’, ‘slope extent’ and ‘distance to the main river’ of each sampling location were included as covariates to control for any potentially confounding effects of these variables. Firstly models were carried out with disturbance history type as a categorical explanatory factor, with the observed sample level richness, abundance and Shannon diversity as the respective response variables. A further set of GLMM’s was then carried out, each containing disturbance history along with one of each of the above potentially confounding factors as a continuous variable, all three together and finally compared to the respective null model. AICc values were compared between the models. A delta of two was used to determine the top models and the most parsimonious of these selected as the final preferred model, which would determine a potential effect of disturbance history as a predictor of sample level richness, abundance and Shannon diversity. Finally, to confirm that any potential spatial auto-correlation between survey locations had been controlled for in the analysis, a Moran's I test was carried out in program R (R Core Team 2012) on the residuals of each preferred model (where preferred to the null) to test if there was any effect from spatial auto-correlation that might lead to pseudo-replication (ape package; Paradis et al. 2004).

In order to assess the structure and evenness of a community, dominance-diversity (Whittaker) plots were produced and compared for all study groups, across CCR and SLR disturbance histories, using the vegan package (Oksanen et al. 2011) in program R (R Core Team 2012). Such plots compare the evenness of a community whereby shallow curves represent a community of many species of similar abundance whereas steep curves represent a skewed assemblage with one or more species in substantially higher relative abundance than the others (Beirne et al. 2013). Significant differences in slope, and therefore significant differences in community evenness, were assessed through the use of a linear model with log relative abundance as the response term and an interaction between species rank and habitat type as continuous and categorical fixed effects, respectively. Results are reported as ΔG which corresponds to absolute change in gradient between disturbance areas, where more negative values denote steeper curves and thus less even assemblages.

Community similarity was investigated by calculating the Morisita-Horn and Bray-Curtis abundance based beta similarity measures in software Estimate S (Colwell 2013). Values were calculated for each study group, to compare communities from CCR, MXD and SLR disturbance areas. Non-metric multidimensional scaling (NMDS; using the Bray-Curtis similarity measure) was conducted to determine the overall differences in assemblage structure and composition for each study group, among survey locations of the three different disturbance areas. All stress values were relatively low (ranging between 0.016 for amphibians to 0.23 for butterflies) and so were displayed within just two dimensions. To assess the statistical significance of observed differences in assemblage composition between different disturbance areas we conducted analysis of similarities tests (ANOSIM; using 999 permutations), which is analogous to an analysis of variance and tests for differences between *a priori*-defined groups of community samples (here, disturbance area) based on a (dis)similarity matrix (see Helbig-Bonitz et al. 2015). NMDS ordinations and ANOSIM tests were carried out in the vegan package (Oksanen et al. 2011), in program R (R Core Team 2012).

Indicator Species, Species of Conservation Concern and Feeding Guilds

We additionally investigated the biodiversity patterns of species of key bird and mammal species of conservation concern and indicator amphibians and birds of primary forest habitat (as identified within chapter one of this thesis); and the patterns of specific feeding guilds within four of our study groups (birds from transects and mist-nets, butterflies and mammals). Bird feeding guilds followed Feeley et al. (2007), with the addition of carnivorous species, and species were assigned using Stotz et al. (1996). Mammal species were assigned to guilds following information gathered from Myers et al. (2016). Butterflies were separated based upon the communities sampled in either fruit or carrion-baited traps.

We created rarefaction curves for each of the groups using the Rich package (Rossi 2011) and plotted using program R (R Core Team 2012). Where the sampling effort detected fewer individuals in one area, we used Estimate S (Colwell 2013) to extrapolate the lower lying curve towards an equal number of individuals for a clearer comparison of richness levels (Colwell et al. 2012).

In order to investigate if differences in observed sample species richness and sample level abundance for each of these groups was significantly different between disturbance areas, a series of Generalized Linear Mixed Models (GLMM's; with either a poisson distrubition, or where overdispersion was detected as a result of zero-inflation, a negative binomial distribution, both using a log link function) were carried out. To account for the repeat measures across sampling locations, sampling location identification was included as a random effect. Mist-netting hours for each survey session were included as an offset for mistnetting data. As in the previous models, 'altitude', 'slope extent' and 'distance to the main river' of each sampling location were included as covariates to control for any potentially confounding effects of these variables and models were compared to the respective null model. AICc values were compared between the models and delta in AICc of two was used to determine the top models and the most parsimonious of these selected as the final preferred model; which would determine a potential effect of disturbance history as a predictor of sample level richness and abundance for each group.

Results

Habitat classification

The habitat classification provided a clear separation between areas of different disturbance type in terms of identifiable features related to overall forest structure. The factor analysis resulted in the original 12 habitat structure variables producing four factors with an eigenvalue >1. These four factors represent 55.7% of variation in the original dataset (factors 1, 2,3 and 4 contained 18.1%, 14.6%, 11.8% and 11.2% of the variation respectively). Factor 1 loaded negatively with increasing upper and mid canopy height, upper canopy coverage, the largest DBH and the number of trees >5cm DBH. Factor 2 loaded positively with leaf litter depth, herb coverage and coarse woody debris coverage and negatively with bare ground coverage. Factor 3 loaded positively with shrub and herb density, whilst factor 4 loaded positively with mid canopy cover and the number of trees >5cm DBH (see Appendix 2A). The first factor separates by the structure of the higher canopy and presence of primary forest features. Factors 3 and 4 relate most to the low-mid levels of the forest structure (understorey). Specifically, SLR habitat has a distinctly higher upper canopy height and greater mid and upper canopy coverage, has trees with larger DBHs and a high number of trees >5cm DBH, clearly separating from both MXD and CCR across factor 1 (Appendix 2C$^{(a)}$). Factor 3 shows a distinct separation between CCR and MXD disturbance history type, with the MXD displaying less dense herb and shrub layers compared to the CCR area (Appendix 2C$^{(c)}$). Factors 2 and 4 show more heterogeneity across the survey region and less distinction between disturbance history types, but SLR again separates from CCR and MXD across factor 2 (see Appendix 2C for a visual representation of all four factors).

The ANOVA analysis between factor scores was statistically different between disturbance areas for the first three factors but not for the fourth (see Appendix 2D for outputs). The first factor showed no overlap in confidence intervals between any of the disturbance areas, the second factor showed no overlap between SLR and the other two areas and the third factor showed no overlap between CCR and the other two areas. This agreed with patterns observed from the kriging maps.

Floristically, SLR, MXD and CCR habitats were shown to be distinct (Pillco-Huarcaya et al. 2014). The tree species richness in SLR and MXD was over double (60 and 65 species respectively), and the number of tree families almost three times greater (31 and 31 families respectively), than that of the CCR habitat (29 species from 11 families; see Table 1). A greater number of individual trees of over 10cm diameter-at-breast-height were encountered in SLR and MXD habitat (80 and 88 respectively), compared to CCR (59).

Table 1. Overall summary information of Gentry plots carried out by Pillco-Hurcaya (2014).

	SLR	MXD	CCR
Number of species	60	65	29
Number of families	31	31	11
Number of individuals	80	88	59
Area sampled	0.1 ha	0.1 ha	0.1 ha

The composition between CCR and SLR was distinct when observing both families and species (see Appendices E and F for a summary of the dominant families and species across disturbance areas). In general the SLR habitat contains many large hardwood species in the families Meliaceae, Moraceae, Sapotaeceae, while the CCR contains many smaller, softwood species and palms in the families Melastomataceae, Rudiaceae and Arecaceae.

Species richness, Shannon diversity and Abundance

The overall observed values of species richness displayed higher observed community level species richness in SLR than CCR disturbance ares for amphibians (23 vs.19), mist-netted birds (88 vs. 70), nocturnal birds (7 vs. 3) and butterflies (136 vs. 109). Mammals showed no difference (21 vs. 21) and diurnal bird transects detected more species in CCR than SLR (108 vs. 95). The observation of the rarefaction curves of these observed values showed that many of the curves failed to reach an asymptote (Figure 3) and that all 84% confidence intervals showed some degree of overlap between CCR and SLR disturbance areas.

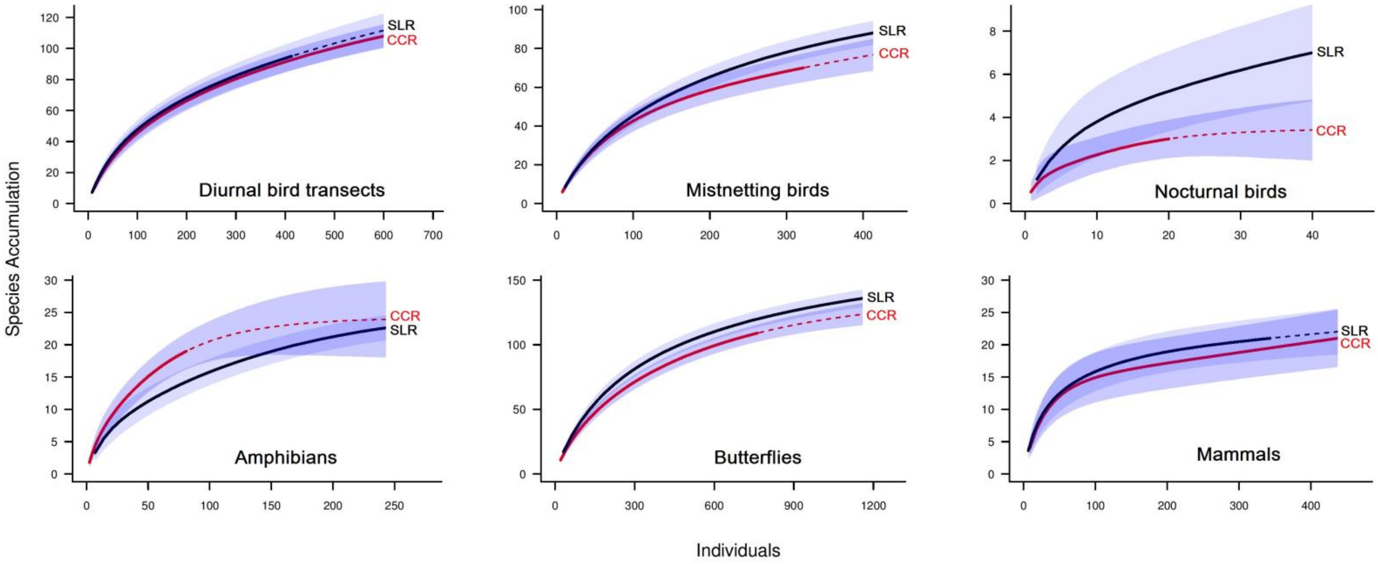


Figure 3. Species rarefaction curves for study groups across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

In total, 469 individuals of 33 different amphibian species were recorded and survey completeness across disturbance history types was relatively high, ranging between 67-85% (Table 2). The average total species richness estimate for amphibians was the same for each disturbance area, with all habitats estimated to contain 27 species. Overall we recorded 1132 mist-net captures representing 122 bird species, 1485 bird records from diurnal transects representing 165 species and 83 bird records from nocturnal transects representing 9 species. Survey coverage across habitats and methodologies ranged between 63-76%. The average richness estimators from mist-net data predicted SLR to contain 116 species whilst CCR was estimated to hold just 102 species (12% fewer than SLR). The average richness estimates for diurnal bird transects predicted SLR to hold 143 species, whilst CCR is estimated to hold 145 species (1% more than SLR). The average estimated species richness for nocturnal birds predicted SLR to contain 10 species, whilst CCR is estimated to hold 4 species (CCR with 60% fewer species than SLR). In total, 2729 individuals of 173 different species of butterfly were recorded and survey coverage across habitats ranged between 74-80%. The average estimated species richness was highest in SLR, containing 170 species and CCR 148 species (13% ±9.8 fewer than SLR across groups). Overall, 1280 medium-to-large terrestrial mammal separate photo records representing 23 species were recorded and survey coverage across habitats ranged between 84-91%. The average species richness estimates were similar with 24 species being estimated in SLR and 25 in CCR. At the overall community level, a greater number of individuals (Table 2) were encountered in SLR than CCR habitat for amphibians (259 vs. 81), mist-netted birds (413 vs. 322), nocturnal birds (40 vs. 20) and butterflies (1162 vs. 763); but a greater number of individulas were recorded in CCR for diurnal bird transects (601 vs. 413) and medium-to-large mammals (437 vs. 343).

Overall community Shannon diversity was higher in SLR habitat for all groups except for mammals, which showed a higher diversity in CCR (Figure 4). However, all groups except for butterflies and mammals displayed a significant degree of overlap between 84% confidence intervals.

General linear mixed models at the sample level showed that the amphibian species richness and abundance (see Appendix 2I for model summaries) were significantly influenced by disturbance area. Assessment of the boxplots from the models shows that SLR had the highest sample species richness and abundance, CCR the lowest values and MXD an intermediate level between these. Sample level species richness, abundance and Shannon diversity for butterflies all showed to be significantly affected by disturbance history. The boxplots showed that SLR displayed the highest levels, CCR the lowest and MXD an intermediate level. Sample level species richness and abundance of nocturnal birds was also influenced by disturbance history. Observation of the boxplots showed that SLR held the highest levels, while CCR and MXD areas displayed similar levels. Birds (mist-nest and diurnal transects) and medium-to-large mammals, showed no significant affect of disturbance history for any of the response variables. Testing of model residuals showed no evidence of spatial auto-correlation between samples with very low correlations (range from -0.001to 0.012) and non-significant (range from p=0.27 to 0.99) observed Moran’s I values for all groups and all response variables (see Appendix 2G).

Table 2. Observed species richness and species richness estimates for rainforest with different historic disturbance histories. Based on six different survey methods targeting four taxonomic groups.

		Disturbance Type	n[a]	Survey effort Samples	Observed Species Richness[b]	Extrapolated Species Richness[c]	Estimated Richness				Coverage (%)[e]	Completeness (%)[f]
							MMMean	*Jacknife 1*	*Chao 2*	**Average**[d]		
Amphibians	**Transects (x5 100m transects/night)**	SLR	259	37 nights	23	23	28	29	25	27	85	70
		CCR	81	34 nights	19	24	29	27	24	27	70	58
		MXD	129	38 nights	18	24	21	27	32	27	67	55
		Total	469	109 nights	33							
Birds	**Diurnal line transects (400m lengths)**	SLR	413	51 samples	95	112	123	136	169	143	66	58
		CCR	601	52 samples	108	108	135	149	150	145	74	65
		MXD	471	54 samples	116	128	157	164	159	160	73	70
		Total	1485	157 samples	165							
	Mist-netting	SLR	413	1109 net hrs	88	88	116	118	112	116	76	72
		CCR	322	1055 net hrs	70	77	93	95	118	102	69	57
		MXD	397	1264 net hrs	75	76	92	100	106	99	76	61
		Total	1132	3428 net hrs	122							
	Nocturnal line transects (500m lengths)	SLR	40	24 samples	7	7	10	10	12	10	70	78
		CCR	20	24 samples	3	3	3	4	4	4	75	33
		MXD	23	24 samples	5	7	7	8	8	8	63	56
		Total	83	72 samples	9							
Butterflies	**Baited traps**	SLR	1162	36 trap weeks	136	136	176	172	162	170	80	79
		CCR	763	36 trap weeks	109	124	161	148	136	148	74	63
		MXD	804	36 trap weeks	114	129	153	154	158	155	74	66
		Total	2729	108 trap weeks	173							
Mammals	**Camera traps**	SLR	343	1400 trap days	21	23	23	25	(24)	24	88	91
		CCR	437	1372 trap days	21	22	21	28	(42)	25	84	91
		MXD	500	1456 trap days	20	20	21	22	(20)	22	91	87
		Total	1280	4228 trap days	23							

[a] Number of individuals encountered or recorded

[b] Number of species observed

[c] Number of species estimated when curves extrapolated to the same number of individuals (extrapolations made only equal to the disturbance history with the highest number of records or to a maximum of three times the number of observed individuals)

[d] Mean estimated species richness - 'classic Chao 2 was used in cases where CV>0.5

[e] Sampling coverage defined as: $^{b}/^{e}*100$

[f] Number of species observed as a percentage of combined species across all habitats

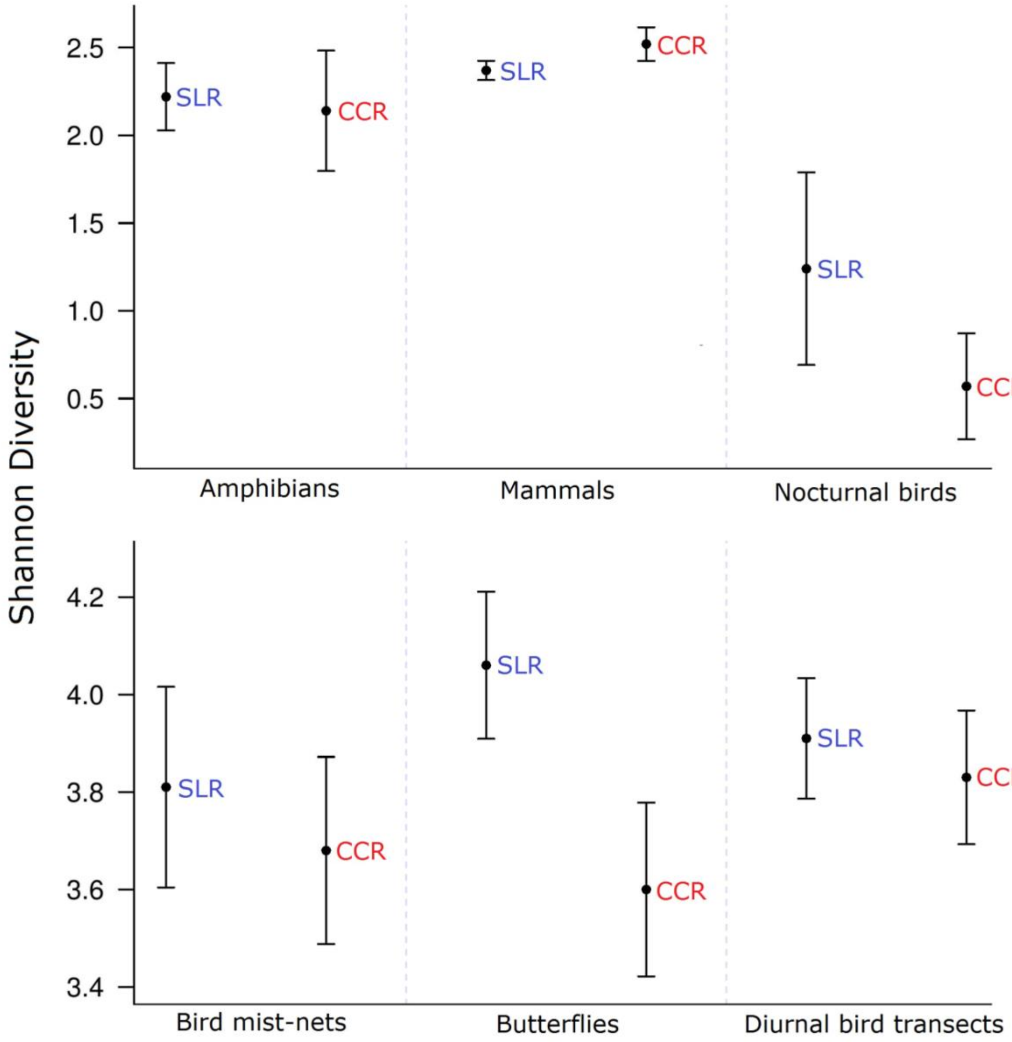


Figure 4. Shannon species diversity estimates across survey locations, with 84% confidence intervals for study groups across the CCR and SLR disturbance types.

Community Evenness, Beta Similarity and Community Composition

Dominance-diversity plots did not display a standard pattern across all groups (Figure 5). Butterflies (ΔG=<-0.004, p=0.002), birds from mist-nets (ΔG=<-0.009, p=<0.0001) and nocturnal birds (ΔG=<-0.878, p=0.01) all showed a significant difference in community evenness between SLR and CCR habitats. The SLR habitat for each of these three groups displayed a more even assemblage (regular intervals between species) with more rare species (increased tail length in community structure plots) than in CCR. However, amphibians (ΔG=0.03, p=0.31), terrestrial mammals (ΔG=-0.02, p=0.30) and overall diurnal birds (ΔG=0.002, p=0.21) showed no statistically significant difference in community structure between SLR and CCR habitats.

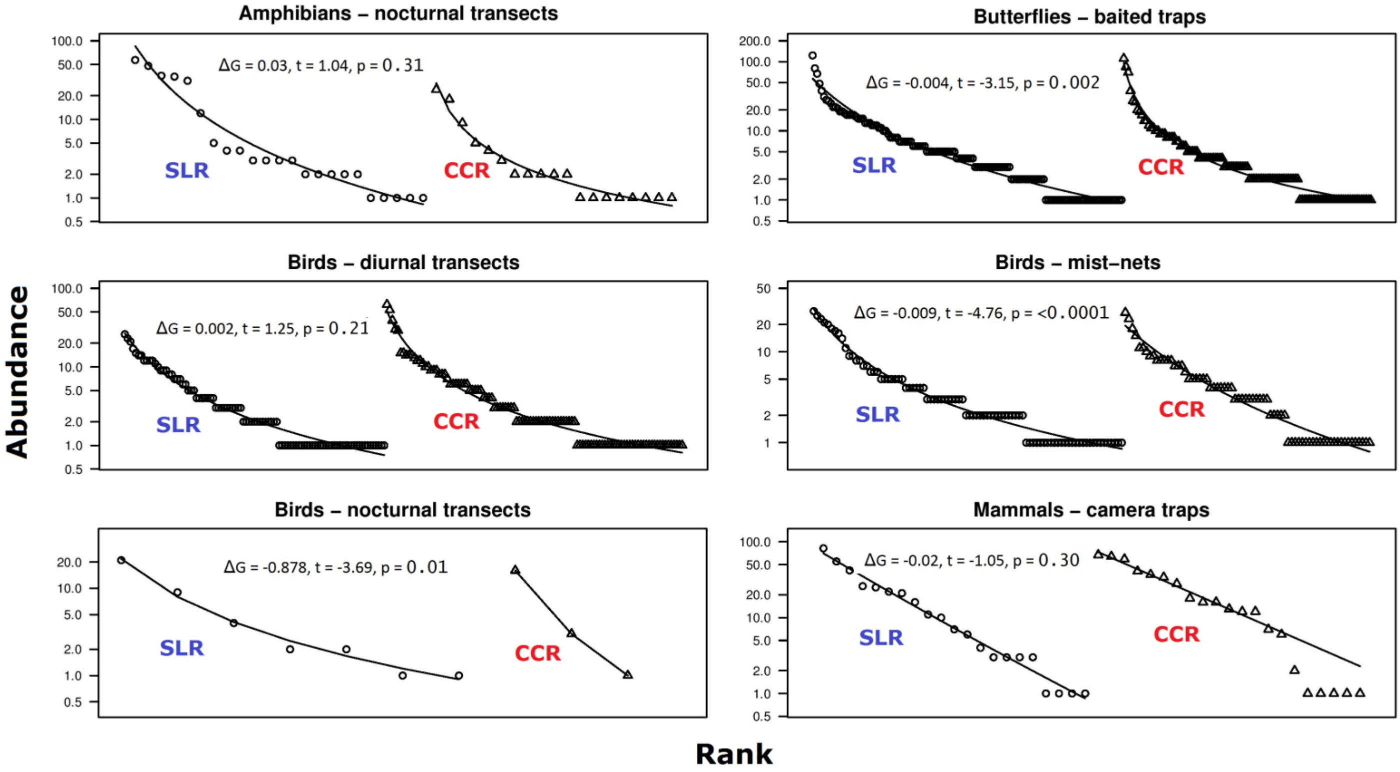


Figure 5. Dominance diversity (Whittaker) plots for faunal study groups comparing curves for SLR (o - left) and CCR (Δ - right) habitat. For each disturbance history the relative abundance of each species (ni/N) was plotted on a logarithmic scale against the species rank ordered from most to least abundant. O = SLR and Δ = CCR. Linear models were used to determine if the slopes were significantly different to one another where ΔG denotes to absolute change in gradient and the symbol denote the level of significance of the deviation where *** = ≥0.001, ** = ≥0.01,* = ≥0.05. Points labelled with letters A-E represent the five most abundant species in SLR habitat and letters following E represent species from the top five in CCR (where different from SLR).

Abundance based beta similarity measures, the Morisita-Horn and Bray-Curtis indices both show that the lowest level of similarity at the overall community level is between SLR and CCR disturbance areas, for all study groups (Table 3). The community composition analysis from NMDS plots and the associated ANOSIM analysis (Figure 6) showed that community composition was significantly different between amphibians (R=0.54, p=0.002) and butterflies (R=0.31, p=0.001) between disturbance areas. Although not significant where p=0.05, all bird groups showed p values <0.1 and no overlap between CCR and SLR ordiellipses (where p=0.05; R=0.30, p=0.07; R=0.33, p=0.056 and R=0.19, p=0.068, for diurnal bird transects, mist-nets and nocturnal bird transects respectively). The anosim for mammals displayed a higher p value than all other groups (0.149) and the lowest R value (0.21), with the exception of nocturnal birds. For individual NMDS plots with the most abundant species labelled for visualisation of which species associate with which disturbance areas, see Appendix 2T, and a list of species corresponding to these codes, see Appendix 2S.

Table 3. Abundance based Beta similarity values for each study group, between disturbance areas. An * denotes the lowest degree of similarity (greatest dissimilarity) between two areas.

		Morisita-Horn similarity		Bray-Curtis similarity	
		MXD	CCR	MXD	CCR
Amphibians	MXD		0.774		0.536
	SLR	0.847	0.670*	0.610	0.329*
Butterflies	MXD		0.804		0.668
	SLR	0.849	0.790*	0.661	0.614*
Mammals	MXD		0.872		0.762
	SLR	0.928	0.744*	0.800	0.664*
Birds transects	MXD		0.722		0.591
	SLR	0.816	0.695*	0.622	0.517*
Birds Mistnetting	MXD		0.78		0.598
	SLR	0.768	0.571*	0.627	0.484*
Nocturnal birds	MXD		0.954		0.837
	SLR	0.890	0.834*	0.603	0.533*

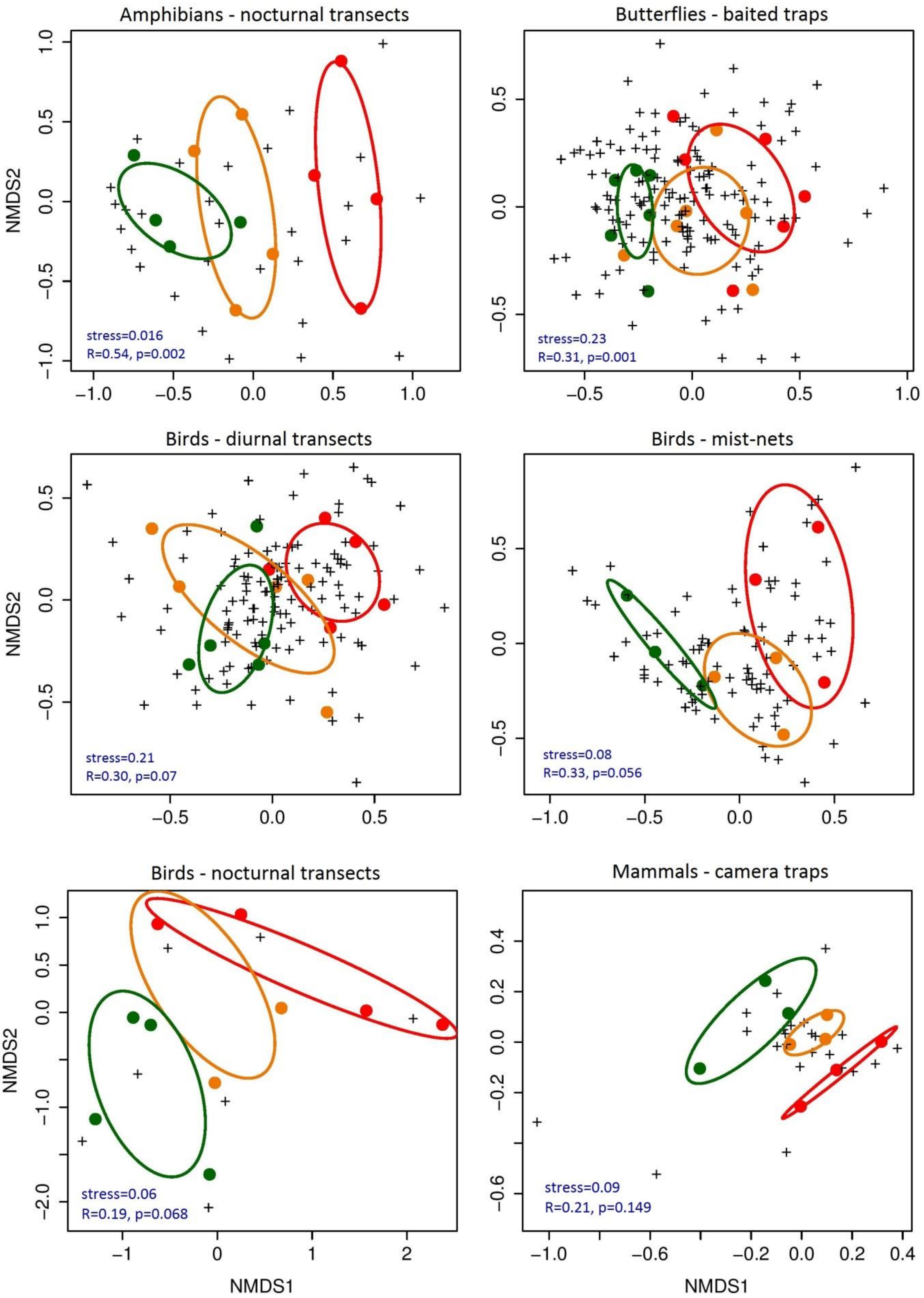


Figure 6. NMDS plot of species composition (species displayed by +) according to disturbance areas (point colours of sampling locations and ellipses; green=SLR, orange=MXD and red=CCR) for each of the six study groups; containing stress values (stress plots provided in Appendix 2U) and the R statistic and p-value from an ANOSIM test of community similarity.

Indicator Species, Species of Conservation Concern and Feeding Guilds

We assessed the indicators of good quality habitat for Southern Amazonian lowland tropical forest birds as described by Stotz et al. (1996), amphibian indicators of good quality habitat as identified by Pearman (1997) and bird and mammal species of special conservation concern (defined in chapter one of this thesis as globally threatened or near-threatened species, according to the IUCN Red List (2013) of threatened species). Only indicator amphibians and indicator birds from diurnal transect surveys displayed higher observed and estimated values of species richness in SLR than CCR habitat (Table 4), and none-overlapping 84% confidence intervls in the species rarefaction curves (Table 7). Indicator amphibian sample species abundance was the only responses supported by the GLMM's, with more individuals encountered per sample in SLR than CCR, with MXD at an intermediate level (see model summaries and box plots in Appendices 2J-M).

Table 4. Observed species richness and species richness estimates for rainforest with different historic disturbance histories. Indicator groups and species of conservation concern.

		Disturbance Type	n[a]	Observed Species Richness[b]	Extrapolated Species Richness[c]	Estimated Species Richness				Coverage (%)[e]	Completeness (%)[f]
						MMMean	*Jacknife 1*	*Chao 2*	**Average**[d]		
Indicator amphibians	Nocturnal transects	SLR	153	10	10	12	12	10	11	91	100
		CCR	42	2	2	2	2	2	2	100	20
		MXD	77	6	7	6	8	6	7	86	60
		Total	272	10							
Indicator birds	Diurnal line transects	SLR	80	15	15	18	21	(33)	20	77	52
		CCR	45	7	7	8	7	7	7	100	24
		MXD	74	14	14	17	20	20	19	74	48
		Total	199	29							
	Mist-netting	SLR	48	14	14	22	16	14	17	82	74
		CCR	32	10	12	19	14	14	15	67	53
		MXD	44	13	13	18	18	18	18	72	68
		Total	124	19							
Birds of Conservation concern	Diurnal line transects	SLR	16	5	5	7	6	5	6	83	31
		CCR	37	7	7	8	8	7	8	88	44
		MXD	32	8	8	10	10	8	10	80	50
		Total	85	16							
Mammals of conservation concern	Camera traps	SLR	111	8	9	8	10	9	9	89	100
		CCR	81	6	6	6	8	7	7	86	75
		MXD	163	7	7	8	7	7	7	100	88
		Total	355	8							

[a] Number of individuals encountered or recorded

[b] Number of species observed

[c] Number of species estimated when curves extrapolated to the same number of individuals (extrapolations made only equal to the disturbance history with the highest number of records or to a maximum of three times the number of observed individuals)

[d] Mean estimated species richness - 'classic Chao 2 was used in cases where CV>0.5

[e] Sampling coverage defined as: $^b/d*100$

[f] Number of species observed as a percentage of combined species across all habitats

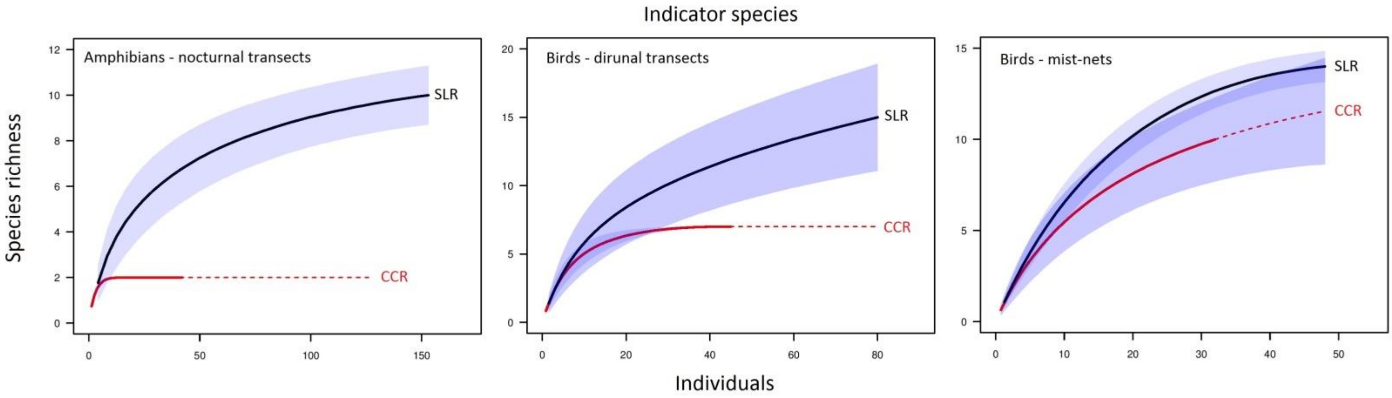


Figure 7. Species rarefaction curves for indicator groups across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

Indicator birds from mist nets, birds of conservation concern (there were too few records and species in mis-nets to perform an analysis) and mammals of conservation concern, each showed no significant difference in richness or abundance (at either community or sample level) between disturbance areas (Table 4; Figure 8 and Appendices 2J-M).

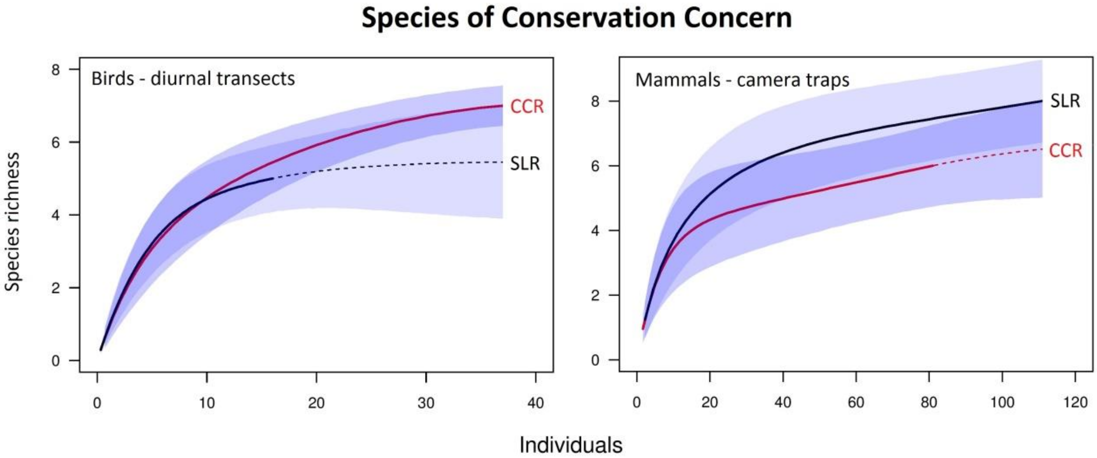


Figure 8. Species rarefaction curves for birds and mammals of conservation concern across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

Table 5. Observed species richness and species richness estimates for rainforest with different historic disturbance histories. Based on feeding guilds of different survey groups.

Survey Group	Feeding Guild	Disturbance Type	n[a]	Observed Species Richness[b]	Extrapolated Species Richness[c]	Estimated Richness				Coverage (%)[e]	Completeness (%)[f]
						MMMean	*Jacknife 1*	*Chao 2*	**Average[d]**		
Birds - diurnal line transects	Carnivorous	SLR	8	5	8	(26)	9	11	10	51	50
		CCR	16	5	5	8	7	7	7	69	50
		MXD	6	5	9	9	9	8	9	58	50
		Total	30	10							
	Frugivorous	SLR	149	28	32	34	34	32	33	84	64
		CCR	297	37	37	41	47	44	44	84	84
		MXD	161	37	42	49	48	42	46	80	84
		Total	607	44							
	Insectivorous	SLR	212	47	53	60	71	95	75	62	53
		CCR	226	52	56	72	75	76	74	70	59
		MXD	266	60	60	81	87	86	84	71	68
		Total	704	88							
	Nectivorous	SLR	4	3	4	5	5	3	5	65	75
		CCR	4	2	2	2	3	2	2	82	50
		MXD	6	3	3	6	4	3	4	69	75
		Total	14	4							
	Omnivorous	SLR	40	12	14	22	18	17	19	63	63
		CCR	58	12	12	15	18	(30)	17	72	63
		MXD	32	11	15	21	17	16	18	61	58
		Total	130	19							
Birds - mist-nets	Carnivorous	SLR	9	2	2	2	2	2	2	99	50
		CCR	5	1	1	1	1	1	1	106	25
		MXD	13	3	3	4	5	4	4	70	75
		Total	27	4							
	Frugivorous	SLR	64	7	7	8	8	7	8	92	70
		CCR	62	8	8	9	10	9	9	86	80
		MXD	73	6	6	7	6	6	6	96	60
		Total	199	10							
	Insectivorous	SLR	228	52	52	71	69	61	67	78	72
		CCR	162	40	47	55	58	(94)	56	71	56
		MXD	220	47	48	61	62	58	60	78	65
		Total	610	72							
	Nectivorous	SLR	82	16	16	21	24	30	25	65	94
		CCR	41	9	9	12	9	9	10	89	53
		MXD	63	11	12	13	15	19	15	71	65
		Total	186	17							
	Omnivorous	SLR	30	11	14	23	16	17	19	59	58
		CCR	28	8	11	17	18	27	20	39	42
		MXD	52	12	12	17	13	11	14	88	63
		Total	110	19							
Butterflies	Fruit feeding	SLR	446	92	92	215	132	129	158	58	77
		CCR	311	58	68	104	84	84	91	64	48
		MXD	308	64	76	110	94	115	106	60	53
		Total	1065	120							
	Carrion feeding	SLR	716	114	114	164	149	137	150	76	79
		CCR	452	90	109	168	131	148	149	60	63
		MXD	496	93	106	158	129	128	138	67	65
		Total	1664	144							
Mammals	Carnivorous	SLR	55	5	6	5	7	6	6	84	100
		CCR	140	4	4	4	5	4	4	94	80
		MXD	110	3	3	3	3	3	3	99	60
		Total	305	5							
	Herbivorous	SLR	161	6	6	6	7	6	6	94	100
		CCR	158	6	6	6	7	6	6	95	100
		MXD	202	5	5	5	5	5	5	98	83
		Total	521	6							
	Insectivorous	SLR	11	2	2	2	3	2	2	81	67
		CCR	17	2	2	2	3	2	2	88	67
		MXD	40	3	3	3	4	3	3	90	100
		Total	68	3							
	Omnivorous	SLR	116	8	8	9	8	8	8	96	89
		CCR	122	9	10	10	13	(15)	11	80	100
		MXD	148	9	9	10	10	9	10	94	100
		Total	386	9							

[a] Number of individuals encountered or recorded

[b] Number of species observed

[c] Number of species estimated when curves extrapolated to the same number of individuals (extrapolations made only equal to the disturbance history with the highest number of records or to a maximum of three times the number of observed individuals)

[d] Mean estimated species richness - 'classic Chao 2 was used in cases where CV>0.5

[e] Sampling coverage defined as: $^{b}/d*100$

[f] Number of species observed as a percentage of combined species across all habitats

The only bird feeding guild that showed a significant difference in species richness at the community level (in terms of non-overlapping 84% confidence intervals) was the nectiverous feeding birds caught in mist-nets, with a greater species richness estimated in SLR habitat (see Table 5 and Figure 10). This was not supported by the GLMM's for sample level abundance or species richness (see Appendices 2P and 2R). However, the sample level abundance of fruit feeding birds from diurnal transects was shown to be higher in CCR disturbance area, lowest in SLR and at an intermediate level in MXD (see Appendices 2O and 2R). Omniverous species caught in mist-nets displayed a significantly higher sample level species richness and abundance wihin CCR disturbance area and lower levels in both SLR and MXD areas (see Appendices 2P and 2R).

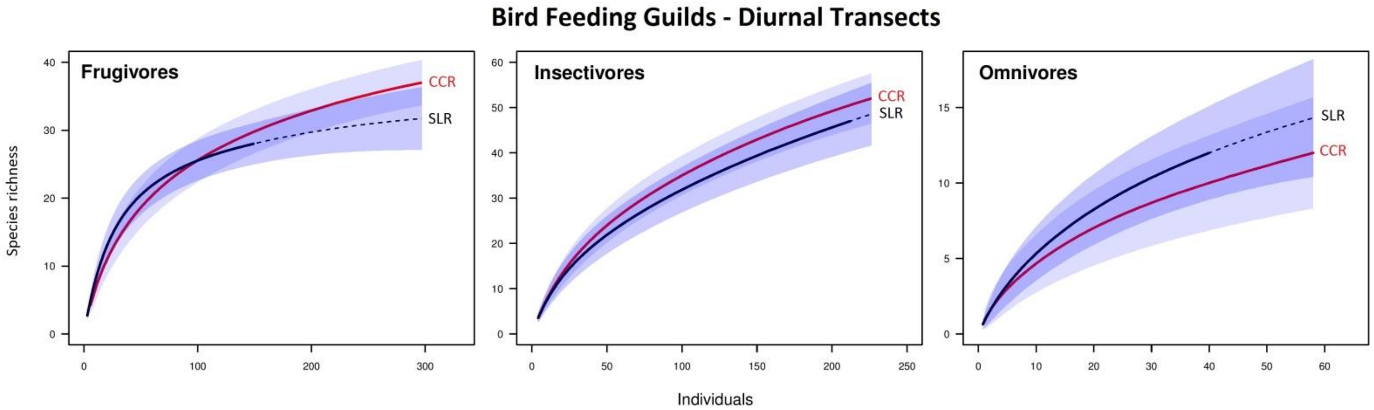


Figure 9. Species rarefaction curves for diurnal bird feeding guilds across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

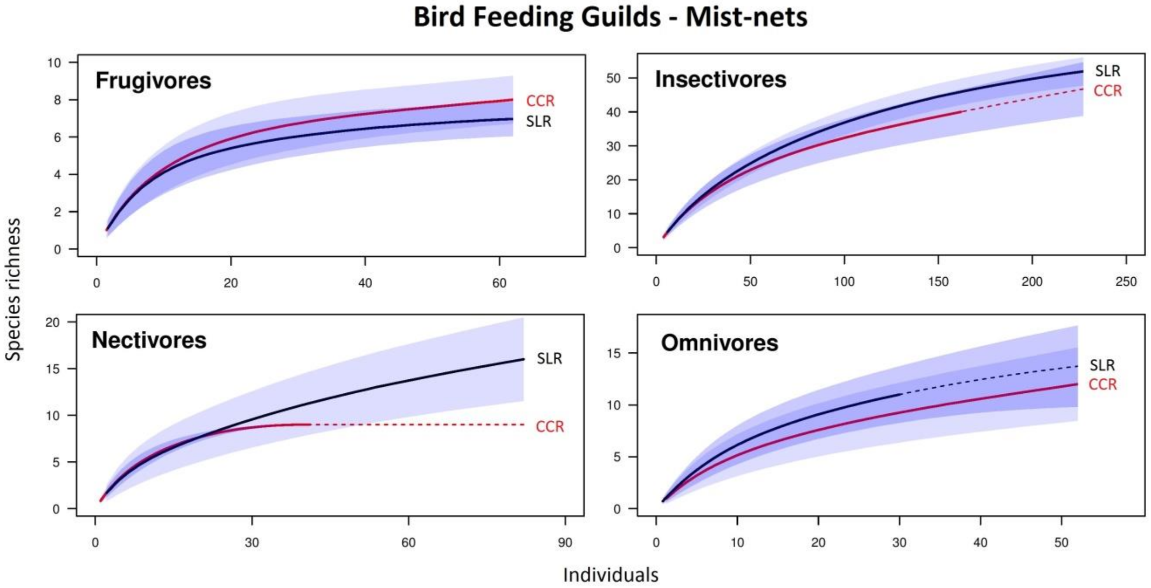


Figure 10. Species rarefaction curves for mist-net feeding guilds across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

In respect to butterfly feeding guilds, fruit-feeding butterflies showed a significant difference in both observed and estimated species richness at the community level between SLR and CCR habitats (Table 5), which was supported by none-overlapping 84% confidence intervals in the rarefaction curves (Figure 11). Fish-feeding butterflies showd no difference, for any response variable between disturbance areas. Model results from GLMM’s showed that only sample level species richness for fruit-feeding butterflies was influenced by disturbance area. Inspection of the box plots showed that sample species richness was highest in SLR, lowest in CCR, and at an intermediate level in MXD habitat (see Appendices 2N and 2R).

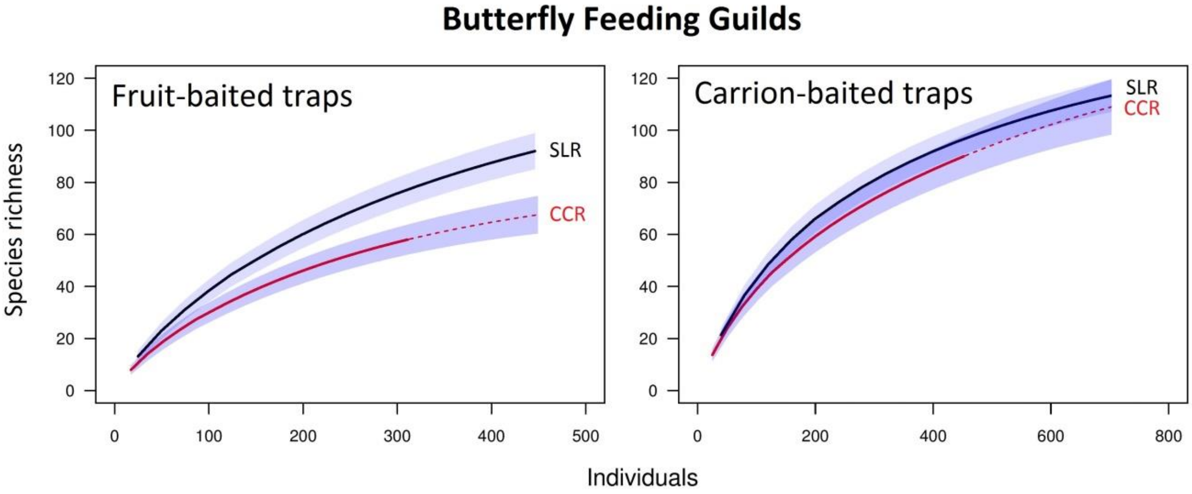


Figure 11. Species rarefaction curves for butterfly feeding guilds across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

Mammal feeding guilds showed no significant difference in species richness between disturbance areas at the community level, with overlapping 84% confidence intervals for all guilds (Figure 12). However, GLMM’s at the sample level show a difference in both sample abundance and species richness for carnivores and insectivores. Both guilds display a higher sample abundance and richness in CCR and MXD disturbance areas than in SLR (see Appendices 2Q and 2R).

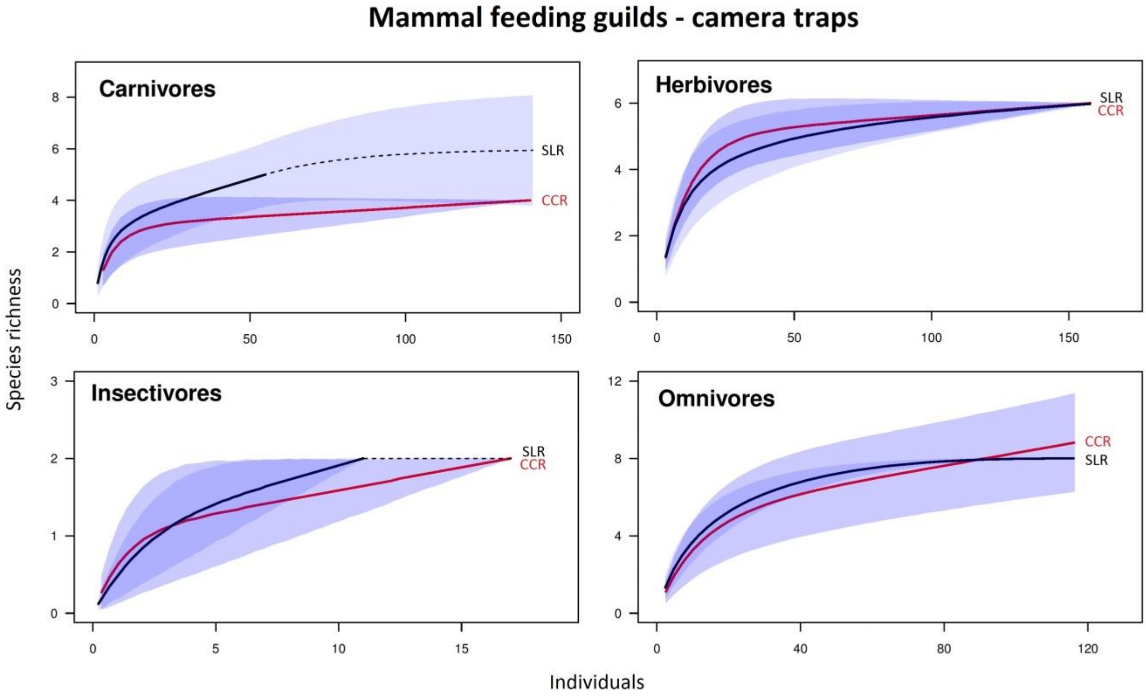


Figure 12. Species rarefaction curves for mammal feeding guilds across SLR and CCR disturbance types. Solid lines represent the observed number of individuals recorded and dashed lines represent projections for habitats with lower numbers of individuals sampled towards the same number of encountered individuals in other habitats. The shaded areas represent 84% confidence intervals.

Discussion

Based on investigation of a within-site scale natural experiment that enabled a first multi-taxa direct comparison of the persistence of human disturbance history effects in an older regenerating forest; the results of this study show that the type of anthropogenic disturbance history still affects current biodiversity levels of multiple taxa even after decades of regeneration. Locations with a history of complete clearance showed lower sample levels of species richness for amphibians, butterflies and nocturnal birds than historically selectively logged locations, while species encounter rates and community composition also continued to show detectable differences in selected taxa. Overall bird communities and medium-to-large terrestrial mammals showed no differences in species richness or encounter rate.

Although previous research has indicated disturbance history to be the most important factor driving species richness levels (Ross et al. 2002), we believe that apart from review papers (Bowen et al. 2007; Dunn 2004a) no previous studies have directly compared (across multiple taxa) the persistence of biodiversity differences in older regenerating forest (>30 years) in relation to differences in historic human disturbance. Valuable although review papers are, when the original data lacks direct (within site) comparisons of historic disturbance types, they can only provide indirect comparisons of the biodiversity value of forest with different historic disturbance types because the data comes from across a variety of landscapes, regions and sites. For example, the two (out of 34) studies assessed by Dunn (2004a) that directly compared more than a single type of forest disturbance, were both carried out on a single taxon, birds (Estrada et al. 1997; Johns 1991). The same was true for four (out of 68) studies that included alternative land uses, assessed by Bowen et al. (2007); ants in the central Amazon (Vasconcelos 1999), saproxylic beetles in Australia (Grove 2002), primates in Costa Rica (Sorensen and Fedigan 2000) and lizards in the Caribbean (Glor et al. 2001). In addition, these studies mostly assessed relatively young regenerating areas (<21 years) and focussed upon comparing the different disturbance types with primary forest, as opposed to directly against one another.

Based largely on less direct comparisons between biodiversity at different sites Berry et al. (2010) estimate faunal biodiversity loss in regenerating selectively logged forest to be ~9% (±3.9); and therefore found that on average, 91% (±3.9) of primary forest species are detected in regenerating selectively logged forests. In contrast, Dent and Wright (2009) focussed on regenerating rainforest with different human disturbance histories associated with clearance activities and categorised sites based on disturbance history into four different prior land-uses. For disturbance histories similar to the CCR in this study, the proportion of primary forest associated species within each area was calculated as follows; pasture or intensive agriculture (46%) and plantation (61%). From these reviews we might therefore have expected the difference between our SLR and CCR disturbance types to be at least 30%, rather than the considerably lower average 13% (±9.8) difference actually found. However, 65 of the 114 (57%) study sites from the review by Dent and Wright (2009) had an age <21 years since abandonment, whereas the findings presented within our study were from forest with over 30 years of regeneration. This suggests that once completely cleared areas have the potential to increase in their biodiversity value given enough time for re-colonisation of once lost species and if provided with sufficient time for regrowth, they would have the potential to contain levels of biodiversity higher than previously expected by the average value suggested by Dent and Wright (2009).

Medium-to-large terrestrial mammals showed little difference at the community or sample level in terms of species richness, diversity or community structure between disturbance areas. The feeding guild level analysis however, showed that carnivores and insectivores were more abundant in areas with a history of complete clearance than historically selectively logged forest. Previous research has shown that mammals in riverine areas and areas previously subjected to low-level logging display similar richness levels to primary forest sites (Salvador et al. 2011). Riverine areas were found to sustain these levels even when terra firme sites became depauperate in the dry season. These data along with our own results, suggest that the overall community of medium-to-large terrestrial mammals may not be useful indicators of different levels of historic anthropogenic disturbance, especially as was the case in our study when near to riverine habitat. Additionally, our results, from a site where animals had been protected from hunting for more than a decade, contrast with those of Burivalova et al. (2014), who found mammals to be the group most sensitive to an increased intensity of logging disturbance. However, Burivalova et al. (2014) acknowledge that although they attempted to exclude studies mentioning current hunting pressures from the meta-analysis, it was possible that hunting was a confounding effect, as many studies did not refer to this factor.

Caveats and limitations

The study site within this study is situated in a very promising context for the potential for both species and community recovery (Morante-Filho et al. 2015). The large tracts of nearby, largely primary forest (Manu National Park and the Amarakaeri Communal Reserve) have likely contributed to the successful recovery of communities within this regenerating forest and as such this case study may not be representative of areas with more intensely fragmented and degraded surrounding landscapes. Large nearby primary fragments likely retain a greater capacity to effect the recruitment of both natural flora and fauna, as they retain functionally influential species. This caveat however does not make our case-study unique and non-comparable. Quite often, abandoned landscapes within tropical regions are situated in close proximity to primary forest areas (Sloan et al. 2015). Sloan et al. (2015) for example, detected that 70% of secondary regenerating forest occurred within 500m of primary forest, and 85% occurred within 1km.

Another possible reason that mammals in the study by Burivalova et al. (2014) displayed a different pattern to that shown in our study is that medium-to-large mammals may be more mobile than the other taxa and over the small spatial scale of this study, may move transiently through all the historically disturbed forest types. This particular caveat is likely true for all groups within this study and is one of the compromises of the choice of a small spatial scale in the avoidance of landscape differences at larger scales; but this effect is likely more significant for groups that are more vagile, such as mammals and birds. This could be a reason why overall differences in species richness, abundance and diversity were detectable in the smaller bodied, less widely ranging groups (amphibians and butterflies) and less detactable in birds and mammals. Even small changes in vegetation structure have resulted in changes in tropical amphibian communities (Cortés-Gómez et al. 2013) and butterflies have been suggested to display significant ecological sensitivity to forest disturbance (Bonebrake et al. 2010), specifically due to their association with specific food plants (Horner-Devine et al. 2003). However, despite patterns not being detectable for the overall groups of birds and mammals (with the exception of nocturnal birds; a relatively small and specialist functional community), specific indicator groups and feeding guilds within birds and mammals allowed for the detection of differences between disturbance areas. However, bird and mammal species of conservation concern displayed no difference between different disturbance areas and were not found to be effective predictors of historic forms of rainforest disturbance, where other groups were.

Indicators, species of Conservation Concern and Feeding Guilds

One potential explanation for the greater encounter rate of insectivorous mammals in the past clear-felled regenerating forest could be the higher abundance of food resources. Insectivorous terrestrial mammals (e.g. giant anteater and giant armadillo) feed primarily on ants, and in one study in Central America ant richness was shown to decline with increasing disturbance, while the abundance of ants was found to decrease with forest maturity (Vasconcelos 1999). As a result, carnivores may be detected more frequently within this area, as many of the cat species have been found to feed primarily upon insectivorous mammals (Foster et al. 2013). In terms of greater encounters of frugivorous and omnivorous birds within the past clear-felled regenerating forest, this could be related once again to a greater abundance of food resources within this highly disturbed and now regenerating habitat. Our inspection of dominant families and tree species from Gentry plots, displayed a high occurrence of shrub-like fruiting trees, potentially providing avourable resources to fruit feeding species. Other studies have found that frugivorous species are often unaffected by habitat disturbance, while insectivorous species in particular are affected (Wolfe et al. 2015). Our study suggests that this effect is still detectable despite >30 years of regeneration following complete clearance. Boxplots for insectivorous bird species captured using mist-nets suggest that greater numbers of insectivores were present in SLR habitat, but this was not supported by the GLMM's. No pattern was observed in insectivorous species along transects, which may be a sign that given sufficient time for regeneration, insectivorous species can recover from even intense forms of clearance.

Conclusions

In conclusion, the direct (within site natural experiment) comparison made here suggests that, in the absence of the confounding effects of on-going disturbance and short regeneration periods, even historically completely cleared forest has the potential to harbour higher levels of biodiversity than previously suggested by average values from studies dominated by younger areas of regenerating forests. However, our results agree with those of Dunn (2004a) which suggests that although species richness levels can recover significantly, community compositions for almost all groups still display a significant despite >30 years of regeneration, despite being surrounded by large tracts of primary forest habitat. We believe it will be beneficial to investigate further within site natural experiments to start to determine how widely applicable the effects we have identified will be and so improve our understanding of the potential value of regenerating rainforest for conservation. While agreeing with other researchers that preventing further impacts on the world's remaining primary tropical forests is vital in order to sustain the highest levels of biodiversity (Gardner et al. 2007a). We suggest that even following complete clearance, regenerating tropical forests could, if managed for conservation, provide important resources for helping retain high levels of tropical biodiversity. This leads us to echo the concerns of Chazdon et al. (2009a) and Edwards et al. (2011) and suggest preventing the further clearance of these potentially valuable regenerating landscapes will be an important priority for future biodiversity conservation of the world's tropical forests.

Chapter 3 – The response of faunal biodiversity to an unmarked road in the Western Amazon.

Authors and contributions

Andrew Whitworth[1,2,3*], Christopher Beirne[1,2,4], Jasmine Rowe[1,2], Fraser Ross[1], Carloine Acton[1], Oliver Burdekin[1,2] and Philip Brown[1,5]

[1] Global Vision International, 3rd Floor, The Senate, Exeter, EX1 1UG, UK.

[2] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.[3] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.

[4] Centre for Ecology & Conservation, School of Biosciences, University Exeter, Cornwall Campus, Cornwall, TR10 9EZ, UK.

[5] School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG.

Designed the study: AW JR

Collected the data: AW CB JR FR CA OB PB

Performed the analysis: AW CB PB

Contributed to writing of the manuscript: AW CB PB OB FR JR

Abstract

Roads are an increasingly common feature of forest landscapes all over the world, and while information accumulates regarding the impacts of roads globally, there remains a paucity of information within tropical regions. Here we investigate the potential for biodiversity impacts from an unmarked road within a rainforest protected area in Western Amazonia. We focus on three key taxonomic groups; amphibians, butterflies and birds, each of which have been shown to be both sensitive and reliable indicators of forest disturbance. In total, 315 amphibians of 26 different species, 348 butterflies of 65 different species, 645 birds representing 77 different species were captured using mist netting and 877 bird records representing 79 different species were recorded using point counts. We provide evidence to show that the presence of a small unmarked road significantly altered levels of faunal species richness, diversity, relative abundance and community structure. This was true to a varying degree for all three taxa, up to and potentially beyond 350m into the forest interior. Responses to the road were shown to be taxon specific. We found increasing proximity to the road had a negative effect on amphibian and understorey bird communities, whilst butterfly and overall diurnal bird communities responded more positively. We show that the impact on biodiversity extends up to at least 32% of the whole reserve area; a serious impact under any scenario. This work provides support for recently voiced calls to consider limiting networks of unmarked roads in order to realistically and effectively preserve natural levels of tropical biodiversity and nature.

Introduction

Roads are an increasingly common feature of forest landscapes all over the world (Caro et al. 2014; Fraser 2014; Laurance & Balmford 2013), with at least 25 million kilometres of new roads anticipated globally by 2015 (Laurance et al. 2014a). Aside from the known direct negative effects of roads through hunting and logging access to remote areas (Coffin 2007; Goosem 2007) and wildlife mortality through vehicle collisions (Bissonette & Rosa 2009), roads also facilitate the spread of exotic flora and fauna (Hulme 2009), influence the spread of disease and danger to public health (Hahn et al. 2014), introduce chemicals (Coffin 2007), alter microclimates (Camargo & Kapos 1995; Fraser 2014), increase the risk of fires (Fraser 2014) and act as dispersal barriers limiting the movements of individuals with and between populations (Bisonette & Rosa 2009; Goosem 2007; Laurance et al. 2014a; Pocock & Lawrence 2005). As such, the negative effects of roads can extend well beyond physical boundaries into the forest landscape.

Whilst information accumulates regarding the impacts of roads worldwide, there remains a paucity of information within tropical regions (Dent & Wright 2009; Stork et al. 2009). To date North America, Europe and Australia have accounted for over 90% of all the studies conducted on the impacts of roads (Taylor & Goldingay, 2010) despite the fact that nine tenths of future global road construction is anticipated to occur within developing nations (Laurance et al. 2014a). Tropical regions such as the Amazon basin are under significant threat from expanding road networks, especially areas within the Western Amazon that had until recently remained largely intact. However, growing pressure on the exploitation of the Western Amazon's resources suggests that this may change and that these regions risk becoming increasingly fragmented (Finer et al. 2008; Laurance et al. 2014b; Oliveira et al. 2007). Large-scale forest damage within the Brazilian Amazon for example, has already been attributed to modern road building and policies supporting the extraction of natural resources (Oliveira et al. 2007). Whilst the expansion of large marked roads, such as the Inter-Oceanic highway connecting Peru and Brazil, has received much attention, an increase in small unmarked roads is potentially just as worrying. According to Brandào Jr. and Souza Jr. (2006), in the Amazon alone the average growth rate for unofficial roads approximately doubled in ten years from 9.85 km/10,000km^2 per year (between 1990-1995) to 19.25 km/10,000km^2 per year (between 1996-2001). Ahmed et al. (2013) showed that the Brazilian road network grew by almost 17,000km per year between 2004 and 2007.

Yet to date there is almost no information available with regards to the impacts of unmarked roads, which provide access for both local people and companies looking to utilise commercially valuable resources (Mäki et al. 2001); but we do know that nearly 95% of all deforestation within the Amazon has occurred within 5.5km of roads or 1km of rivers (Barber et al. 2014). These issues must be addressed in order to provide timely research based evidence related to the potential impacts of these roads both globally and specifically to the rainforest of Western Amazonia (Laurance et al. 2012b).

One limiting factor hindering assessments of the impact of roads in hyper bio-diverse regions like the Amazon is that the majority of studies are single taxon orientated (Anand et al. 2010; Gardner et al. 2008). Focussing on single groups can lead to erroneous conclusions of overall faunal and floral responses and research has shown that a multi-taxa approach is essential for monitoring changes in biodiversity (Lawton et al. 1998). Taylor and Goldingay (2010) found a taxonomic bias within research assessing the impacts of roads towards mammals in particular (53% of studies), with just 10% of studies focussing on birds, 8% on amphibians and only 20% of studies including multiple taxonomic data. An excellent example regarding this bias within tropical forests is highlighted by Dunn (2004a) in which a review of 34 research projects found just two studies that provided multi-taxa information. One recent case study from Yasuní Biosphere Reserve in Ecuador (Suárez et al. 2013) studied just two groups, large birds and medium-large terrestrial mammals (both associated with hunting pressures) to show the effectiveness of access control along two oil-roads. Despite the reduced species richness losses along the managed road compared to an unmanaged road, several species still showed a reduction in their populations, likely due to increased hunting pressure from native populations.

In response to the lack of multi-taxon research related to unmarked roads in the Western Amazon, we provide an investigation of the potential for biodiversity impacts from an unmarked road within a rainforest protected area. The study focuses on three key taxonomic groups; amphibians, butterflies and birds, each of which have been shown to be both sensitive and reliable indicators of forest disturbances (Findlay & Bourdages 2000; Hill et al. 2001; Hopkins 2007; Laurance 2004; Lindell et al. 2007; Schlaepfer & Gavin, 2001; Schulze et al. 2004). Specifically we ask three key questions: 1. Are faunal species richness and diversity affected by the presence of an unmarked road within the forest interior of a lowland tropical rainforest? 2. Is faunal community composition and abundance affected by the presence of an unmarked road within the forest interior? 3. Do all taxa respond in the same way to this unmarked road, or are such responses taxon specific?

Methods

Study site

All research was conducted in the Yachana Reserve between October 2009 and November 2011 (Figure 1). The reserve is situated within the Napo province in the Amazonian region of Ecuador (77°13'43.9"W, 0°50'45.281"S; 300-350m altitude). The study site is a legally-designated Bosque Protector (Protected Forest), consisting of approximately 1000 hectares of lowland rainforest, bisected by a small unmarked road typical of many in the region. The reserve is surrounded by a mosaic landscape consisting of pasture land, small active cacao farms and forest. The road was constructed circa 2005 and measures 8-10 meters wide, plus an additional 2-3 metres of partially cleared vegetation either side. It is large enough to permit buses and trucks to pass through but remained as an unpaved gravel road throughout the research period. During the study period traffic on the road was sparse but usually consisted of 2-4 buses, 4-8 motorbikes and 2-6 trucks passing per day. This limited amount of traffic relates to the purpose of the road; a dead end road constructed simply to serve as access for a small number of remote communities within the area to connect them with the wider region.

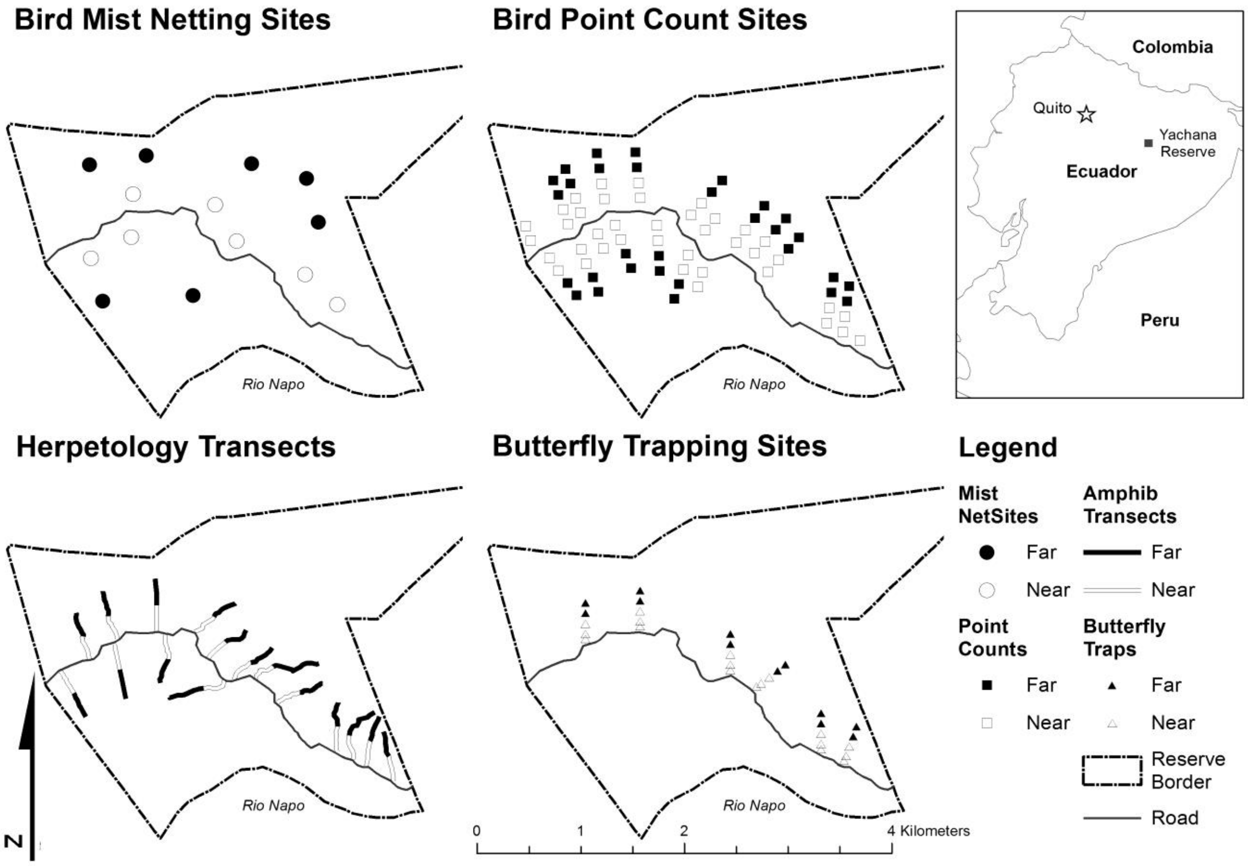


Figure 1. Location of the study site, east of the Ecuadorian Andes, in western Amazonia. Example layouts for survey sites and transects for each methodology are shown with their respective category as ‘Near’ or ‘Far’ for analysis.

Field Survey methodologies

In order to ensure that any potential results were related directly to the road and not differences in habitat, all survey sites were located in the same type of forest habitat. Other sites with different anthropogenic disturbance histories were avoided in site selection, as they are known to possess different species compositions and relative abundances (Beirne et al. 2013). As the study site is a private protected area with effective protection, the potential for hunting effects along the road to contribute towards any differences detected was minimal. Amphibians were surveyed along 16 nocturnal transects (500m long x 6m wide) starting both from the road into the forest interior and on other nights from the forest interior towards the road. 0-250m was designated as near to the road and 251-500m designated as far. Understory birds were targeted using nine mist-net (MN) sites located within 0–350m from the road designated as near and a further seven sites located within 350–700m from the road designated as far. To survey the overall diurnal bird community, point count (PC) surveys were conducted at 69 independent points (separated by at least 100m); 39 near points (located between 1-350m from the road) and 30 far points (located between 351-700m from the road). The majority of points were repeated on at least two occasions and for half of the mornings points began close to the road and for the other half points were started far from the road, in order to avoid temporal bias in species detection. Fruit baited traps were used to survey butterflies along six linear transects; two traps were placed at each study site comprising of one ground trap (1m above the ground) and one sub-canopy trap (10-15m from the ground). Sites at 50, 100 and 200m were designated as near from the road and sites at 300 and 400m as far. In order to ensure that any differences detected within the results were due to the road presence and were not due to seasonal variances both near and far habitats were surveyed evenly throughout dry and wet seasons.

Forman and Deblinger (2000) detected that direct maximum ecological effects from the edge of the road surface extended to an average of just over 300m, particularly for birds and large mammals. We therefore determined 350m to act as the differential point between near and far for birds. Near and far survey areas were set at a slightly reduced scale for amphibians and butterflies (with 250m as the differential between near and far), as birds are generally a more mobile group (Eglington et al. 2012) and we wanted to be confident that any smaller scale effect would be detected for less mobile amphibians and butterflies. A more detailed summary of field methodologies and survey design is provided in Appendix 3A.

Analyses

Species richness and diversity

In order to assess the extent to which our study effort had detected as many species likely to be found within the near and far study areas, we created rarefaction curves of observed species richness levels for each sampling methodology using the Rich package (Rossi 2011) and plotted these using program R (R Core Team 2012). Where the sampling effort detected fewer individuals in one area, we extrapolated the lower lying curve towards an equal number of individuals for a clearer comparison of where observed richness values would have projected given a detection of an equal number of individuals. We then used three estimators of species richness (Jack 1, Chao 2 and Mmmeans) that have previously been shown to be effective for birds and butterflies (Ferman et al. 2005; Herzog et al. 2002; Hughes et al. 2002; Ramesh et al. 2010), whilst amphibians remain relatively unresolved as to which estimators work most effectively (Veith et al. 2004). We therefore used these three estimators to determine a comparable average value of estimated species richness for each survey method, in both near and far study areas.

Species diversity was defined as the Simpson diversity index, a measure shown to be robust when applied to even relatively small sample sizes (Barlow et al. 2007b; Billeter et al. 2008; Hamer et al. 2003; Lande et al. 2000). Repeating the analyses using Fisher's Alpha Shannon and Shannon Exponential diversity indices did not change the results and as such are not presented. All richness and diversity estimators were calculated in Estimate S v9 (Colwell 2013).

Encounter rates and community composition

Where survey effort was equal between near and far samples, relative encounter rates are presented as the number of individuals encountered per single sample (individuals per 250m for amphibians, individuals per sampling site for butterflies and individuals per point count for birds). For mist-netting, where sampling effort varied between samples (owing to weather constraints), relative abundance represents the estimated number of individuals caught per 100 mist net hours. Comparisons of relative encounter rates between near and far areas were performed using linear models in R. Mixed models with 'transect identity' as a random effect were used for methodologies which involved the repeated sampling of the same transects (amphibians and butterflies) or sampling stations (point counts) to account for non-independence between samples. As many mist-netting sites were only sampled once (rendering the mixed-effects modelling approach inappropriate), standard linear models were used. A Poisson link-function was used for all amphibian, butterfly and point-count models, whereas mist-net models used the Gaussian link-function.

Community compositions and structures were compared by producing dominance-diversity (Whittaker) plots for all study methodologies, across both near and far areas using the vegan package (Oksanen et al. 2011) in program R (R Core Team 2012). Such plots compare the evenness of a community whereby shallow curves represent a community of many species of similar abundance, whereas steep curves represent a skewed assemblage with one or more species in substantially higher relative abundance than the others (as in Beirne et al. 2013). Significant differences in slope, and therefore significant differences in community evenness, were assessed through the use of a linear model with log relative abundance as the response term and an interaction between species rank and habitat type as continuous and categorical fixed effects, respectively. Results are reported as ΔG which corresponds to absolute change in gradient between forest and the modified habitats; more negative values denote steeper curves and thus less even assemblages.

Results

Species richness and diversity

In total, 315 amphibians of 26 different species, 348 butterflies of 65 different species, 645 birds representing 77 different species (from Mist Nets) and 877 bird records representing 79 different species (from Point Counts) were recorded (Table 1). Survey coverage across all survey sites and groups was over 67% (ranging up to 99%). Far habitat harboured the highest frequency of exclusive amphibians and birds captured in MN (nine and 33 species respectively) and the highest proportions of the total species (completeness) observed for amphibians and birds from MN (85% and 86% respectively). Near habitat harboured the highest frequency of exclusive butterfly and bird species recorded by PC (25 and 21 respectively) and the highest proportions of total species encountered (completeness) observed for butterflies and birds from PC (88% and 87% respectively).

Table 1. Capture/encounter frequency, survey effort, observed and estimated species richness, sample coverage and sample completeness per distance classification for each of the four study methodologies.

Taxa	Habitat class	n[a]	Survey effort	Species[b]	Species richness estimates: Jack 1	Chao 2	Mmmeans	Average[c]	Coverage (%)[d]	Exclusive species[e]	Completeness (%)[f]
Amphibians	Near	165	x30 transects	17	21.83	20.13	20.09	**21**	82	4	65
	Far	150	x30 transects	22	32.63	37.13	26.92	**32**	68	9	85
	Total	315	x60 transects	26							
Butterflies	Near	218	X288 site check days	57	57.96	50.91	63.62	**57**	99	25	88
	Far	130	x216 site check days	38	52.93	51.06	53.18	**52**	73	8	58
	Total	348	X504 site check days	65							
Birds - Mist netting	Near	203	x836 net-hrs	44	63.62	77.33	57.47	**66**	67	11	57
	Far	442	x1178 net-hrs	66	93.6	105.2	76.28	**92**	72	33	86
	Total	645	x2014 net-hrs	77							
Birds – Point counts	Near	472	x75 counts	69	91.69	102.06	75.78	**90**	77	21	87
	Far	405	x60 counts	58	69.8	63.41	65.11	**66**	88	10	73
	Total	877	x135 counts	79							

[a] Number of individuals encountered

[b] Number of species observed

[c] Mean estimated species richness 'classic Chao 2 was used due in cases where CV>0.5'

[d] Sampling coverage defined as: ${}^{b}/{}^{c}$*100

[e] Number of species found exclusively within a given habitat

[f] Number of species observed as a percentage of combined species across all habitats

Estimated species richness, whether using richness estimators (Table 1) or individual rarefaction curves (Figure 2), showed the same general trends; amphibian and understorey bird richness decreased with increasing proximity to the road, whereas butterfly and overall diurnal bird richness increased. For amphibians, habitat near the road was estimated to support 11 fewer species (-34%) than habitat far from the road. For understorey birds, habitat near the road was estimated to support 26 fewer species (-28%) than habitat far from the road. Estimated butterfly richness suggests that habitat near the road contains five more species (+10%) than far from the road. Overall diurnal birds from PC richness estimates suggest that near habitat supports 24 more species (+36%) than far habitat. Although many of the rarefied curves suggest the difference in values to be non-significant, it is also clear that the majority of curves have failed to reach a plateau, as is common in many tropical studies; and certainly for amphibians and point-counts, the observed difference between the curves is widening as accumulation of individuals increases (along with the width of the confidence intervals).

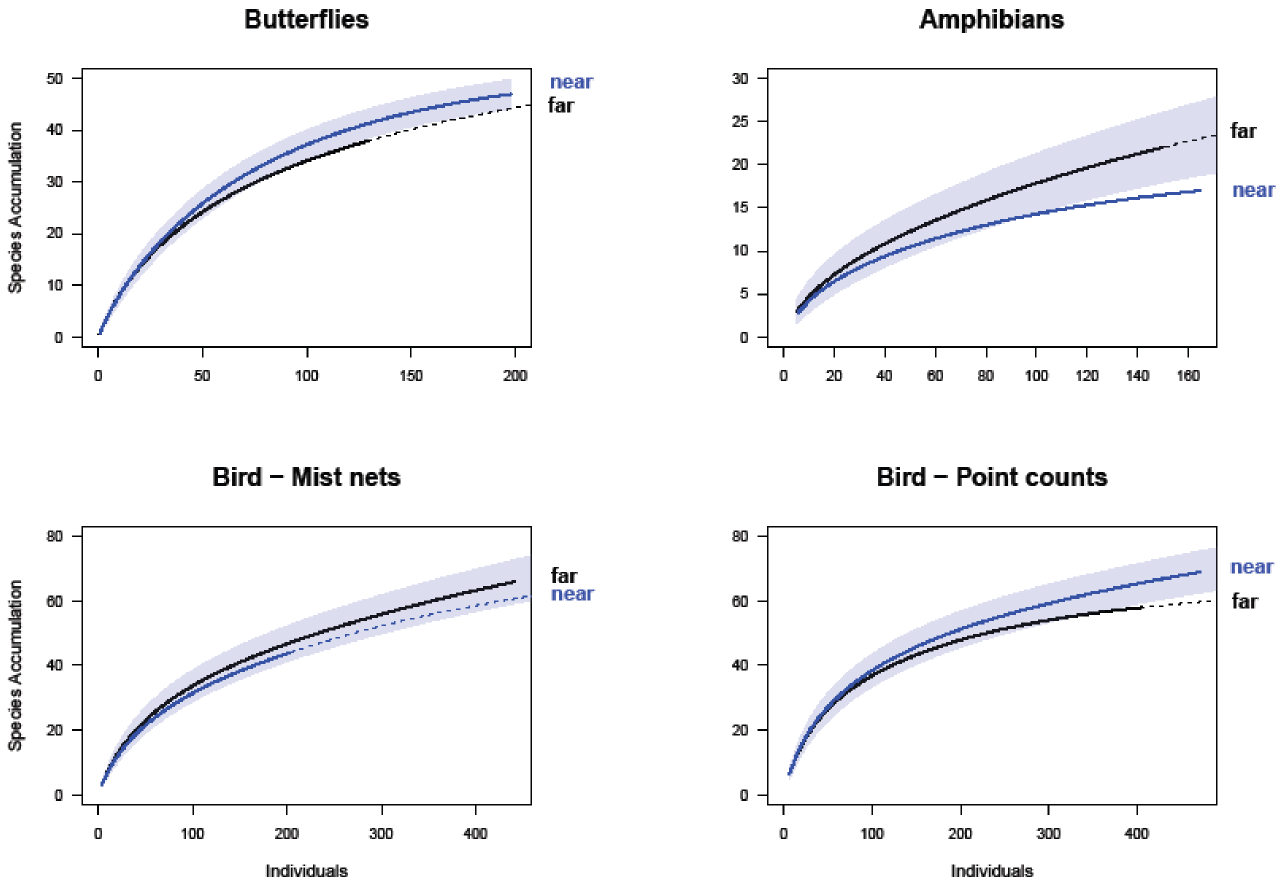


Figure 2. Rarefaction curves for butterflies, amphibians, mist net-surveyed birds and point count-surveyed birds for near and far areas to the road. Solid lines represent true observations and dashed lines represent a projection. Grey areas represent 84% confidence intervals for the highest projected survey area.

Mean Simpson diversity estimates indicate that habitats far from the road are the most diverse for butterflies, amphibians and understorey birds (Figure 3). However, examination of confidence intervals suggests that this trend is only significant for understorey birds. The overall diurnal birds show the opposite trend with a lower mean diversity found farther from the road but there is high overlap between confidence intervals suggesting that this was not a significant pattern. The same patterns were found for other diversity estimators: Shannon, Shannon Exponential and Fisher's alpha (data therefore not shown).

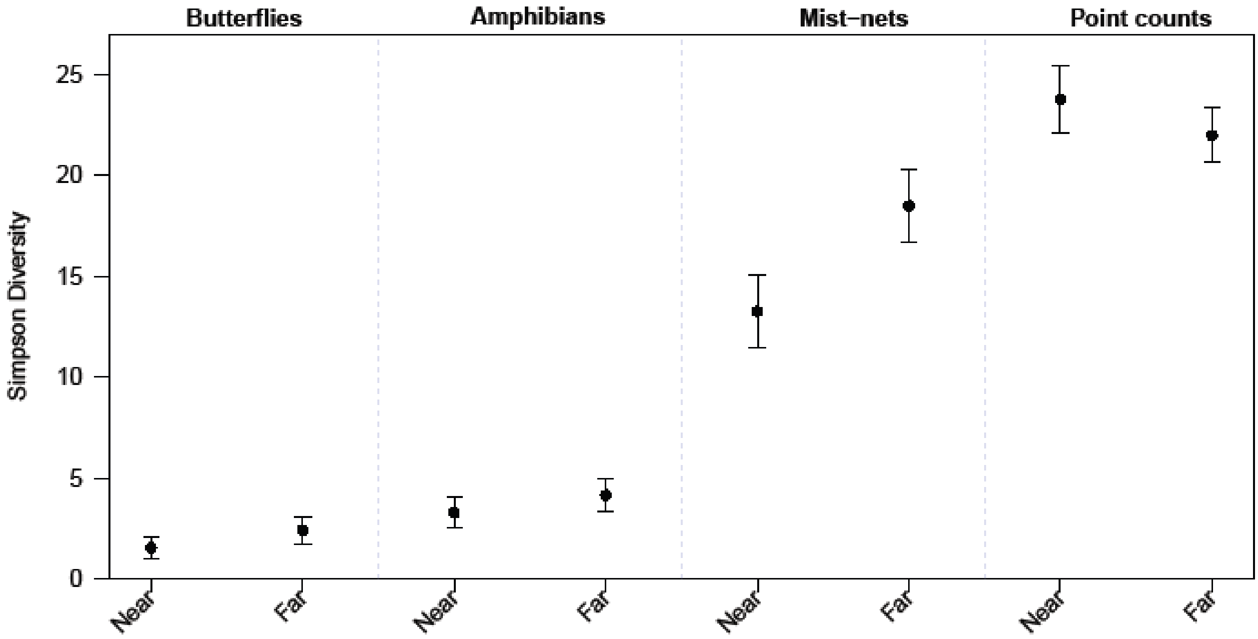


Figure 3. Simpson species diversity estimates with 84% confidence intervals for butterflies, amphibians, MN caught birds and PC detected birds for near and far distances from the road.

Encounter rates and community composition

The only instance in which an overall relative encounter rate of the four survey methodologies was found to show significant difference in capture rate was for understorey birds (p=0.02). Approximately 12 more individuals (33% more) were captured for every 100 net hours in habitat far from the road (Table 2).

Table 2. The estimated relative encounter/capture for near and far habitat from the road, for each survey methodology. Where; 'Near' and 'Far' columns present the estimated relative abundance for each area; n = the total number of individuals encountered by the particular survey method; *p* = probability that the relative abundance estimates significantly differ (determined through ANOVA comparison of models including, and excluding distance from the road), * = p<0.05.

Study group	Near	Far	Relative rate	n	*p*
Amphibians	4.9	4.5	Individuals / 250m walked	315	0.40
Butterflies	6.3	5.1	Individuals / 9 trapping days (x2 nets/site)	348	0.06
Bird - mist-netting	24.8	37.0	Capture rate per 100 net hours	645	**0.02***
Bird - point counts	6.3	6.7	Records / point count	877	0.33

All other survey methodologies showed no significant difference in overall encounter rates; butterflies showed higher capture rates closer to the road but this was found to be non-significant (p=0.06). Dominance-diversity plots demonstrate that for both amphibians and understorey birds captured by MN, habitat farther from the road supports a significantly more even assemblage (regular intervals between species) and more rare species (increased tail length) than habitat near to the road (Figure 4). No significant difference in the evenness of the species composition was found for butterflies, whilst overall diurnal birds showed a significantly more even assemblage near to the road.

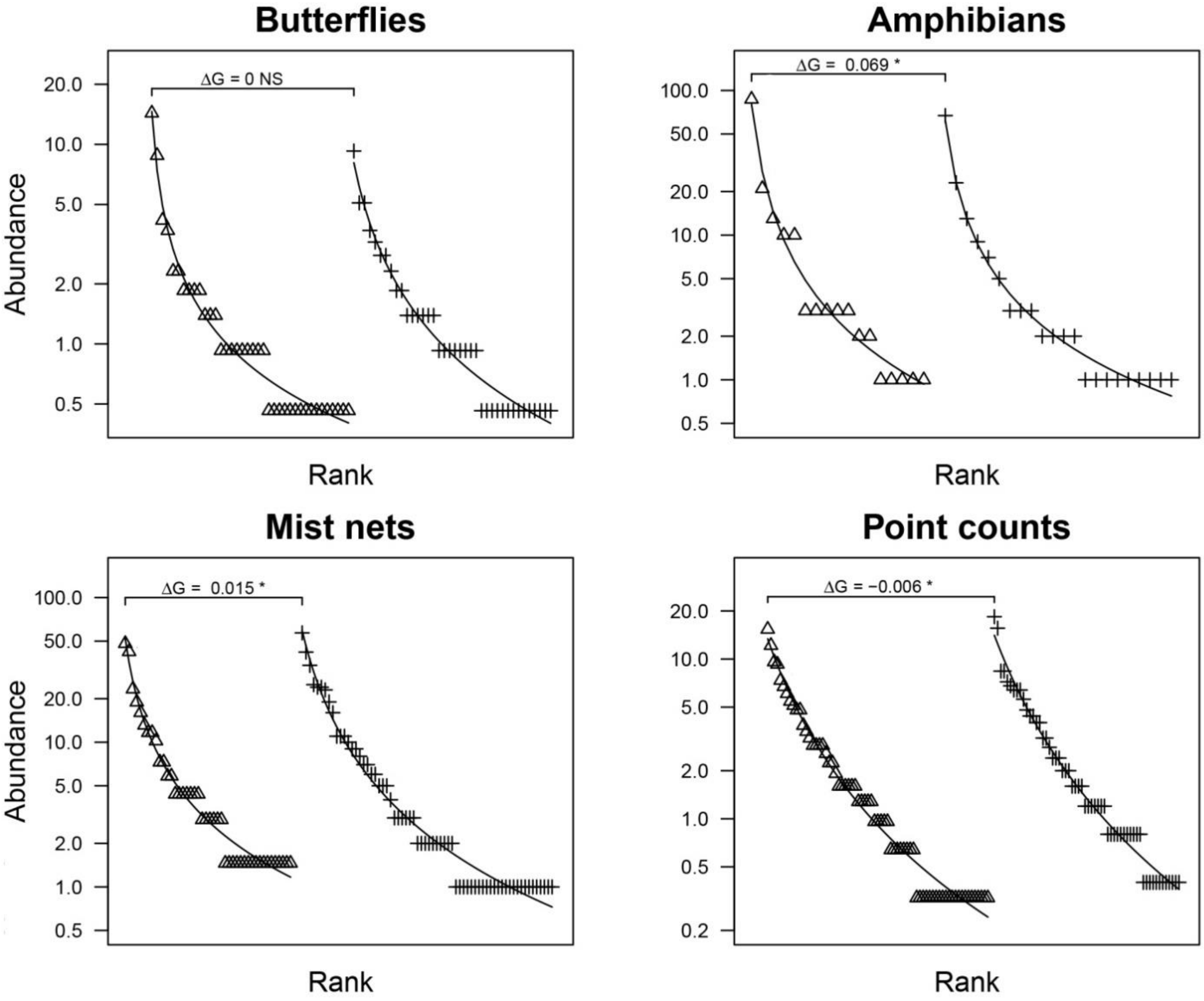


Figure 4. Dominance diversity (Whittaker) plots for butterflies, amphibians, mist net-caught birds and point count-caught birds. Each plot displays curves for both far (on the left) and near (on the right) habitats. For each habitat the relative abundance of each species (ni/N) was plotted on a logarithmic scale against the species rank ordered from most to least abundant. Δ = near and + = far. Linear models were used to determine if the slopes of near and far habitat were significantly different, where ΔG denotes to absolute change in gradient and the symbol denote the level of significance of the deviation where * <= 0.05 and NS = not significant.

Discussion

The analyses presented in this study provide evidence to show that the presence of a small unmarked road significantly altered levels of faunal species richness, diversity, abundance and community structure. This was true to a varying degree for at least three different taxa, up to at least 350m into the forest interior. The general response has shown to be both taxa and species specific with some species increasing encounter rates near to the road, whilst others higher encounter rates far from the road. We found that a small unmarked road can have an adverse effect on amphibians and understorey bird communities in particular, whilst butterflies and overall diurnal bird communities may respond positively near to the road.

Our results suggest that birds targeted by two different survey methods respond oppositely. Understorey birds were found to have a higher estimated richness, higher diversity, and a more even community structure containing more specialists. Adversely, the PC methodology targeting the overall diurnal bird community showed higher observed and estimated richness and diversity levels, a more balanced and even community structure closer to the road. This finding is congruent with other researchers that have found understorey birds to be particularly sensitive to habitat disturbance (Banks-Leite et al. 2010), whereas overall bird communities respond positively when dominated by habitat generalists (Burivalova et al. 2014).

Butterflies have previously been shown to be effective in detecting ecological change due to their sensitivity to forest disturbance (Lewis 2001; Hamer et al. 2003). The slightly higher species richness and higher encounter rate for butterflies in habitat near to the road (albeit non-significant) are likely driven by physical properties altered by the road, such as light. A more open canopy and therefore increased light has been shown to be positively correlated with butterfly species diversity by previous studies (Hamer et al. 2003; Willott et al. 2000). The presence of the road did not appear to alter the overall community structure or overall encounter rate of butterflies deep into the forest (>200m). Despite this unaltered community structure beyond 200m, there may be differences found at a finer scale, closer to the road-forest boundary itself, which were not detected within this study.

Amphibians showed changes in observed and estimated species richness near to the road and also a shift in overall community structure. Despite this they did not display any differentiation in overall capture rate. It seems that the forest near to the road has the potential to hold comparable numbers of individuals but becomes unsuitable for some of the more rarely encountered species. Even small changes in vegetation structure have recently been shown to create significant changes to community structure of amphibians within tropical forests (Cortés-Gómez et al. 2013).

Whilst utilising a combination of methods to expand the scope of research and target whole community structures, it is paramount that methods employed are appropriate to ensure susceptible groups are well represented. This study has identified that in terms of birds the most vulnerable group susceptible to the impact of small roads on forest habitat are understorey species that reside in the first three meters above the ground. As different taxa have shown a mixed response towards the impact of a small unmarked rainforest road in Western Amazonia, this study has shown that multi taxa approaches are necessary to provide a more detailed insight into habitat disturbance and responses shown by faunal groups. This suggests that different taxa or groups may not produce good surrogate indicators for one another. The factors influencing different taxonomic responses are likely wide and varied, potentially including: traffic mortality (Bissonette & Rosa 2009), boundary or barrier limitations (Goosem 2007; Laurance et al. 2014a; Pocock & Lawrence 2005), changes in physical environment (Camargo & Kapos 1995; Fraser 2014), changes in biological diversity of host or food plants (Lewis 2001; Hamer et al. 2003), all of which relate directly to the presence of the road itself.

Caveats and limitations

Although within this study we were able to detect differences in biodiversity patterns, and attribute this to the presence of the road, we didn’t investigate the specific drivers that relate to these differences that are likely caused by the road itself. Future research into the impacts of roads within the western Amazon could identify the specific changes to forest structure, environmental conditions, levels of mortality and community changes in plant communities, in order to better understanding these specific drivers of change. This might provide useful insights in how to develop mitigation strategies where roads are necessary, as in the case of environmental bridges to avoid collisions (Glista et al. 2009).

Another potential limitation within this study is the situation of the road itself. The road in this case-study follows a natural ridge line through the reserve, as this was likely the easiest, cheapest or safest route for the development of the road; a potential common practice for many pioneer roads in remote regions. It may be therefore possible that some differences observed close to the road might be attributed to more general landscape features related to natural contour that were not measured within this particular study. This possibility could be tested by finding a nearby natural undeveloped ridge line and assessing the biodiversity close the apex where a road might be situated if developed, and then compared to the 'near' habitat close to an existing road.

Implications for conservation and conclusions

First and foremost this study adds to the empirical evidence towards the potential for small unmarked roads to significantly alter and impact faunal biodiversity within tropical forests. This was true for all four survey methodologies and across all three taxa assessed within this research, albeit to a varying degree and pattern. Although groups could not be used as surrogates for patterns of change (as they did not display a standardised response), they all demonstrated some level of disruption, which would lead us to believe that this case is likely true for a variety of flora and fauna. This stands to reason when we consider that the groups studied here provide integral roles into the functioning of a healthy tropical forest.

In terms of land management implications, we can see that a 4.5km stretch of road (<16m width as in this study) does not only have an impact on biodiversity over the area of the road itself (in this case an area of 7.2ha) but potentially up to and beyond a much greater area (in the case of this study up to 322ha). When applying these figures to the whole of the reserve (~1000ha in size), then the first value (road area) would equate to an impact on biodiversity at a scale of <1% of the total reserve area. However, in the case of the much wider impact that this study has detected upon biodiversity (>350m) we can see that the impact on biodiversity extends up to 32% of the whole reserve area, almost 45 times greater than managers may have predicted and a significant area of impact under any scenario. Worryingly, at least 21.9% of the world's tropical and sub-tropical national parks in 2006 were shown to be traversed by main roads, a further 8.4% by highways and an unknown value by unmarked roads (Caro et al. 2014). The scale of ecological disruption to national parks of these regions could therefore be extensive and fast increasing.

In light of other amalgamating pressures not recorded within this particular study, such as mortality figures due to the road itself and anthropogenic pressures through agriculture and hunting, then small reserves that exist primarily to preserve pockets of biodiversity are under serious threat in failing to achieve their conservation goals. Land managers should be made increasingly aware of these impacts to tropical biodiversity, especially in the case of intact areas of the Amazon that to date remain free from extensive road networks. A large-scale zoning programme for roads such as the one recently laid out by Laurance et al. (2014a) could help to mitigate biodiversity losses and ecological destruction whilst maximising agricultural production. Our results only allow us to echo the concerns laid out recently by Fraser (2014) and Laurance et al. (2009) that efforts should be made towards limiting the network of unmarked roads to realistically and effectively preserve tropical nature.

Chapter 4 - Past human disturbance effects upon biodiversity are greatest in the canopy; a case study on rainforest butterflies.

Authors and contributions

Andrew Whitworth[1,2], Jaime Villacampa[1], Alice Brown[1], Ruthmery Pillco Huarcaya[1,3], Roger Downie[2] and Ross MacLeod[2]

[1] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.
[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.

[3] Universidad Nacional San Antonio Abad del Cusco (UNSAAC), Perú.

Designed the study: AW RM

Collected the data: AW JV RPH AB

Performed the analysis: AW AB RPH

Contributed to writing of the manuscript: AW JV RM RD

Abstract

A key part of tropical forest spatial complexity is the vertical stratification of biodiversity, with widely differing communities found in higher rainforest strata compared to terrestrial levels. Despite this, our understanding of how human disturbance may differentially affect biodiversity across vertical strata of tropical forests has been slow to develop. For the first time, how the patterns of current biodiversity vary between three vertical strata within a single forest, subject to three different types of historic anthropogenic disturbance, was directly assessed. In total, 229 species of butterfly were detected, with a total of 5219 individual records. Butterfly species richness, species diversity, abundance and community evenness differed markedly between vertical strata. We show for the first time, for any group of rainforest biodiversity, that different vertical strata within the same rainforest, responded differently in areas with different historic human disturbance. Differences were most notable within the canopy. Regenerating forest following complete clearance had 47% lower estimated canopy species richness than regenerating forest that was once selectively logged, while the reduction in the mid-storey was 33% and at ground level, 30%. These results also show for the first time that even long term regeneration (over the course of 30 years) may be insufficient to erase differences in biodiversity linked to different types of human disturbance. We argue, along with other studies, that ignoring the potential for more pronounced effects of disturbance on canopy fauna, could lead to the underestimation of the effects of habitat disturbance on biodiversity, and thus the overestimation of the conservation value of regenerating forests more generally.

Introduction

Tropical forests provide habitats of exceptional spatial complexity, which contribute significantly to global biodiversity, while making them vulnerable to human disturbance that disrupts this complexity (Gardner et al. 2009; Gibson et al. 2011). A key part of tropical forest spatial complexity is the vertical stratification of biodiversity, with widely differing communities often found in higher rainforest strata compared to terrestrial levels (DeVries et al. 1997; Dumbrell & Hill 2005; Maguire et al. 2014; Tregidgo et al. 2010). Despite this, understanding of how human disturbance may differentially affect biodiversity across vertical zones or strata of tropical forests has been slow to develop (DeVries et al. 1997; Dumbrell & Hill 2005; Haefke et al. 2013; Tregidgo et al. 2010).

Biodiversity differences across vertical levels have been detected for a variety of both vertebrate and invertebrate taxa. In vertebrates for example, fruit bats from Malaysian rainforest displayed higher species diversity in the canopy than the understorey (Francis 1994), while Neotropical birds showed a pronounced vertical layering of species (Walther 2002). Understorey birds were found to occupy a wider vertical niche and therefore forage in a greater variety of light levels than either canopy or terrestrial species (Walther 2002). In addition to vertical differences in rainforest vertebrates, a number of invertebrate groups, including ants, butterflies and dung beetles, have also been found to display differences in vertical levels of biodiversity (Dumbrell & Hill 2005; Klimes et al. 2012 and Tregidgo et al. 2010). Therefore, despite being less well studied it has been suggested that understanding vertical differences will be as important, or perhaps of even greater importance for understanding biodiversity patterns than more traditional assessments along the horizontal gradient (de Faria Lopes et al. 2014).

Despite evidence for differences in biodiversity patterns between vertical layers there remains disagreement as to which vertical zones contain the most biodiversity. For example in one of the best studied indicator taxa for tropical forests, butterflies, DeVries et al. (1997) found that estimated species richness of butterflies in the Ecuadorian Amazon was higher in the canopy than in the terrestrial community. In addition, Ribeiro and Freitas (2012), found in the Brazilian Amazon that the canopy community was significantly richer and more species diverse than the terrestrial layer. In contrast, other studies of tropical forest butterflies have found terrestrial or understorey layers to hold higher levels of biodiversity than those detected in the canopy (Barlow et al. 2007a; DeVries & Walla 2001; Fermon et al. 2005). Regardless of this lack of consensus, the differences between vertical zones suggest that sampling within a single stratum could lead to under or over estimation of true overall levels of biodiversity within rainforest habitats, and therefore bias judgements about the relative conservation value of different areas.

As many of the world's tropical forests are being rapidly modified through ongoing anthropogenic disturbance (FAO 2010; Gardner 2012) there is a pressing need to understand how biodiversity at different vertical levels responds to such disturbance (de Faria Lopes et al. 2014; DeVries et al. 1997). Any bias resulting from single stratum assessments has the potential to be of particular importance in studies which intend to assess the conservation and biodiversity value of secondary rainforest (Edwards et al. 2014b; Gibson et al. 2011; Letcher & Chazdon 2009), specifically because biodiversity could be under or overestimated and therefore lead to an under or overestimation of the conservation and biodiversity value of such forests (Ribeiro et al. 2015). For example, Dumbrell and Hill (2005) have shown for butterflies in a Southeast Asian rainforest that terrestrial species diversity of regenerating forest (15 years since logging) was similar to primary forest. However, when canopy sampling data were included they found the disturbed habitat to be significantly less biodiverse than undisturbed forest controls. Canopy dwelling specialists can play an integral role in forest regeneration through the provision of essential ecosystem services, but are often overlooked within habitat disturbance assessments (de la Peña-Domene et al. 2014; de Faria Lopes et al. 2014). Despite the importance for conservation about the differential effects of habitat disturbance upon rainforest biodiversity across vertical layers, research remains very sparse.

In this study we use Neotropical butterfly communities to assess the differential impact of habitat disturbance history upon biodiversity across vertical layers. Butterflies are key components within their ecosystems and are effective in detecting ecological change due to their sensitivity to forest disturbance (Bonebrake et al. 2010; Hamer et al. 2003), specifically through association with specific food plants (Horner-Devine et al. 2003). Butterfly biodiversity assessments are therefore well suited to assess changes in biodiversity due to anthropogenic habitat disturbance. Previous studies using terrestrial based sampling designs have often suggested that butterfly biodiversity does not show a significant degree of difference between human disturbed and primary forest (Devy & Davidar 2001; Hamer et al. 2003; Kudavidanage et al. 2012; Lewis 2001; Posa & Sodhi 2006). However, the relatively few studies including canopy level data have suggested that disturbance effects may be significant at higher levels. This makes butterflies an ideal group to start investigating if biodiversity at different levels shows differential responses to human disturbance type (Barlow et al. 2007a; Dumbrell & Hill 2005; Fermon et al. 2005; Ribeiro & Freitas 2012; Ribeiro et al. 2015). Further, Barlow et al. (2007) suggest there is an over-emphasis on the high conservation value of regenerating forest for butterflies, likely due to the failure to consider different vertical strata (amongst other factors, such as a lack of seasonal replication and small sample sizes; Ribeiro et al. 2015). In addition, rotten fruit bait is more commonly and widely used in studies of tropical butterflies (Barlow et al. 2007a; DeVries & Walla 2001; Fermon et al. 2005), despite rotten fish bait being shown to capture a greater number of individuals and provide wider coverage of the butterfly community (Austin & Riley 1995; Hall & Willmott 2000; Sourakov & Emmel 1995). As such, we investigate the potential difference in patterns detected as a result of utilising these different bait types.

Here, for the first time, we directly assess how patterns of current biodiversity vary between three vertical strata, within a single forest subject to three different types of historic anthropogenic disturbance. Although a number of studies to date have compared primary forest with logged forest, or forest that has started to regenerate after complete clearance, very few studies have assessed biodiversity within a forest once subjected to different types of disturbance (Bowen et al. 2007). In this study we assess butterfly communities in a regenerating rainforest study site located in one of the world’s most biodiverse and important conservation areas: the Manu Biosphere Reserve, a UNESCO World Heritage Site designated to protect the globally important Amazon rainforest and its biodiversity. Specifically, we quantified and compared species richness, diversity, abundance and community structure of butterflies across three vertical zones, between areas regenerating after three different types of historic human disturbance. The aim of which was to answer the following questions; *i)* How do patterns of biodiversity differ between vertical zones of this regenerating rainforest study site?, *ii)* How do areas that differ in historic human disturbance differ in current biodiversity between vertical zones of this regenerating rainforest?, and *iii)* How does the use of different bait types (fruit or carrion) affect the patterns of biodiversity beween strata and across disturbance areas?

Methods

Study site

The study was carried out at the Manu Learning Centre (MLC) research station in the Peruvian Amazon (71°23'28"W 12°47'21"S; Figure 1); owned and operated by conservation NGO the Crees Foundation. Beyond the study site to the west lies the core area of the Manu National Park, (over 1.5 million ha of mainly primary tropical forest) whilst to the east of the reserve lies the second largest protected area in the biosphere reserve; the Amarakaeri Communal Reserve (402,335 ha of forest reserve, created in 2002). The Manu Biosphere Reserve consists of a network of core protected areas surrounded by areas designated as cultural buffer zones due to historically high human impact, including extensive logging or clearance for subsistence agriculture.

The study site lay within one of these cultural buffer zones. It consists of ~800ha of regenerating lowland tropical forest accessed by a 20km trail system, and covers an altitudinal range of 450-740m asl. During the period of the study (2011-2014) the average daily wet season (October-March) temperature was 24.78^{o}C (average high of 27.89^{o}C; average low of 22.19^{o}C), the average humidity was 90.58% (average high of 96.32%; average low of 69.26%) and the average seasonal rainfall was 3098mm. The average dry season (April-September) temperature was 23.74^{o}C (average high of 27.17^{o}C; average low of 20.95^{o}C), humidity was 84.89% (average high of 94.54%; average low of 66.16%) and the average seasonal rainfall was 1557mm (weather data collected as part of this research).

A key feature of the study site for this research was a known history of where within the site three different anthropogenic disturbance types had occurred. These disturbance types were 1) selective logging (identified herein with the acronym SLR – selectively logged and now regenerating forest), 2) complete clearance due to conversion to agriculture for coffee, cacao and other subsistence crops such as banana (identified herein with the acronym CCR – completely cleared and now regenerating forest). 3) a mixed area that had historically consisted of a mosaic of small completely cleared areas used for subsistence agriculture combined with selective logging of the adjacent forest (identified herein as MXD – mixed disturbance and now regenerating forest). Major human disturbance had started ~50 years prior to the study and lasted for 30 years before systematic human disturbance activities were abandoned in the 1980s. For 30 years following abandonment the site was left to regenerate, and from 2003 the site was actively protected from further human disturbance. At the time of the study the whole area was covered by closed canopy regenerating tropical forest.

Disturbance history habitat classification

Initially the boundaries between the three different disturbance history types were identified by two of the authors visiting the site to visually inspect it, which allowed points of transition between distinct forest disturbance types to be identified based on subjective observation of forest structure. These observations were confirmed by consulting local guides who had expert local knowledge related to historic land-use of the study site. Independent of the authors’ observations, the guides were asked to point out areas of different historic land use and indicate from memory where transitions between areas of different disturbance types had been. Each approach identified consistent transitional points which were marked as the boundaries of the different disturbance histories. A systematic vegetation structure survey was then carried out to assess specific structural forest differences and confirm the subjective observations of differences in forest structure. The following seven parameters were measured: upper canopy height in meters; canopy coverage (to the nearest 5%); leaf-litter depth (to the nearest 0.5cm); the number of trees with a diameter at breast height (DBH) >10cm/100m^2; shrub layer and herb density;

and epiphyte cover, estimated using the DAFOR scale (5 = dominant, 4 = abundant, 3 = frequent, 2 = occasional and 1 = rare; Affre et al. 2010).

In order to compare structural features between disturbance areas, average values for each structural habitat parameter were calculated per butterfly trap location from ten sample points surrounding each trap site in CCR, MXD and SLR areas. A multivariate factor analysis was then performed using Minitab analysis software (v14.12) in order to detect if there was separation of disturbance types by their specific habitat variables (Beirne et al. 2013; Hilje & Aide 2012). Factor scores were sorted both without and with rotation (quartimax) in order to provide the most logical representation of the data visually.

The factor analysis resulted in the original variables reducing to three factors with an eigenvalue greater than one (see Appendix 4A for factor analysis results). These three factors represent 72.7% of variation in the original data set (Factors 1, 2 and 3 contained 33%, 24% and 15.6% of variation respectively). Factor 1 loaded positively with a denser herb layer, shrub layer and increased epiphyte cover and negatively with leaf litter depth. Factor 2 loaded positively with epiphyte cover, canopy height and canopy cover and negatively with leaf litter depth. Factor 3 loaded negatively with the number of trees>10cm DBH. Factor scores were plotted against each other in a correlation matrix (see Appendix 4B) in order to demonstrate the structural differences between the habitat disturbance type classifications. As illustrated in Appendix 4B, the SLR and CCR survey locations separated out with no overlap when factors 1 and 2 were plotted against one another, whilst MXD sampling locations lay between CCR and SLR. The factor analyses demonstrated that even after 30 years of regeneration the SLR forest had a higher forest canopy and greater canopy cover with an increased occurrence of epiphytes, whereas CCR habitat was characterised by the opposite trends and a deeper leaf litter.

Study approach, smapling design and sampling effort

The study was planned to focus on the potential for biodiversity to utilise different vertical levels of anthropogenic-disturbed rainforest following a long period of regeneration. The initiation of a human designed experiment waiting 30 years before investigating the impacts was deemed impractical. As such, a natural (or measurative) experiment approach (Hurlbert 1984) was used. An appropriate regenerating rainforest study site was chosen where historic human disturbance had varied across a relatively small area (~800 ha). Studying within site differences in biodiversity distribution across this small spatial scale were used to avoid confounding effects of large scale drivers of spatial auto-correlation, such as climatic differences or differences in physical geography. In addition, we were confident that butterflies were not hindered in dispersing across the site, as there were no geographic barriers (such as large rivers or mountain ranges). We predicted that in the absence of any effects of differences in historic disturbance ("treatment"), biodiversity would be distributed randomly across the site. Therefore, if human disturbance history differentially impacted on biodiversity distribution patterns we would see systematic differences at different vertical levels and across areas once subjected to different forms of disturbance. To test this, butterflies were surveyed across 18 sampling locations, six in each of the three regenerating disturbance areas (Figure 1). All survey locations were situated a minimum distance of 200m apart to ensure sampling independence (Barlow et al. 2007a; Lewis 2001).

Three traps were suspended at each location to represent three vertical zones of forest structure: terrestrial zone (1-2m), understorey zone (6-10m) and canopy zone (>16m). At each of the 18 locations two bait types (rotten banana and rotten fish) were used. Rotten fish bait was used in addition to the more widely used rotting fruit bait because fish bait has been shown to capture a greater number of individuals and provide wider coverage of the butterfly community (Austin & Riley 1995; Hall & Willmott 2000; Sourakov & Emmel 1995). Total trapping effort over a 12 month period was 2160 trap days (April 2013 – March 2014; 720 trap days per disturbance type). This overall sampling effort consisted of 120 trap days (40 trap days from each of the three vertical zones) at each individual sampling location. At each sampling location the traps in the three vertical zones were set to collect simultaneously with each trap operated twice in each of four three month periods, once with banana and once with fish bait. Each of these trapping sessions lasted five days: accumulating to four sessions with banana (20 days) and four sessions with fish bait (20 days) for each trap over the 12 months.

Field survey methodology

Butterflies were surveyed using Van Someren-Rydon traps (Hughes et al. 1998). These simple cylindrical baited traps have been used successfully by previous studies on butterflies in the tropics (Hughes et al. 1998; DeVries & Walla 2001; Armstrong 2010; Hill et al. 2001). Traps were checked daily between 0900 and 1500 with a randomized site visiting sequence to avoid any systematic bias (Barlow et al. 2007a). Bait was replaced every day to ensure similar bait freshness across all sites (DeVries & Walla 2001; Hughes et al. 1998). The number of butterflies of each species at each site was recorded; individuals large enough and without transparent wings were marked with a non-toxic silver marker, to allow the identification of recaptures, which were excluded from the analysis in order to avoid double counting within sessions. Species moving between strata were also excluded after a first trap encounter within a session, to avoid pseudoreplication in recounting individuals (in total 1.43% of records were excuded recaptures). The rotting banana bait was prepared following the methods by DeVries et al. (1999) and the rotten fish bait was prepared a week prior to sampling (Austin and Riley 1995; Hughes et al. 1998). Butterflies were identified using field plates from The Field Museum (2014) and the development of an internal identification guide, in which species codes were assigned to any species that were not immediately identifiable. Photographs were taken to aid further identification and verification once out of the field by experts from the Department of Entomology at the Natural History Museum of San Marcos in Lima. All individuals were later released.

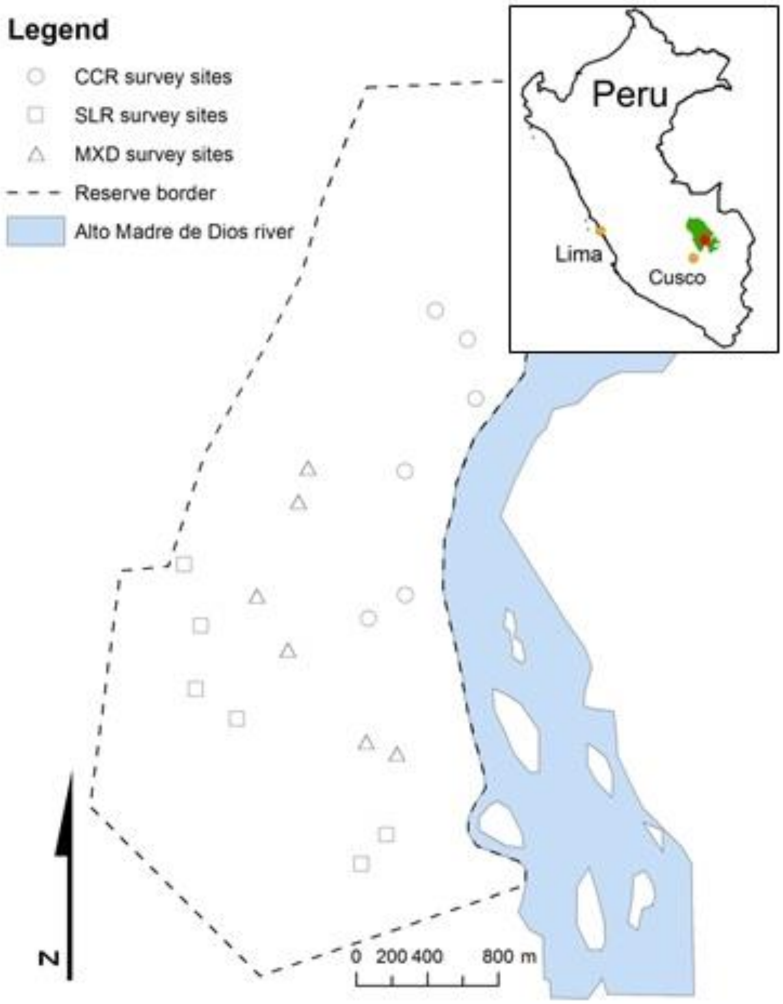


Figure 1. The context of the study site (as indicated by a red circle) in the Manu Biosphere Reserve in SE Peru, and the study site highlighting butterfly sampling locations.

Analyses methodologies

In order to investigate differences in biodiversity patterns at different vertical levels and in forest with differences in disturbance history we assessed species richness, species diversity, and community structure and composition (Bruton et al. 2013; Hu et al. 2013). To assess species richness levels and the extent to which our effort had detected as many species as were likely to be found within each disturbance area, we plotted rarefaction curves for each sampling methodology using the Rich package (Rossi 2011) and presented these graphically along with 84% confidence intervals, using program R (R Core Team 2012). Where sampling effort detected fewer individuals in one area, we extrapolated the lower lying curve towards an equal number of individuals for a clearer comparison of where our observed richness accumulation curves would have projected given detection of an even number of individuals (Colwell et al.

2012). The following estimators of species richness, which have previously been utilised for butterflies (Koh 2008; Posa & Sodhi 2006) were calculated: Abundance-based Coverage Estimator (ACE), Incidence-based Coverage Estimator (ICE), Chao1 estimator, Chao2 estimator, Jack1 estimator, Jack2 estimator and Michaelis–Menten Means estimator (Gotelli & Colwell 2011). The average of these estimators was calculated for each habitat as the understanding of their relative performance is still poorly unknown (Reese et al. 2014).

To ensure comparability with previous studies on butterflies, species diversity was assessed using the Shannon diversity index (Seshadri 2014; Trimble & van Aarde 2014). Repeating the analyses using Fisher's Alpha, Simpson's and Shannon Exponential diversity indices did not change the pattern of results and are therefore not presented. All richness and diversity estimators were calculated using EstimateS software (Colwell 2013). Species abundance was recorded as the number of individuals caught in each trap per 40 trapping days.

Community structure was compared by producing dominance-diversity (Whittaker) plots using the vegan package (Oksanen et al. 2011) in program R (R Core Team 2012). Such plots compare the evenness of a community, with shallow curves representing a community of many species of similar abundance, whereas steep curves represent a skewed assemblage with one or more species in substantially higher abundance than others. Significant differences in slope, and therefore significant differences in community evenness, were assessed through the use of a linear model with log relative abundance of species as the response term, and an interaction between species rank and disturbance history or vertical zone as continuous and categorical fixed effects respectively (Oksanen et al. 2011; vegan package, function 'rad.zipfbrot'; see Beirne et al. 2013). Results are reported as ΔG, which corresponds to absolute change in gradient between disturbance areas and vertical zones, whereby more negative values denote steeper curves and thus less even assemblages (Beirne et al. 2013).

As this was a natural experiment and not a human designed one, it was not possible to intersperse independent sampling locations to guarantee treatment replication (in addition to the sampling replication described). It was recently highlighted that nearly all tropical forest studies investigating effects of human disturbance on biodiversity due to logging have the potential for pseudo-replication (Ramage et al. 2013; in agreement with Hurlbert 1984, Heffner et al. 1996). However, Ramage et al. (2013) also point out that whilst interspersion is a desired goal where human designed experiments are practical, natural experiments still provide useful scientific evidence if potential causes of spatial variation (other than the potential "treatment" effect) are investigated and controlled for where necessary. Pseudo-replication only occurs if the results are over generalised (Ramage et al. 2013). We agree with Ramage (2013) and Hulbert (1984) and therefore included additional environmental data as control variables in our analysis, utilised spatial statistics to confirm the absence of spatial auto-correlation (that might create pseudo-replication) and finally, considered the likelihood of potential alternative inferences from the results. Therefore, in order to investigate if differences in average estimated species richness, Shannon diversity and abundance between SLR, CCR and MXD survey locations and across vertical zones were significant a series of linear models were carried out. Where both habitat and vertical zone were found to be significant, an interaction between 'disturbance history' and 'vertical zone' was included. Having excluded most potential large scale causes of spatial auto-correlation by choice of a small scale study area, we considered if there were any consistent local scale differences between the sampling locations. As a result of a general trend for altitude to increase north to south and distance from the river to increase east to west, the local environmental variables 'altitude' and 'distance to the main river' of each sampling location were included as covariates to control for any potential spatial auto-correlation that might make either of these variables confounding effects. We utilised a dredge of the global model, followed by a top model averaging approach (on models where ΔAICc <2), to determine relative variable importance. Finally, to confirm that any potential spatial auto-correlation between survey locations had been controlled for in the analysis, a Moran's I test was carried out in program R (R Core Team 2012) on the residuals of each model (ape package; Paradis et al. 2004).

Differences in fruit and carrion-feeding communites

Individual records and the number of species detected overall, were calculated for both fruit-baited and carrion-baited traps, and stratum specialist species (*i.e.* consisting of; 1. specialist species, determined as those significantly more abundant in a particular stratum, confirmed by an ANOVA test, and 2. species that were exclusively caught within a single stratum; see Aduse-Poku et al. 2012) were calculated for each vertical strata.In order to investigate if bait type was significatly correlated with the observed weekly sample species richness and abundance Generalized Linear Mixed Models (GLMM's; with a negative binomial distribution as overdispersion was detected as a result of zero-inflation; and using a log link function) were carried out. To account for the repeat measures across sampling locations, sampling location identification was included as a random effect and the models were compared with the null model containing only this random effect. We then ran a further set of GLMM's to investigate disturbance history and vertical strata, for fruit and fish baited traps separately (once again with sampling location identification included as a random effect and the models were compared with null model and model AICc values compared to assess the top-model; with a ΔAICc<2). Finally, to confirm that any potential spatial auto-correlation between survey locations had been controlled for in the analysis, a Moran's I test was carried out in program R (R Core Team 2012) on the residuals of each preferred model to test if there was any effect from spatial auto-correlation that might lead to pseudo-replication (ape package; Paradis et al. 2004).

Non-metric multidimensional scaling (NMDS; using the Bray-Curtis similarity measure) was conducted to determine differences in community composition for fruit and carrion-feeding butterfly communities in each disturbance area, for community composition differences between disturbance areas for fruit and carrion-feeding communities separately, and finally to assess community composition differences between vertical strata for fruit and carrion-feeding butterflies separately. All stress values were relatively low (ranging between 0.11 to 0.25) and so were displayed within just two dimensions. To assess the statistical significance of observed differences in assemblage composition between different disturbance areas we conducted analysis of similarities tests (ANOSIM; using 999 permutations), which is analogous to an analysis of variance and tests for differences between *a priori*-defined groups of community samples (here, disturbance area) based on a (dis)similarity matrix (see Helbig-Bonitz et al. 2015). NMDS ordinations and ANOSIM tests were carried out in the vegan package (Oksanen et al. 2011), in program R (R Core Team 2012).

Results

Species Richness

In total 229 species of butterfly were detected (see Appendix 4G), with a total of 5219 individual records (Table 1). Fish baited traps constituted almost 60% of the records with 3127 individuals recorded and 2092 individuals recorded in banana-baited traps. Species richness was highest in the understorey community (193 species) and decreased with sampling height, with 167 and 115 species detected in the midstorey and canopy strata respectively. The greatest number of stratum specialist species overall (see Aduse-Poku et al. 2012) was encountered within the understorey (93 species; 48% of species encountered in the understorey), followed by the midstorey (30 species; 18% of species encountered within the midstorey), and with the canopy stratum containing only 11 stratum specialist species (just 10% of species encountered within the canopy). When combining values for the midstorey and canopy, 41 stratum specialist species were detected above the understorey, representing 31% of stratum specialist species detected within the study overall (134 stratum specialist species). Results were similar when considering fruit and carrion-baited trap data separately, but with a slightly higher percentage of stratum specialist species within the canopy for fruit-baited traps (17%), compared with carrion-baited traps (11%).

Table 1 – Summary table; individual records and the number of species detected overall, and for both fruit-baited and carrion-baited traps separately. Stratum specialist species are those that are significantly (ANOVA test) more abundant in a particular stratum (Specialist species) or were exclusively caught in one of the strata (as in Aduse-Poku et al. 2012).

	Fruit-baited traps			Carrion-baited traps			Overall (Fruit + Carrion)		
	Understorey	Midstorey	Canopy	Understorey	Midstorey	Canopy	Understorey	Midstorey	Canopy

Number of records	1198	556	338	1788	905	434	2986	1461	772
Species richness	138	115	72	170	145	99	193	167	115
Specialist species	21	4	4	38	5	5	50	10	8
Exclusively in one stratum	44	17	8	44	24	6	43	20	3
Stratum specialist species	65	21	12	82	29	11	93	30	11
Percentage (%) of stratum specialists	47	18	17	48	20	11	48	18	10

Overall, observed species richness was a high proportion of the averaged estimated species richness (74% ±2.43%; ranging between 57-88%). In contrast to some previous studies, species richness was highest in the terrestrial community (193 species) and decreased with sampling height, with 167 and 115 species detected in the understorey and canopy zones respectively. Observed species richness was lowest in forest that had regenerated after a history of disturbance due to complete clearance, compared to forest that had regenerated after disturbance by selective logging, with intermediate species richness levels observed in the mixed disturbance history type (Table 2).

Table 2. Capture frequency, survey effort, observed, extrapolated and estimated species richness and sample completeness per disturbance history. O = Overall community, T = Terrestrial community, U = Understorey community and C = Canopy community.

Strata	Past disturbance area	Number of individuals recorded	Survey effort: samples	Observed species	Extrapolated species[a]	Species richness estimates									% average estimated species richness compared to SLR	Coverage (%)[c]	Completeness (%)[d]
						ACE	*ICE*	*Chao1*	*Chao2*	*Jacknife1*	*Jacknife2*	*Bootstrap*	*MMMean*	Average[b]			
O	SLR	2399	720	207	207	230	233	233	236	248	262	227	223	237		88	90
O	CCR	1215	720	145	163	165	172	163	167	181	190	163	165	171	72	85	63
O	MXD	1605	720	176	197	217	220	237	238	227	257	199	196	224	95	79	77
O	Total	5219	2160	229													
T	SLR	1299	240	168	168	211	220	203	214	221	245	192	196	213		79	87
T	CCR	883	240	117	148	143	153	141	152	155	174	134	137	149	70	79	61
T	MXD	804	240	116	159	153	156	170	171	158	184	134	141	158	74	73	60
T	Total	2986	720	193													
U	SLR	701	240	127	127	171	176	179	193	175	207	148	163	176		72	76
U	CCR	249	240	80	111	119	130	112	118	117	136	96	121	118	67	68	48
U	MXD	511	240	115	130	155	174	147	170	163	190	136	165	162	92	71	69
U	Total	1461	720	167													
C	SLR	399	240	86	86	116	119	112	117	120	137	101	113	117		74	75
C	CCR	83	240	35	51	60	61	58	51	53	61	43	103	61	53	57	30
C	MXD	290	240	77	88	119	117	121	113	111	129	92	114	115	98	67	67
C	Total	772	720	115													

NB: [a] Number of species estimated when curves extrapolated to the same number of individuals; [b] Mean estimated species richness - 'classic Chao values were used in cases where CV>0.5; [c] Sampling coverage defined as: (observed species richness/average estimated species richness)*100; [d] Number of species observed as a percentage of combined species across all habitats.

Extrapolated rarefaction curves based on observed species richness (Figure 2) show similar patterns both overall (with 207 species in SLR v 145 species in CCR), and in each sampling height separately (terrestrial butterfly community, 168 species in SLR v 117 species in CCR; understorey butterfly community, 127 species in SLR v 80 species in CCR; canopy butterfly community, 86 species in SLR v 35 species in CCR). For all but the understorey community the non-overlapping 84% confidence intervals suggest these differences are significant.

Average estimated terrestrial species richness was highest in the forest regenerating after selective logging (an average estimated 213 ±11.56 species) and 30% lower in forest regenerating after complete clearance (149 ±8.82

species). For the understorey butterfly community the difference between disturbance types increased slightly to 33%, with average estimated understorey butterfly species richness higher in SLR (an average estimated 176 ±12.33 species) than CCR (118 ±8.11 species). The canopy understorey community showed a much larger difference between disturbance types with a drop of 47% between SLR and CCR. The average estimated canopy butterfly species richness was higher in SLR, with an average estimated 117 ±6.88 species in SLR and just 61 ±12.54 species in CCR.

The linear modelling showed that these differences in estimated species richness patterns were significant. Both vertical level and historic disturbance history type as key predictors of butterfly species richness across the study site, each showing full support with relative variable importance = 1 within top models where ΔAICc <2; see Table 3. There was no evidence to suggest that there was an interaction between strata and disturbance type or that there was any influence from distance to the main river (neither variable within the top models where ΔAICc <2) and only weak support that increasing altitude had a negative effect on species richness (relative variable importance = 0.38 within the top models where ΔAICc <2; see Table 3). Testing of the model residuals showed no evidence of spatial auto-correlation between samples with a very low and non-significant observed Moran's I value of -0.04, s.d. = 0.02, p = 0.42 (see Appendix 4F).

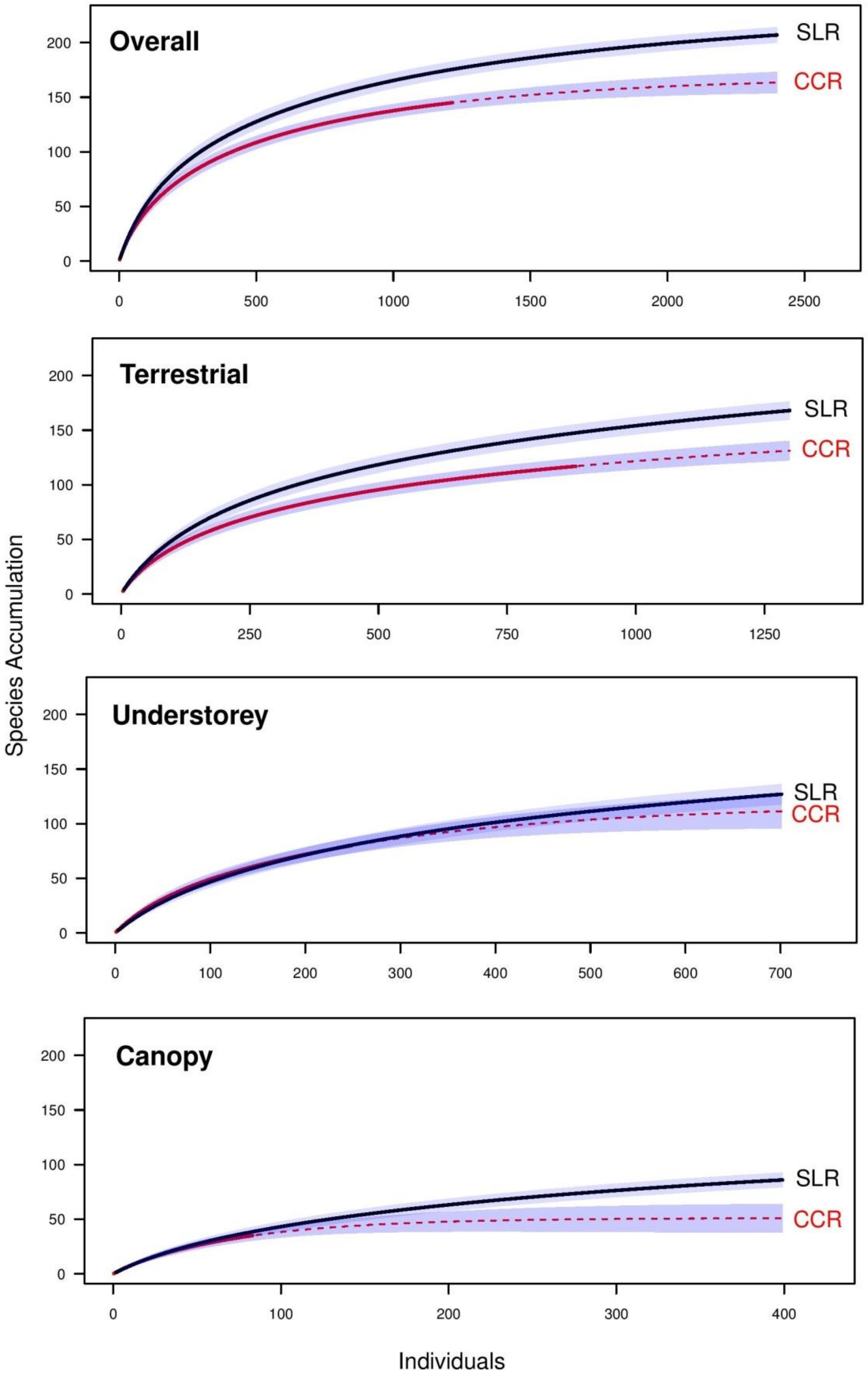


Figure 2. Butterfly species richness of regenerating rainforest with different disturbance histories. Solid lines represent the observed number of individuals recorded and dashed lines represent extrapolated species richness. The grey shades represent 84% confidence intervals. Mean species accumulation lines falling outside of this envelope are statistically significant. (a) the overall community, (b) the terrestrial community, (c) the understorey community and (d) the canopy community

Table 3. Candidate models explaining variation in estimated species richness, Shannon diversity and abundance of butterflies, ranked according to increasing value of delta AICc. See Appendix 4E for top model averaged co-efficients. df= degrees of freedom; logLik = maximum log likelihood; delta AICc = AICci – AICcmin and weight = Akaike weights; + = inclusion within a given model.

Response variable	Model #	Intercept	Altitude	Disturbance history	Distance to river	Vertical zone	Disturbance history * Vertical zone	df	logLik	AICc	delta AICc	Weight
Estimated species richness	11	35.14		+		+		6	-235.2	484.2	0.0	0.340
	12	143.9	-0.21	+		+		7	-234.4	485.2	1.0	0.210
	15	37.46		+	-0.01	+		7	-234.9	486.3	2.0	0.123
	27	25.58		+		+	+	10	-230.6	486.3	2.1	0.120
	28	134.4	-0.21	+		+	+	11	-229.6	487.5	3.2	0.067
	16	136.8	-0.20	+	0.00	+		8	-234.4	487.9	3.7	0.053
	31	27.9		+	-0.01	+	+	11	-230.2	488.8	4.5	0.035
	10	-88.62	0.25			+		5	-239.2	489.6	5.4	0.023
	32	127.2	-0.20	+	0.00	+	+	12	-229.6	490.8	6.5	0.013
	13	36.32			0.02	+		5	-240.2	491.7	7.5	0.008
	14	-73.2	0.22		0.00	+		6	-239.2	492.1	7.9	0.007
	9	48.81				+		4	-243.3	495.5	11.3	0.001
Relative variable importance from top models			0.38	1		1						
Shannon diversity	27	2.188		+		+	+	10	4.4	16.3	0	0.519
	31	2.238		+	-1.70E-04	+	+	11	5.5	17.4	1.1	0.299
	28	2.727	-0.00106	+		+	+	11	4.6	19.1	2.9	0.123
	32	2.021	0.000434	+	-1.83E-04	+	+	12	5.5	20.7	4.3	0.058
	11	2.479		+		+		6	-7.8	29.3	13.1	0.001
Relative variable importance from top models				1	0.37	1	1					
Abundance	16	312.4	-0.5579	+	-0.05	+		8	-263.1	545.4	0.0	0.383
	15	33.59		+	-0.07	+		7	-264.6	545.6	0.2	0.349
	32	312.5	-0.5579	+	-0.05	+	+	12	-258.3	548.1	2.7	0.099
	31	33.69		+	-0.07	+	+	11	-260.0	548.3	2.9	0.090
	12	507.2	-0.9701	+		+		7	-266.3	549.0	3.6	0.064
	28	507.3	-0.9701	+		+	+	11	-262.0	552.3	6.9	0.012
	11	13.74		+		+		6	-271.2	556.2	10.8	0.002
Relative variable importance from top models			0.52	1	1	1						

Butterfly Diversity, Abundance and Community Evenness

Shannon diversity was found to be higher in SLR than CCR, and in SLR was higher in the terrestrial zone than understorey but not for CCR (Figure 3); MXD values (not illustrated) were intermediate. The understorey zone was more diverse than the canopy zone in both CCR and SLR.

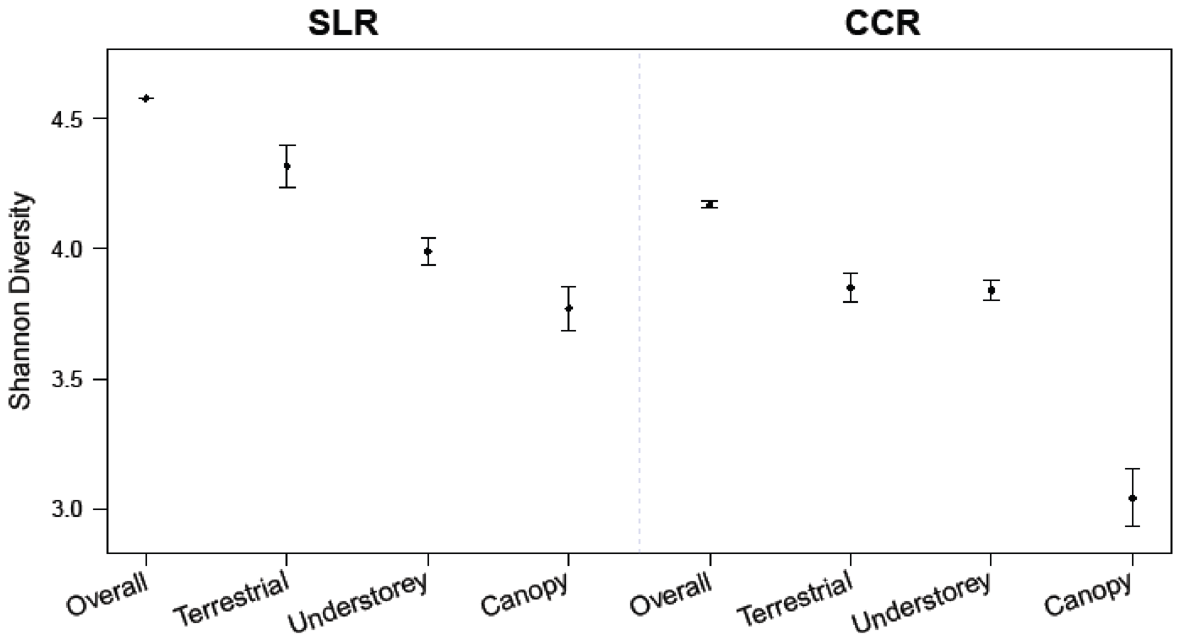


Figure 3. Shannon species diversity of overall, terrestrial, understorey and canopy strata of butterflies in regenerating rainforest with different disturbance histories. Error bars are 84% confidence intervals.

Both vertical level and disturbance history were found to predict Shannon diversity of regenerating forest butterflies within the linear models, along with evidence to suggest that diversity differed across vertical strata between habitats (each showing full support for relative variable importance = 1 within the top models where ΔAICc <2; see Table 3). Shannon diversity of the canopy was therefore affected to a greater extent than terrestrial diversity by differing historic human disturbance. There was no influence from altitude upon Shannon diversity (not represented within the top models where ΔAICc <2) and only weak support for any effect from distance to the main river (relative variable importance = 0.37 within the top models where ΔAICc <2; see Table 3). Testing of the model residuals showed no evidence of spatial auto-correlation with a very low and non-significant observed Moran's I value of -0.02, s.d. = 0.02, p = 0.93 (see Appendix 4F).

Overall butterfly abundance was found to be higher in SLR than CCR, being highest in the terrestrial zone and lowest in the canopy (the understorey zone was intermediate; see Appendix 4C). Results from the linear models showed that vertical zone, disturbance history and distance from the river were found to influence abundance (each showing full support for relative variable importance = 1 within the top models where ΔAICc <2; see Table 3). Although abundance was higher in SLR habitat there was no evidence to suggest that butterfly abundance differed across strata between habitats or that there was any influence from altitude (not represented within the top models where ΔAICc <2). Testing of the model residuals showed no evidence of spatial auto-correlation with a very low and non-significant observed Moran's I value of -0.04, s.d. = 0.02, p = 0.46 (Appendix 4F).

Dominance-diversity plots between disturbance histories demonstrate that SLR supports a significantly more even community assemblage than CCR (Appendix 4D) for overall (ΔG=−0.005, p=<0.001), terrestrial (ΔG=−0.008, p=<0.001), understorey (ΔG =−0.006, p=<0.001) and canopy zones (ΔG=−0.02, p=<0.001).

Dominance-diversity plots between SLR vertical layers demonstrate that the terrestrial layer supports a significantly more even community assemblage than both understorey (ΔG=−0.003, p=<0.001) and canopy zones (ΔG=−0.014, p=<0.001), and that the understorey supports a significantly more even community assemblage than the canopy zone (Figure 4a; ΔG=−0.011, p=<0.001). Dominance-diversity plots between CCR zones demonstrate that the terrestrial zone supports a significantly more even assemblage than the canopy zone (ΔG=−0.03, p=<0.001) and that the understorey also supports a significantly more even community assemblage than the canopy (ΔG=−0.03, p=<0.001) but as with the Shannon diversity results there is no significant difference between the community evenness of the terrestrial and understorey zones (Figure 4b; ΔG=−0.001, p=0.47).

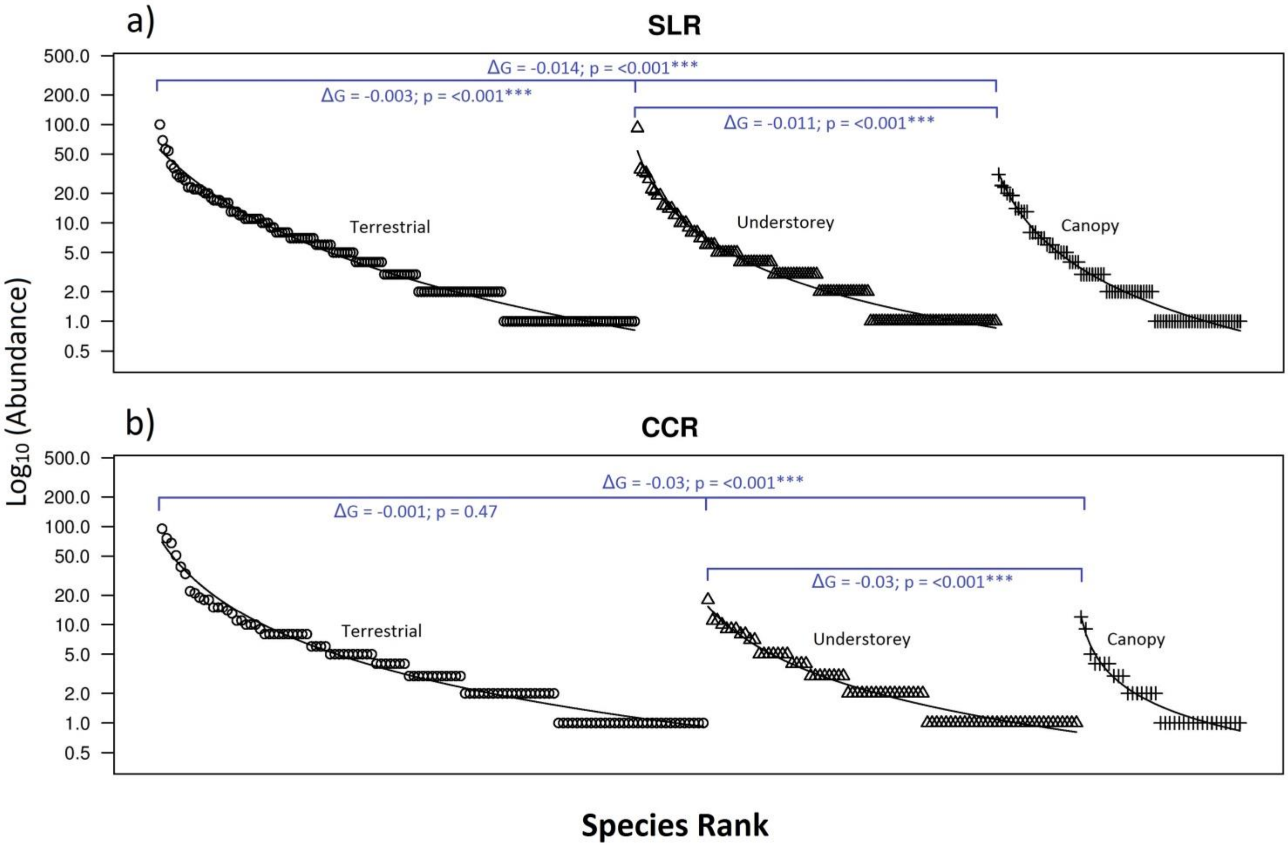


Figure 4. Dominance-diversity (Whittaker) plots for terrestrial, understorey and canopy butterfly communities in regenerating rainforest with different disturbance histories; (a) SLR and (b) CCR. Species are represented by points. For each habitat the relative abundance of each species (ni/N) was plotted on a logarithmic scale against the species rank ordered from most to least abundant. O = Terrestrial, Δ = Understorey and + = Canopy. Linear models were used to determine if the slopes of terrestrial, understorey and canopy communities were significantly different, where ΔG denotes to absolute change in gradient from the predicted line and the symbol denote the level of significance of the deviation where *** = <0.001, ** = <0.01, * = <0.05 and blank = not significant.

Bait Types and Community Compositions

Bait type was found to be a significant predictor of both species abundance and richness at the weekly sample level (ANOVA's against the null models were displayed p-value<0.001 for both observed species richness and abundance; see Appendix 4H for model outputs), with a greater number of species and individulas captured using carrion-bait (Figure 5).

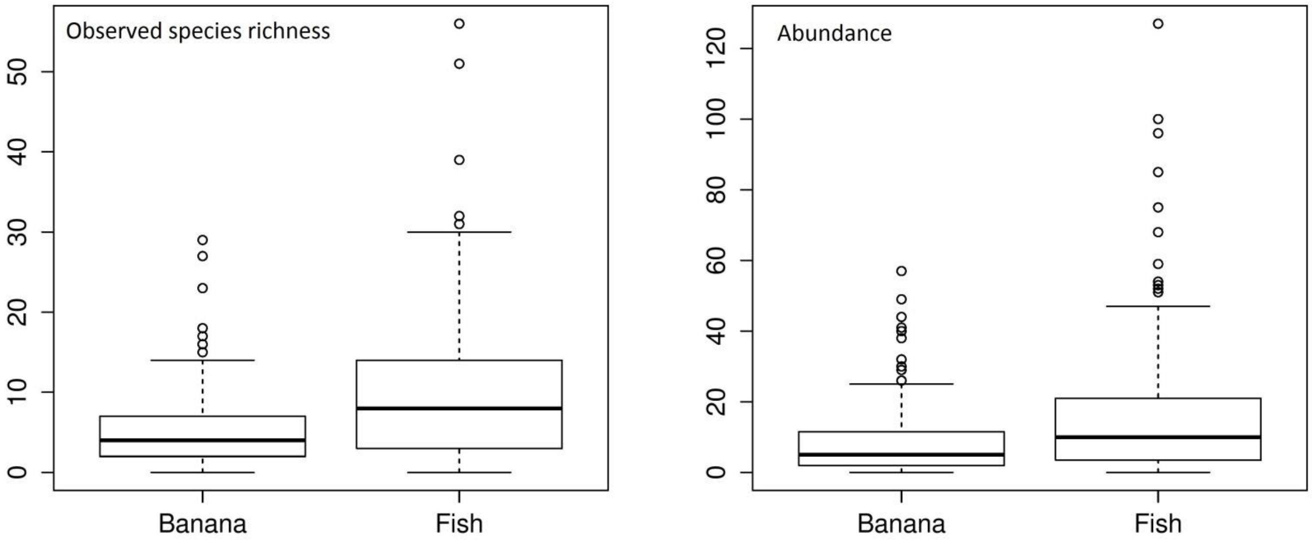


Figure 5. Box plots of observed species richness and abundance for different bait types.

The community composition analysis from NMDS plots and the associated ANOSIM analysis (Figure 6) showed that community composition was significantly different between fruit and carrion-baited traps for all disturbance areas. The smaller ellipses (set at p=0.05) and tighter grouping of carrion-baited sites suggest that carrion-communities are more homogenous than for fruit-baited trap sites (displaying larger ellipses and spread of sites). The lowest degree of dissimilarity was observed in CCR habitat (although still significant; R=0.47, p=0.005) and the highest in MXD habitat (R=0.84, p=0.004).

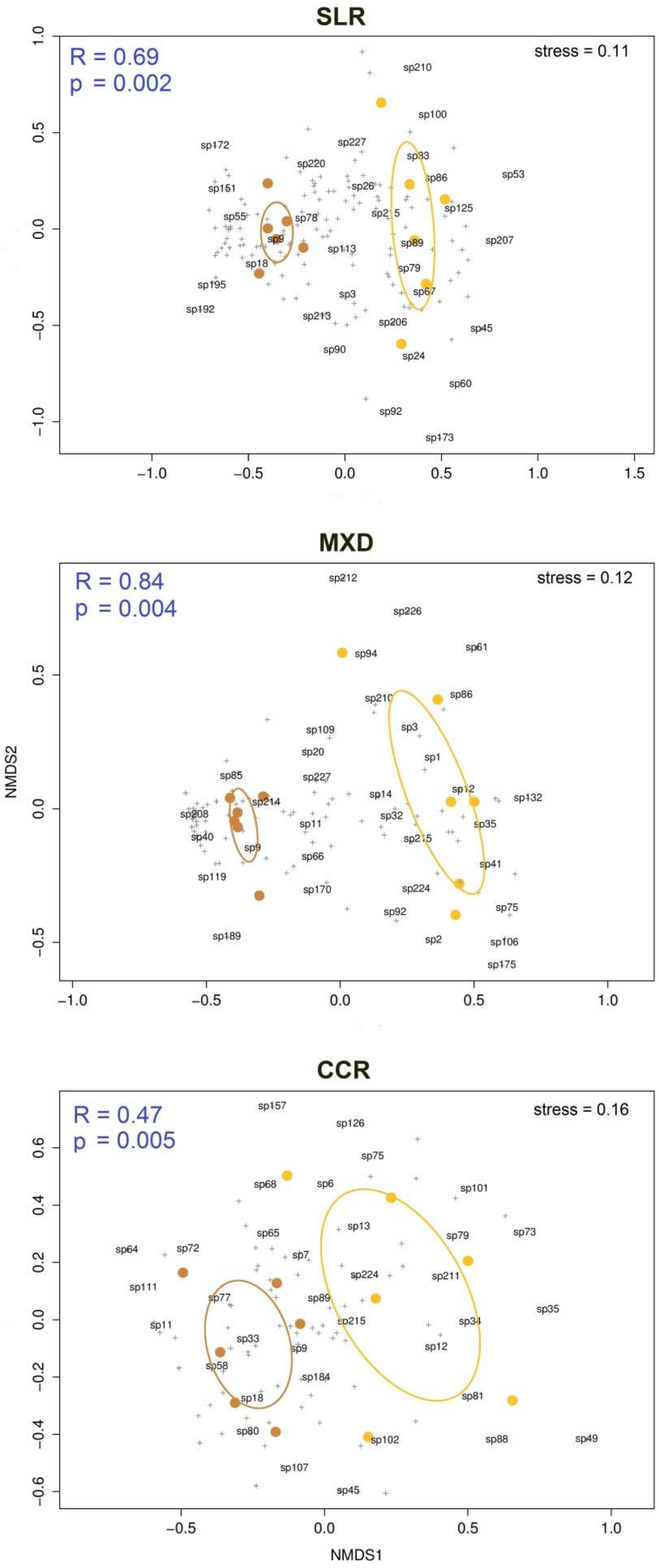


Figure 6. NMDS plots (species codes presented, with priority for those most abundant where points overlap) for communities captured using different baited traps; fish-baited traps in brown and banana-baited traps in yellow, across each disturbance area.

The GLMM results for both fruit and banana-baited traps showed that both vertical strata and disturbance history were significant predictors of both observed butterfly species richness and abundance (ANOVA's against the null models were displayed p-value<0.001 for both observed species richness and abundance; see Appendix 4I for model outputs and model selection tables). Although significant for all levels and predictors, the fish-baited traps appeared to show a greater effect from disturbance history, with significantly more species and more individuals being captured in SLR, an intermediate level in MXD and the lowest levels in CCR habitat (Figure 7). The greatest number of species and individuals were encountered in the terrestrial traps, an intermediate level in understorey traps and the lowest levels were in the canopy traps. Testing of model residuals showed no evidence of spatial auto-correlation between samples with very low correlations (range from -0.002 to 0.006) and non-significant (range from p=0.07 to 0.68) observed Moran's I values.

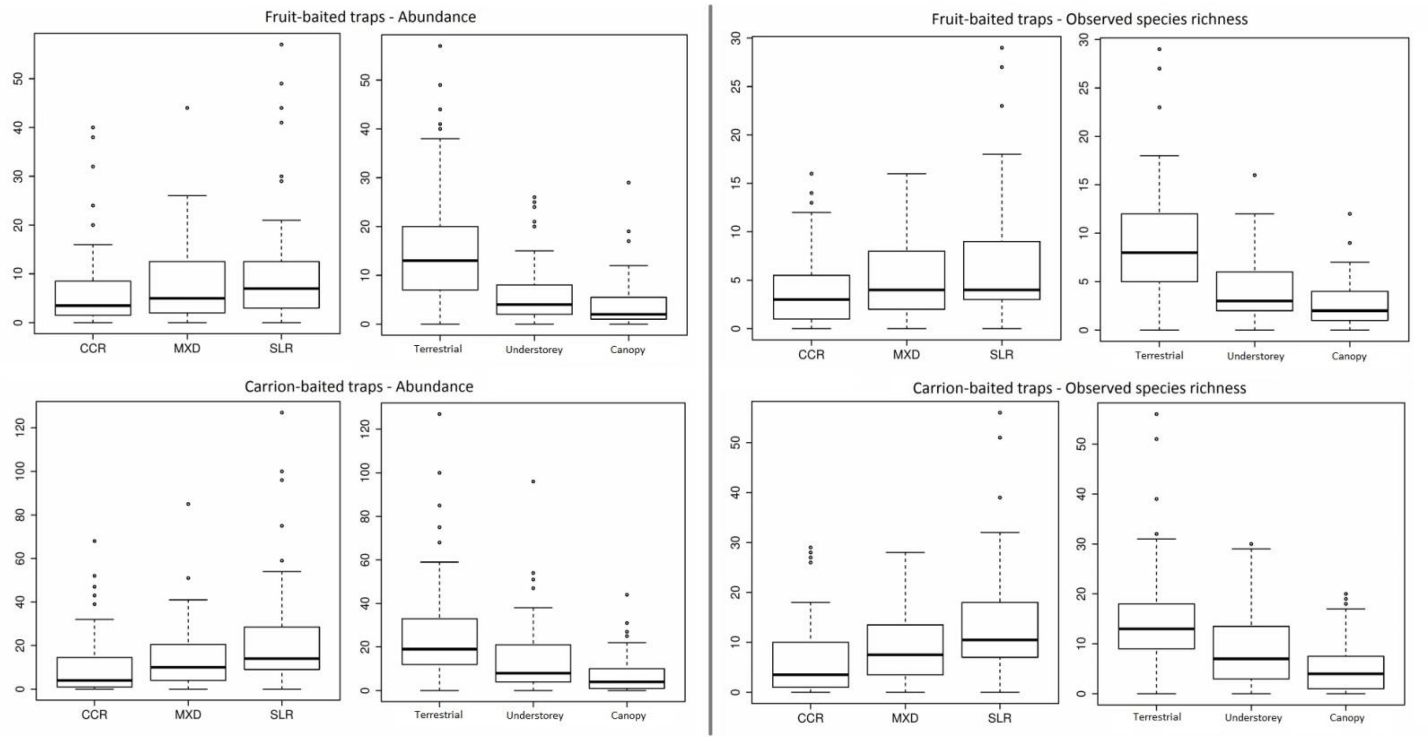


Figure 7. Box plots of observed species richness and abundance for both bait types; with disturbance history and vertical strata as predictors.

The community composition analysis from NMDS plots and the associated ANOSIM analysis showed that community composition was significantly different between disturbance areas for all three vertical strata when using carrion-bait, but only within the canopy for fruit-baited traps (Figure 8). The greatest degree of dissimilarity was observed in the canopy for both fruit and carrion-baited traps (R=0.39, p=0.003 and R=0.39, p=0.003 respectively).

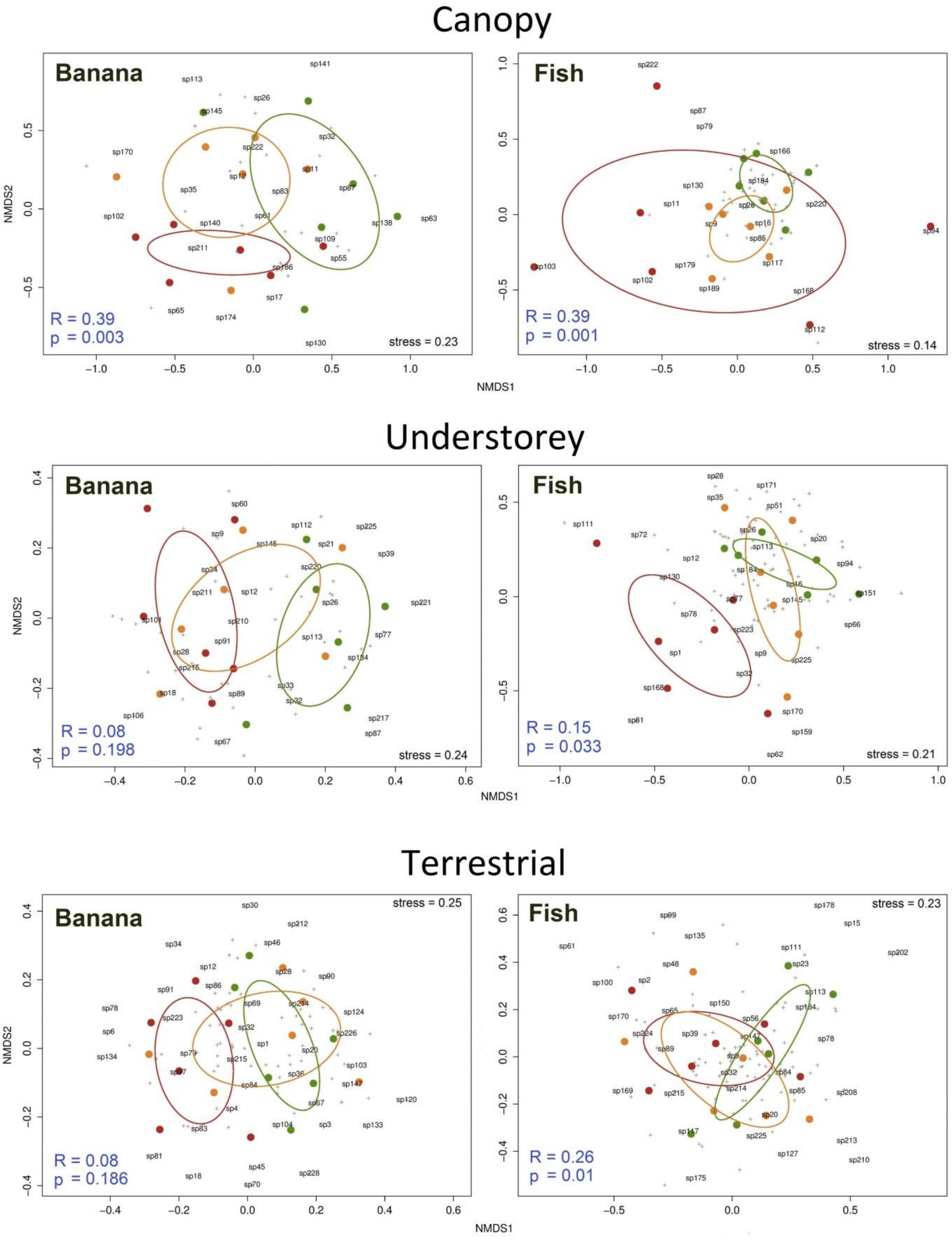


Figure 8. NMDS plots (species codes presented, with priority for those most abundant where points overlap) for communities captured in different disturbance areas; red circles=CCR, orange circles=MXD and green circles=SLR sampling locations; for each vertical strata and different bait types.

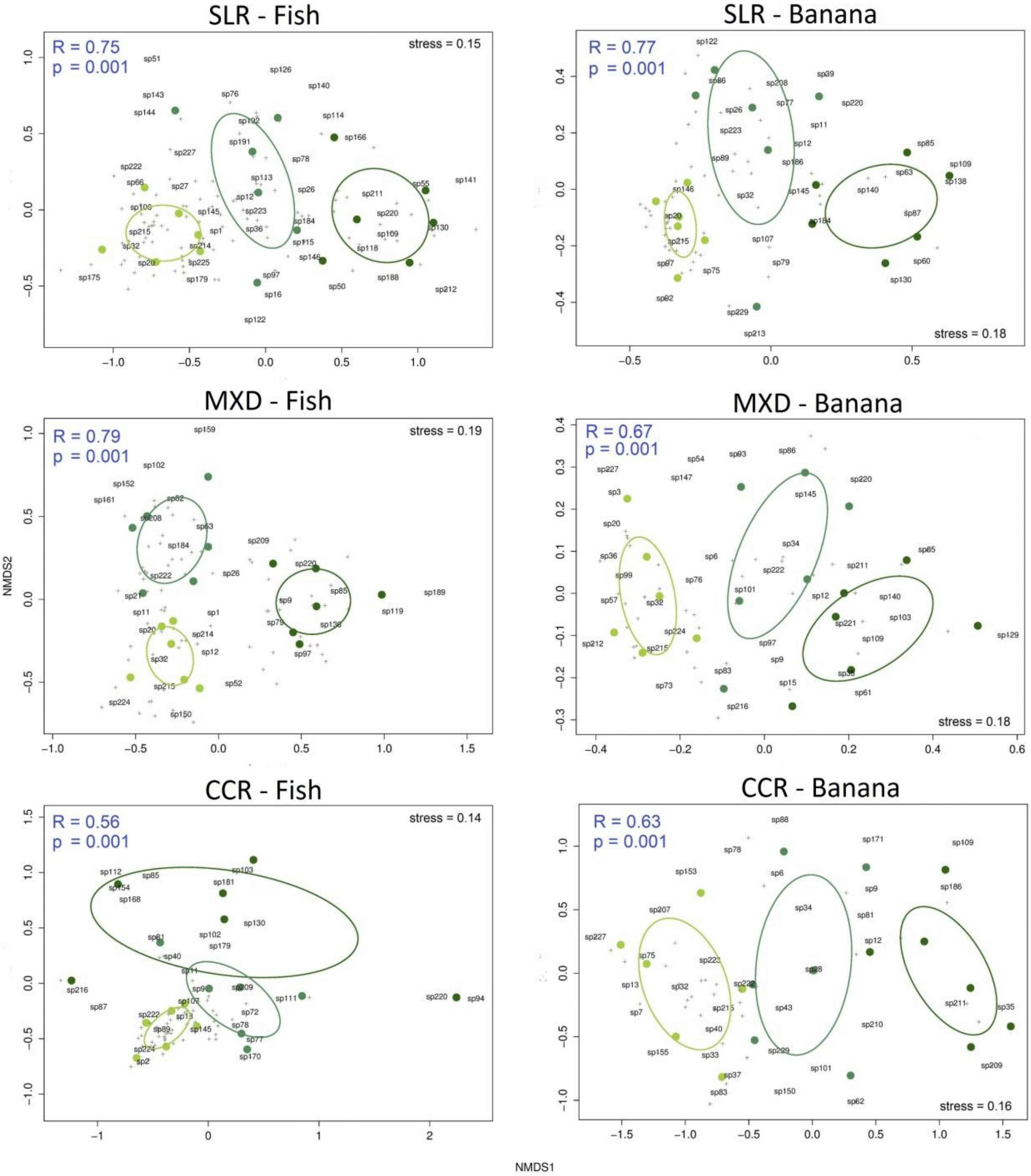


Figure 9. NMDS plots (species codes presented, with priority for those most abundant where points overlap) for communities captured in different vertical strata; light green circles=terrestrial traps, mid-gree circles=understorey traps and dark green circles=canopy traps; for each disturbance area and different bait types.

The community composition analysis from NMDS plots and the associated ANOSIM analysis also showed that community composition was significantly different between vertical strata for all disturbance areas when using both carrion and fruit-baited traps (Figure 9). The lowest degree of dissimilarity (although still significantly different) was observed in the CCR disturbance area for both carrion and fruit-baited traps ($R=0.56$, $p=0.001$ and $R=0.63$, $p=0.001$ respectively). The ΔR for fish-baited traps between SLR and CCR was 0.19 but for fruit-baited traps, 0.13, suggesting a greater disruption to community structure for the carrion-feeding butterfly community.

Discussion

Our results show that butterfly communities within a regenerating tropical forest displayed many marked differences between vertical strata, with species richness, species diversity, species abundance and community structure and composition all differing significantly. The terrestrial community was the most biodiverse, followed by the understorey and finally, the canopy community displayed the lowest species richness, diversity and abundance. The canopy also displayed a less evenly balanced community structure and greatest degree of difference in community composition for both fruit and carrion-feeding butterflies. Although more stratum specialist species were found at the terrestrial level, 31% were detected above the ground (within the midstorey and the canopy), and would therefore have been either underrepresented, or for some species, undetected had only an understorey assessment been carried out. Further, we show for the first time for any group of rainforest biodiversity, that different vertical zones within the same rainforest responded differently to areas differing in historic human disturbance. We found that differences were most notable within the canopy. Comparing forest regenerating after only selective logging (SLR) with forest regenerating after complete clearance (CCR) showed that there was a 17% greater reduction in canopy species richness in CCR compared with the terrestrial community, and significant differences in species diversity, species abundance and community structure. Our results also show for the first time that even long term regeneration (over the course of 30 years) was insufficient to erase differences in butterfly biodiversity linked to different types of human disturbance.

The general pattern of biodiversity differences between vertical levels in this study showed that the butterfly fauna was greatest terrestrially, followed by the understorey and finally, the canopy; a result in contrast with DeVries et al. (1997) and Ribeiro and Freitas (2012). Although DeVries et al. (1997) found estimated canopy species richness to be highest, DeVries and Walla (2001) subsequently showed that accumulation of species was faster in the canopy over short-term assessments but that terrestrial communities displayed higher species richness given longer-term sampling. As such, long-term studies like ours, which account for annual variation, should provide more complete outcomes related to lepidopteran biodiversity across strata (Barlow et al. 2007a; Ribeiro et al. 2015). We therefore conclude that the pattern we show of higher butterfly species richness in the terrestrial zone is unlikely to be driven by seasonal difference. Further, the survey coverage within this study overall was very high with 84% (±2.65) of estimated species detected over 2160 trap-days; higher than many previous studies including for example the detailed study by Ribeiro et al. (2015), who found that 1435 trap-days in Central Amazonian forest detected 74% of butterfly fauna. We also show that within vertical levels (720 trap-days per level) coverage was high, with 77% (±2.0) for the terrestrial community, 70.33% (±1.2) for the understorey community and 66% (±4.93) for the canopy community. Although this suggests that different survey effort may be required in order to equally assess biodiversity patterns between vertical levels, coverage was still high for each vertical level within this study, and it is therefore unlikely that our results were driven by insufficient survey effort.

Our results also showed that bait type was an important predictor of both observed species richness and abundance. Rotten fish bait has previously been shown to capture a greater number of individuals and provide wider coverage of the butterfly community (Austin & Riley 1995; Hall & Willmott 2000; Sourakov & Emmel 1995), but despite this, rotten fruit bait is more commonly and widely used in studies of tropical butterflies (Barlow et al. 2007a; DeVries & Walla 2001; Fermon et al. 2005). In addition to detecting a greater number of individuals and greater observed species richness at the sample level, the carrion-bait butterfly community displayed a greater sensitivity to habitat disturbance in terms of composition. As such, we suggest that the effects of habitat disturbance might be better detected by assessing carrion-feeding butterflies, at least in addition to the more commonly surveyed fruit-feeding Nymphalidae.

This study was deliberately designed to investigate only biodiversity differences over a small scale (~800ha) so that any differences detected could be more clearly linked to historic disturbance type and not due to differences in the landscape more generally. Over a small scale butterfly species can move easily and select between areas of different types of disturbance, so we can be confident that differences were not due to larger patters of heterogeneity that are often present in landscape ecology scale studies (Barlow et al. 2007a). Landscape studies, in which survey areas are kept spatially separate, often >10km apart (e.g. Hill et al. 1995), address questions over much larger regions and seek to include the effects of natural heterogeneity due to locality differences in climate, soil types and general topography so that these effects can be investigated. In contrast, in order to answer specific questions about differences between one type of treatment and another; as in the case of on-trail vs. off-trail (von May & Donnelly 2009), near to a road vs. far from a road (Chapter three of this thesis) or high altitude vs. lower altitude (Linden et al. 2014), a within-site scale approach of the type we adopted here is often more desirable, as it eliminates large scale drivers of heterogeneity.

Caveats and limitations

One potential difficulty of a small spatial scale is that transient species may enter adjacent treatment types temporarily (Barlow et al. 2007a). Individuals may therefore not necessarily be able to survive in a given habitat where detected but risk being recorded. However, in this study this is true of all three disturbance types and as such, should not significantly affect the detection of overall differences in biodiversity patterns between disturbance areas.As few studies have assessed the effects of habitat disturbance or vertical stratification of carrion-feeding butterflies, it makes the comparison of our findings difficult to place into a wider context for this particular group. Howeverm we did also study the fruit-feeding community, which allowed us to place our findings for this community into context, but at the same time suggest that future studies on the carrion-feeding butterflies would allow confirmation and wider generality in our findings for this specific community. Although we accounted for landscape differences, in terms of altitude and the distance to the main river, we didn not account for other features that may have had some influence on the biodiversity patterms of butterflies. For example, other topographic features, such as streams, environmental factors, such as rainfall or seasonality (Barlow et al. 2007a), might have had an influence on biodiversity patterns, but were not featured within this study. Future work should certainly investigate seasonal effects on carrion-feeding communities, as this has been found to be a significant effect in fruit-feeding Nymphalidae (Barlow et al. 2007a).

As this study used a natural experiment approach, we followed the recommendations of Ramage et al. (2013) for avoiding potential pseudo-replication problems in tropical forest ecology. This was achieved by including environmental factors in the analytical models and examining whether spatial-auto correlation of the sampling locations could be driving the biodiversity patterns detected. Our autocorrelation analysis confirmed that biodiversity patterns detected were not being driven by spatial autocorrelation. Our results therefore provide evidence that two common land uses within the cultural zone of the Manu Biosphere Reserve (and common in rainforest ecosystems more generally) display different potential to sustain levels of butterfly biodiversity, despite a significant time for natural regeneration (30 years). The forest that was once selectively logged for the removal of commercially valuable hardwood trees displayed higher levels of biodiversity than forests that were once cleared for agriculture. Even small changes in rainforest vegetation structure have been shown to create significant changes to biodiversity (Cortés-Gómez et al. 2013). Considering that butterflies are known to be sensitive to forest disturbance (Hamer et al. 2003), largely through the association with specific food plants (Horner-Devine et al. 2003), it seems likely that this relates to the significant difference in butterfly biodiversity between regenerating areas.

The differences we have shown in the responses of butterfly biodiversity at different vertical levels in this regenerating rainforest contribute to a growing body of evidence that canopy dwelling species are likely under greater threat than other communities, due to anthropogenic habitat change (de la Peña-Domene et al. 2014; Klimes et al. 2012; Tregidgo et al. 2010). Invertebrates; including butterflies, ants, and dung beetles have all been shown to display increased sensitivity to human disturbance in the canopy. It therefore seems likely that other groups yet to be assessed may be similarly affected. For vertebrates, fruit bats from Malaysian rainforest showed species diversity and capture rates (100 times greater) to be higher in the arboreal layer (Francis 1994), and as such it was suggested they would be severely affected by habitat modification of the canopy (Tregidgo et al. 2010). Together these results suggest that we will need to improve our understanding of how canopy and arboreal biodiversity respond to human disturbance if we are to have an accurate picture of the conservation value, and develop appropriate management strategies for human disturbed tropical forests. Further significant impacts upon arboreal species could subsequently negatively affect natural forest regeneration processes, especially considering the key role of many canopy dwelling specialists as rainforest pollinators and seed dispersers (de la Peña-Domene et al. 2014; Kays & Allison 2001; Lowman 2009). We suggest future research should aim to assess these patterns more widely and determine the impact of habitat change at different vertical levels for a variety of taxa. This is especially true for vertebrate groups such as amphibians, birds, mammals and reptiles, which to date remain largely understudied (Kays & Allison 2001).

Conclusions

To our knowledge only this study and Fermon et al. (2005) consider the effects of habitat change upon biodiversity at more than two vertical levels. Had we utilised only terrestrial and canopy traps and not included the understorey, we could not have detected the degrees of difference between vertical levels of once cleared forest. Both Fermon et al. (2005) (working on butterfly assemblages in natural forests of Indonesia) and this study, show clear differences between vertical zones; but Fermon found the difference was no longer pronounced in human disturbed forest, whereas we found that even though the difference was less in the area with the most pronounced historic human disturbance type, there was still a significant difference. We would therefore suggest that future studies assessing vertical biodiversity patterns should assess more than only terrestrial and upper canopy communities. Rainforests are, after all, complex structurally and floristically diverse three-dimensional environments; from the ground, to the herb and shrub layer, to the lower and upper canopy, right through to the emergent trees above the canopy itself (Lowman 2009). Understanding biodiversity patterns for a variety of taxa, across a variety of vertical levels will be important for effective conservation decision making about the value of regenerating rainforest. If coupled with detailed assessments of how human habitat modification differentially impacts these vertical environments of tropical forests, conservation managers and decision makers can become better informed as to which forests are most important for biodiversity conservation.

Chapter 5 - Out on a limb: Arboreal camera traps as an emerging methodology for inventorying elusive rainforest mammals.

Authors and contributions

Andrew Whitworth[1,2], Laura Dominie Braunholtz[1,2], Ruthmery Pillco Huarcaya[1,3], Ross MacLeod[2] and Christopher Beirne[1,4]

[1] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.

[3] Universidad Nacional San Antonio Abad del Cusco (UNSAAC), Perú.

[4] Centre for Ecology & Conservation, School of Biosciences, University Exeter, Cornwall Campus, Cornwall, TR10 9EZ, UK.

Designed the study: AW LDB RPH RM CB

Collected the data: AW LDB RPH

Performed the analysis: AW CB

Contributed to writing of the manuscript: AW LDB RM CB

Abstract

Traditionally, arboreal rainforest mammals have been inventoried using ground-based survey techniques. However, given the success of camera traps in detecting secretive terrestrial rainforest mammals, camera trapping could potentially also provide a valuable tool for inventorying arboreal species. Here we assess, for the first time, the effectiveness of arboreal camera traps for inventorying arboreal rainforest mammals and compare to results from other methods. We do so in one of the world's most biodiverse conservation areas, the Manu Biosphere Reserve. We accumulated 1201 records of 24 arboreal mammal species; 18 species were detected by arboreal cameras, seven by diurnal line transects, six by nocturnal transects and 18 species through incidental observations. Comparing arboreal camera traps with traditional ground-based techniques therefore suggests camera traps are an effective tool for inventorying secretive arboreal rainforest mammal communities and detect a higher number of cryptic species compared with other methodologies. Daily patterns in detection frequency were also found to differ between ground-based techniques and arboreal cameras. Finally, a cost-effort analysis indicated that despite greater upfront costs in equipment and training for arboreal camera trapping, when accounting for the additional survey hours that would be needed to provide similar numbers of records using ground-based methods, overall costs were similar. Our work demonstrates that arboreal camera trapping is likely to be a powerful technique for inventorying canopy mammals. The method also has considerable potential for studying charismatic and threatened arboreal mammal species that would otherwise risk remaining largely unknown, and could quietly disappear from the world's tropical forests.

Introduction

Rainforest habitats are spatially complex environments (Davis et al. 2011) that contribute significantly to global biodiversity (Haefke et al. 2013; DeVries et al. 1997). Part of this complexity is evident in the vertical stratification of different faunal communities between terrestrial and canopy layers (DeVries et al. 1997; Dumbresll & Hill 2005; Maguire et al. 2014; Malcolm 1991; Tregidgo et al. 2010). Arboreal rainforest mammal species are high conservation priorities as it has been suggested that habitat alteration due to anthropogenic impacts likely causes greater disruption to arboreal than to terrestrial biodiversity (Dumbresll & Hill 2005; Francis 1994; Klimes et al. 2012; Kurten et al 2015; Tregidgo et al. 2010; Walther 2002) and as they are often impacted by additional human disturbance in the form of hunting.

Improving our understanding of arboreal rainforest mammals is crucial as they serve as charismatic flagship species for conservation (Kays & Allison 2001), are integral dispersers of fruits and seeds (Kurten et al. 2015; Vieira & Izar 1999), act as key rainforest pollinators (Ganesh & Devy 2000) and are essential ecosystem engineers (Chapman et al. 2013). Despite their importance, knowledge of the ecology and distribution of many arboreal rainforest mammals often remains sparse due to secretive, cryptic and nocturnally active behaviours making them particularly difficult to survey (Kays & Allison 2001).

Traditionally medium-large arboreal rainforest mammals have been assessed utilising ground-based survey techniques, such as line transects, visual searches and acoustic surveys (Bennett et al. 1991; Laurance 1990; de Thoisy et al. 2008; Umapathy & Kumar 2000). However, attempting to see through dense understorey into the upper reaches of 20-40 metre high rainforest canopy is challenging, especially for inconspicuous, cryptic and nocturnal species (Munari et al. 2011). As a result the majority of studies on arboreal rainforest mammals focus on diurnal, vocal, conspicuous primates leading to incomplete studies of arboreal mammal communities (Kays & Allison 2001). Additionally, using human observers to address questions related to hunting pressure can introduce unknown biases related to the differential degree of avoidance to observers between hunted and non-hunted areas (Bshary 2001; Carrillo et al. 2000; Croes et al. 2007; Wright et al. 2001). Similar issues have been successfully addressed with the use of terrestrial camera traps on medium-large mammals within tropical forests (Balme et al. 2009; Espartosa et al. 2011; Munari et al. 2011; Rovero & Marshall 2004), particularly in the detection and assessment of elusive, nocturnal and hunted rainforest species (Azlan & Lading 2006; Datta et al. 2008; Rao et al. 2010; Rao et al. 2005; Rovero et al. 2005; Tobler et al. 2008). Whilst the benefits of terrestrial camera trapping are well documented, the potential effectiveness of using camera traps in the canopy to survey arboreal mammals remains largely unknown.

The success stories from terrestrial camera trapping projects suggest that there could be several potential benefits to arboreal camera trapping. Firstly, arboreal camera traps could have the potential to rapidly inventory arboreal medium-large rainforest mammal communities as they function 24 hours a day, enabling them to detect both diurnal and nocturnal species. Second, they can be left for extended periods in-situ (potentially several months), providing long time periods to enable maximum detection opportunities. Third, they have the potential to provide novel ecological information, as behaviours only rarely detectable to human observers can be recorded; and fourth, cameras could provide an unbiased means of assessment within hunted areas, as animals are unlikely to associate traps with hunters and should therefore be less susceptible to displaying avoidance behaviour. Despite these potential benefits, arboreal camera traps have so far only been utilised successfully to study single species behaviour (Kierulff et al. 2004; Olson et al. 2012; Otani 2001; Schipper 2007), frugivore feeding preferences (Javasekara et al. 2007; Otani 2001) and document the use of natural crossing points over a gas pipeline clearing (Gregory et al. 2014). No studies to date have assessed arboreal camera traps for effective inventorying of arboreal mammal communities within typical tropical forest habitat.

In this study we therefore assess, for the first time, the effectiveness of arboreal camera traps to inventory medium-large arboreal rainforest mammals. We did so in one of the world’s most biodiverse and important conservation areas, the Manu Biosphere Reserve, a UNESCO World Heritage Site designated to protect the globally important Amazon rainforest and its biodiversity. Specifically we aimed to: 1) Compare arboreal medium-large mammal inventories obtained by classical ground-based approaches with inventories by arboreal camera traps, 2) Determine the potential of arboreal camera traps to record difficult to detect species, 3) Determine whether there is a difference between detection rates between cameras located within the lower (8-12m) and upper canopy (18-33m), 4) Compare the cost and effort involved in using arboreal camera traps with classical ground-based survey approaches and 5) Assess the potential of arboreal camera traps for obtaining useful ecological information.

Methods

Study sites

This study was carried out at two sites within the Manu Biosphere Reserve in south-eastern Peru. The first of these was the Manu Learning Centre (MLC) research station owned and operated by conservation NGO the Crees Foundation (71°23'28"W 12°47'21"S; Figure 1A). The study site consists of regenerating lowland tropical forest (~800ha) covering an altitude range of 450-740m asl. The MLC reserve has a known history of different anthropogenic disturbances, ranging from complete clearance for intensive agriculture in some areas, to selective logging for the most commercially valuable timber in others. Regeneration of the forest at the site has been on-going for >30 years and since 2002 the site has been strictly protected from hunting and other human impacts. Biodiversity studies have been undergoing at the site since 2003 and a thorough inventory of many taxa including amphibians, butterflies, birds, mammals and reptiles already exists (see Chapter 1 of this thesis).

The second site was an area of land owned by the Native Community of Shipetiari (71°9'59"W 12°28'60"S; Figure 1B), an area of land 26800ha split into different land uses. One zonation of this land has been set aside as a Tourism and Conservation area, where this research was carried out. Within this, a small lodge has been built and the forest in this zone has undergone minimum logging activities with only narrow access trails cut into the forest. Other zones within Shipetiari’s land have undergone disturbance activities such as conversion to small-scale agriculture, clearing of land for constructing houses that make up the community, and subsistence logging. Shipetiari community is made up of ~120 inhabitants (of ~24 families) who practice subsistence hunting, increasingly moving away from traditional methods of bow and arrows, to using shotguns. Few biodiversity studies have ever been conducted near to the community

(Salvador et al. 2011) and as such, prior to this study no mammal species list existed for the site. The key features for inventorying within these two study sites were the differences in current anthropogenic pressures and intensity of prior research.

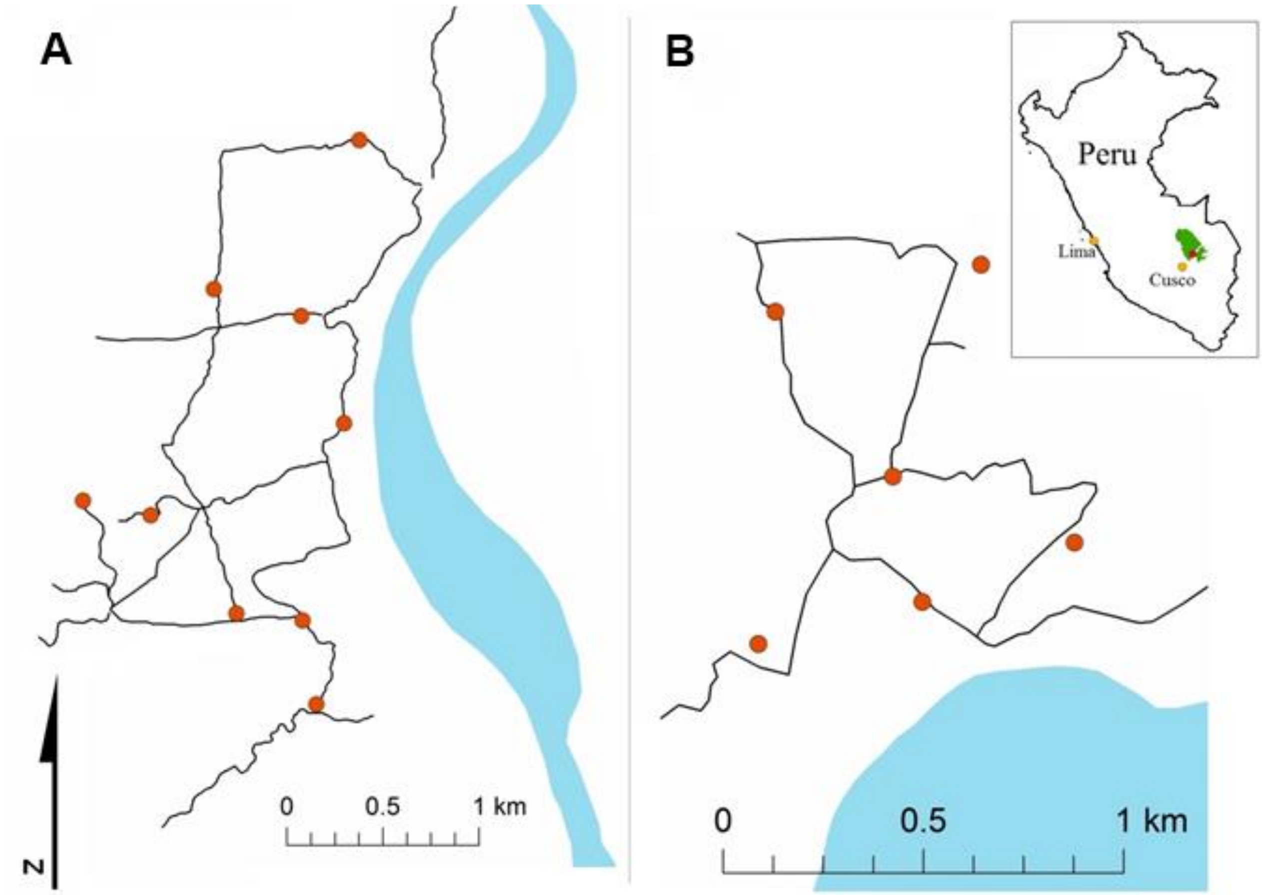


Figure 1. Study area. Map inlay shows the location of Manu Biosphere Reserve (green) in south-eastern Peru; A) shows the trail system used to survey the Manu Learning Centre Reserve (MLC); and B) the trail system to survey Shipetiari. Red circles indicate arboreal camera trap survey locations.

Data collection – Camera traps

Thirty camera traps triggered by a motion detector (Model - Bushnell 119438 Natureview Cam 8mp) were deployed across 15 arboreal sampling locations; nine at the MLC and six at Shipetiari. Each sampling location contained two camera traps, set at two heights; a mid-canopy camera (8-12m) and a high canopy camera (18-33m). Camera traps were programmed to work 24 hours per day and to take 1 photo followed by a 30 second video. An interval of 30s between sets of photos and videos was set and date and time were automatically stamped on videos and photos. The trees selected for camera trap placement were situated a minimum of 400 and maximum of 800m apart with close access from existing trails. Traps were set up in mid-June 2014 and removed before the onset of the wet season at the start of October 2014. Not all camera traps worked for the entirety of the time they were in the field; resulting in a total of 1413 mid canopy trap days and 1503 high canopy trap days (2916 total camera days; well within the 1000-2000 needed to accumulate 60-70% of tropical terrestrial community species richness, as suggested by Rovero et al. 2013). Of these, 876 mid canopy trap days and 892 high canopy trap days came from the MLC and 537 mid height trap days and 611 high canopy trap days from Shipetiari. Setting up and taking down the cameras from both sites took a team of three people 21 days (12 at the MLC and nine at Shipetiari), equating to ~756 person working hours (based on an eight hour working day).

Data collection - Traditional methods; transects and incidental observations

Between the 15th January 2014 and the 27th of December 2014, thirty-nine timed morning transects (0530-0800) were performed across 11 different 2km transects at the MLC. Survey teams consisted of two trained observers. Each transect was walked between three and five occasions and took on average 128 minutes (sd = 25 mins). In total, these transect surveys represented 166 observer hours of effort. In addition to the timed morning transects, all incidental mammal observations made whilst performing an array of other surveys (nocturnal and diurnal), were recorded. Whilst it is difficult to quantify the effort from incidental records, permanently employed MLC research staff worked extensively in the forest, day and night, all year round. Nocturnal transect and incidental data was also gathered from the MLC during the dry season of 2013 (between the 18th March and 20th August). This represented 249 nocturnal transect observer hours carried out along the same trail system as diurnal surveys in 2014.

Between the 13th of November 2014 and the 30th of November 2014 a rapid biological survey expedition visited the Shipetiari region. Pairs of trained observers performed ten timed morning transects (0530-0810am) across four 2km transects, totalling 57 hours of observer effort. Incidental arboreal mammal records include observations recorded outside of the survey periods. In addition, 48 hours of survey effort (between 12th and 26th July 2014) was carried out by an experienced primatologist and their assistant searching morning and afternoon, specifically for woolly monkeys but recording all other arboreal mammal species.

Analysis

We compared arboreal medium-large mammal inventories obtained by classical ground-based approaches with inventories by arboreal camera traps to determine the potential of arboreal camera traps to record difficult to detect species. To do this, at both sites we compiled detections of arboreal mammals from diurnal transects, nocturnal transects (only from the MLC study site), incidental observations and data from arboreal camera traps in 2014. Camera trap detections were designated as separate events if there was at least a 30 minute interval between captures of the same species (Srbek-Araujo & Chiarello 2005). The percentage of species detected by each methodology was calculated for the MLC site against the long term ten-year species list for the site, and for the Shipetiari site against the total number of species recorded at the site in 2014 (comprising of detections from all methodologies used). The number of species which were uniquely detected by each survey methodology was also determined.

The cost and effort involved with classical ground-based approaches and arboreal camera traps were compared by calculating the financial costs involved in terms of necessary training, equipment required and related field site costs appropriate to the person hours required to provide an equivalent number of detections for each survey methodology (diurnal transects, nocturnal transects and arboreal camera traps – based upon information from the MLC study site, which had the more intensive survey effort).

In order to determine if there was any difference in detection frequency of arboreal mammals between mid- and upper-canopy camera traps, we implemented a linear mixed effects model with a normal error structure using the ‘lme4’ package within the R statistical environment (R Core Team 2012). We used tree ID as a random effect in order to account for the non-independence of cameras within the same tree. The significance of camera trap height was assessed using a likelihood ratio test. We also indicate the potential temporal coverage per day and detection biases related to traditional transect surveys compared with arboreal camera traps. Daily patterns in detection frequency between traditional survey methods and arboreal cameras were assessed through the production of activity pattern charts using the package ‘activity’ within the R statistical environment (R Core Team 2012). We then used a Wald test to determine if the two activity profiles were significantly different to each other in the R package ‘activity’, with 1000 bootstrap repetitions. Finally, observations of special interest were highlighted in order to assess the potential of arboreal camera traps for obtaining useful ecological information.

Results

Overall we detected 24 arboreal mammal species, based on 1201 separate records, 339 of which were from arboreal camera traps. In total 18 species of arboreal mammal were detected by arboreal camera traps, seven species were detected by diurnal line transects, six species were detected by nocturnal transects and 18 species were detected incidentally (Table 1; see Appendix 5A for number of detections per species). At the MLC this represented 15, four, six and 16 species for each methodology respectively. At the Shipetiari site this represented 12, six and eight species, for camera traps, diurnal transects and incidental records respectively.

Whereas arboreal camera trapping resulted in the detection of four unique species at the MLC and six unique species at Shipetiari, no unique species were detected using diurnal visual encounter surveys (Table 1). Incidental records provided five unique species detections at the MLC and a single species from Shipetiari (Table 1). Nocturnal transects added one unique species detection at the MLC (Table 1). Comparison with the full MLC species list suggests that several species known to be present in the area were not detected by the arboreal cameras: Bolivian bamboo rat, brown titi monkey, margay, short-furred woolly mouse opossum and southern Amazonian red squirrel. However, despite ten years of surveying at the MLC (see Chapter 1 of this thesis); arboreal camera trapping resulted in the addition of the silky pygmy anteater to the species list (Figure 3).

Table 1. Comparison of arboreal mammal species inventories using camera traps and traditional survey techniques. Where IUCN RL = IUCN Red List; LC = Least Concern; NT = Near Threatened; E = Endangered; D = Decreasing; S = Stable; U = Unknown; ACT = Arboreal Camera Traps; DT = Diurnal transects; NT = Nocturnal Transects; and INC = Incidentals.

					Manu Learning Centre					Shipetiari N. C.			
Common name	**Species name**	**IUCN RL status**	**IUCN population trend**	**Diurnal or Nocturnal**	**Ten year species list**	**ACT**	**DT**	**NT**	**INC**	**Total from 2014**	**ACT**	**DT**	**INC**
Allen's olingo	*Bassaricyon alleni*	LC	D	N	✓	✓							
Bicolour-spined porcupine	*Coendou bicolor*	LC	U	N	✓	✓		✓	✓				
Black-eared common oppossum	*Didelphis marsupialis*	LC	S	N	✓					✓	✓		
Black-faced spider monkey	*Ateles chamek*	E	D	D	✓	✓			✓	✓	✓		
Bolivian bamboo rat	*Dactylomys boliviensis*	LC	S	N	✓			✓		✓			✓
Bolivian red howler monkey	*Alouatta sara*	LC	D	D	✓	✓	✓		✓	✓	✓	✓	
Bolivian squirrel monkey	*Saimiri boliviensis*	LC	D	D	✓	✓			✓	✓	✓	✓	✓
Brown titi monkey	*Callicebus brunneus*	LC	U	D	✓		✓		✓	✓		✓	✓
Brown-eared woolly opossum	*Caluromys lanatus*	LC	D	N	✓	✓				✓	✓		
Four-eyed opossums (Brown/Gray)	*Metachirus nudicaudatus / Philander opossum*	LC	S	N	✓	✓							
Gray monk saki monkey	*Pithecia irrorata*	-	-	D						✓	✓		
Hoffman's two-toed sloth	*Choloepus hoffmanni*	LC	U	N	✓	✓		✓	✓				
Kinkajou	*Potos flavus*	LC	D	N	✓	✓		✓	✓	✓	✓		
Large-headed capuchin	*Sapajus macrocephalus*	LC	D	D	✓	✓	✓		✓	✓	✓	✓	✓
Margay	*Leopardus wiedii*	NT	D	N/D	✓				✓				
Peruvian night monkey	*Aotus nigriceps*	LC	U	N	✓	✓		✓	✓	✓	✓		✓
Peruvian woolly monkey	*Lagothrix cana*	E	D	D	✓	✓	✓		✓				
Saddleback tamarin	*Saguinus fuscicollis*	LC	D	D	✓				✓	✓	✓	✓	✓
Short-furred woolly mouse oppossum	*Micoureus regina*	LC	S	N	✓			✓	✓				
Silky pygmy anteater	*Cyclopes didactylus*	LC	U	N	✓	✓							
Southern Amazonian red squirrel	*Sciurus spadiceus*	LC	U	D	✓				✓	✓		✓	✓
Southern tamandua	*Tamandua tetradactyla*	LC	U	N/D	✓				✓	✓	✓		
White-fronted capuchin	*Cebus albifrons*	LC	D	D						✓	✓		✓
White-bellied slender mouse opossum	*Marmosops noctivagus*	LC	S	N	✓				✓				
Observed species					22	13	4	6	16	15	12	6	8
% detected of total list						59	18	27	73		80	40	53
Unique species detected						4	0	1	5		6	0	1
Person working hours in the forest						432	166	249	na		324	105	Na

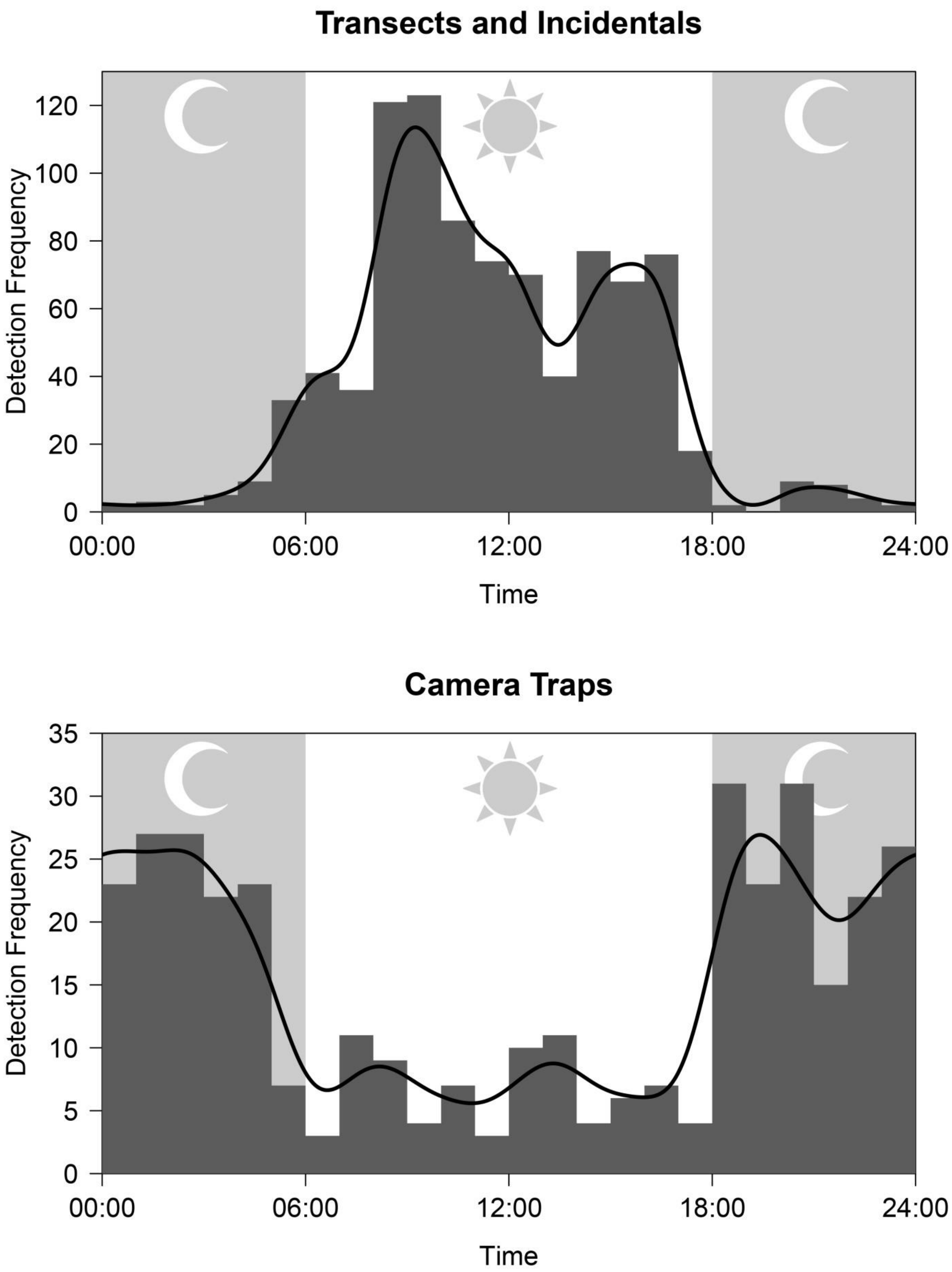


Figure 2. Daily average detection frequency patterns of arboreal rainforest mammals; using transects and incidental data from the MLC, compared with data gathered from arboreal camera traps. The histogram denotes raw detection frequency and the black line denotes the fitted spline. The Wald test used to assess statistically the two activity profiles showed that the patterns were significantly different (W=29.5, p=<0.0001).

Daily patterns in detection frequency were found to be strikingly different between data gathered from arboreal cameras and data gathered from traditional transects and incidental records (Figure 2). Data gathered incidentally and through both diurnal and nocturnal transect surveys displayed more observations of diurnal species; whereas camera traps displayed a greater frequency of detections nocturnally than diurnally. The Wald test used to assess statistically the two activity profiles showed that the patterns were significantly different (W=29.5, p=<0.0001).

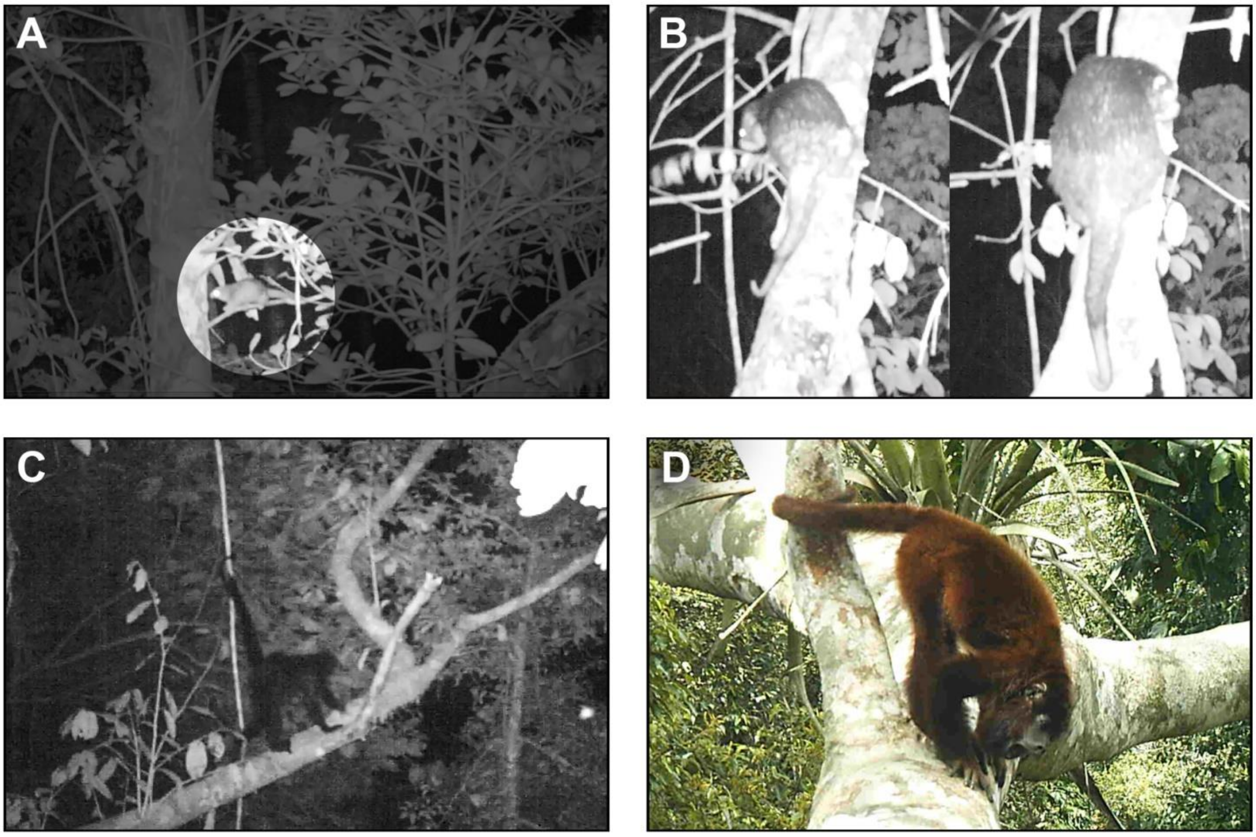


Figure 3. Species detected on arboreal cameras. A) silky pygmy anteater from the MLC, the first detection for the reserve in over 10 years of biodiversity research at the site (Chapter 1 of this thesis); B) a pair of bicolour-spined porcupines from the MLC, both detected in the same tree, as observed in related species (Moreau et al. 2003); C) first record of nocturnal activity of the endangered black-faced spider monkey, detected at the MLC and D) Bolivian red howler monkey attempting to call but making no sound from Shipetiari, suggesting potential human avoidance behaviour due to hunting at the site (Bshary 2001; Croes et al. 2007).

Upper canopy camera traps were found to result in significantly more mammal detections than those placed in the mid-canopy (p=0.008; see Appendix 5B for full model outputs). On average, upper-canopy traps were predicted to result in 21.0 mammal detections per 100 days, whereas mid-canopy traps resulted in 0.7 detection's per 100 days (see Appendix 5C for complete species detections by vertically stratified camera location). Six arboreal mammal species were detected on mid-canopy camera traps, while 18 species were detected on upper canopy cameras. All of the species recorded on mid canopy traps were also recorded in the upper canopy, and in all but one case (saddleback tamarin) species were detected in higher frequencies in the upper canopy (Appendix 5C). Tree ID accounted for none of the variance in arboreal mammal detection frequency.

The cost effort analysis indicated that upfront costs in terms of training and equipment for arboreal camera trapping were greater than those for traditional transect surveys (Table 2; \$10,367 vs. \$1178). However, when considering the total expense necessary to cover field station costs related to the person hours needed to provide equivalent numbers of observations (note: not an equivalent number of species), the overall costs balanced out considerably (\$1913 to gather equivalent diurnal data plus \$8499 to gather equivalent nocturnal data; \$10,412 vs. a total of \$11,457 for arboreal camera trapping which collected data both diurnally & nocturnally).

Table 2. Cost-effort analysis of arboreal camera trapping vs. traditional transect techniques for a single field season. Note: this relates to effort to gather an equivalent number of observations and not an equivalent number of species.

Method	**Line transect**	**Camera traps**
Number of detections / 100 person hours	8 nocturnal 22 diurnal	77 nocturnal 22 diurnal
Daily Coverage	6hrs of survey effort per day	24hrs
Estimated field station days needed to gather equivalent number of observations (to camera trapping in this study)	181 nocturnal 12 diurnal	12
Training costs USD	Training period for seven days; research station fee for two people $424	BCAP Climbing course $1080
Equipment costs USD	Recording equipment, microphone, head torch. $754 total	Climbing kit: - $2027 Camera traps / unit - $242 (x30 for this study = $7261 total
Total field station costs (to gather an equivalent number of observations) USD	$7321 nocturnal $735 diurnal	$1089
Total projected cost (to gather equivalent number of observations) USD	$8499 nocturnal $1913 diurnal **$10,412 overall**	**$11,457 overall**

Discussion

Our results suggest that arboreal camera traps are an effective tool for inventorying secretive rainforest mammal communities within the canopy. Cameras detected a greater number of species than either diurnal or nocturnal transects; only incidental records provided greater numbers of detections and detected a comparable number of unique species. Arboreal traps also detected a higher number of secretive rainforest mammal species than more traditional methodologies. Whilst tradition techniques tend to focus on subsets of the overall mammal community (diurnal or nocturnal), arboreal camera traps allowed for 24 hour detection of species.

Arboreal camera traps were not only useful in the detection of active-larger bodied species of high conservation concern (Kays & Allison 2001), such as the endangered black-faced spider monkey and Peruvian woolly monkey, but also in detecting lesser known, cryptic species, such as the bicolour-spined porcupine (de Freitas et al. 2013; Voss et al. 2013) and silky pygmy anteater (Superina et al. 2010; see Figure 3). Although recorded from a number of locations throughout Amazonia, detailed information about the ecology and distribution of both species is currently limited. Biologists have been carrying out biodiversity surveys at the MLC since 2004, through biodiversity surveys by expedition groups and more consistently since 2010 with an all-year round field team dedicated to surveying the biodiversity of the reserve both day and night. Despite ten years of on-going research and assessment, the nocturnal and inconspicuous, silky pygmy anteater (Munari et al. 2011; Superina et al. 2010) had evaded detection (see Chapter 1 of this thesis). However, in just three months, cameras at the MLC captured two separate records of this elusive species from two trees (>400m apart; Figure 3). This provided a clear demonstration of the ability of arboreal cameras to collect novel distribution and ecological data, especially for species where this has proven difficult or impossible using traditional survey techniques.

A further effective use of arboreal camera traps identified within this study is the ability of cameras to detect species in hunted areas. Mammals are often difficult to detect in hunted areas using traditional methodologies, due to human avoidance behaviours as a result of hunting pressure (Bshary 2001; Croes et al. 2007). For example, at Shipetiari where hunting for subsistence is common, spider monkey, woolly monkey and howler monkeys were not recorded despite extensive searches by research groups visiting the site in 2014. However, the cameras in this study detected both howler and spider monkeys within 1.5km of the community (Figure 3).

Comparison of detection frequency and species richness between upper and mid canopy cameras suggests that upper canopy traps were more effective for rapid species inventorying than those placed lower down, with upper canopy traps accruing thirty times more detections than those placed in the mid canopy. Rather than this reflecting increased use of the upper canopy in comparison to mid-canopy, this may have arisen due to the selection of high trap locations primarily for large horizontal limbs whilst mid traps were just placed in the best possible location upon descent. We would therefore recommend further investigation into the best approach to locating mid-level traps.

Caveats and limitations

Despite the potential benefits in utilising arboreal camera traps to survey arboreal rainforest mammals, there are, as with any method, a number of potential limitations and advantages in favour of traditional ground-based survey techniques. Direct observations, for example, may be more effective at identifying the number of individuals within a group and they could also facilitate the use of distance-sampling techniques to calculate density estimates. Under the right circumstances animals can also be followed to gather detailed information on movement patterns, competition and feeding behaviours. However, arboreal camera trapping remains in its infancy as a survey and monitoring technique, and as with terrestrial based camera traps, there is the potential to further develop analysis techniques and sampling regimes that can provide density estimates (e.g. Azlan & Lading 2006; Datta et al. 2008; Rao et al. 2010; Rao et al. 2005; Rovero et al. 2005; Rovero & Marshall 2009; Tobler et al. 2008) and in doing so, gather more detailed ecological information about elusive arboreal mammals (Javasekara et al. 2007; Gregory et al. 2014; Lowman 2009).

In addition, arboreal camera trapping might initially seem unattractive to money-constrained conservation scientists due to the large up-front investment required in terms of training and equipment costs (Gradner et al. 2008; Lawton et al. 1998). However, cost estimates here refer to a single field season of data collection and since training is typically a one-off investment (unless further skills are being developed or technique refreshment is needed) and equipment can be re-used in future assessments (only needing re-investment due to 'wear and tear' or breakages) costs dived over multiple field seasons would be much lower. As traditional survey methods require longer field stays in order to provide equivalent size data sets, the costs of using traditional techniques are often likely to outweigh the larger initial investment required for arboreal camera surveys in the long-term. In addition, although longer stays may be able to provide equivalent records, we still cannot be certain that they will record the same number of species as arboreal cameras or provide detections of rare or cryptic species as effectively.

Finally, our study is a pilot work which would benefit from further research across multiple sites, in different regions and different forests from around the world. This would provide greater support to the preliminary findings that we have suggested in this study.

Implications for conservation

In a rapidly changing era, currently acknowledged by many as the anthropocene, when the condition of the world's tropical forests is being modified at an alarming rate (Gardner et al. 2009), rapid and cost effective survey techniques can provide invaluable tools for understanding how tropical fauna are responding to such changes. This can consequently facilitate increased awareness about the biodiversity and conservation value of both primary and regenerating tropical forests (Chazdon et al. 2009b). Understanding the effects of human caused disturbance to canopy environments is particularly important given that a number of different taxonomic assessments which have suggested that biodiversity within canopy strata is under greatest threat due to habitat modification (Dumbrell & Hill 2005; Francis 1994; Klimes et al. 2012; Kurten et al. 2015; Tregidgo et al. 2010; Walther 2002). Here we suggest that the arboreal camera trapping method we have investigated can be both useful and cost-effective in the long term for conservation and will provide opportunities to learn more about some of the most charismatic (Kays & Allison 2001) and threatened species in the world (Gregory et al. 2014; Jayasekara et al. 2007; Lowman 2009); which otherwise risk remaining largely unknown and could quietly disappear from our planet.

Chapter 6 - Methods matter: Different biodiversity survey methodologies identify contrasting biodiversity patterns in a human modified rainforest.

Authors and contributions

Andrew Whitworth[1,2], Jaime Villacampa[1,2], Shirley Jennifer Serrano Rojas[1,3], Roger Downie[2] and Ross MacLeod[1]

[1] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.

[3] Universidad Nacional San Antonio Abad del Cusco (UNSAAC), Perú.

Designed the study: AW RM JV

Collected the data: AW JV SJSR

Performed the analysis: AW SJSR JV

Contributed to writing of the manuscript: AW RM RD JV

Abstract

Understanding how well tropical forest biodiversity can recover following habitat change is often difficult due to conflicting assessments arising from different studies. One often overlooked potentially confounding factor that may influence assessments of biodiversity response to habitat change, is the possibility that different survey methodologies, targeting sub-sets of a community, may identify different patterns and so lead to different conclusions. Here we investigated whether two different but commonly used survey methodologies used to assess amphibian communities, pitfall trapping and nocturnal transects, indicate the same or different responses of amphibian biodiversity to historic human induced habitat change. We did so in a regenerating rainforest study site located in one of the world's most biodiverse and important conservation areas: the Manu Biosphere Reserve. We show that the two survey methodologies tested identified contrasting biodiversity patterns in a human modified rainforest. Nocturnal transect surveys indicated biodiversity differences between forest with different human disturbance histories, whereas pitfall trap surveys suggested no differences between forest disturbance types, except for community composition. This pattern was true for species richness, diversity, overall abundance and community evenness and structure. For some fine scale metrics, such as species specific responses and abundances of family groups, both methods detected differences between disturbance types. However, the direction of differences was inconsistent between methods. We highlight that for assessments of rainforest recovery following disturbance, survey methods do matter and that different biodiversity survey methods can identify contrasting patterns in response to different types of historic disturbance.

Introduction

There is a pressing need to better understand future biodiversity and conservation value of tropical rainforest following human disturbance (Dent & Wright 2009; Kinnaird et al. 2003; Peres et al. 2006), especially as the Global Forest Resources Assessment (FAO 2010) classifies just 36% of global forest cover as primary. Despite regenerating landscapes representing the majority of remaining tropical forest, the potential of such human-modified forests to provide important habitat for rainforest biodiversity is contentious (Chazdon et al. 2009a, 2009b; Gibson et al. 2011). As human populations in tropical countries increase and primary forest is converted to agricultural land and later abandoned, some authors suggest that secondary forests will become increasingly important for conservation (Anand et al. 2010; Chazdon et al. 2009b; Durães et al. 2013; Irwin et al. 2010; Letcher & Chazdon 2009; Norris et al. 2010; Tabarelli et al. 2010), while others suggest that the major conservation priority is to protect remaining primary forest (Barlow et al. 2007a; Gibson et al. 2011; Sodhi et al. 2010).

Determining how well tropical forest biodiversity can recover is difficult (Gardner et al. 2010) as studies from different locations often produce contrasting results. There are many potential reasons for different studies to identify contrasting patterns; including geographic context, study scale, potential on-going human impacts, timeframe since disturbance (Chazdon et al. 2009b) and a tendency to focus on overall species richness patterns (Anand et al. 2010; Barlow at al. 2007a). However, one factor often overlooked is the potential for different survey methodologies, targeting sub-sets of a community, to provide different results on the response of biodiversity to habitat change (Barlow et al. 2007b). In the case of butterflies for example, line transect studies carried out in a number of locations have suggested that butterfly biodiversity does not show a significant degree of difference between human disturbed and primary forest (Devy & Davidar 2001; Kudavidanage et al. 2012; Posa & Sodhi 2006). In contrast, studies using traps undertaken at other sites suggest that butterfly biodiversity does show a significant degree of difference between human disturbed and primary forest (Dumbrell & Hill 2005; Ribeiro & Freitas 2012; Ribeiro et al. 2015). Additionally, bird studies carried out in different locations and based upon different survey methodologies have also found contrasting patterns (Barlow et al. 2007b). In some locations secondary forests display similar biodiversity levels to primary forest based on mist net methodologies (Barlow et al. 2007b; Srinivasan et al. 2015; Waltert et al. 2005), but other studies using point counts have suggested that secondary forest may have significantly lower levels than primary (Barlow et al. 2007b; Carillo-Rubio et al. 2014). Understanding more about how these contrasting patterns might relate to differences due to survey methodologies can therefore help to improve our ability to assess the true value of regenerating tropical forests and better understand the response of specific communities. Otherwise, assessments of a specific community may under or overestimate the potential biodiversity value for such forests, especially if the results from single surveys are over generalised (Barlow et al. 2007b).

One key taxonomic group utilised to study the impacts of habitat disturbance in tropical forests are amphibians, chosen due to their high conservation importance (31% of evaluated species are threatened with extinction; IUCN 2013), and because they are key components within their ecosystems (Ficetola et al. 2014; Hocking & Babbitt 2014). Amphibians display a high level of sensitivity to disturbance due to low mobility, limited dispersal capacity and narrow ecological requirements (Lawler et al. 2010). Habitat change is therefore likely to affect amphibians more severely than other vertebrate groups (Ficetola et al. 2014), especially as small changes in vegetation structure can create significant alterations to amphibian communities (Cortés-Gómez et al. 2013). As a result, habitat destruction and fragmentation are among the leading causes of the global threat to amphibians (Catennazi & Von May 2014; Eigenbrod et al. 2008), especially in tropical regions where levels of diversity are highest (Ficetola et al. 2015).

So far, investigations using amphibians to assess rainforest biodiversity response to habitat change often use different survey methodologies and describe contrasting patterns from different locations. Hilje and Aide (2012), for example, utilised diurnal and nocturnal visual searches and acoustic surveys in Costa Rica and found that even young regenerating forest had similar amphibian species richness and composition to primary forest. In contrast, Gardner et al. (2007a), using terrestrial traps and diurnal visual searches to target leaf litter amphibians in Brazil, found just two-thirds of primary forest amphibian species in regenerating forest. Finally, Seshadri (2014) utilised quadrats to assess amphibian biodiversity in selectively logged forests of southern India, detecting a 42% lower density of amphibians than in primary forest; and even though species richness and composition were converging with primary forest levels, the effects of logging were still detectable. These results therefore raise the question of whether the lack of a consistent pattern in detected amphibian responses is driven by site specific factors or whether such differences could be caused by different methods that focus on different groups of amphibian communities.

Here we investigate whether two different but commonly used biodiversity survey methodologies, pitfall trapping and nocturnal transects (Doan 2003; Dodd 2010), find the same or different responses of amphibian biodiversity in areas with different historic human induced habitat change. We do so in a regenerating rainforest study site located in one of the world's most biodiverse and important conservation areas, the Manu Biosphere Reserve, a UNESCO World Heritage Site designated to protect the globally important Amazon rainforest and its biodiversity. Specifically, we quantified and compared species richness, diversity, abundance, community structure and composition of amphibian communities using both pitfall traps and nocturnal transect surveys, between areas of old regenerating forest, following different types of historic human disturbance.

Methods

Study site

The study was carried out at the Manu Learning Centre (MLC) research station in the Peruvian Amazon (71°23'28"W 12°47'21"S). The site (described in Chapter 2 of this thesis) is within the Manu Biosphere Reserve, which consists of a network of core protected areas surrounded by areas designated as cultural buffer zones due to historically high human impact, including extensive logging or clearance for subsistence agriculture. The study site lay within one of these cultural buffer zones. It consists of ~800ha of regenerating lowland tropical forest.

Three different anthropogenic disturbance types had occurred: 1) selective logging (SLR – selectively logged and now regenerating forest), 2) complete clearance due to conversion to agriculture for coffee and cacao (CCR – completely cleared and now regenerating forest), and 3) a mixed area that had historically consisted of a mosaic of small completely cleared areas used for agriculture combined with selective logging of the adjacent forest (MXD – mixed disturbance and now regenerating forest). Major human disturbance had started ~50 years prior to the study and lasted for 20 years before systematic human disturbance activities were abandoned in the 1980s. For 30 years following abandonment the site was left to regenerate, and from 2003 the site was actively protected from further human disturbance. At the time of the study the whole area was covered by closed canopy regenerating tropical forest.

Study approach, sampling design and sampling effort

In order to test whether different methodologies indicate the same or different responses of biodiversity to historic human induced habitat change, we used two different biodiversity survey methods to compare detected within-site differences in relation to known differences in human disturbance history. A regenerating rainforest study site was chosen where historic human disturbance had varied across a relatively small area (~800ha). Studying within site differences in biodiversity distribution across this small spatial scale was used to avoid potential confounding effects due to large scale differences in climatic variables or physical geography. We were confident that amphibians were not hindered in dispersing across the site, as there were no geographic barriers, such as large rivers or mountains dividing the site. We predicted that in the absence of any effects of differences in historic disturbance, biodiversity would be distributed randomly across the site. As such, if different historic human disturbance differentially impacted biodiversity patterns, we would expect to see differences in current patterns across areas once subjected to different forms of disturbance. To test whether different methodologies would detect different biodiversity patterns, amphibians were surveyed across 36 sampling locations, 12 in each of the three regenerating disturbance areas (Figure 1). Each sampling location was a minimum of 200m apart to ensure spatial independence of sites (see Ribeiro-Junior et al. 2008).

Surveying was conducted through both wet and dry seasons between March 2012 and May 2014 in order to obtain an annual representation of community structures. Methodologies were conducted simultaneously in order to avoid any bias in capturing a temporally different community due to the trapping method used at any particular time. Sampling locations were situated at least 70m from a clear habitat edge or water body to reduce the influence of edge effects (Demaynadier and Hunter 1998). Due to the steep nature of the terrain and dense forest habitat, sample sites were placed in areas that were accessible, yet away from existing trails, in order to avoid known detection biases associated with pre-existing trails (von May and Donnelly 2009).

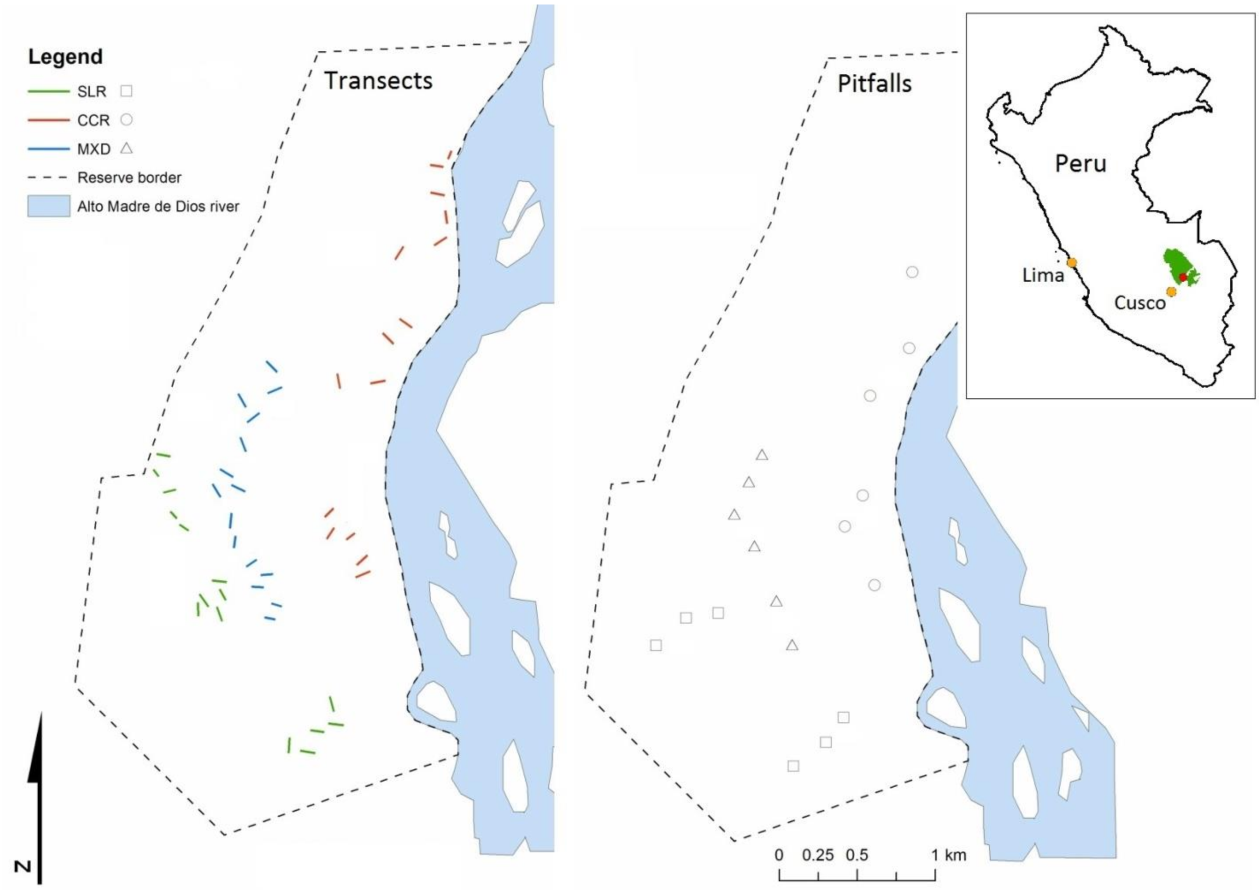


Figure 1. The context of the study site (as indicated by a red circle) in the Manu Biosphere Reserve in SE Peru, and the study site highlighting amphibian transect and pitfall trap sampling locations.

Pitfall traps were utilised due to their effectiveness in sampling terrestrial herpetofauna (e.g. Beirne et al. 2013). Eighteen pitfall sampling locations were established throughout the reserve: six within SLR, six within CCR and, and six within MXD forest. The 25 m long arrays consisted of four 25-litre buckets connected by eight metre lengths of drift fence, 40 cm in height. Pitfall traps were opened for a period of five days in each trapping session. Each site had similar, although not identical level of survey effort due to weather and other constraints, with a total effort of between 85 to 140 days of trapping at each site (675 days of trapping per disturbance area; accumulating to 2025 trap days overall). The difference resulted due to logistic constraints; some traps were first installed during 2012 and the remainder were installed in 2013. This meant that some traps were more intensively surveyed than others, but since they were spread evenly between the disturbance types following a balanced design this would not be expected to influence the patterns identified.

Nocturnal transects were used due to their known effectiveness in sampling arboreal and semi-arboreal species of tropical forest herpetofauna (e.g. Bell and Donnelly 2006; Doan 2003) and are known to provide higher yields per unit effort than other sampling methods (Bell and Donnelly 2006; Rödel and Ernst 2004). Nine sampling locations (each consisting of five 100m long x 4m wide transects; surveyed up to two metres in height; Folt and Reider 2013) were established throughout the reserve: fifteen transects within SLR, fifteen within MXD and fifteen within CCR. All transects were surveyed at night, commencing at 20.00 h±15 mins. Transects were surveyed by a pair of searchers over a period of 25 mins (accumulating to ~117 observer hours for the study). Each transect was surveyed between 13-22 occasions to build a picture of the biodiversity at each survey location. The difference resulted due to logistic constraints; some transects were first installed during 2012 and the remainder were installed in 2013. This meant that some transects were more intensively surveyed than others, but since they were spread evenly between the disturbance types following a balanced design this would not be expected to influence the patterns identified. All transects, were studied throughout both dry (April-September) and wet (October-March) seasons to avoid any potential temporal biases, and the order in which transects were searched was randomised to avoid systematic sampling bias (Beirne et al. 2013).

Field survey methodologies

Pitfall and transect search teams consisted of one experienced herpetologist and a trained conservation volunteer. All amphibians encountered were identified in the field where possible or later at the field centre (using the following resources: AmphibiaWeb 2013; Beirne & Whitworth 2011; The Field Museum 2014). Open pitfall traps were checked once daily between 08.00h and 13.00h. Lids were placed 10 cm above the buckets to prevent flooding during prolonged periods of heavy rain during the trapping periods and then closed tight between sessions. Individuals caught in pitfall traps were released approximately 40 m away from the trap site to reduce the probability of recapture (Beirne et al. 2013; Trimble & van Aarde 2014). Individuals captured during nocturnal transects were released behind the searchers, so that the same individual could not be encountered twice within a survey (Beirne et al. 2013). Unidentifiable species were given a temporary species label (e.g. "*Pristimantis spA*") and a small number of individuals (n≤4) of each unidentifiable species were anaesthetised with Lidocaine and fixed with 10% formalin, then subsequently identified and stored at the herpetology department of the Natural History Museum of the University of San Marcos in Lima (del Departamento de Herpetología del Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos – UNMSM). Owing to the previous detection of a limited number of cases of chytridiomycosis within the study site (Kosch et al. 2012) codes of good practice to prevent disease transmission were strictly adhered to. This was achieved by the systematic cleaning of tools and equipment, and sterile bags were used when handling amphibians and small reptiles.

Disturbance history habitat classification

Initially the boundaries between the three different disturbance history types were identified by two of the authors visiting the site to visually inspect it, with confirmation by local guides who had expert local knowledge related to historic land-use of the study site. A systematic vegetation structure survey was then carried out to assess specific structural forest differences of sampling locations and this confirmed the subjective observations of consistent differences in forest structure (see Chapter 2 of this thesis).

Analysis methodologies

In order to test whether different methods detected significantly different responses in areas with different disturbance history we calculated species richness, diversity, community structure, community composition and overall abundance for each disturbance history type, and compared the patterns detected by each survey methodology (Bruton et al. 2013; Hu et al. 2013). Species strongly associated with either wetland or large river habitat were excluded from analysis due to the presence of significant wetland habitat within CCR disturbance type and more of the main large river habitat being located along the outer edge of the CCR disturbance type (see Appendix 6A for details of excluded species). This enabled the analysis to focus upon forest interior associated species. Transect nights or pitfall survey sessions were used as the sample units for calculating species richness estimates, species diversity and rarefaction curves (Beirne et al. 2013).

To assess observed species richness levels and the extent to which survey effort had detected as many species as were likely to be found within each disturbance type, we plotted rarefaction curves for each sampling methodology using the Rich package (Rossi 2011) and presented these graphically using program R (R Core Team 2012). Where sampling effort detected fewer individuals in one area, we extrapolated the lower lying curves towards an equal number of individuals for a clearer comparison of where observed richness values would have projected given detection of an even number of individuals. Since the issue of which species richness estimators are most effective for amphibians remains unresolved (Veith et al. 2004), various estimators of species richness were calculated; ACE, ICE Chao 1 and 2, Jacknife 1 and 2, Bootstrap and MMMeans. The average of these estimators was then calculated for each methodology across each disturbance type. Following Altman and Bland (2011), and Gotelli and Colwell (2011) the 84% confidence intervals for the average estimated species richness were calculated for each group in CCR, MXD and SLR disturbance types, as for pair-wise comparison, non-overlapping intervals at this level suggests differences that would be significant at $p=<0.05$ (MacGregor-Fors and Payton 2013). In order to verify any patterns statistically we carried out a linear model for both pitfall traps and nocturnal transects, with average estimated richness as the response term and disturbance history as a categorical fixed effect and accounted for any effect from imbalance of survey effort between sampling locations by including survey effort as a fixed effect (using package lme4, program R).

Species diversity was defined as the Shannon diversity index (Seshadri 2014; Trimble and van Aarde 2014). Repeating the analyses using Fisher’s Alpha, Simpson's and Shannon Exponential diversity indices all showed the same pattern of results and therefore are not presented. All richness and diversity estimators were calculated in Estimate S (Colwell 2013). Data was analysed with linear models for both pitfall traps and nocturnal transects, with Shannon diversity as the response term and disturbance history as a categorical fixed effect and again accounted for any effect from imbalance of survey effort between sampling locations by including survey effort as a fixed effect ((using package lme4, program R).

In order to confirm that any potential spatial auto-correlation between survey locations had been controlled for in the analysis, a Moran's I test was carried out in program R (R Core Team 2012) on the residuals of each preferred model (where preferred to the null) to test if there was any effect from spatial auto-correlation that might lead to pseudo-replication (ape package; Paradis et al. 2004).

Community structure was compared by producing dominance-diversity (Whittaker) plots using the vegan package (Oksanen et al. 2011) in program R (R Core Team 2012). Such plots compare the evenness of a community (e.g. Beirne et al 2013). Significant differences in slope, and therefore significant differences in community evenness, were assessed through the use of a linear model with log relative abundance as the response term and an interaction between species rank and habitat type as continuous and categorical fixed effects, respectively (Beirne et al. 2013). Results are reported as ΔG which corresponds to absolute change in gradient between disturbance areas, whereby more negative values denote steeper curves and thus less even assemblages.

Community composition between disturbance areas for each of the two survey methodologies was assessed using non-metric multidimensional scaling (NMDS; using the Bray-Curtis similarity measure). All stress values were relatively low (0.14 for transects and 0.22 for pitfall tarps) and so were displayed within just two dimensions. To assess the statistical significance of observed differences in assemblage composition between different disturbance areas we conducted analysis of similarities tests (ANOSIM; using 999 permutations, see Helbig-Bonitz et al. 2015). NMDS ordinations and ANOSIM tests were carried out in the vegan package (Oksanen et al. 2011), in program R (R Core Team 2012). For this part of the analysis a standardised survey effort across all sampling locations was utilised, as compositions were assessed based upon sampling locations and so included only data collected in 2013 and 2014.

In order to determine whether methods detected different changes in amphibian abundance patterns, relative abundance values of the overall community and family groups were calculated. Amphibian transect abundances were calculated as the number of individuals encountered over 100 transect-set nights and pitfall trap abundances were calculated as the number of individuals encountered per trap site, based on 200 nights of trapping. We assessed whether abundances could be predicted by disturbance history through the use of linear mixed models (only conducted where over 30 detections were made of a particular family). A Poisson family distribution was utilised initially (package lme4; glmer function) but significant levels of overdispersion were detectable within the models, a common feature in count data (Zuur et al. 2009). A negative binomial family distribution with a log link function was therefore used to account for this overdispersion (packages - R2admb and glmmADMB; using function glmmadmb) (Trimble & van Aarde 2014; Zuur et al. 2009). To account for repeat measures from transect groups and from pitfall arrays, transect group or pitfall identification was added as a random effect. An analysis of variance (ANOVA) test on the log likelihoods of models including and excluding disturbance history was used to determine the significance of disturbance history as a predictor of abundance for each method. As the family Craugastoridae, containing the genus *Pristimantis* (previously *Eleutherodactylus*), have previously been identified as a key indicator group of good quality habitat by Pearman (1997), we used the same GLMM model structure to assess the observed species richness encountered within this group between disturbance areas, in addition to testing the abundance of the group.

Results

Following the exclusion of wetland and large river associated amphibian species, 968 individuals of 34 species were recorded (Table 1). These included 551 individuals of 30 species from nocturnal transects and 417 individuals of 21 species from pitfall traps.

Table 1. Observed and estimated species richness patterns based on different survey methodologies. A comparison of nocturnal transects and pitfall traps as amphibian survey methodologies.

		Habitat class	n^a	Survey effort: samples	Observed species	Extrapolated species^b	Species richness estimates									Coverage (%)^d	Completeness (%)^e	Unique species
							ACE	*ICE*	*Chao 1*	*Chao 2*	*Jacknife 1*	*Jacknife 2*	*Bootstrap*	*MMMean*	Average^c			
Amphibians	**Nocturnal transects**	SLR	284	47	22	22	24	24	23	23	26	25	24	24	**24**	92	73	5
		CCR	74	44	13	15	17	16	14	14	17	17	15	17	**16**	94	43	3
		MXD	193	49	23	27	31	38	28	32	33	39	27	26	**32**	84	77	1
		Total	551	140	30													
	Pitfall traps	SLR	112	135	12	14	16	16	18	15	16	19	14	13	**16**	88	57	3
		CCR	164	135	15	15	20	22	17	19	20	22	17	16	**19**	79	71	2
		MXD	141	135	13	14	19	19	17	17	17	19	15	14	**17**	82	62	2
		Total	417	405	21													

[a] Number of individual records

[b] Number of species estimated when curves extrapolated to the same number of encounters

[c] Mean estimated species richness - 'classic Chao values were used in cases where CV>0.5

[d] Sampling coverage defined as: $^{b/d}$*100

[e] Number of species observed as a percentage of combined species across all habitats

The average estimated species richness from nocturnal transects was highest in MXD disturbance type (32 ±6.61species; 84% confidence intervals), followed by the SLR disturbance type with an estimated 24 ±1.09 species and just 16 ±1.68 species in CCR (34% lower than SLR; Table 1). With non-overlapping 84% confidence intervals of average estimated species richness, except for a small margin of overlap between SLR and MXD disturbance areas (SLR lower ci 22.62, upper ci 25.62; MXD lower ci 25.04, upper ci 38.26; CCR lower ci 14.19, upper ci 17.55), these differences appear to be significant ($p<0.05$). Figure 2 shows that based on the extrapolated rarefaction curves of predicted species richness (Table 2), MXD and CCR species richness values lie outside the 84% CI for the SLR curve. Linear modelling showed that using the transect methodology, disturbance history type was a good predictor of estimated species richness with 60.6% of variation explained and that SLR locations had on average 10.3 (± 4.4 s.e) more estimated species than CCR locations (d.f.=6, f=4.62, p=0.06). Survey effort across sampling locations was also found to have some effect on estimated species richness (p=0.09) but did not significantly change the effect size of disturbance history.

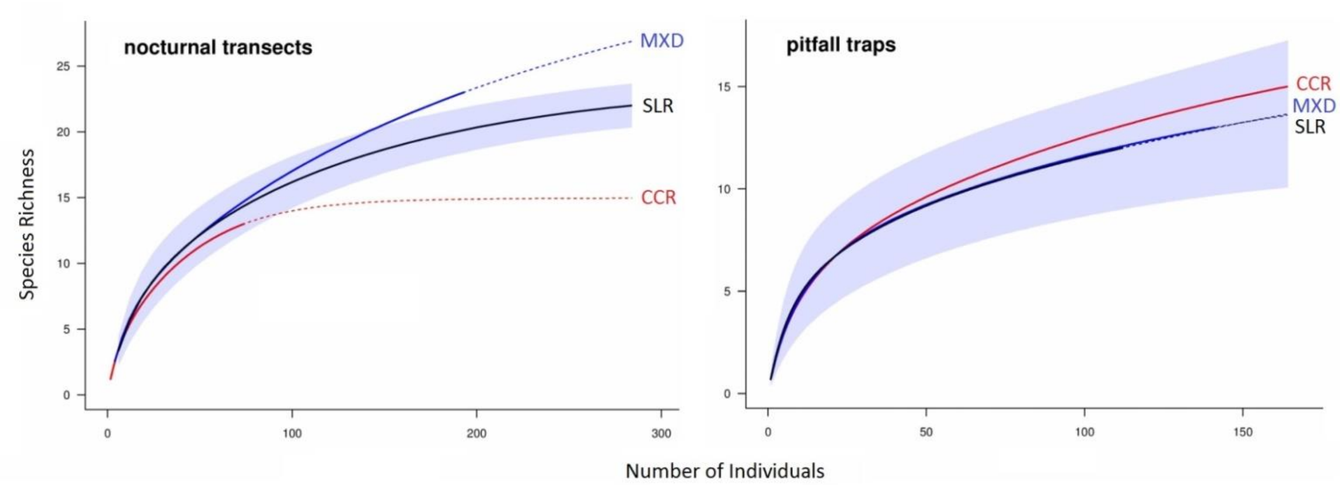


Figure 2. Amphibian species richness of regenerating rainforest with different disturbance histories for amphibian communities from the same area based on nocturnal transects and pitfall traps. Solid lines represent the observed number of individuals recorded and dashed lines represent predicted species richness based on extrapolated rarefaction curves. The grey shades represent 84% confidence intervals for SLR disturbance type, only the confidence intervals for SLR are plotted to provide clearer graphs; for transects, CCR and MXD predicted species richness lie outside the SLR confidence intervals and are therefore likely to be significantly different while for pitfalls there is no difference.

The average estimated species richness from pitfall traps did not appear to show any difference between disturbance areas; CCR = 19 ±2.32 species, MXD = 17 ±1.95 species and SLR = 16 ±2.05 species, with overlap between 84% confidence intervals (p>0.05; SLR lower ci 12.96, upper ci 18.59; MXD lower ci 14.34, upper ci 19.68; CCR lower ci 15.98, upper ci 22.34). Disturbance history type was also a good predictor of species richness for pitfalls, explaining 22.05% of variation explained, but suggested the opposite patterns with on average 2.6 (± 1.60 s.e) fewer species in SLR than CCR (d.f. = 15, f = 2.12, p = 0.15). As the two methods predicting different directions for the species richness pattern this difference is significant (p=0.01). Survey effort across sampling locations was found to have no effect on estimated species richness for pitfalls.

Overall Shannon diversity from nocturnal transects was higher in SLR than in CCR (Fig. 3) but not for pitfall traps. The MXD habitat displayed intermediate values of Shannon diversity between SLR and CCR disturbance areas. Linear modelling showed that using the transect methodology, disturbance history type explained 35.4% of variation for Shannon diversity (d.f. = 6, f = 1.65, p = 0.27). Survey effort across sampling locations was also found to have an effect but did not significantly change the effect size of disturbance history. However, linear modelling showed that when using the pitfall methodology, disturbance history type explained just 0.04% of variation for Shannon diversity (d.f. = 15, f = 0.27, p = 0.76). Although the two methods predict different directions for the diversity pattern, this difference was not significant (p=0.21).

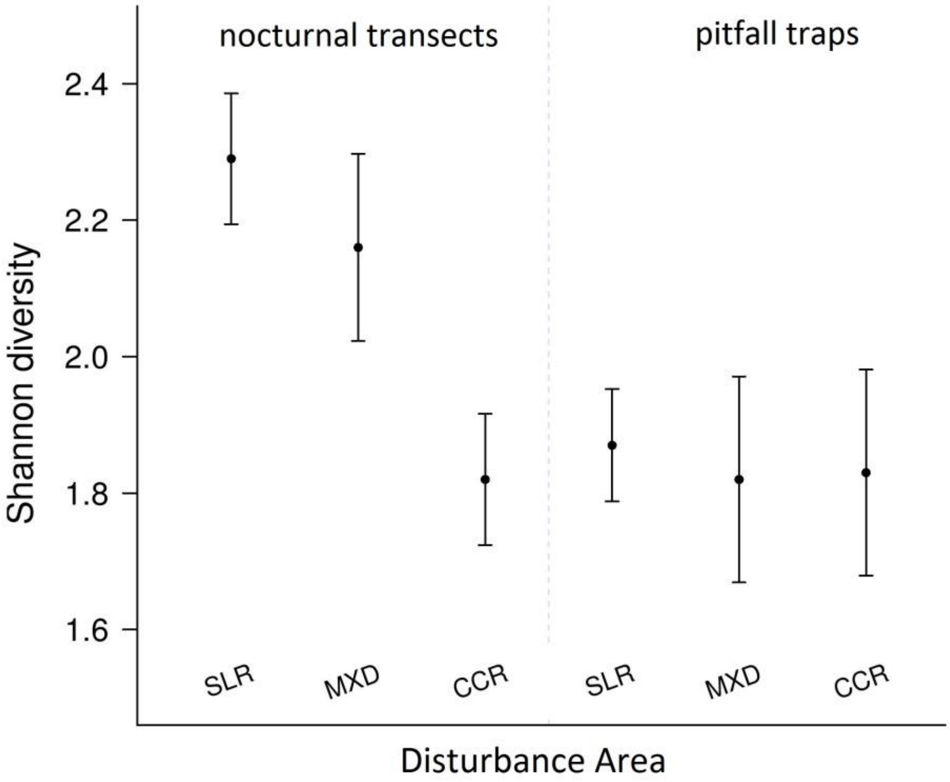


Figure 3. Shannon species diversity of regenerating rainforest with different disturbance histories for amphibian communities from the same area based on nocturnal transects and pitfall traps; with 84% confidence intervals.

Testing of species richness and species diversity model residuals, from both transect and pitfall date, showed no evidence of spatial auto-correlation between samples with very low correlations (range from -0.22 to -0.09) and non-significant observed Moran's I values (range from p=0.18 to 0.65).

Dominance-diversity plots demonstrated that the amphibian community recorded by nocturnal transects supports a significantly more even assemblage (regular intervals between species) with more rare species (increased tail length) in SLR and MXD habitat, than in CCR (Fig. 4). This difference was significant between SLR and CCR ($\Delta G = -0.08$, $t = -2.25$, $p=0.03$), and between

MXD and CCR (ΔG = -0.10, t = -3.00, p=0.004). The only significant difference for pitfall traps was a more even community structure in CCR than SLR habitat (ΔG = 0.08, t = 2.14, p=0.04).

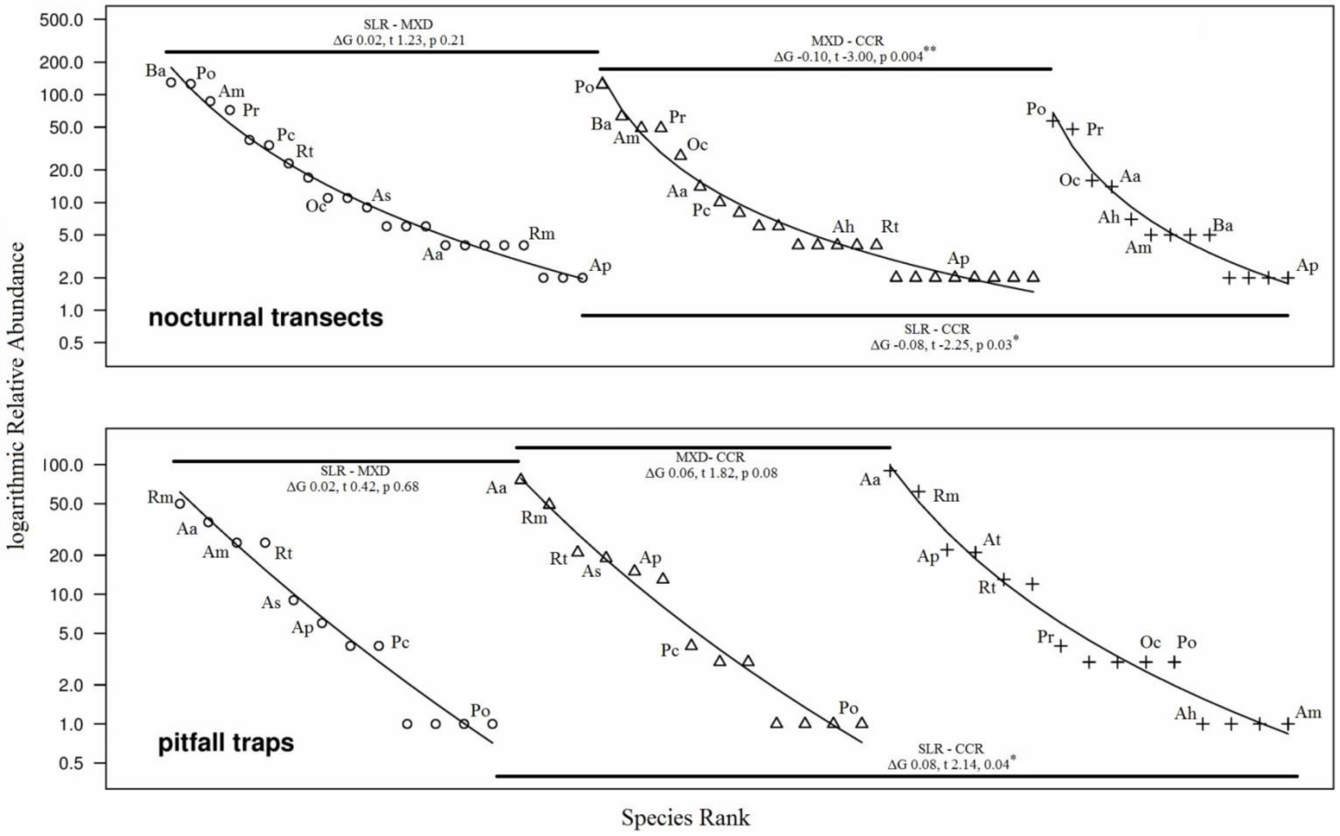


Figure 4. Dominance-diversity (Whittaker) plots for nocturnal transects and pitfall trap amphibian communities in regenerating rainforest with different disturbance histories. Species are represented by points. For each habitat the relative abundance of each species (ni/N) was plotted on a logarithmic scale against the species rank ordered from most to least abundant. O = SLR, Δ = MXD and + = CCR. Linear models were used to determine if the slopes of SLR, MXD and CCR were significantly different, where ΔG denotes to absolute change in gradient from the comparative gradient and the * symbol denote the level of significance of the deviation where * = 0.05, ** = 0.01. The most dominant five species from SLR were indicated on each of the curves, along with any of the five most dominant species from MXD and CCR in order to investigate compositional shifts in the most frequently encountered or dominant species from each habitat.

The community composition analysis from NMDS plots and the associated ANOSIM analysis (Fig. 5) showed that community composition between disturbance areas was significantly different for both nocturnal transects (R=0.47, p=0.01) and pitfall trap (R=0.16, p=0.03) methodologies. However, the R statistic, which denotes the degree of difference between communities, is higher for the communities assessed using nocturnal transects and although the communities assessed by pitfall traps shows a significant p-value, the R statistic is relatively low. The NMDS plots (Fig. 5) show that the SLR community for nocturnal transects is completely distinct from both CCR and MXD communities (which show a significant degree of overlap), whilst for pitfalls, the SLR community is only distinct from the CCR community but shares some degree of overlap with the MXD community.

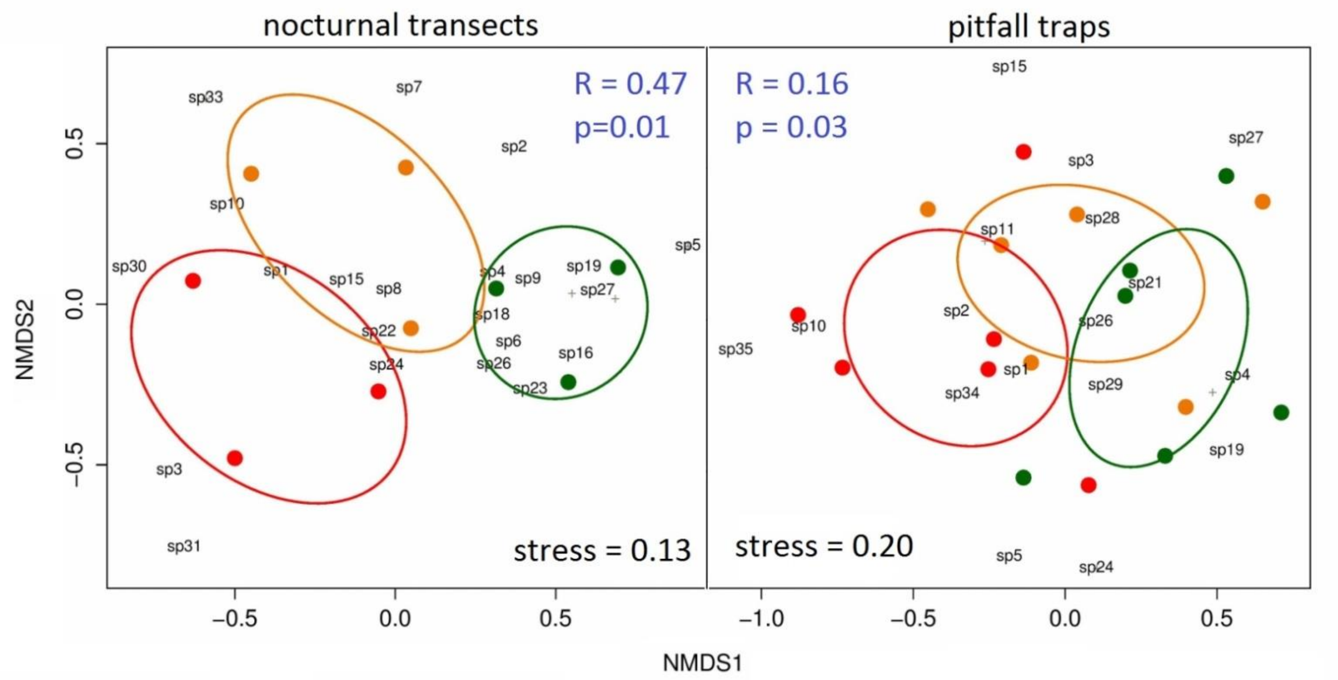


Figure 5 - Community composition NMDS plots of regenerating rainforest with different disturbance histories for amphibian communities from the same area, based on nocturnal transects and pitfall traps; species and corresponding codes are provided in Appendix 6A. The red circles = CCR sampling locations, orange circles = MXD sampling locations, and green circles = SLR sampling locations. Species points (+) and labels (e.g. sp1) were plotted using function orditorp in vegan package (Oksanen et al. 2011). This function will label an item only if this can be done without overwriting previous labels. If an item could not be labelled with text (priority was given to the most abundant species), it was marked as a point. Function ordiellipse in vegan package (Oksanen et al. 2011) was used to draw 95% confidence interval for disturbance classifications assigned to sampling locations. Stress values of the NMDS for two-dimensions are displayed, along with the respective R statistic and p-values from the associated ANOSIM analyses.

Differences in the most frequently encountered species between habitats were visible from the dominance-diversity plots (Fig. 4) and the NMDS community composition plots for both survey methodologies (for observed records and relative abundances of all species, see Appendix A). For example, *Ameerega macero* (Am - both transects and pitfalls), *A. sp1* (As – pitfalls), *Pristimantis carvalhoi* (Pc – transects) and the salamander, *Bolitoglossa caldwellae* (Ba - transects) all displayed a reduction in both abundance and community rank with increasing disturbance (i.e. disturbance sensitive species). However, other species such as *P. ockendeni* (Po - transects) and *P. reichlei* (Pr - transects) retained a high species rank despite decrease in abundance (i.e. habitat generalists) and an association with more intensely disturbed habitats. Some species, such as *Rhinella marina* (Pitfalls - Rm), *R. margaritifera* (Rt – Pitfalls), *Osteocephalus castaneicola* (Oc – transects) and *Adenomera andrea* (Aa - pitfalls) not only retained species rank but increased in abundance slightly in the habitat with the most intense historic disturbance (i.e. disturbance specialists).

The overall relative abundance of amphibians from nocturnal transects was significantly different between disturbance areas, highest in SLR and lowest in CCR (n/20ha; n_{CCR}=168, n_{MXD}=394, n_{SLR}=604; p=<0.001), whilst overall amphibian abundance from pitfall traps showed no difference (n/200 trap days; n_{CCR}=243, n_{MXD}=209, n_{SLR}=166; p=0.19; Table 2). When considering different families encountered along nocturnal transects, Dendrobatidae (p=0.001) and Plethodontidae (p=<0.001) both displayed a significantly different abundance between disturbance areas, the Craugastoridae were very close to significant (p=0.051), whilst Hylidae and Leptodactylidae were not. Each group that displayed a difference was in highest abundance in SLR and lowest in CCR. Pitfall traps found Dendrobatidae (p=0.003) and Leptodactylidae (p=0.03) to display significantly different abundances but in opposite directions, with Dendrobatidae in higher abundance in SLR and Leptodactylidae in higher abundance in CCR. Bufonidae displayed no difference in abundance between disturbance areas (p=0.93). The key indicator group of good quality habitat (Pearman 1997), the Craugastoridae, not only displayed a higher abundance in the less intensely disturbed habitat (SLR) but also displayed a higher observed species richness at the survey level in SLR forest; a result detectable from transects data (ANOVA result between disturbance history and the null model, p=0.04), but not from pitfall traps (ANOVA result between disturbance history and the null model, p=0.85).

Table 2. The mean relative abundances for amphibian families in each disturbance type. Nocturnal transect relative abundances (RA) represent the number of individuals encountered per 100 transect set nights surveyed per night; pitfall trap relative abundances (RA) represent the number of individuals encountered per 200 trapping days at a given site. n = number of encounters; p = p-value relates to an ANOVA test on the log likelihoods of models including and excluding disturbance history; the * symbol denotes the level of significance where * = <0.01 and ** = <0.001; only conducted where Total n>30.

Family	Nocturnal transects								Pitfall traps							
	CCR		MXD		SLR		Total		CCR		MXD		SLR		Total	Statistical test
	n	RA	n	RA	N	RA	n	p	n	RA	n	RA	n	RA	n	p
Craugastoridae	67	102	122	191	243	363	432	**0.051**	5	6	7	8	6	7	18	-
Hylidae	27	32	23	33	12	15	62	0.41	2	2	-	-	-	-	2	-
Dendrobatidae	8	12	31	48	72	107	111	**0.02***	4	4	15	17	32	36	51	**0.003***
Centrolenidae	-	-	4	6	6	9	10	-	-	-	-	-	-	-	-	-
Aromobatidae	-	-	-	-	-	-	-	-	16	18	4	4	1	1	21	-
Leptodactylidae	17	26	11	17	11	16	39	0.70	114	127	83	92	41	46	238	**0.03***
Bufonidae	1	2	2	3	17	25	20	-	65	72	58	64	60	67	183	0.93
Plethodontidae	2	3	34	53	88	131	124	**<0.001****	-	-	-	-	-	-	-	-
Total	**116**	**176**	**227**	**355**	**449**	**670**	**792**	**<0.001****	**206**	**229**	**168**	**187**	**140**	**156**	**514**	**0.19**

Discussion

We show that two different but commonly utilised survey methodologies identify contrasting biodiversity patterns in a human modified rainforest, decades after initial disturbance. The occurrence of contrasting patterns depending on methodology held true for a variety of frequently utilised biodiversity measures; species richness, diversity, abundance, community structure and community composition. Using nocturnal transects to assess amphibian biodiversity suggested that historic clearance of tropical forest resulted in lower levels of amphibian biodiversity and a greater disruption to community evenness and composition, compared with forest once subjected to selective logging. Whereas pitfall traps indicated no difference in amphibian species richness, diversity or abundance, and a lower level of dissimilarity in community composition between disturbance areas than nocturnal transects. The community evenness structure plots actually showed the opposite pattern to transects, with a more evn community structure in CCR than SLR habitat. These results show how assessing the same taxonomic group, at the same site, using different methods can suggest different relative biodiversity value between disturbance types, which could ultimately therefore lead to over or underestimation of the conservation value of different types of regenerating tropical forests.

Although previous studies have identified that survey methodologies often target subsets of faunal communities (Sparrow et al. 1994) and have investigated the most efficient methods (Doan 2003), few studies have systematically assessed the potential for different methodologies to lead to contrasting conclusions in relation to biodiversity and conservation value of regenerating forests (Barlow et al. 2007b). The results reported here focused on the effect of methodology on detecting patterns in amphibian biodiversity; however, several previous studies on other taxonomic groups, which focused on other questions, suggest that such methodological effects may be important for biodiversity assessments more generally. For example, Barlow et al. (2007b) utilised mist nets and point counts to assess the response of bird communities to tropical forest disturbance in Brazil and found a contrasting response of bird species richness. Primary forest bird species richness was higher than in secondary forest when point counts were used but was equal to primary forest when mist nets were utilised. In another avian study, mist netting found a negative response to the presence of an unmarked Amazonian forest road, whilst bird point counts detected the opposite pattern, with a greater biodiversity detected near to the road (Chapter 3 of this thesis).In addition to amphibians and birds, similar methodological patterns have been found for butterflies but were conducted at different survey sites (Kudavidanage et al. 2012, Ribeiro et al. 2015).

A key aspect of the approach in this study is that the type of contrasting patterns identified here can only be linked to methodological effects, because they were carried out within the same study site. If data on different methods had come from different study sites then it would have been much more difficult to disentangle the effects of study location. For example, contrasting results from studies upon butterflies have been found across a variety of locations (Devy and Davidar 2001; Dumbrell and Hill 2005; Kudavidanage et al. 2012; Posa and Sodhi 2006; Ribeiro et al. 2015; Ribeiro and Freitas 2012), making it difficult to robustly conclude that opposing patterns are related to landscape differences and not due to the sampling methodology utilised.

Caveats and limitations

Although confident that observed differences are an effect of different methodologies, one alternative explanation for the results is the potential that the pitfalls and transects are in different locations, and so results could be due to systematic differences in locations rather than methods, however, since the pitfalls are interspersed within the network of transects this should be unlikely. Although we may also consider factors other than disturbance history type as the cause of the difference, in this specific study we are interested specifically in what patterns the methods detect, and not the cause of the differences. However, this study utilised a natural experiment approach in order to follow the recommendations of Ramage et al. (2013) for avoiding potential pseudo-replication problems in tropical forest ecology, and we also examined whether spatial autocorrelation of the sampling locations could be driving the biodiversity patterns detected. The autocorrelation analysis confirmed that biodiversity patterns detected were not being driven by spatial autocorrelation.

In addition to assessing overall patterns of biodiversity, we also investigated fine scale metrics of the amphibian community, in the form of species specific response patterns and abundances of family groups (as opposed to overall community structure and overall abundance patterns). Although there is a growing body of literature investigating species specific and functional groups in tropical forests for birds (De Coster et al. 2015; Edwards et al. 2013; Hidasi-Neto et al. 2012; Newbold et al. 2013) and plants (Ding et al. 2012; Carreño-Rocabado et al. 2012), few studies exist for amphibians (Trimble and van Aarde 2014). These fine scale metrics allowed for the detection of community structure and composition differences between disturbance areas using both survey methodologies; with increases/decreases in rank and abundance for some species. Although overall abundance of the amphibian community from pitfalls did not show a difference between disturbance areas, two of the three families tested did, albeit in opposite directions. We suggest therefore, that fine scale metrics, which assess species specific responses or patterns of families, may be less susceptible to show contrasting patterns between methodologies. However, this should be considered with caution and requires further investigation, specifically in light of concerns over the application of community similarity metrics to assess disturbance patterns across landscapes, as suggested by Ramage et al. (2013).

In addition to simply identifying that the different levels of impact upon biodiversity linked to habitat change may be the result of alternative methodologies, it is also important to understand more about why alternative methods indicate different patterns. Within this study for example, we utilised two commonly used methodologies which target distinct subsets of the overall amphibian community. Pitfall traps better target the terrestrial amphibian community (i.e. Dendrobatidae, Leptodactylidae and Bufonidae; e.g. Beirne et al. 2013), whereas nocturnal transects have been shown to be more efficient in detecting a wider representation of the amphibian community (e.g. Beirne et al. 2013; Doan 2003), including both terrestrial (i.e. Dendrobatidae and Leptodactylidae) and arboreal groups (i.e. Craugastoridae, Hylidae and Plethodontidae). Our results related to overall patterns may therefore suggest that arboreal amphibian communities are more sensitive to habitat disturbance than terrestrial communities. This is a pattern that has been detected for a variety of invertebrates within tropical forests, including ants (Klimes et al. 2012), dung beetles (Tregidgo et al. 2010), and butterflies (Chapter four of this thesis). The known key indicator group of good quality habitat (Pearman 1997), the Craugastoridae, are more commonly encountered via transects as opposed to terrestrial based techniques, and are a mostly semi-arboreal group (comprising mostly *Pristimantis sp.*). However, the mostly terrestrial Dendrobatidae appeared well represented by both methodologies and indicated the same abundance patterns in relation to historic disturbance. Contrastingly, Leptodactylidae were better surveyed by pitfall traps, with only this method detecting a significantly different abundance related to historic disturbance. This may be due to the detectability differences within these families, which relates to morphological and functional traits (Mouillot et al. 2013). Dendrobatids are often bright coloured and conspicuous, therefore easy to spot on the ground at night; whereas Leptodactylidae are brown cryptically coloured frogs, often located in holes, more difficult to detect at night in the leaf-litter.

Conclusions

In conclusion, we show that the choice of survey methodology, for the same taxonomic group, can suggest different biodiversity values of regenerating tropical forest, and as such, methods matter in assessments of habitat disturbance upon biodiversity. This suggests that the use of different methods could be an important factor as to why there are conflicting results and therefore conclusions regarding the biodiversity value of secondary regenerating tropical forests (Chazdon et al. 2009a; Sloan et al. 2015). Increasing our understanding about different methodologies and the patterns they suggest can probably be best achieved by conducting side-by-side comparisons of survey methodologies at the same study locations. Such studies are likely to be important if we intend to better unravel the factors relating to how well tropical forest biodiversity can recover from environmental change.

Thesis Discussion

In this discussion I aim to highlight the three main themes described and assessed within the six data chapters of this thesis. To do this I reiterate the primary results and place these within context to related literature. In addition to discussing the six main data chapters, a further five manuscript papers from the appendices are also discussed within the overall context of this thesis. Each of these is related in some way towards assessing the effects of habitat disturbance to biodiversity in tropical forest, the novel testing and trialling of survey methodologies and the use of limited numbers of observation records to generate up-to-date distribution maps for species with few or occasional records. For more detail on these papers and details for their inclusion, please see the outline section of this thesis.

The biodiversity and conservation value of regenerating rainforest

Using a best-case scenario, case-study approach, the results of this thesis suggest that regenerating rainforest, in the absence of on-going human impacts, may have the potential to hold high levels of tropical faunal biodiversity. These levels were shown to be higher than the average value approach used by other review studies (Barlow et al. 2007; Chazdon et al. 2009a; Gibson et al. 2011), which often assess mostly young regenerating areas (<30 years) and can include forests that continue to experience on-going human impacts, through low-level logging or hunting. In the absence of these potentially confounding factors, the best-case scenario approach presented here, not only shows that regenerating forest might have the potential to successfully conserve high levels of biodiversity but that they can also include a high proportion of species of conservation concern. The results also suggest that commonly used historic land uses at a within-site spatial scale (Ramage et al. 2013) affect biodiversity to a varying degree and that these differences may still be observed even decades after initial human disturbance for some biodiversity groups (amphibians, butterflies and nocturnal birds) but not others (diurnal birds and medium-to-large terrestrial mammals). This could be important in understanding how current land uses being utilised within cultural buffer areas (a key feature of many Biosphere Reserves) will affect future levels of biodiversity (Bowen et al.

2007; Dunn 2004a). This is a key point when we consider that the success of core protected areas, such as Manu, Sumaco and Yasuni, has been suggested to depend upon the success of surrounding buffer areas (Laurance et al. 2012a).

Understanding the potential biodiversity and conservation value of secondary regenerating landscapes and the impacts of current human disturbances to biodiversity could be essential if we hope to create successfully managed buffer zones (Laurance et al. 2012a); which in turn will allow for the successful protection of primary forest and preserve the areas shown to be of highest biodiversity and conservation value (Chazdon et al. 2009a, 2009b; Laurance et al. 2012a). This could be achieved by increased realisation of the potential of secondary regenerating forests, in the absence of on-going disturbances; and the use of regenerating lands to increase the connectivity of primary areas and help to buffer the threat and pressure to primary forest areas. Placing a greater importance on these regenerating forests and abandoned lands could prove essential if we hope to prevent the further degradation and clearance of these areas for the introduction of extremely intensive, monoculture practices; such as palm oil, which have shown to contain extremely low levels of biodiversity (Chazdon et al. 2009a; Edwards et al. 2011; de Queiroz et al. 2014). In assessing a case study forest site under the best-case of scenarios, i.e. contiguous to large tracts of primary forest, in the absence of on-going human impacts and with decades for regeneration; this thesis has been able to highlight this high potential value for biodiversity conservation. This could be an important tool for local reserves and native communities to put into context their own biodiversity and conservation value if they don’t have resources, in terms of funds, access or time (Lawton et al. 1998; Gardner et al. 2008). Especilly as assessments are essential to local reserves in understanding biodiversity and conservation value to attract potential visitors, or in developing payment services systems related to landscape preservation (CBD 2010; Phelps et al. 2012; Sommerville et al. 2012).

Current and recent disturbances to biodiversity within tropical rainforest

Another important finding from this thesis relates to current forms of on-going or recent disturbance to tropical rainforest. This first of these is about the impacts to biodiversity by an unmarked Amazonian road. Roads have been highlighted as a major source for deforestation, with nearly 95% of all deforestation within the Amazon occurring within 5.5km of roads or 1km of rivers (Barber et al. 2014). The study presented within this thesis was the first muti-taxa faunal study to be carried out on a small unmarked road within the western Amazon and suggested that the impacts are greater than land managers may have expected. This could be of great importance to future planning and land management within regions of the western Amazon, which to date have remained relatively free from the fragmentation and dissection of roads (Finer et al. 2008; Finer et al. 2014). These largely ‘roadless’ areas however, are quickly changing, as already observed throughout regions of Brasil (Ahmed et al. 2013), where road networks have expanded rapidly; creating significant ‘fishbone’ effects of small unmarked and pioneering roads stemming from larger highways.

Despite the effect appearing as a ‘fishbone’ from satellite or drone imagery from above, the effects upon biodiversity run much farther into the forest and do not just affect the direct physical area in which the road is located; as suggested by the case study from chapter three of this thesis. If land managers wish to successfully preserve primary forest areas, (mostly contained within protected areas) then understanding how road networks surrounding these areas, and potentially within them, could impact biodiversity is essential (Epps et al. 2015; Laurance et al. 2012a; Laurance et al. 2014b). For example, the cultural zone within Manu has a road situated between the Manu National Park and the Amarakaeri Reserve (Pieck 2013). The road currently stems from the nearby city of Cusco in the Andes, terminating at the small jungle village of Nuevo Eden. However, there are plans to expand this road and create access right through the Biosphere, so that Manu will eventually be directly connected to the growing jungle town of Puerto Maldonado. This could have drastic effects in terms of connectivity of forest in Manu, causing significant changes to surrounding biodiversity and ultimately cause a greater influx of colonisers and threats to the region (Monteiro et al. 2014). These threats stem from the desire to extract natural resources such as timber, gold, coca and expand land for agriculture. Roads assist in decreasing the transport costs in difficult to reach places. This means that areas where less profitable timbers, which may have been left standing in areas that have been cleared of more profitable timbers, may also become financially viable to extract and result in further, if not complete clearance of forest within these regions.

The second assessment of more recently degraded forest habitat was added as Appendix Manuscript 1. This study, carried out at the same study site as the road assessment, surveyed amphibian and reptiles communities within recently abandoned (~10 years) grassland and plantation habitats and compared them both against one another and with surrounding areas of forest. As these areas were only recently abandoned, the forest habitat had not had time to regenerate and create a closed canopy cover over the abandoned disturbed areas, as with the site surveyed in Manu. Although recently abandoned plantations (of coffee, cacao and guyaba) supported similar numbers of individuals to forest habitat, they contained fewer species. The opposite was true for the abandoned grasslands, which contained higher levels of biodiversity than the abandoned plantation (still lower than the forest habitat) but higher numbers of individuals. However, the reserve as a whole, a matrix of forest and abandoned human altered habitats, contained relatively high levels of biodiversity (71 amphibian and 72 reptile species) when compared with another well surveyed site from primary forest in the protected area of Yasuni National Park (105 amphibian and 80 reptile species). As such, this study showed that even a small reserve located within a buffer region, comprising of a matrix of forest and human disturbed landscapes, did contain high levels of herpetological biodiversity despite relatively recent disturbance (Gillespie et al. 2015; Riemann et al. 2015). When we consider that the first two chapters of this thesis show a high potential future value for once disturbed areas, if this was a general pattern, then we might predict that given a significant time for regeneration and adequate protection, then the biodiversity of this Ecuadorian reserve might also continue to increase and potentially reach comparable levels to primary forest.

The effects of methodologies, survey design and the development of new survey techniques

This thesis investigated two potentially confounding factors of tropical rainforest biodiversity assessments in relation to methodologies and study design. The first of these highlighted the chance for different potential values to be suggested depending upon whether assessments were made along the ground or at different vertical levels within the canopy (DeVries et al. 1997; Dumbrell & Hill 2005; Haefke et al. 2013; Tregidgo et al. 2010).

In terms of butterfly survey assessments, results showed that at least three trapping levels across vertical strata were necessary to observe the disruption to vertical strata of biodiversity between regenerating forests of different original human disturbance. The third level in the understorey allowed the observation of a disruption to biodiversity that might not have been detectable had only terrestrial and upper canopy traps been used; a pattern also detected by Fermon et al. (2005), who assessed diversity across five vertical strata. Although the stratification of biodiversity across vertical levels has been highlighted for various taxa, the differential disruption as a result of habitat disturbance has only yet been verified by studying invertebrates (DeVries et al. 1997; Dumbrell & Hill 2005; Maguire et al. 2014; Tregidgo et al. 2010). If we are losing greater amounts of biodiversity higher within the canopy, while the majority of assessments are made along the ground (Lowman 2009), then the impacts of habitat disturbance to overall biodiversity are likely being underestimated (Fermon et al. 2005). This was true for butterflies within this thesis, despite utilising the same methodology and trapping design, at three different vertical strata, across each of the areas of different historic disturbance.

Second to this, this thesis highlights how different survey methodologies, which often target different subsets of communities, can potentially provide contrasting patterns of biodiversity in response to habitat degradation and subsequent regeneration. Understanding how different methods may under or overestimate biodiversity values in regenerating areas is important in understanding contrasting patterns between studies and can be done directly at a within-site scale (Barlow et al. 2007b). Although this has been observed and suggested previously, comparisons are often noted between different survey regions or have simply assessed the effectiveness of methodologies. The sixth chapter of this thesis provided a direct assessment of two methods to provide contrasting patterns at the same site and in reference to how these methods could provide contrasting patterns of the response of biodiversity to habitat disturbance. This migh suggest that the factor of methodology selection and utilisation of different methods could be a contributing factor to the varying values related to the biodiversity and conservation value of human disturbed landscapes more widely; but this should be further tested to see if this is a general pattern.

Finally, in this thesis I have investigated and tested new survey techniques in order to better understand the biodiversity within tropical forest landscapes. The results in the fifth chapter of this thesis assessed the effectiveness for camera traps to be used within the trees to detect arboreal mammals. This relatively novel use of camera traps proved effective in detecting cryptic arboreal species when compared to traditional methodologies and as such, could prove a useful tool for future assessments to understand how arboreal mammals are being affected by habitat change, degradation and subsequent forest regeneration. This is an important tool for conservation, as many arboreal mammals, such as primates for example, are viewed as important charismatic flagship species for conservation (Kays & Allison 2001; Whitworth 2012). Using camera traps in the canopy for example, could potentially gather exciting footage of these charismatic animals and contribute information of little known cryptic and nocturnal species that are otherwise extremely difficult to detect from the ground (Lowman 2009). This might provide the opportunity to increase our knowledge about species ecology, distributions and responses to disturbance; and also provides the opportunity to rally support and interest in rainforest biodiversity and conservation to a wider public audience (Kays & Allison 2001). Further developing such novel and remote technologies are an important way of advancing our understanding of tropical rainforest biodiversity in general and which can then be used as useful assessment techniques (Pimm et al. 2015).

One other methodology presented in appendix manuscript two, applied for the first time on hereptofauna in a tropical forest, was the use of thread-bobbins.

The method has been used previously for other taxa, and for reptiles in different environments but never within a rainforest for amphibians and reptiles (Tozetti & Martins 2007). The method was found particularly effective for gathering detailed ecological information over a relatively short time frame and proved to be a very cost-effective solution. Developing such cost-effective methods to understand more about the ecology of these groups is essential to learning more about highly threatened, yet poorly known tropical forest species (Böhm et al. 2013; Lawton et al. 1999).

In appendix manuscript three, bamboo traps were used to detect the presence of two poorly known Amazonian amphibian species; providing new information regarding the breeding behaviour of one (*Osteocephalus castaneicola*) and use of bamboo as important refugia for a second species (*Pristimantis olivaceus*). Finally, in appendix manuscripts four and five, the distributions of two Amazonian species, a tree frog (*Osteocephalus mimeticus*) and the hummingbird, the blue-fronted lancebill (*Doryfera johannae*) were predicted using presence based records, each of which was detected at the regenerating study site in the Manu Biosphere Reserve. This methodology through the use of relatively small numbers of records for each species, combined with climatic data layers, allowed the prediction of species presence and production of maps of estimated distributions. Understanding basic ecological and distributional information is essential in understanding the potential effects of habitat change or loss, especially from the super biodiverse tropics where much of this information is still absent (Böhm et al. 2013).

Only through the development and testing of new methodologies, such as the arboreal camera traps, thread-bobbins and others presented in this thesis, can we improve our ability to assess the impacts of habitat change upon species distributions and populations (Böhm et al. 2013; Lawton et al. 1999).

Additional/future themes and conclusions

Overall in this thesis I have investigated the conservation and biodiversity value of regenerating and human disturbed tropical forest and utilised and tested methods of biodiversity assessment within them. The results add to current understanding around this topic of research, specifically through the use of a within-site scale approach. However, the case studies presented here should be trialled and tested across a greater number of sites to see if the findings are specific to the study areas within this thesis or are more widely and generally representative of regenerating tropical forests both in the neotropics and globally. For example, although this thesis provides the first study on the impacts of an unmarked road to faunal biodiversity in the western Amazon, future studies assessing multiple roads would confirm these results more widely. Future studies could also assess the effects of different sized roads in the western Amazon, assess the impact of traffic intensity, assess how the disturbance to fauna around these roads will likely affect the floristic structure and services directly surrounding them and finally, develop and assess mitigation strategies and the effectiveness of these around the roads (Gregory et al. 2014).

Additionally, although not specifically assessed within this thesis, understanding how the disruption to rainforest habitats and biodiversity has resulted in altered functions within tropical forest is a fast growing field of research. For example, large frugivorous birds have been shown to facilitate functional connectivity of fragmented forests (Mueller et al. 2014) and the impacts of hunting by humans has been shown to alter the seedling functional trait composition of neotropical forests (Kurten et al. 2015). Assessments of indicators and functional groups under a best-case scenario approach could allow us to see how the degradation of forests could alter the potential functions of the forest and the ecosystem services that these forests could provide in the future.

In conclusion, through the development of new survey techniques we can increase our understanding of biodiversity patterns more generally. Additionally, through alternative ways of assessing regenerating forests biodiversity and conservation value, there may be the opportunity to generate a greater body of evidence to support the further degradation of these potentially valuable complementary habitats. In terms of a wider context, a reduction in biodiversity signifies not only a reduction in species richness or disruption to communities but also a degradation of ecosystem functions (Edwards et al. 2014) and services to human wellbeing in a global context (Balvanera et al. 2014; CBD 2015; Woodley et al. 2012).

References – main data chapters

Aduse-Poku, K., William, O., Oppong, S. K., Larsen, T., Ofori-Boateng, C., and Molleman, F. (2012). Spatial and temporal variation in butterfly biodiversity in a West African forest: lessons for establishing efficient rapid monitoring programmes. African Journal of Ecology, 50(3), 326-334.

Affre, L., Suehs, C. M., Charpentier, S., Vila, M., Brundu, G., Lambdon, P., and Hulme, P. E. (2010). Consistency in the habitat degree of invasion for three invasive plant species across Mediterranean islands. Biological Invasions, 12(8), 2537-2548.

Ahmed, S. E., Souza Jr, C. M., Riberio, J., and Ewers, R. M., (2013). Temporal patterns of road network development in the Brazilian Amazon. Regional Environmental Change, 13(5), 927-937.

Aide, T. M., Clark, M. L., Grau, H. R., López-Carr, D., Levy, M. A., Redo, D., and Muñiz, M. (2013). Deforestation and reforestation of Latin America and the Caribbean (2001–2010). Biotropica, 45(2), 262-271.

Altman, D. G., and Bland, J. M. (2011). How to obtain the confidence interval from a P value. BMJ, 343, d2090.

AmphibiaWeb. (2013). Berkeley, California: AmphibiaWeb. www.amphibiaweb.org/ Accessed: March 2013.

Anand, M. O., Krishnaswamy, J., and Das, A. (2008). Proximity to forests drives bird conservation value of coffee plantations: implications for certification. Ecological Applications, 18(7), 1754-1763.

Anand, M. O., Krishnaswamy, J., Kumar, A., and Bali, A. (2010). Sustaining biodiversity conservation in human-modified landscapes in the Western Ghats: remnant forests matter. Biological Conservation, 143(10), 2363-2374.

Ansell, F. A., Edwards, D. P., and Hamer, K. C. (2011). Rehabilitation of Logged Rain Forests: Avifaunal Composition, Habitat Structure, and Implications for Biodiversity-Friendly REDD+. Biotropica, 43(4), 504-511.

Armstrong C. (2010) The effect of forest edges on the community structure of tropical fruit-feeding butterflies. The Plymouth Student Scientist. 3(2): 3-17.

Austin, G. T., and Riley, T. J. (1995). Portable bait traps for the study of butterflies. Tropical Lepidoptera, 6(1), 5-9.

Azlan, J. M. and Lading, E. (2006). Camera trapping and conservation in Lambir Hills National Park, Sarawak. Raffles Bulletin of Zoology 54: 469-475.

Balme, G. A., Hunter, L. T. B. and Slotow, R. (2009). Evaluating methods for counting cryptic carnivores. Journal of Wildlife Management 73: 433-441.

Balvanera, P., Siddique, I., Dee, L., Paquette, A., Isbell, F., Gonzalez, A.,and Griffin, J. N. (2014). Linking biodiversity and ecosystem services: current uncertainties and the necessary next steps. BioScience, 64(1), 49-57

Banks-Leite, C., Ewers, R. M., and Metzger, J. P. (2010). Edge effects as the principal cause of area effects on birds in fragmented secondary forest. Oikos, 119(6), 918-926.

Barber, C. P., Cochrane, M. A., Souza Jr, C. M., and Laurance, W. F., (2014). Roads, deforestation, and the mitigating effect of protected areas in the Amazon. Biological Conservation, 177, 203-209.

Barlow, J., Gardner, T. A., Araujo, I. S., Ávila-Pires, T. C., Bonaldo, A. B., Costa, J. E. and Peres, C. A. (2007a). Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. Proceedings of the National Academy of Sciences, 104(47), 18555-18560.

Barlow, J., Mestre, L. A., Gardner, T. A., and Peres, C. A. (2007b). The value of primary, secondary and plantation forests for Amazonian birds. Biological Conservation, 136(2), 212-231.

Barlow, J., Gardner, T. A., Louzada, J., and Peres, C. A. (2010). Measuring the conservation value of tropical primary forests: the effect of occasional species on estimates of biodiversity uniqueness. PLoS One, 5(3), e9609.

Bass, M. S., Finer, M., Jenkins, C. N., Kreft, H., Cisneros-Heredia, D. F., McCracken, S. F. and Kunz, T. H. (2010). Global conservation significance of Ecuador's Yasuní National Park. PloS one, 5(1), e8767.

Beck, H., Thebpanya, P., and Filiaggi, M. (2010). Do Neotropical peccary species (Tayassuidae) function as ecosystem engineers for anurans? Journal of Tropical Ecology, 26(4), 407.

Beck, M. J., and Vander Wall, S. B. (2010). Seed dispersal by scatter-hoarding rodents in arid environments. Journal of Ecology, 98(6), 1300-1309.

Beirne, C., Burdekin, O., and Whitworth, A. (2013). Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in Eastern Ecuador. The Herpetological Journal, 23(4), 209-219.

Bell, K. E., and Donnelly, M. A. (2006). Influence of forest fragmentation on community structure of frogs and lizards in northeastern Costa Rica. Conservation Biology, 20(6), 1750-1760.

Bennett, A. F., Lumsden, L. F., Alexander, J. S. A., Duncan, P. E., Johnson, P. G., Robertson, P. and Silveira, C. E. (1991). Habitat use by arboreal mammals along an environmental gradient in North-eastern Victoria. Wildlife Research 18: 125-146.

Berry, N. J., Phillips, O. L., Lewis, S. L., Hill, J. K., Edwards, D. P., Tawatao, N. B. and Hamer, K. C. (2010). The high value of logged tropical forests: lessons from northern Borneo. Biodiversity and Conservation, 19(4), 985-997.

Bibby, C. J., Burgess, N. D., Hill, D. A., and Mustoe, S. H. Bird Census Techniques, (2000). Academic New York.

Billeter, R., Liira, J., Bailey, D., Bugter, R., Arens, P., Augenstein, I., and Edwards, P. J. (2008). Indicators for biodiversity in agricultural landscapes: a pan-European study. J Applied Ecology, 45(1), 141-150.

Bissonette, J. A., and Rosa, S. A. (2009). Road zone effects in small-mammal communities. Ecology and society, 14(1), 27.

Blake, J. G., and Loiselle, B. A. (2001). Bird assemblages in second-growth and old-growth forests, Costa Rica: perspectives from mist nets and point counts. The Auk, 118(2), 304-326.

Blanche, K. R., Ludwig, J. A., and Cunningham, S. A. (2006). Proximity to rainforest enhances pollination and fruit set in orchards. Journal of Applied Ecology, 43(6), 1182-1187.

Blicharska, M., and Grandin, U. (2015). Why protect biodiversity? Perspectives of conservation professionals in Poland. International Journal of Biodiversity Science, Ecosystem Services and Management, 1-14.

Böhm, M., Collen, B., Baillie, J.E.M., Chanson, J., Cox, N., Hammerson, G., Hoffmann, M., Livingstone, S.R. and Ram, M. (2013). The Conservation Status of the World's Reptiles. Biological Conservation, 157, 372–385.

Bonebrake, T. C., Ponisio, L. C., Boggs, C. L., and Ehrlich, P. R. (2010). More than just indicators: a review of tropical butterfly ecology and conservation. Biological Conservation, 143(8), 1831-1841.

Bowen, M. E., McAlpine, C. A., House, A. P., and Smith, G. C. (2007). Regrowth forests on abandoned agricultural land: a review of their habitat values for recovering forest fauna. Biological Conservation, 140(3), 273-296.

Bradshaw, C. J., Sodhi, N. S., and Brook, B. W. (2008). Tropical turmoil: a biodiversity tragedy in progress. Frontiers in Ecology and the Environment, 7(2), 79-87.

Brandão Jr, A. O. and Souza Jr, C. M., (2006). Mapping unofficial roads with Landsat images: a new tool to improve the monitoring of the Brazilian Amazon rainforest. International Journal of Remote Sensing, 27(1), 177-189.

Broadbent, E.N., Zambrano, A.M.A., Dirzo, R., Durham, W.H., Driscoll, L., Gallagher, P., Salters, R., Schultz, J., Colmenares, A. and Randolph, S.G., (2012). The effect of land use change and ecotourism on biodiversity: a case study of Manuel Antonio, Costa Rica, from 1985 to 2008. Landscape ecology, 27(5), pp.731-744.

Bruton, M. J., McAlpine, C. A., and Maron, M. (2013). Regrowth woodlands are valuable habitat for reptile communities. Biological Conservation, 165, 95-103.

Bshary, R. (2001). Diana monkeys, Cercopithecus diana, adjust their anti-predator response behaviour to human hunting strategies. Behavioral Ecology and Sociobiology 50: 251-256.

Burivalova, Z., Şekercioğlu, Ç. H., and Koh, L. P. (2014). Thresholds of logging intensity to maintain tropical forest biodiversity. Current Biology, 24(16), 1893-1898.

Butt, N., Beyer, H. L., Bennett, J. R., Biggs, D., Maggini, R., Mills, M., and Possingham, H. P. (2013). Biodiversity risks from fossil fuel extraction. Science, 342(6157), 425-426.

Camargo, J. L. C., and Kapos, V. (1995). Complex edge effects on soil moisture and microclimate in central Amazonian forest. Journal of Tropical Ecology, 11(02), 205-221.

Caro, T., Dobson, A., Marshall, A. J., and Peres, C. A. (2014). Compromise solutions between conservation and road building in the tropics. Current Biology, 24(16), R722-R725.

Carreño-Rocabado, G., Peña-Claros, M., Bongers, F., Alarcón, A., Licona, J. C., and Poorter, L. (2012). Effects of disturbance intensity on species and functional diversity in a tropical forest. Journal of Ecology, 100(6), 1453-1463.

Carrillo, E., Wong, G. and Cuarón, D. (2000). Monitoring mammal populations in Costa Rican protected areas under different hunting restriction. Conservation Biology 14: 1580-1591.

Carrillo-Rubio, E., Kery, M., Morreale, S. J., Sullivan, P. J., Gardner, B., Cooch, E. G., and Lassoie, J. P. (2014). Use of multispecies occupancy models to evaluate the response of bird communities to forest degradation associated with logging. Conservation Biology, 28(4), 1034-1044.

Cassano, C. R., Barlow, J., and Pardini, R. (2012). Large mammals in an agroforestry mosaic in the Brazilian Atlantic Forest. Biotropica, 44(6), 818-825.

Catenazzi, A., and von May, R. (2014). Conservation Status of Amphibians in Peru 1. Herpetological Monographs, 28(1), 1-23.

CBD (2010) Access via: https://www.cbd.int/sp/targets/

CBD (2015) Access via: https://www.cbd.int/health/stateofknowledge and https://www.cbd.int/getattachment/health/stateofknowledge/SOK-Summary-Finalv4_reduced-(1).pdf

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., and Palmer, T. M. (2015). Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science advances, 1(5), e1400253.

Chapman, C. A., Bonnell, T. R., Gogarten, J. F., Lambert, J. E., Omeja, P.A., Twinomugisha, D., Wasserman, M. D. and Rothman J. M. (2013). Are primates ecosystem engineers? International Journal of Primatology 34: 1-14.

Chazdon, R. L. (2014). Second growth: The promise of tropical forest regeneration in an age of deforestation. University of Chicago Press.

Chazdon, R. L., Harvey, C. A., Komar, O., Griffith, D. M., Ferguson, B. G., Martínez-Ramos, M. and Philpott, S. M. (2009a). Beyond reserves: A research agenda for conserving biodiversity in human-modified tropical landscapes. Biotropica, 41(2), 142-153.

Chazdon, R. L., Peres, C. A., Dent, D., Sheil, D., Lugo, A. E., Lamb, D. and Miller, S. E. (2009b). The potential for species conservation in tropical secondary forests. Conservation Biology, 23(6), 1406-1417.

Coffin, A. W. (2007). From roadkill to road ecology: a review of the ecological effects of roads. Journal of transport Geography, 15(5), 396-406.

Colwell, R. K., Chao, A., Gotelli, N. J., Lin, S. Y., Mao, C. X., Chazdon, R. L., and Longino, J. T. (2012). Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. Journal of Plant Ecology, 5(1), 3-21.

Colwell, R.K. (2013). Estimate S: Statistical estimation of species richness and shared species from samples. Version 9.0. and earlier. User's Guide and application. URL http://viceroy.eeb.uconn.edu/estimates

Cortés-Gómez, A. M., Castro-Herrera, F., and Urbina-Cardona, J. N. (2013). Small changes in vegetation structure create great changes in amphibian ensembles in the Colombian Pacific rainforest. Tropical Conservation Science, 6(6).

Croes, B. M., Laurance, W. F., Lahm, S. A., Tchignoumba, L., Alonso, A., Lee, M. E. and Buij, R. (2007). The influence of hunting on antipredator behavior in Central African monkeys and duikers. Biotropica 39: 257-263.

Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation, 128(2), 231-240.

Daily, G. C., Ceballos, G., Pacheco, J., Suzán, G., and Sanchez-Azofeifa, A. (2003). Countryside biogeography of neotropical mammals: conservation opportunities in agricultural landscapes of Costa Rica. Conservation Biology, 17(6), 1814-1826.

Datta, A., Anand, M. O. and Naniwadekar, R. (2008). Empty forests: Large carnivore and prey abundance in Namdapha National Park, northeast India. Biological Conservation 141: 1429-1435.

Davis, A. J., Sutton, S. L. and Brendell, M. J. D. (2011). Vertical distribution of beetles in a tropical rainforest in Sulawesi: the role of the canopy in contributing to biodiversity. Sepilok Bulletin 13-14: 59-83.

De Coster, G., Banks-Leite, C., and Metzger, J. P. (2015). Atlantic forest bird communities provide different but not fewer functions after habitat loss. Proceedings of the Royal Society, 282(1811), 20142844). The Royal Society.

de Faria Lopes S, Vale VS, Schiavini I, Júnior JAP, Oliveira AP, and Arantes CS. (2014). Canopy stratification in tropical seasonal forests: how the functional traits of community change among the layers. Bioscience Journal, 30(5).

de Freitas , M. A., de França, D. P. F. and Veríssimo, D. (2013). First record of the Bicoloured-spined Porcupine Coendou bicolor (Tschudi, 1844) for Brazil. Check List 9: 94-96.

de la Peña-Domene M, Martínez-Garza C, Palmas-Pérez S, Rivas-Alonso E, and Howe HF. (2014). Roles of birds and bats in early tropical-forest restoration. PloS one, 9(8): e104656.

de Queiroz, J. S., Silva, F., Ipenza, C., Hernick, C., Batallanos, L., Griswold, D., and Rogers, A. E. (2014). Peru tropical forest and biodiversity assessment.

de Thoisy, B., Brosse, S. and Dubois, M. A. (2008). Assessment of large vertebrate species richness and relative abundance in Neotropical forest using line-transect censuses: what is the minimal effort required? Biodiversity and Conservation 17: 2627-2644

DeClerck, F. A., Chazdon, R., Holl, K. D., Milder, J. C., Finegan, B., Martinez-Salinas, A., and Ramos, Z. (2010). Biodiversity conservation in human-modified landscapes of Mesoamerica: Past, present and future. Biological Conservation, 143(10), 2301-2313.

Demaynadier, P. G., and Hunter, M. L. (1998). Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. Conservation Biology, 12(2), 340-352.

Dent, D.H. and Wright, J.S. (2009). The future of tropical species in secondary forests: A quantitative review. Biological Conservation, 142, 2833–2843.

Devries, P. J., and Walla, T. R. (2001). Species diversity and community structure in neotropical fruit-feeding butterflies. Biological journal of the Linnean Society, 74(1), 1-15.

DeVries, P. J., Lande, R., and Murray, D. (1999). Associations of co-mimetic ithomiine butterflies on small spatial and temporal scales in a neotropical rainforest. Biological Journal of the Linnean Society, 67(1), 73-85.

DeVries, P. J., Murray, D. and Lande, R. (1997). Species diversity in vertical, horizontal, and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. Biological Journal of the Linnean Society 6: 343-364.

Devy, M. S., and Davidar, P. (2001). Response of wet forest butterflies to selective logging in Kalakad-mundanthurai tiger reserve: implications for conservation. Current Science, 80(3), 400-405.

Ding, Y., Zang, R., Letcher, S. G., Liu, S., and He, F. (2012). Disturbance regime changes the trait distribution, phylogenetic structure and community assembly of tropical rain forests. Oikos, 121(8), 1263-1270.

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J., and Collen, B. (2014). Defaunation in the Anthropocene. Science, 345(6195), 401-406.

Doan, T. (2003). Which methods are most effective for surveying rain forest herpetofauna? Journal of Herpetology, 37, 72–81.

Dodd, C. K. (2010). Amphibian ecology and conservation: a handbook of techniques. Oxford University Press.

Dumbrell, A. J., and Hill, J. K. (2005). Impacts of selective logging on canopy and ground assemblages of tropical forest butterflies: implications for sampling. Biological Conservation, 125(1), 123-131.

Dunn, R. R. (2004a). Managing the tropical landscape: a comparison of the effects of logging and forest conversion to agriculture on ants, birds, and lepidoptera. Forest Ecology and Management, 191(1), 215-224.

Dunn, R. R. (2004b). Recovery of faunal communities during tropical forest regeneration. Conservation Biology, 18(2), 302-309.

Durães, R., Carrasco, L., Smith, T. B., and Karubian, J. (2013). Effects of forest disturbance and habitat loss on avian communities in a Neotropical biodiversity hotspot. Biological Conservation, 166, 203-211.

Edwards, D. P., Larsen, T. H., Docherty, T. D., Ansell, F. A., Hsu, W. W., Derhé, M. A. and Wilcove, D. S. (2011). Degraded lands worth protecting: the biological importance of Southeast Asia's repeatedly logged forests. Proceedings of the Royal Society B: Biological Sciences, 278(1702), 82-90.

Edwards, D. P., Woodcock, P., Edwards, F. A., Larsen, T. H., Hsu, W. W., Benedick, S., and Wilcove, D. S. (2012). Reduced-impact logging and biodiversity conservation: a case study from Borneo. Ecological Applications, 22(2), 561-571.

Edwards, D.P., and Laurance, W.F. (2013). Biodiversity despite selective logging. Science, 339, 646-647.

Edwards, F. A., Edwards, D. P., Hamer, K. C., and Davies, R. G. (2013). Impacts of logging and conversion of rainforest to oil palm on the functional diversity of birds in Sundaland. Ibis, 155(2), 313-326.

Edwards, D. P., Tobias, J. A., Sheil, D., Meijaard, E., and Laurance, W. F. (2014a). Maintaining ecosystem function and services in logged tropical forests. Trends in ecology and evolution, 29(9): 511-520.

Edwards, F. A., Edwards, D. P., Larsen, T. H., Hsu, W. W., Benedick, S., Chung, A., and Hamer, K. C. (2014b). Does logging and forest conversion to oil palm agriculture alter functional diversity in a biodiversity hotspot?. Animal Conservation, 17(2), 163-173.

Edwards, D. P., Magrach, A., Woodcock, P., Ji, Y., Lim, N. T. L., Edwards, F. A. & Chung, A. Y. (2014c). Selective-logging and oil palm: multitaxon impacts, biodiversity indicators, and trade-offs for conservation planning.*Ecological Applications*, *24*(8), 2029-2049.

Eglington, S. M., Noble, D. G., and Fuller, R. J., (2012). A meta-analysis of spatial relationships in species richness across taxa: Birds as indicators of wider biodiversity in temperate regions. Journal for Nature Conservation, 20(5), 301-309.

Eigenbrod, F., Hecnar, S. J., and Fahrig, L. (2008). The relative effects of road traffic and forest cover on anuran populations. Biological Conservation, 141(1), 35-46.

Eilers, E. J., Kremen, C., Greenleaf, S. S., Garber, A. K., and Klein, A. M. (2011). Contribution of pollinator-mediated crops to nutrients in the human food supply. PLoS one, 6(6), e21363.

Epps, C. W., Nowak, K., and Mutayoba, B. (2015). Unfenced reserves, unparalleled biodiversity and a rapidly changing landscape: roadways and wildlife in east Africa. Handbook of Road Ecology, 448.

Espartosa, K. D., Pinotti, B. T. and Pardini, R. (2011). Performance of camera trapping and track counts for surveying large mammals in rainforest remnants. Biodiversity Conservation 20: 2815-2829.

Estrada, A., Coates-Estrada, R., and Meritt, D. A. (1997). Anthropogenic landscape changes and avian diversity at Los Tuxtlas, Mexico. Biodiversity and conservation, 6(1), 19-43.

Fahrig, L. (2001). How much habitat is enough?. Biological Conservation, 100(1), 65-74.

FAO (2001) “Food and Agricultural Organization” Committee on World Food security Rome 28, May-June.

FAO (2010). The Global Forest Resources Assessment http://www.fao.org/forestry/fra/fra2010/en/ (Accessed March 2013)

FAO. (2012). State of the world’s forests. www.fao.org Date consulted March 2013.

Feeley, K. J., Gillespie, T. W., Lebbin, D. J., and Walter, H. S. (2007). Species characteristics associated with extinction vulnerability and nestedness rankings of birds in tropical forest fragments. Animal Conservation, 10(4), 493–501. http://doi.org/10.1111/j.1469-1795.2007.00140.x

Fermon, H., Waltert, M., Vane-Wright, R. I., and Mühlenberg, M. (2005). Forest use and vertical stratification in fruit-feeding butterflies of Sulawesi, Indonesia: impacts for conservation. Biodiversity and Conservation, 14(2), 333-350.

Ferraz, S. F., Ferraz, K. M., Cassiano, C. C., Brancalion, P. H. S., da Luz, D. T., Azevedo, T. N. and Metzger, J. P. (2014). How good are tropical forest patches for ecosystem services provisioning?. Landscape Ecology, 29(2), 187-200.

Ficetola, G. F., Rondinini, C., Bonardi, A., Baisero, D., and Padoa-Schioppa, E. (2015). Habitat availability for amphibians and extinction threat: a global analysis. Diversity and Distributions, 21(3), 302-311.

Ficetola, G. F., Rondinini, C., Bonardi, A., Katariya, V., Padoa-Schioppa, E., and Angulo, A. (2014). An evaluation of the robustness of global amphibian range maps. Journal of biogeography, 41(2), 211-221.

Findlay, C. S. and J. Bourdages., (2000). Response time of wetland biodiversity to road construction on adjacent lands. Conservation Biology 14(1), 86-94.

Finer, M., Jenkins, C. N., Pimm, S. L., Keane, B., and Ross, C., (2008). Oil and gas projects in the western Amazon: threats to wilderness, biodiversity, and indigenous peoples. PloS One, 3(8), e2932.

Finer, M., and Orta-Martínez, M. (2010). A second hydrocarbon boom threatens the Peruvian Amazon: trends, projections, and policy implications.Environmental research letters, 5(1), 014012.

Finer, M., Jenkins, C. N., Sky, M. A. B., and Pine, J. (2014). Logging concessions enable illegal logging crisis in the Peruvian Amazon. Scientific reports, 4.

Folt, B., and Reider, K. E. (2013). Leaf-litter herpetofaunal richness, abundance, and community assembly in mono-dominant plantations and primary forest of northeastern Costa Rica. Biodiversity and conservation, 22(9), 2057-2070.

Fonseca, C. R., Ganade, G., Baldissera, R., Becker, C. G., Boelter, C. R., Brescovit, A. D., and Joner, F. (2009). Towards an ecologically-sustainable forestry in the Atlantic Forest. Biological Conservation, 142(6), 1209-1219.

Forman, R. T., and Deblinger, R. D., (2000). The ecological road-effect zone of a Massachusetts (USA) suburban highway. Conservation Biology, 14(1), 36-46.

Francis, C. M. (1994). Vertical stratification of fruit bats in lowland rain forests of Malaysia. Journal of Tropical Ecology 10: 523-530.

Fraser, B., (2014). Carving up the Amazon. Nature, 509, 418-419.

Ganesh, T. and Devy M. S. (2000). Flower use by arboreal mammals and its consequence in the pollination of a rainforest tree in the south Western Ghats, India. Selbyana 21: 60-65.

Gardner T (2010) Monitoring forest biodiversity. Improving conservation through ecologically responsible management. Earthscan, London

Gardner, T. A., Barlow, J., Araujo, I. S., Ávila-Pires, T. C., Bonaldo, A. B., Costa, J. E., and Peres, C. A. (2008). The cost-effectiveness of biodiversity surveys in tropical forests. Ecology Letters, 11(2), 139-150.

Gardner, T. A., Barlow, J., Chazdon, R., Ewers, R. M., Harvey, C. A., Peres, C. A., and Sodhi, N. S. (2009). Prospects for tropical forest biodiversity in a human-modified world. Ecology Letters, 12(6), 561-582.

Gardner, T. A., Barlow, J., Sodhi, N. S., and Peres, C. A. (2010). A multi-region assessment of tropical forest biodiversity in a human-modified world. Biological Conservation, 143(10), 2293-2300.

Gardner, T. A., Ribeiro-Junior, M. A., Barlow, J. O. S., Ávila-Pires, T. C. S., Hoogmoed, M. S., and Peres, C. A. (2007a). The value of primary, secondary, and plantation forests for a Neotropical herpetofauna. Conservation Biology, 21(3), 775-787.

Gardner, T.A., Barlow, J. and Peres, C.A. (2007b). Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. Biological Conservation, 138, 166–179.

Gardner, T.A., Hernández, M.I.M., Barlow, J. Peres, C.A., (2008). Understanding the biodiversity consequences of habitat change: the value of secondary and plantation forests for neotropical dung beetles. Journal of Applied Ecology, 45, 883–893.

Gascon, C., Malcolm, J. R., Patton, J. L., da Silva, M. N., Bogart, J. P., Lougheed, S. C., and Boag, P. T. (2000). Riverine barriers and the geographic distribution of Amazonian species. Proceedings of the National Academy of Sciences, 97(25), 13672-13677.

Gasparri, N. I., Grau, H. R., and Angonese, J. G. (2013). Linkages between soybean and neotropical deforestation: coupling and transient decoupling dynamics in a multi-decadal analysis. Global environmental change, 23(6), 1605-1614.

Gibson, L., Lee, T. M., Koh, L. P., Brook, B. W., Gardner, T. A., Barlow, J., and Sodhi, N. S. (2011). Primary forests are irreplaceable for sustaining tropical biodiversity. Nature, 478(7369), 378-381.

Gillespie, G. R., Ahmad, E., Elahan, B., Evans, A., Ancrenaz, M., Goossens, B., and Scroggie, M. P. (2012). Conservation of amphibians in Borneo: Relative value of secondary tropical forest and non-forest habitats. Biological Conservation, 152, 136-144.

Gilroy, J. J., Woodcock, P., Edwards, F. A., Wheeler, C., Medina Uribe, C. A., Haugaasen, T., and Edwards, D. P. (2014). Optimizing carbon storage and biodiversity protection in tropical agricultural landscapes. Global change biology, 20(7), 2162-2172.

Glista, D. J., DeVault, T. L., and DeWoody, J. A. (2009). A review of mitigation measures for reducing wildlife mortality on roadways. Landscape and Urban Planning, 91(1), 1-7.

Glor, R. E., Flecker, A. S., Benard, M. F., and Power, A. G. (2001). Lizard diversity and agricultural disturbance in a Caribbean forest landscape. Biodiversity and Conservation, 10(5), 711-723.

Golden, C. D., Fernald, L. C., Brashares, J. S., Rasolofoniaina, B. R., and Kremen, C. (2011). Benefits of wildlife consumption to child nutrition in a biodiversity hotspot. Proceedings of the National Academy of Sciences, 108(49), 19653-19656.

Gómez, C., Wulder, M. A., Montes, F., and Delgado, J. A. (2012). Modeling forest structural parameters in the Mediterranean pines of central Spain using QuickBird-2 imagery and Classification and Regression Tree Analysis (CART). Remote Sensing, 4(1), 135-159.

Goosem, M., (2007). Fragmentation impacts caused by roads through rainforests. Current Science, 93(11), 1587-1595.

Gotelli, N. J. and R. K. Colwell. (2011). Estimating species richness. Pages 39-54 in A. E. Magurran and B. J. McGill, editors. Frontiers in measuring biodiversity. Oxford University Press, New York.

Goyette, J.L, Howe, R.W., Wolf, A.T., and W.D. Robinson. (2011). Detecting tropical nocturnal birds using automated audio recordings. Journal of Field Ornithology, 82(3), 279-287.

Grau, H. R., and Aide, M. (2008). Globalization and land-use transitions in Latin America. Ecology and Society, 13(2), 16.

Gregory, T., Rueda, F. C., Deichmann, J. L., Kolowski, J. and Alonso, A. (2014). Arboreal camera trapping: taking a proven method to new heights. Methods in Ecology and Evolution 5: 443-451.

Grove, S. J. (2002). The influence of forest management history on the integrity of the saproxylic beetle fauna in an Australian lowland tropical rainforest. Biological Conservation, 104(2), 149-171.

Haefke, B. J., Spiers, A. I., Miller, W. R. and Lowman, M. D. (2013). Tardigrades in the Canopy: Using double rope techniques to conduct sampling along vertical transects. Transactions of Kansas Academy of Science 116: 119-124.

Hahn, M. B., Gangnon, R. E., Barcellos, C., Asner, G. P., Patz, J. A., (2014). Influence of deforestation, logging, and fire on malaria in the Brazilian Amazon. PloS one, 9(1), e85725.

Hall JP and Willmott KR (2000). Patterns of feeding behaviour in adult male riodinid butterflies and their relationship to morphology and ecology. Bioll J Linn Soc; 69(1): 1-23.

Hamer, K.C., Hill, J.K., Benedick, S., Mustaffa, N., Sherratti, T.N., Maryati, M., and Chey, V.K. (2003). Ecology of butterflies in natural and selectively logged forests of northern Borneo: the importance of habitat heterogeneity. Journal of Applied Ecology, 40, 150-162.

Hamer, K. C., Newton, R. J., Edwards, F. A., Benedick, S., Bottrell, S. H., and Edwards, D. P. (2015). Impacts of selective logging on insectivorous birds in Borneo: the importance of trophic position, body size and foraging height. Biological Conservation, 188, 82-88.

Hayes, T. B., Falso, P., Gallipeau, S., and Stice, M. (2010). The cause of global amphibian declines: a developmental endocrinologist's perspective. The Journal of Experimental Biology, 213(6), 921-933.

Heffner, R. A., Butler, M. J. and Reilly, C. K. (1996). Pseudoreplication revisited. Ecology, 77, 2558-2562.

Helbig-Bonitz, M., Ferger, S. W., Böhning-Gaese, K., Tschapka, M., Howell, K. and Kalko, E. K. V. (2015), Bats are Not Birds – Different Responses to Human Land-use on a Tropical Mountain. Biotropica, 47: 497–508.
doi: 10.1111/btp.12221

Herzog, S. K., Kessler, M., and Cahill, T. M. (2002). Estimating species richness of tropical bird communities from rapid assessment data. The Auk, 119(3), 749-769.

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. A. C., and Foster, M. S. (1994) Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press.

Hidasi-Neto, J., Barlow, J., and Cianciaruso, M. V. (2012). Bird functional diversity and wildfires in the Amazon: the role of forest structure. Animal Conservation, 15(4), 407-415.

Hilje, B., and Aide, M. T. (2012). Recovery of amphibian species richness and composition in a chronosequence of secondary forests, northeastern Costa Rica. Biological Conservation, 146(1), 170-176.

Hill, J.K., Hamer, K.C., Tangah, J., and Dawood, M., (2001). Ecology of tropical butterflies in rainforest gaps. Oecologia, 128(2), 294–302.

Hocking, D. J., and Babbitt, K. J. (2014). Amphibian contributions to ecosystem services. Herpetological Conservation and Biology, 9(1), 1-17.

Hopkins, W. A. (2007). Amphibians as models for studying environmental change. Ilar Journal, 48(3), 270-277.

Horner-Devine, M.C., Daily, G.C., Ehrlich, P.R., and Boggs, C.I. (2003). Countryside biogeography of tropical butterflies. Conservation Biology. 17(1), 168-177.

Hu, Y., Magaton, S., Gillespie, G., and Jessop, T. S. (2013). Small reptile community responses to rotational logging. Biological Conservation, 166, 76-83.

Hughes, J. B., Daily, G. C., and Ehrlich, P. R. (1998). Use of fruit bait traps for monitoring of butterflies (Lepidoptera: Nymphalidae). Revista de Biología Tropical, 46(3), 697-704.

Hughes, J. B., Daily, G. C., and Ehrlich, P. R. (2002). Conservation of tropical forest birds in countryside habitats. Ecology Letters, 5(1), 121-129.

Hulme, P.E., (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. Journal of Applied Ecology, 46, 10–18.

Hunt, C. A., Durham, W. H., Driscoll, L., and Honey, M. (2015). Can ecotourism deliver real economic, social, and environmental benefits? A study of the Osa Peninsula, Costa Rica. Journal of Sustainable Tourism,23(3), 339-357.

Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. Ecological monographs, 54(2), 187-211.

Hurst, J.M. and Allen, R.B. (2007). A permanent plot method for monitoring indigenous forests – field protocols. Manaaki Whenua – Landcare Research, Lincoln, NZ.

Irwin, M. T., Wright, P. C., Birkinshaw, C., Fisher, B. L., Gardner, C. J., Glos, J., and Ganzhorn, J. U. (2010). Patterns of species change in anthropogenically disturbed forests of Madagascar. Biological Conservation, 143(10), 2351-2362.

IUCN. (2013). The IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org. Accessed: 02 July 2013.

Jayasekara, P., Weerasinghe, U. R., Wijesundara, S. and Takatsuki S. (2007). Identifying diurnal and nocturnal frugivores in the terrestrial and arboreal layers of a tropical rain forest in Sri Lanka. Ecotropica 13: 7-15.

Johns, A. D. (1991). Responses of Amazonian rain forest birds to habitat modification. Journal of Tropical Ecology, 7(04), 417-437.

Kati, V., Devillers, P., Dufrêne, M., Legakis, A., Vokou, D., and Lebrun, P. (2004). Testing the value of six taxonomic groups as biodiversity indicators at a local scale. Conservation Biology, 18(3), 667-675.

Kays R and Allison A. (2001). Arboreal tropical forest vertebrates: current knowledge and research trends. In Tropical Forest Canopies: Ecology and Management. (pp. 109-120). Springer Netherlands.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., and Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature, 468(7324), 647-652.

Kierulff, M. C. M., dos Santos, G. R., Canale, G., Guidorizzi, C. E. and Cassano, C. (2004). The use of camera-traps in a survey of the buff-headed capuchin monkey, Cebus xanthosternos. Neotropical Primates 12: 256-59.

Kinnaird, M. F., Sanderson, E. W., O'Brien, T. G., Wibisono, H. T., and Woolmer, G. (2003). Deforestation trends in a tropical landscape and implications for endangered large mammals. Conservation Biology, 17(1), 245-257.

Klimeš P., Idigel C., Rimandai M., Fayle T.M., Janda M., Weiblen G.D. and Novotný V. (2012). Why are there more arboreal ant species in primary than secondary tropical forests? Journal of Animal Ecology 81: 1103–1112.

Koh, L. P. (2008). Can oil palm plantations be made more hospitable for forest butterflies and birds?. Journal of Applied Ecology, 45(4), 1002-1009.

Kosch, T. A., Morales, V., and Summers, K. (2012). Batrachochytrium dendrobatidis in Peru. Herpetological Review, 43(2), 150-159.

Kudavidanage, E. P., Wanger, T. C., Alwis, C., Sanjeewa, S., and Kotagama, S. W. (2012). Amphibian and butterfly diversity across a tropical land-use gradient in Sri Lanka; implications for conservation decision making. Animal Conservation, 15(3), 253-265.

Kurten, E. L., Wright, S. J., and Carson, W. P. (2015). Hunting alters seedling functional trait composition in a Neotropical forest. Ecology, 96(7), 1923-1932.

Lande, R., DeVries, P. J., and Walla, T. R., (2000). When species accumulation curves intersect: implications for ranking diversity using small samples. Oikos, 89(3), 601-605.

Laporta, G. Z., Lopez de Prado, P. I., Kraenkel, R. A., Coutinho, R. M., and Sallum, M. A. M. (2013). Biodiversity can help prevent malaria outbreaks in tropical forests. PLoS Negl Trop Dis, 7(3), e2139.

Laurance, W. and Edwards, D. (2014). Saving logged tropical forests. Frontiers in Ecology and the Environment, 12.3, 147-147.

Laurance, W.F. (1990). Comparative responses of five arboreal marsupials to tropical forest fragmentation. Journal of Mammalogy 71: 641-653.

Laurance, S.G., (2004). Responses of understory Rain Forest Birds to Road Edges in Central Amazonia. Ecological Applications, 14(5), 1344–1357.

Laurance, W.F., Goosem, M., and Laurance, S.G., (2009). Impacts of roads and linear clearings on tropical forests. Trends Ecol Evolution, 24(12), 659–669.

Laurance, W. F., Useche, D. C., Rendeiro, J., Kalka, M., Bradshaw, C. J., Sloan, S. P. and Plumptre, A. (2012a). Averting biodiversity collapse in tropical forest protected areas. Nature, 489(7415), 290-294.

Laurance, W. F., Koster, H., Grooten, M., Anderson, A. B., Zuidema, P. A., Zwick, S. Anten, N. P., (2012b). Making conservation research more relevant for conservation practitioners. Biological Conservation, 153, 164-168.

Laurance, W. F., Balmford, A., (2013). Land use: a global map for road building. Nature, 495(7441), 308-309.

Laurance, W. F., Clements, G. R., Sloan, S., O'Connell, C. S., Mueller, N. D., Goosem, M. and Arrea, I. B., (2014a). A global strategy for road building. Nature.

Laurance, W. F., Sayer, J., and Cassman, K. G. (2014b). Agricultural expansion and its impacts on tropical nature. Trends in ecology and evolution, 29(2), 107-116.

Lawler, J. J., Shafer, S. L., Bancroft, B. A., and Blaustein, A. R. (2010). Projected climate impacts for the amphibians of the Western Hemisphere. Conservation Biology, 24(1), 38-50.

Lawler, J. J., White, D., Sifneos, J. C., and Master, L. L. (2003). Rare species and the use of indicator groups for conservation planning. Conservation Biology, 17(3), 875-882.

Lawton, J. H., Bignell, D. E., Bolton, B., Bloemers, G. F., Eggleton, P., Hammond, P. M., and Watt, A. D. (1998). Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature, 391(6662), 72-76.

Le Saout, S., Hoffmann, M., Shi, Y., Hughes, A., Bernard, C., Brooks, T. M. and Rodrigues, A. S. (2013). Protected areas and effective biodiversity conservation. Science, 342(6160), 803-805.

Letcher, S. G. and R. L. Chazdon (2009). Rapid Recovery of Biomass, Species Richness, and Species Composition in a Forest Chronosequence in Northeastern Costa Rica. Biotropica, 41(5), 608-617.

Lewis, O.T., (2001). Effect of experimental selective logging on tropical butterflies. Conservation Biology, 15(2), 389-400.

Lindell, C. A., Riffell, S. K., Kaiser, S. A., Battin, A. L., Smith, M. L., and Sisk, T. D. (2007). Edge responses of tropical and temperate birds. The Wilson Journal of Ornithology, 119(2), 205-220.

Linden, V. M., Weier, S. M., Gaigher, I., Kuipers, H. J., Weterings, M. J., and Taylor, P. J. (2014). Changes of bat activity, species richness, diversity and community composition over an altitudinal gradient in the Soutpansberg range, South Africa. Acta Chiropterologica, 16(1), 27-40.

Lowman, M. D. (2009). Canopy research in the twenty-first century: a review of Arboreal Ecology. Tropical Ecology 50: 125-136.

Louzada, J., Gardner, T., Peres, C., and Barlow, J. (2010). A multi-taxa assessment of nestedness patterns across a multiple-use Amazonian forest landscape. Biological Conservation, 143(5), 1102-1109.

Mackey, B., DellaSala, D. A., Kormos, C., Lindenmayer, D., Kumpel, N., Zimmerman, B. and Watson, J. E. (2014). Policy Options for the World's Primary Forests in Multilateral Environmental Agreements. Conservation Letters, 8(2), 139-147.

MacGregor-Fors I, Payton ME (2013) Contrasting Diversity Values: Statistical Inferences Based on Overlapping Confidence Intervals. PLoS ONE 8(2): e56794. doi:10.1371/journal.pone.0056794

Maclaurin, J., and Sterelny, K. (2008). What is biodiversity?. University of Chicago Press.

Maguire D.Y., Robert, K., Brochu, K., Larrivée, M., Buddle, C. M. and Wheeler, T. A. (2014). Vertical stratification of Beetles (Coleoptera) and Flies (Diptera) in temperate forest canopies. Environmental Entomology 43: 9-17.

Magurran, A. E. (2004). Measuring biological diversity. African Journal of Aquatic Science, 29(2), 285-286.

Magurran, A. E. (2013). Measuring Biological Diversity. John Wiley and Sons.

Magurran, A. E., and McGill, B. J. (2011). Biological diversity: frontiers in measurement and assessment. Oxford University Press.

Mäki, S., Kalliola, R., and Vuorinen, K., (2001). Road construction in the Peruvian Amazon: process, causes and consequences. Environmental Conservation, 28(03), 199-214.

Malcolm, J. R. (1991). Comparative abundances of Neotropical small mammals by trap height. Journal of Mammalogy 72: 188-192.

McCallum, M. L. (2015). Vertebrate biodiversity losses point to a sixth mass extinction. Biodiversity and Conservation, 1-23.

Melo, F. P., Arroyo-Rodríguez, V., Fahrig, L., Martínez-Ramos, M., and Tabarelli, M. (2013). On the hope for biodiversity-friendly tropical landscapes. Trends in Ecology and Evolution, 28(8), 462-468.

Miranda, J. J., Corral, L., Blackman, A., Asner, G., and Lima, E. (2016). Effects of Protected Areas on Forest Cover Change and Local Communities: Evidence from the Peruvian Amazon. World Development, 78, 288-307.

Miranda, J. J., Corral, L., Blackman, A., Asner, G., and Lima, E. (2014). Effects of Protected Areas on Forest Cover Change and Local Communities: Evidence from the Peruvian Amazon. Inter-American Development Bank.

Monteiro, M. S., Seixas, S. R. D. C., and Vieira, S. A. (2014). The politics of Amazonian deforestation: environmental policy and climate change knowledge. Wiley Interdisciplinary Reviews: Climate Change, 5(5), 689-701.

Morante-Filho JC, Faria D, Mariano-Neto E, Rhodes J (2015) Birds in Anthropogenic Landscapes: The Responses of Ecological Groups to Forest Loss in the Brazilian Atlantic Forest. PLoS ONE 10(6): e0128923. doi:10.1371/journal.pone.0128923

Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. Trends in Ecology and Evolution, 28(3), 167-177.

Mueller, T., Lenz, J., Caprano, T., Fiedler, W., and Böhning-Gaese, K. (2014). Large frugivorous birds facilitate functional connectivity of fragmented landscapes. Journal of applied ecology, 51(3), 684-692.

Munari, D. P., Keller, C., and Venticinque, E. M. (2011). An evaluation of field techniques for monitoring terrestrial mammal populations in Amazonia. Mammalian Biology-Zeitschrift für Säugetierkunde, 76(4), 401-408.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853-858.

Myers, S. S., Gaffikin, L., Golden, C. D., Ostfeld, R. S., Redford, K. H., Ricketts, T. H., and Osofsky, S. A. (2013). Human health impacts of ecosystem alteration. Proceedings of the National Academy of Sciences, 110(47), 18753-18760.

Myers, P., R. Espinosa, C. S. Parr, T. Jones, G. S. Hammond, and T. A. Dewey. (2016). The Animal Diversity Web (online). Accessed at http://animaldiversity.org.

Newbold, T., Scharlemann, J. P., Butchart, S. H., Şekercioğlu, Ç. H., Alkemade, R., Booth, H., and Purves, D. W. (2013). Ecological traits affect the response of tropical forest bird species to land-use intensity. Proceedings of the Royal Society of London B: Biological Sciences, 280(1750), 20122131.

Norden, N., Chazdon, R. L., Chao, A., Jiang, Y. H., and Vílchez-Alvarado, B. (2009). Resilience of tropical rain forests: tree community reassembly in secondary forests. Ecology Letters, 12(5), 385-394.

Norris, K., Asase, A., Collen, B., Gockowksi, J., Mason, J., Phalan, B., and Wade, A. (2010). Biodiversity in a forest-agriculture mosaic–The changing face of West African rainforests. Biological Conservation, 143(10), 2341-2350.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B. and Wagner, H. (2011). Vegan: community ecology package version 2.0-2. R CRAN package.

Oldekop, J. a, Bebbington, A.J., Truelove, N.K., Tysklind, N., Villamarín, S. and Preziosi, R.F. (2012). Co-occurrence patterns of common and rare leaf-litter frogs, epiphytic ferns and dung beetles across a gradient of human disturbance. PloS One, 7, e38922

Oldekop, J. A., Holmes, G., Harris, W. E., and Evans, K. L. (2015). A global assessment of the social and conservation outcomes of protected areas. Conservation Biology.

Oliveira, P. J., Asner, G. P., Knapp, D. E., Almeyda, A., Galván-Gildemeister, R., Keene, S., Smith, R. C., (2007). Land-use allocation protects the Peruvian Amazon. Science, 317(5842), 1233-1236.

Olson, E. R., Marsh, R. A., Bovard, B. N., Randrianarimanana, H. L. L., Ravaloharimanitra, M., Ratsimbazafy, J. H. and King, T. (2012). Arboreal camera trapping for the Critically Endangered greater bamboo lemur Prolemur simus. Oryx 46: 593-597.

Otani, T. (2001). Measuring fig foraging frequency of the Yakushima macaque by using automatic cameras. Ecological Research 16: 49-54.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A. and Hayes, D. (2011). A large and persistent carbon sink in the world's forests. Science, 333(6045), 988-993.

Paradis, E., Claude, J., and Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20(2), 289-290.

Pardini, R., Faria, D., Accacio, G. M., Laps, R. R., Mariano-Neto, E., Paciencia, M. L., and Baumgarten, J. (2009). The challenge of maintaining Atlantic forest biodiversity: a multi-taxa conservation assessment of specialist and generalist species in an agro-forestry mosaic in southern Bahia. Biological Conservation, 142(6), 1178-1190.

Pearman, P.B. (1997). Correlates of amphibian diversity in an altered landscape of Amazonian Ecuador. Conservation Biology, 11, 1211–1225.

Pereira, H. M., Navarro, L. M., and Martins, I. S. (2012). Global biodiversity change: the bad, the good, and the unknown.

Peres, C. A., Barlow, J., and Laurance, W. F. (2006). Detecting anthropogenic disturbance in tropical forests. Trends in Ecology and Evolution, 21(5), 227-229.

Peres, C. A., Gardner, T. A., Barlow, J., Zuanon, J., Michalski, F., Lees, A. C. and Feeley, K. J. (2010). Biodiversity conservation in human-modified Amazonian forest landscapes. Biological Conservation, 143(10), 2314-2327.

Phelps, J., Friess, D. A., and Webb, E. L. (2012). Win–win REDD+ approaches belie carbon–biodiversity trade-offs. Biological Conservation, 154, 53-60.

Phillips, O. and Miller, J. S. (2002). *Global Patterns - Alwyn H. Gentry's Forest Transect Data Set*. (V. C. Hollowell, Ed.). St. Louis, Missouri U.S.A: Missouri Botanical Garden Press.

Pieck, S. K. (2013). Asphalt Dreams: Road Construction and Environmental Citizenship in Peru. Development and Change, 44(5), 1039-1063.

Pimm, S. L., Alibhai, S., Bergl, R., Dehgan, A., Giri, C., Jewell, Z., and Loarie, S. (2015). Emerging Technologies to Conserve Biodiversity. Trends in ecology and evolution, 30(11), 685-696.

Pocock, Z. and Lawrence, R.E., (2005). How far into a forest does the effect of a road extend? Defining road edge effect in eucalypt forests of South-Eastern Australia. IN: Proceedings of the 2005 International Conference on Ecology and Transportation, Eds. Irwin CL, Garrett P, McDermott KP. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, 397-405.

Posa, M. R. C., and Sodhi, N. S. (2006). Effects of anthropogenic land use on forest birds and butterflies in Subic Bay, Philippines. Biological Conservation, 129(2), 256-270.

Putz, F. E., Blate, G. M., Redford, K. H., Fimbel, R., and Robinson, J. (2001). Tropical forest management and conservation of biodiversity: an overview. Conservation Biology, 7-20.

Putz, F. E., Zuidema, P. A., Synnott, T., Peña-Claros, M., Pinard, M. A., Sheil, D., and Zagt, R. (2012). Sustaining conservation values in selectively logged tropical forests: the attained and the attainable. Conservation Letters, 5(4), 296-303.

R Core Team. (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Ramage, B. S., Sheil, D., Salim, H. M., Fletcher, C., Mustafa, N. Z. A., Luruthusamay, J. C., and Potts, M. D. (2013). Pseudoreplication in tropical forests and the resulting effects on biodiversity conservation. Conservation Biology, 27(2), 364-372.

Ramesh, T., Hussain, K. J., Selvanayagam, M., Satpathy, K. K., and Prasad, M. V. R. (2010). Patterns of diversity, abundance and habitat associations of butterfly communities in heterogeneous landscapes of the department of atomic energy (DAE) campus at Kalpakkam, South India. International Journal of Biodiversity and Conservation, 2(4), 75-85.

Rao M, Htun S, Zaw T. and Myint T. (2010). Hunting, livelihoods and declining wildlife in the Hponkanrazi Wildlife Sanctuary, North Myanmar. Environmental Management 46: 143-153.

Rao, M., Myint, T., Zaw, T. and Htun, S. (2005). Hunting patterns in tropical forests adjoining the Hkakaborazi National Park, north Myanmar. Oryx 39: 292-300.

Reddington, C. L., Butt, E. W., Ridley, D. A., Artaxo, P., Morgan, W. T., Coe, H., and Spracklen, D. V. (2015). Air quality and human health improvements from reductions in deforestation-related fire in Brazil. Nature Geoscience, 8(10), 768-771.

Reese, G. C., Wilson, K. R., and Flather, C. H. (2014). Performance of species richness estimators across assemblage types and survey parameters. Global Ecology and Biogeography, 23(5), 585-594.

Ribeiro, D. B., and Freitas, A. V. (2012). The effect of reduced-impact logging on fruit-feeding butterflies in Central Amazon, Brazil. Journal of Insect Conservation, 16(5), 733-744.

Ribeiro, D. B., Williams, M. R., Specht, A., and Freitas, A. V. (2015). Vertical and temporal variability in the probability of detection of fruit-feeding butterflies and moths (Lepidoptera) in tropical forest. Austral Entomology. doi: 10. 1111/aen. 12157

Ribeiro-Júnior, M. a, Gardner, T. a, and Ávila-Pires, T. C. (2008). Evaluating the Effectiveness of Herpetofaunal Sampling Techniques across a Gradient of Habitat Change in a Tropical Forest Landscape. Journal of Herpetology, 42(4), 733. http://doi.org/10.1670/07-097R3.1

Ricketts, T. H., Daily, G. C., Ehrlich, P. R., and Fay, J. P. (2001). Countryside biogeography of moths in a fragmented landscape: biodiversity in native and agricultural habitats. Conservation Biology, 15(2), 378-388.

Riemann, J. C., Ndriantsoa, S. H., Raminosoa, N. R., Rödel, M. O., and Glos, J. (2015). The value of forest fragments for maintaining amphibian diversity in Madagascar. Biological Conservation, 191, 707-715.

Rödel, M. O., and Ernst, R. (2004). Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. Ecotropica, 10, 1-14.

Roldán, A. I., and Simonetti, J. A. (2001). Plant-Mammal Interactions in Tropical Bolivian Forests with Different Hunting Pressures. Conservation Biology, 15(3), 617-623.

Ross, K. A., Fox, B. J., and Fox, M. D. (2002). Changes to plant species richness in forest fragments: fragment age, disturbance and fire history may be as important as area. Journal of Biogeography, 29(5-6), 749-765.

Rossi, J. P. (2011). rich: an R package to analyse species richness. Diversity, 3(1), 112-120.

Rovero, F. and Marshall, A. R. (2009). Camera trapping photographic rate as an index of density in forest ungulates. Journal of Applied Ecology 46: 1011-1017.

Rovero, F. and Marshall, A. R. (2004). Estimating the abundance of forest antelope by using line transect techniques: a case from the Udzungwa Mountains of Tanzania. Tropical Zoology 17: 267-277.

Rovero, F., Jones, T. and Sanderson, J. (2005). Notes on Abbott's duiker (Cephalophus spadix True 1890) and other forest antelopes of Mwanihana Forest, Udzungwa Mountains, Tanzania, as revealed by camera-trapping and direct observations. Tropical Zoology 18: 13-23.

Rovero, F., Zimmermann, F., Berzi, D. and Meek, P. (2013). "Which camera trap type and how many do I need?" A review of camera features and study designs for a range of wildlife research applications. Hystrix, the Italian Journal of Mammalogy 24: 148-156.

Salvador, S., Clavero, M., and Leite Pitman, R. (2011). Large mammal species richness and habitat use in an upper Amazonian forest used for ecotourism. Mammalian Biology-Zeitschrift für Säugetierkunde, 76(2), 115-123.

Sberze, M., Cohn-Haft, M., and G. Ferraz. (2010). Old growth and secondary forest site occupancy by nocturnal birds in a neotropical landscape. Animal Conservation, 13, 3-11.

Schipper, J. (2007). Camera-trap avoidance by Kinkajous Potos flavus: rethinking the "non invasive" paradigm. Small Carnivore Conservervation 36: 38-41.

Schlaepfer, M. A., and Gavin, T. A. (2001). Edge effects on lizards and frogs in tropical forest fragments. Conservation Biology, 15(4), 1079-1090.

Schulenberg, T. S., Stotz, D. F., Lane, D. F., O'Neill, J. P., and Parker III, T. A. (2010). Birds of Peru: revised and updated edition. Princeton University Press.

Schulze, C.H., Waltert, M., Kessler, P.J.A., Pitopang, R., Veddeler, D., Mühlenberg, M., Gradstein, S.R., Leuschner, C., Steffan-Dewenter, I., Tscharntke, T., (2004). Biodiversity indicator taxa of tropical land-use systems, comparing plants, birds, and insects. Ecological Applications, 14(5), 1321–1332.

Seshadri, K. S. (2014). Effects of Historical Selective Logging on Anuran Communities in a Wet Evergreen Forest, South India. Biotropica 46(5), 615-623.

Sharma, R. K., Jhala, Y., Qureshi, Q., Vattakaven, J., Gopal, R., and Nayak, K. (2010). Evaluating capture–recapture population and density estimation of tigers in a population with known parameters. Animal Conservation, 13(1), 94-103.

Silveira, J. M., Louzada, J., Barlow, J., Andrade, R., Mestre, L., Solar, R., and Cochrane, M. A. (2015). A Multi-Taxa Assessment of Biodiversity Change After Single and Recurrent Wildfires in a Brazilian Amazon Forest.Biotropica.

Sloan, S., Goosem, M., and Laurance, S. G. (2015). Tropical forest regeneration following land abandonment is driven by primary rainforest distribution in an old pastoral region. Landscape Ecology, 31(3), 601–618. http://doi.org/10.1007/s10980-015-0267-4

Sodhi, N. S., Koh, L. P., Clements, R., Wanger, T. C., Hill, J. K., Hamer, K. C., and Lee, T. M. (2010). Conserving Southeast Asian forest biodiversity in human-modified landscapes. Biological Conservation, 143(10), 2375-2384.

Sommerville, M. M., Milner-Gulland, E. J., and Jones, J. P. (2011). The challenge of monitoring biodiversity in payment for environmental service interventions. Biological Conservation, 144(12), 2832-2841.

Sorensen, T. C., and Fedigan, L. M. (2000). Distribution of three monkey species along a gradient of regenerating tropical dry forest. Biological Conservation, 92(2), 227-240.

Soulé, M. (2013). The “new conservation.” Conservation Biology 27:895–897.

Soulé, M. E., and Sanjayan, M. A. (1998). Ecology: Conservation Targets: Do They Help?. Science, 279(5359), 2060-2061.

Sourakov, A. and Emmel, T. C. (1995). Bait trapping for butterflies in Kenya. Tropical Lepidoptera, 6(1), 1-2.

Sparrow, H. R., Sisk, T. D., Ehrlich, P. R., and Murphy, D. D. (1994). Techniques and guidelines for monitoring neotropical butterflies. Conservation Biology, 8(3), 800-809.

Srbek-Araujo, A. C. and Chiarello, A. G. (2005). Is camera-trapping an efficient method for surveying mammals in Neotropical forests? A case study in south-eastern Brazil. Journal of Tropical Ecology 21: 121-125.

Srinivasan, U., Hines, J. E., and Quader, S. (2015). Demographic superiority with increased logging in tropical understorey insectivorous birds. Journal of Applied Ecology, 52, 1374-1380.

Steege, H., Pitman, N. C., Sabatier, D., Baraloto, C., Salomão, R. P., Guevara, J. E., and Fine, P. V. (2013). Hyperdominance in the Amazonian tree flora. Science, 342(6156), 1243092.

Stork, N. E., Coddington, J. A., Colwell, R. K., Chazdon, R. L., Dick, C. W., Peres, C. A., and Willis, K., (2009). Vulnerability and Resilience of Tropical Forest Species to Land-Use Change. Conservation Biology, 23(6), 1438-1447.

D.F. Stotz, J.W. Fitzpatrick, T.A. Parker, and D.K. Moskovitz. (1996). Neotropical birds: ecology and conservation. University of Chicago Press.

Stratford, J. A., and Robinson, W. D. (2005). Gulliver travels to the fragmented tropics: geographic variation in mechanisms of avian extinction. Frontiers in Ecology and the Environment, 3(2), 85-92.

Suárez, E., Zapata-Ríos, G., Utreras, V., Strindberg, S., and Vargas, J. (2013). Controlling access to oil roads protects forest cover, but not wildlife communities: a case study from the rainforest of Yasuní Biosphere Reserve (Ecuador). Animal Conservation, 16(3), 265-274.

Superina, M., Miranda, F. R. and Abba, A. M. (2010). The 2010 anteater red list assessment. Edentata 11: 96-114.

Suzán, G., Marcé, E., Giermakowski, J. T., Mills, J. N., Ceballos, G., Ostfeld, R. S., and Yates, T. L. (2009). Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. PloS one, 4(5), e5461.

Swenson, J. J., Carter, C. E., Domec, J. C., and Delgado, C. I. (2011). Gold mining in the Peruvian Amazon: global prices, deforestation, and mercury imports. PloS one, 6(4), e18875.

Tabarelli, M., Aguiar, A. V., Ribeiro, M. C., Metzger, J. P., and Peres, C. A. (2010). Prospects for biodiversity conservation in the Atlantic Forest: lessons from aging human-modified landscapes. Biological Conservation, 143(10), 2328-2340.

Taylor, B. D., and Goldingay, R. L., (2010). Roads and wildlife: impacts, mitigation and implications for wildlife management in Australia. Wildlife Research, 37(4), 320-331.

The Field Museum. (2014). Available at: http://fieldguides.fieldmuseum.org/guides; Accessed December 2014.

The Reptile Database. (2013). Information on reptile biology and conservation www.reptile-database.reptarium.cz/; Accessed March 2013.

Tobler, M. W., Carrillo-Percastegui, S. E., Leite Pitman, R., Mares, R., and Powell, G. (2008). An evaluation of camera traps for inventorying large-and medium-sized terrestrial rainforest mammals. Animal Conservation, 11(3), 169-178.

Torres-Sovero, C., González, J. A., Martín-López, B., and Kirkby, C. A. (2012). Social–ecological factors influencing tourist satisfaction in three ecotourism lodges in the southeastern Peruvian Amazon. Tourism Management, 33*(3),* 545-552.

Tozetti, A.M., and M. Martins. (2007). A technique for external radio-transmitter attachment and the use of thread-bobbins for studying snake movements. South American Journal of Herpetology 2:184–190.

Tregidgo, D. J., Qie, L., Barlow, J., Sodhi, N. and Lim, S. L. (2010). Vertical stratification responses of an arboreal dung beetle species to tropical forest fragmentation in Malaysia. Biotropica 42: 521-525.

Trimble, M. J., and Aarde, R. J. (2014). Amphibian and reptile communities and functional groups over a land-use gradient in a coastal tropical forest landscape of high richness and endemicity. Animal Conservation, 17(5), 441-453.

Umapathy, G. and Kumar, A. (2000). Impacts of the habitat fragmentation on time budget and feeding ecology of lion-tailed macaque (Macaca silenus) in rain forest fragments of Anamalai Hills, South India. Primate Report 58: 67-82.

Urquiza-Haas, T., Peres, C. A., and Dolman, P. M. (2011). Large vertebrate responses to forest cover and hunting pressure in communal landholdings and protected areas of the Yucatan Peninsula, Mexico. Animal Conservation, 14(3), 271-282.

Vasconcelos, H. L. (1999). Effects of forest disturbance on the structure of ground-foraging ant communities in central Amazonia. Biodiversity and Conservation, 8(3), 407-418.

Veith, M., Lötters, S., Andreone, F., and Rödel, M. O. (2004). Measuring and monitoring amphibian diversity in tropical forests. II. Estimating species richness from standardized transect censing. Ecotropica, 10(2), 85-99.

Vieira, E. M. and Izar, P. (1999). Interactions between aroids and arboreal mammals in the Brazilian Atlantic rainforest. Plant Ecology 145: 75-82.

von May, R., and Donnelly, M. A. (2009). Do trails affect relative abundance estimates of rainforest frogs and lizards? Austral Ecology, 34(6), 613-620.

Voss, R. S., Hubbard, C. and Jansa, S. A. (2013). Phylogenetic relationships of New World porcupines (Rodentia, Erethizontidae): implications for taxonomy, morphological evolution, and biogeography. American Museum Novitates 3769: 1-36.

Wake, D. B., and Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences, 105(Supplement 1), 11466-11473.

Waltert, M., Mardiastuti, A., and Mühlenberg, M. (2005). Effects of deforestation and forest modification on understorey birds in Central Sulawesi, Indonesia. Bird Conservation International, 15(03), 257-273.

Walther, B. A. (2002). Vertical stratification and use of vegetation and light habitats by Neotropical forest birds. Journal of Ornithology 143: 64-81.

Whitworth, A. W. (2012). An investigation into the determining factors of zoo visitor attendances in UK zoos. PloS one, 7(1), e29839.

Willott, S.J., Lim, D.C., Compton, S.G. and Sutton, S.L. (2000) Effects of selective logging on the butterflies of a Bornean rainforest. Conservation Biology, 14, 1055–1065.

Wilson, E. O. (1992). The diversity of life. New York and London.

Woodley, S., Bertzky, B., Crawhall, N., Dudley, N., Londoño, J. M., MacKinnon, K., and Sandwith, T. (2012). Meeting Aichi Target 11: what does success look like for protected area systems?. Parks, 18(1), 23-36.

Wright, B. A., Rodgers, E. D. and Backman, K. F. (2001). Assessing the temporal stability of hunting participation and the structure and intensity of constraints: a panel study. Journal of Leisure Research 33: 450-469.

Wright, S. J. (2010). The future of tropical forests. Annals of the New York Academy of Sciences, 1195(1), 1-27.

Wright, S.J. (2005). Tropical forests in a changing environment. Trends in Ecology and Evolution, 20, 553–560.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. New York: Springer.

Appendices

Chapter 1

Appendix 1A. Comparative study sites (with references)

The existing primary forest field sites used as a comparison to herpetofaunal levels found at the Manu Learning Centre (MLC) were from Cocha Cashu (studies conducted in ~1000ha), Pakitza (studies conducted in <4000ha) and Los Amigos Biological Station (studies conducted in <2000ha). Herpetological work at Cocha Cashu consisted of 395 person days [1-3], Pakitza, 286 person days [3], through eight field visits between 1987 and 1993 for project BIOLAT [4] and the Los Amigos list was developed from 711 person days between 2001 and 2007, with seven sampling periods and a variety of nocturnal survey methods, including visual encounter surveys, leaf litter plots and pitfall traps. Just like the herpetological research at the MLC (consisting of ~300 person days) all of these studies were conducted in both wet and dry seasons. Cocha Cashu is one of the richest and most well studied sites in the Amazon basin, in which bird and mammal research began in 1973 [5-7] and the mist netting data, for example has gathered over 5000 captures representing over 260 species. Los Amigos Biological Station is one of the most active research stations in the Amazon [8], established in 2000 with over five years of intensive mammal studies including camera trapping, censuses, incidentals, trapping and interviews [9, 10] and has a particularly well developed bird list [11, 12]. Mammal field work has also been conducted at a tourist lodge known as Bonanza (study conducted in <2715ha), consisting of diurnal line transects, making up 310 records from 12 three km transects. This resulted in a total of 62 standardised surveys and 186km of trails walked and a further 84km walked from non-standardised surveys. Medium-large mammal lists therefore included combined field data from Bonanza, Cocha Cashu and Los Amigos and bird lists from Cocha Cashu and Los Amigos.

[1] Rodríguez, L. B., and Cadle, J. E. (1990). A preliminary overview of the herpetofauna of Cocha Cashu, Manu National Park, Peru. *Four Neotropical Rainforests*, 410-425.

[2] Rodríguez, L. O. (1992). Structure et organisation du peuplement d'anoures de Cocha Cashu, Parc National Manu, Amazonie péruvienne.

[3] von May, R., Siu-Ting, K., Jacobs, J. M., Medina-Müller, M., Gagliardi, G., Rodríguez, L. O., and Donnelly, M. A. (2009). Species diversity and conservation status of amphibians in Madre de Dios, Southern Peru. *Herpetological Conservation and Biology*, *4*(1), 14-29.

[4] Morales, V. R., and McDiarmid, R. W. (1996). Annotated checklist of the amphibians and reptiles of Pakitza, Manu National Park Reserve Zone, with comments on the herpetofauna of Madre de Dios, Peru. *Manu. The biodiversity of Southeastern Perú. Smithsonian Institution Press. Washington, DC*, 503-522.

[5] Peres, C. A. (1999). The structure of nonvolant mammal communities in different Amazonian forest types. *Mammals of the Neotropics*, *3*, 564-581.

[6] Terborgh, J., Fitzpatrick, J. W., and Emmons, L. (1984). *Annotated checklist of bird and mammal species of Cocha Cashu Biological Station, Manu National Park, Peru*. Chicago: Field Museum of Natural History.

[7] Voss, R.S. and Emmons, L.H. (1996) *Mammalian diversity in Neotropical lowland rain- forests: a preliminary assessment*. Bulletin of the American Museum of Natural History 230, 1–115.

[8] Salvador, S., Clavero, M., and Pitman, R. L. (2011). Large mammal species richness and habitat use in an upper Amazonian forest used for ecotourism. *Mammalian Biology-Zeitschrift für Säugetierkunde*, *76*(2), 115-123.

[9] Pitman, R. L., Beck, H., and Velazco, P. M. (2003). Mamíferos terrestres y arbóreos de la selva baja de la Amazonía Peruana; entre los ríos Manu y Alto Purús. *Alto Purus: Biodiversidad, Conservación y Manejo. Center for Tropical Conservation, Nicholas school of the environment, Duke University, Lima*, 109-122.

[10] Nuñez-Iturri, G., and Howe, H. F. (2007). Bushmeat and the fate of trees with seeds dispersed by large primates in a lowland rain forest in western Amazonia. *Biotropica*, *39*(3), 348-354.

[11] Lebbin, D. J., Hosner, P. A., Andersen, M. J., Valdez, U., and Tori, W. P. (2007). First description of nest and eggs of the White-lined Antbird (Percnostola lophotes), and breeding observations of poorly known birds inhabiting Guadua bamboo in southeastern Peru. *Boletin de la Sociedad Antioquena de Ornitologia*, 17, 119-132.

[12] Tobias, J. A., Aben, J., Brumfield, R. T., Derryberry, E. P., Halfwerk, W., Slabbekoorn, H., and Seddon, N. (2010). Song divergence by sensory drive in Amazonian birds. *Evolution*, 64(10), 2820-2839.

Appendix 1B. Sources for generating the primary forest predicted diversity lists (with references)

The sources for generating the primary forest base lists and distributional data were as follows: the baseline primary forest comparison site list for the amphibians was taken from von May et al. [1] and amended to include species unique to the regenerating forest site, as some species found in the regenerating site were potential undescribed species but closely related to those found in nearby primary forest sites. The reptile site lists were developed by the authors by combining inventories from literature to date [2-4] and the first publication of reptile lists from four field sites within the Madre de Dios region (see Appendix H). These new site lists include the Los Amigos Research Centre (CICRA is the Spanish acronym), 12°34′07″S 70°05′57″W, 270 m elevation; Centro de Monitoreo 1 (CM1), 12°34′17″S 70°04′29″W, ca. 250 m elevation; and Centro de Monitoreo 2 (CM2), 12°26′57″S 70°15′06″W, 260 m elevation and finally the MLC regenerating forest area. The baseline primary forest comparison site lists for the birds were taken from the Manu National Park list [5] and lists from other well known sites in the region [6-9]. The base mammal list was taken from Salvador et al. [10].

[1] von May, R., Siu-Ting, K., Jacobs, J. M., Medina-Müller, M., Gagliardi, G., Rodríguez, L. O., and Donnelly, M. A. (2009). Species diversity and conservation status of amphibians in Madre de Dios, Southern Peru. Herpetological Conservation and Biology, 4(1), 14-29.

[2] Rodríguez, L. B., and Cadle, J. E. (1990). A preliminary overview of the herpetofauna of Cocha Cashu, Manu National Park, Peru. Four Neotropical Rainforests, 410-425.

[3] Duellman, W. E., and Salas, A. W. (1991). Annotated checklist of the amphibians and reptiles of Cuzco Amazonico, Peru. University of Kansas.

[4] Morales, V. R., and McDiarmid, R. W. (1996). Annotated checklist of the amphibians and reptiles of Pakitza, Manu National Park Reserve Zone, with comments on the herpetofauna of Madre de Dios, Peru. Manu. The biodiversity of Southeastern Perú. Smithsonian Institution Press. Washington, DC, 503-522.

[5] Walker, B., Stotz, D. F., Pequeno, T., and Fitzpatrick, J. W. (2006). Birds of the Manu Biosphere Reserve. Fieldiana Zoology, 23-49.

[6] Terborgh, J., Fitzpatrick, J. W., and Emmons, L. (1984). Annotated checklist of bird and mammal species of Cocha Cashu Biological Station, Manu National Park, Peru. Chicago: Field Museum of Natural History.

[7] Tambopata Bird list 2010 A checklist of the Birds of the Tambopata Reserved Zone. www.explorersinn.com/tambopata-rainforest/species-list-class-birds-en Date consulted March 2013.

[8] Manu Wildlife Centre 2013 Bird list. www.manuwildlifecenter.com/manu-wildlife-center-birding-list.htm. Date consulted March 2013.

[9] Los Amigos Bird list 2013 Lista Anotada de las Aves de Los Amigos/Annotated List of the Birds of Los Amigos. www.zoo.ox.ac.uk/egi/wp-content/uploads/2012/03/Los-Amigos-birdlist.xls. Date consulted March 2013.

[10] Salvador, S., Clavero, M., and Pitman, R. L. (2011). Large mammal species richness and habitat use in an upper Amazonian forest used for ecotourism. Mammalian Biology-Zeitschrift für Säugetierkunde, 76(2), 115-123.

Appendices 1C-J. Available via Datacite DOI:
http://dx.doi.org/10.5525/gla.researchdata.239

Chapter 2

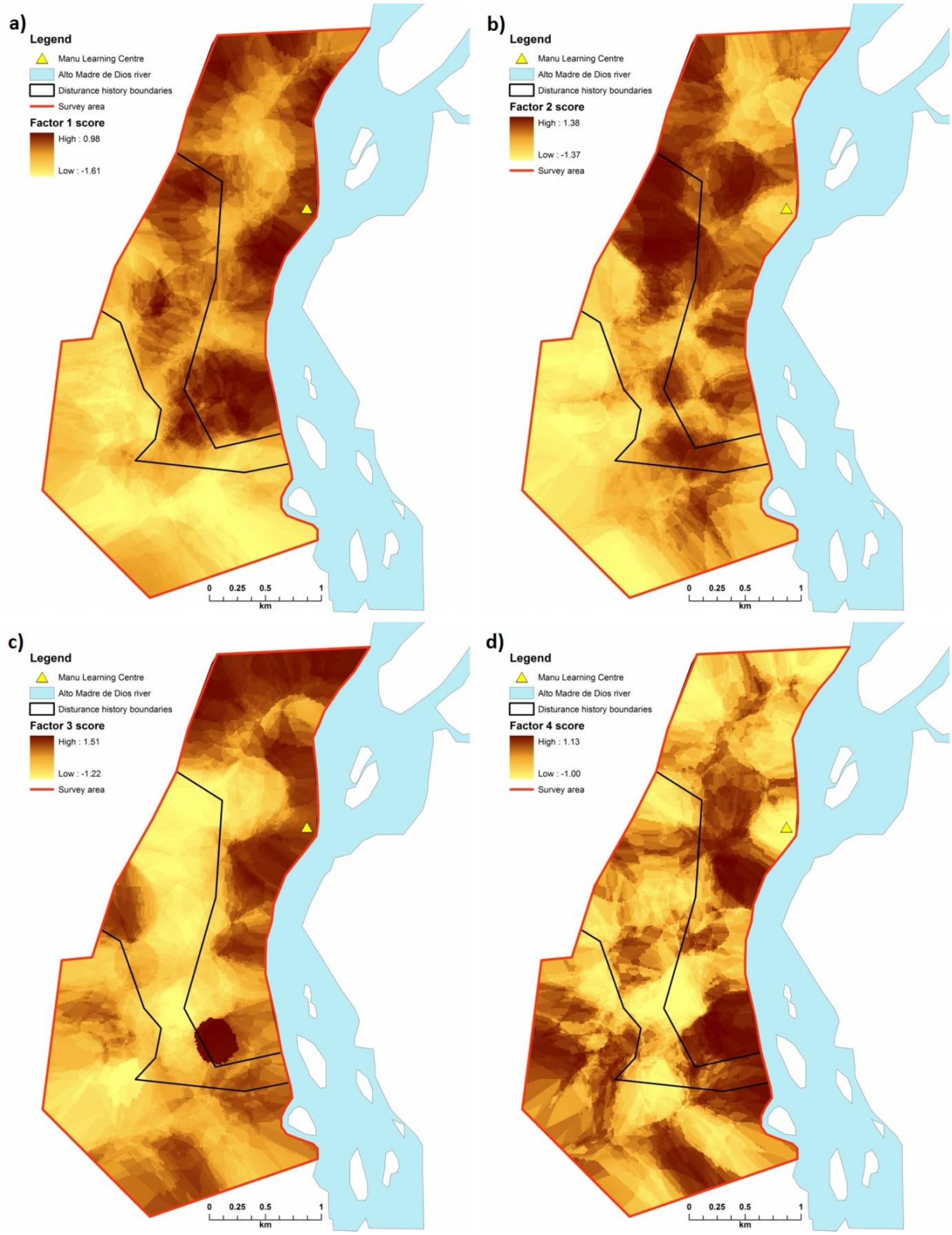
Appendix 2A – Principal Component Factor Analysis and loadings, based upon vegetation structure features of the study site.

```
Unrotated Factor Loadings and Communalities
Variable  Factor1  Factor2  Factor3  Factor4  Communality
CH         -0.615   -0.376    0.062    0.111        0.536
CC         -0.559   -0.523   -0.000   -0.342        0.702
MCC        -0.195    0.448    0.501    0.537        0.779
MCH        -0.626   -0.204   -0.061    0.223        0.487
LLD         0.625   -0.304    0.299   -0.049        0.575
big_DBH    -0.536   -0.239    0.330   -0.203        0.494
trees_5    -0.499    0.066    0.512    0.243        0.574
SL          0.489   -0.402   -0.187    0.512        0.698
HL          0.236   -0.622   -0.068    0.400        0.608
HC          0.370   -0.256    0.331   -0.199        0.352
BGC        -0.270    0.236   -0.604    0.047        0.496
CWD         0.415    0.081    0.396   -0.226        0.387

Variance   2.7246   1.4875   1.3895   1.0854       6.6871
% Var       0.227    0.124    0.116    0.090        0.557
```

```
Rotated Factor Loadings and Communalities
Quartimax Rotation
Variable  Factor1  Factor2  Factor3  Factor4  Communality
CH         -0.684   -0.212    0.118    0.099        0.536
CC         -0.778   -0.040   -0.066   -0.303        0.702
MCC         0.066   -0.046   -0.076    0.875        0.779
MCH        -0.542   -0.393    0.104    0.166        0.487
LLD         0.207    0.658    0.295   -0.109        0.575
big_DBH    -0.659    0.083   -0.210    0.097        0.494
trees_5    -0.433    0.004   -0.125    0.609        0.574
SL          0.255    0.085    0.789   -0.053        0.698
HL         -0.112    0.135    0.755   -0.083        0.608
HC          0.013    0.577    0.079   -0.110        0.352
BGC         0.099   -0.666   -0.087   -0.186        0.496
CWD         0.225    0.558   -0.154    0.036        0.387

Variance   2.1723   1.7567   1.4125   1.3456       6.6871
% Var       0.181    0.146    0.118    0.112        0.557
```

```
Sorted Rotated Factor Loadings and Communalities
Variable  Factor1  Factor2  Factor3  Factor4  Communality
CC         -0.778    0.000    0.000    0.000        0.702
CH         -0.684    0.000    0.000    0.000        0.536
big_DBH    -0.659    0.000    0.000    0.000        0.494
MCH        -0.542    0.000    0.000    0.000        0.487
BGC         0.000   -0.666    0.000    0.000        0.496
LLD         0.000    0.658    0.000    0.000        0.575
HC          0.000    0.577    0.000    0.000        0.352
CWD         0.000    0.558    0.000    0.000        0.387
SL          0.000    0.000    0.789    0.000        0.698
HL          0.000    0.000    0.755    0.000        0.608
MCC         0.000    0.000    0.000    0.875        0.779
trees_5    -0.433    0.000    0.000    0.609        0.574

Variance   2.1723   1.7567   1.4125   1.3456       6.6871
% Var       0.181    0.146    0.118    0.112        0.557
```

Appendix 2B – Kriging layers of the four Principal Component Factors applied to the study site; for detailed loadings of habitat structure features on each factor see Appendix 2A.



Appendix 2C – Detailed survey methodologies

Amphibians

Amphibians were surveyed using sets of five 100m length, 3 metre wide, two metre high nocturnal visual encounter transects (Beirne et al. 2013; Doan 2003), located within 50m of one another, running in a variety of directions. Twelve sets of transects were set up over the whole study area, four within each habitat type. All sites were surveyed within both wet and dry season. All transects were surveyed at night, commencing at approximately 2000h and were surveyed by two searchers over a period of ~25 minutes per transect (0410 observer hrs/night). Search teams consisted of one experienced herpetologist and one trained international conservation volunteer. Diurnal VES transects were not performed as nocturnal VES sampling has previously been shown to be the most efficient method in herpetological inventorying and still allowed us to detect resting diurnal species (Doan 2003). Five species encountered were omitted from the analysis within this study due to their association and breeding with a large permanent open body of water located within CCR habitat. As this feature is not related to the forest structure due to disturbance of the area as it existed prior to disturbance this was deemed appropriate. Owing to two previous detections of chytridiomycosis within the study site (Kosch et al. 2012), codes of good practice to prevent disease transmission were adhered to. This was achieved by the systematic cleaning of tools and equipment. Sterile bags were used when handling amphibians. All amphibians encountered were identified in the field where possible and any unidentifiable individuals were anaesthetised with Lidocaine and fixed with 10% formalin then subsequently identified and stored in the Herpetological department of the Natural History Museum with the Universidad Nacional Mayor de San Marcos – UNMSM (MUSM) in Lima.

Birds

Due to the known high bird species richness at the site, we utilised three different methodologies to study different bird communities. This included mist-netting to target understorey cryptic species (Blake & Loiselle 2001), nocturnal line transects to gather information on the relatively understudied nocturnal bird community (Goyette et al. 2011) and diurnal line transects were carried out to detect vocal and conspicuous species in all strata of the forest (Bibby 2000). Mist-netting has been found to be generally less efficient than other survey methods (Blake & Loiselle 2001; Barlow et al. 2006; Whitman et al. 1997) but offers a method less implicated by observer bias. It is also a useful and standardised technique to compare understory avifaunal communities composed of cryptic species, but is restricted by not including canopy avifauna when traps are not elevated into the canopy (Blake & Loiselle 2000; Barlow et al. 2006). This study used a combination of both low level mist netting and line-transect methods so the advantages of one would counteract the weaknesses of the other (Rappole et al. 1998). Birds were identified visually using Schulenberg et al. (2010), the most authoritative and up to date knowledge on bird identification and distributions in Peru and by calls with the aid of Schulenberg et al. (2000).

Diurnal line transects

In total x15 line transects (400m length) were walked throughout the survey site (5 located in CCR, 5 in MXD and 5 in SLR) to monitor all bird species both visually and by call (Bibby et al. 1998; Bibby et al. 2000).. Each transect was walked between 9 and 12 times to build up a record of the bird community around each transect location. Transects were conducted at various times of the year, in both wet and dry seasons, between October 2011 to October 2013.

Mist-netting

A total of 9 mist net sites were surveyed (three located within CCR, three within MXD and three within SLR), using between four-six mist-nets (ten meters long and two and a half meters high) placed in a randomised array with at least 20m between each net.. Sites were surveyed between May 2012 and October 2013 on a rotation basis so that each site was sampled during various times of the year, in both wet and dry seasons. Nets were opened at first light (~0530-0600h) and closed at ~1030h to allow for optimised capture rates during periods of high activity (Blake 1992). Nets were checked every 25 minutes.

Nocturnal line transects

Nocturnal birds were surveyed along 500m length transects, both visually and by call. Each transect was walked six times by paired observers (one of whom conducted all transects to provide standardisation to data collection), three times between the hours of 2000-0000h and three times between the hours 0300-0600h; between June-September 2013.

Butterflies

A total of 18 survey sites were used for butterfly trapping, six in each of the three distinct habitat types. Each of the 18 sites was subjected to the use of two different bait types (rotten banana and rotten fish), accumulating in 450 trap days within a 12 month period (Jan–Dec 2013) . Many studies have targeted just fruit-feeding Nymphalidae butterflies, however in order to represent a fuller representation of overall community structure we also utilised rotten fish bait, which has shown to capture a greater number of individuals than rotting fruit baits (Hall & Willmott 2000). We utilised rotting banana bait prepared following the methods by DeVries and Walla (1999) and rotten fish bait prepared a week prior to sampling; butterflies were caught using Van Someren-Rydon traps measuring 1m in length and 0.40m in diameter (Austin & Riley 1995; Hughes et al. 1998). These simple baited traps have been used successfully by previous studies on butterflies in the tropics (Hughes et al. 1998; DeVries & Walla 2001; Armstrong 2010; Hill et al. 2001). Each trapping session ran for five consecutive days and traps were checked daily between 0900 and 1500 with a randomized site visiting sequence to avoid any systematic bias (Barlow et al. 2007). Bait was replaced every second day to ensure bait freshness across all sites (DeVries & Walla 2001; Hughes et al. 1998). The number of butterflies of each species at each site was recorded and individuals large enough and without transparent wings were marked, using a non-toxic pen with a simple dot code. Marking appropriate individuals allowed differentiation between recaptures and newly caught individuals in order to avoid pseudoreplication.

Mammals

Nine camera trap sites (three within each disturbance type) were used, culminating in 151 camera trap months (50 in SLR, 52 in PCR and 49 in CCR) or 4228 camera trap days. Trapping was conducted between March 2012 and October 2013, in both wet and dry seasons. Terrestrial medium-to-large mammals were the target group for this study, therefore excluding arboreal species, small rodents and highly aquatic or riverine species (Munari et al. 2011; Silveira et al. 2003; Tobler et al. 2008). Traps were Bushnell Trophycams (models 119437 and 119436). Sites were spaced between 0.75-1.5km apart to ensure there were no major gaps in the sampled areas (Sharma et al. 2010) and sites were changed every 3 months by a distance no greater than 50m, to ensure that cameras were not located in front of the path of a particular individual passing to and from a sleeping site over prolonged periods. Cameras were set at an average height of 40cm above the ground at a distance of 3m from forest trails to obtain good quality full-frame pictures and were left un-baited (Sharma et al. 2010; Tobler et al. 2008). The delay between pictures was set to 30 seconds (Dillon & Kelly 2007) and the sensitivity of the infrared sensor was set to normal. Cameras were operating 24h a day and were checked every 7-12 days to replace SD cards and batteries if necessary. Obvious Photos of the same individual appearing within a five minute period were removed for the analysis (Liu et al. 2013)

Appendix 2C references

Armstrong, C. (2010). The effect of forest edges on the community structure of tropical fruit-feeding butterflies. The Plymouth Student Scientist, 3(2), 3-17.

Austin, G. T., & Riley, T. J. (1995). Portable bait traps for the study of butterflies. Tropical Lepidoptera, 6(1), 5-9.

Barlow, J., Peres, C. A., Henriques, L. M. P., Stouffer, P. C., & Wunderle, J. M. (2006). The responses of understorey birds to forest fragmentation, logging and wildfires: an Amazonian synthesis. Biological Conservation, *128*(2), 182-192.

Barlow, J., Gardner, T. A., Araujo, I. S., Ávila-Pires, T. C., Bonaldo, A. B., Costa, J. E. and Peres, C. A. (2007a). Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. Proceedings of the National Academy of Sciences, 104(47), 18555-18560.

Beirne, C. Burdekin, O. & Whitworth, A. (2013) Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in Eastern Ecuador. Herpetological Journal. 23, 209–219.

Bibby C, J. (2000). Bird census techniques. Elsevier.

Bibby C, J. (2000). Bird census techniques. Elsevier.

Bibby, C. J., Marsden, S., & Jones, M. (1998). Bird surveys. Expedition Advisory Centre.

Blake, J. G., & Loiselle, B. A. (2001). Bird assemblages in second-growth and old-growth forests, Costa Rica: perspectives from mist nets and point counts. The Auk, 118(2), 304-326.

Blake, J.G. (1992). Temporal variation in point counts of birds in a lowland wet forest in Costa Rica. The Condor. 94:265-275.

Devries, P. J., & Walla, T. R. (2001). Species diversity and community structure in neotropical fruit-feeding butterflies. *Biological journal of the Linnean Society*, *74*(1), 1-15.

Dillon, A., & Kelly, M. J. (2007). Ocelot Leopardus pardalis in Belize: the impact of trap spacing and distance moved on density estimates. Oryx, 41(04), 469-477.

Doan, T. (2003). Which methods are most effective for surveying rain forest herpetofauna? Journal of Herpetology, 37, 72–81.

Goyette, J.L, Howe, R.W., Wolf, A.T., & W.D. Robinson. (2011). Detecting tropical nocturnal birds using automated audio recordings. Journal of Field Ornithology. 82 (3): 279-287.

Hall JP, Willmott KR. Patterns of feeding behaviour in adult male riodinid butterflies and their relationship to morphology and ecology. Bioll J Linn Soc, 2000; 69(1): 1–23.

Hill, J., Hamer, K., Tangah, J., & Dawood, M. (2001). Ecology of tropical butterflies in rainforest gaps. Oecologia, 128(2), 294-302.

Hughes, J. B., Daily, G. C., & Ehrlich, P. R. (1998). Use of fruit bait traps for monitoring of butterflies (Lepidoptera: Nymphalidae). *Revista de biología tropical*, *46*(3), 697-704.

Kosch, T. A., V. Morales, & K. Summers. (2012). Batrachochytrium dendrobatidis in Peru. Herpetological Review 43.2: 288.

Liu, X., Wu, P., Songer, M., Cai, Q., He, X., Zhu, Y., & Shao, X. (2013). Monitoring wildlife abundance and diversity with infra-red camera traps in Guanyinshan Nature Reserve of Shaanxi Province, China. Ecological Indicators, 33, 121-128.

Munari, D. P., Keller, C., & Venticinque, E. M. (2011). An evaluation of field techniques for monitoring terrestrial mammal populations in Amazonia. Mammalian Biology-Zeitschrift für Säugetierkunde, 76(4), 401-408.

Rappole, J. H., Winker, K., & Powell, G. V. (1998). Migratory Bird Habitat Use in Southern Mexico: Mist Nets versus Point Counts (Hábitat Utilizado por las Aves Migratorias en México: Redes vs. Conteos de Punto). Journal of Field Ornithology, 635-643.

Schulenberg, T. S., Stotz, D. F., Lane, D. F., O'Neill, J. P., & Parker III, T. A. (2010). *Birds of Peru: revised and updated edition*. Princeton University Press.

Schulenberg, T.S., Marantz, C.A., & P.H. English. (2000). Voices of Amazonian Birds, Vol 1-3. [CD]. Cornell Laboratory of Ornithology.

Sharma, R. K., Jhala, Y., Qureshi, Q., Vattakaven, J., Gopal, R., & Nayak, K. (2010). Evaluating capture–recapture population and density estimation of tigers in a population with known parameters. Animal Conservation, 13(1), 94-103.

Silveira, L., Jacomo, A. T., & Diniz-Filho, J. A. F. (2003). Camera trap, line transect census and track surveys: a comparative evaluation. Biological Conservation, 114(3), 351-355.

Tobler, M. W., Carrillo-Percastegui, S. E., Leite Pitman, R., Mares, R., & Powell, G. (2008). An evaluation of camera traps for inventorying large-and medium-sized terrestrial rainforest mammals. Animal Conservation, 11(3), 169-178.

Whitman, A. A., Hagan, J. M., & Brokaw, N. V. (1997). A comparison of two bird survey techniques used in a subtropical forest. The Condor, 99(4), 955-965.

Appendix 2D – Output summaries of ANOVA analysis of the factor scores generated by the PCA (see Appendix 2A and 2B), across disturbance areas.

One-way ANOVA: PC1 versus dist_type

```
Source      DF       SS      MS      F      P
dist_type    2  101.039  50.520  61.26  0.000
Error      565  465.960   0.825
Total      567  567.000
S = 0.9081   R-Sq = 17.82%   R-Sq(adj) = 17.53%


                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level    N     Mean   StDev  ---+---------+---------+---------+------
CCR    240   0.3684  0.8641                             (---*--)
MXD    168   0.0904  0.9783                     (---*---)
SLR    160  -0.6475  0.8960  (---*----)
                             ---+---------+---------+---------+------
                             -0.70     -0.35      0.00      0.35
Pooled StDev = 0.9081
```

One-way ANOVA: PC2 versus dist_type

```
Source      DF       SS      MS      F      P
dist_type    2   61.801  30.901  34.56  0.000
Error      565  505.198   0.894
Total      567  567.000
S = 0.9456   R-Sq = 10.90%   R-Sq(adj) = 10.58%


                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level    N     Mean   StDev   --+---------+---------+---------+-------
CCR    240   0.1980  0.9471                          (---*---)
MXD    168   0.2187  0.9476                          (---*----)
SLR    160  -0.5266  0.9412   (---*----)
                              --+---------+---------+---------+-------
                             -0.60     -0.30      0.00      0.30
Pooled StDev = 0.9456
```

One-way ANOVA: PC3 versus dist_type

```
Source      DF       SS      MS      F      P
dist_type    2   48.544  24.272  26.45  0.000
Error      565  518.456   0.918
Total      567  566.999
S = 0.9579   R-Sq = 8.56%   R-Sq(adj) = 8.24%


                             Individual 95% CIs For Mean Based on Pooled
                             StDev
Level    N     Mean   StDev     +---------+---------+---------+---------
CCR    240   0.3284  1.1514                              (----*----)
MXD    168  -0.3443  0.7611     (-----*-----)
SLR    160  -0.1311  0.8121              (-----*-----)
                                +---------+---------+---------+---------
                             -0.50     -0.25      0.00      0.25
Pooled StDev = 0.9579
```

One-way ANOVA: PC4 versus dist_type

```
Source      DF       SS     MS     F      P
dist_type    2    4.619  2.309  2.32  0.099
Error      565  562.381  0.995
Total      567  567.000
S = 0.9977   R-Sq = 0.81%   R-Sq(adj) = 0.46%


                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level    N     Mean   StDev  --------+---------+---------+---------+-
CCR    240  -0.0007  1.0712            (-------*-------)
MXD    168  -0.1153  0.9837  (---------*---------)
SLR    160   0.1221  0.8921                  (---------*---------)
                             --------+---------+---------+---------+-
                                  -0.15      0.00      0.15      0.30
Pooled StDev = 0.9977
```

Appendix 2E - Summary information of the dominant 10 families within each disturbance area; data from Gentry plots carried out by Pillco-Hurcaya (unpublished 2014).

SLR		MXD		CCR	
Family	N° ind. 0.1 ha	Family	N° ind. 0.1 ha	Family	N° ind. 0.1 ha
Meliaceae	13	Lauraceae	11	Lauraceae	14
Icacinaceae	9	Urticaceae	8	Melastomataceae	10
Moraceae	8	Arecaceae	7	Rubiaceae	8
Euphorbiaceae	5	Sapotaceae	7	Fabaceae	7
Fabaceae	4	Bignoniaceae	6	Arecaceae	5
Rubiaceae	4	Fabaceae	6	Bignoniaceae	5
Annonaceae	3	Myristicaceae	5	Urticaceae	5
Salicaceae	3	Rubiaceae	5	Annonaceae	2
Sapotaceae	3	Elaeocarpaceae	3	Clusiaceae	1
Violaceae	3	Melastomataceae	3	Hypericaceae	1

Appendix 2F - Summary information of the dominant 10 species, and their respective fmilly association, within each disturbance area; data from Gentry plots carried out by Pillco-Hurcaya (unpublished 2014).

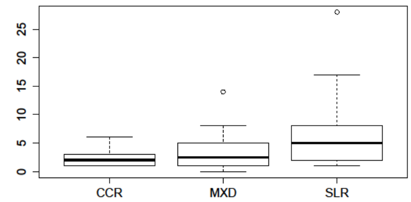
Species	Family	N° ind./ 0.1 ha
SLR		
Calatola costaricensis	Icacinaceae	9
Guarea kunthiana	Meliaceae	3
Lunania parviflora	Violaceae	3
Rinorea viridiflora	Violaceae	3
Iriartea deltoidea	Arecaceae	2
Guarea macrophylla	Meliaceae	2
Trichilia sp. 1	Meliaceae	2
Brosimum parinarioides	Moraceae	2
Sorocea sp. 1	Moraceae	2
Otoba parviflora	Myristicaceae	2
MXD		
Jacaranda copaia	Bignoniaceae	6
Iriartea deltoidea	Arecaceae	4
Pleurothyrium sp. 1	Lauraceae	3
Virola sebifera	Myristicaceae	3
Pourouma cecropiifolia	Urticaceae	3
Sloanea sp. 1	Elaeocarpaceae	2
Miconia sp. 2	Melastomataceae	2
Ladenbergia oblongifolia	Rubiaceae	2
Pouteria sp. 2	Sapotaceae	2
Pourouma sp. 1	Urticaceae	2
CCR		
Ladenbergia oblongifolia	Rubiaceae	8
Jacaranda copaia	Bignoniaceae	5
Socratea exorrhiza	Arecaceae	4
Ocotea sp. 1	Lauraceae	4
Miconia sp. 3	Melastomataceae	4
Ocotea sp. 2	Lauraceae	3
Cecropia sp. 1	Urticaceae	3
Inga sp. 3	Fabaceae	2
Miconia sp. 2	Melastomataceae	2
Cecropia polystachya	Urticaceae	2

Appendix 2G – A summary of Morans I test for spatio-autocorrelation against GLMM model residuals. A p-value of >0.05 indicates no significant affect from spatio-auto correlation within model residuals.

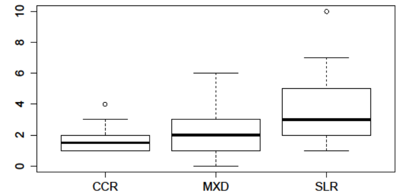
Survey group	Response variable	observed	expected	sd	p-value
Amphibians	Sample richness	-0.010	-0.009	0.007	0.93
	Sample abundance	-0.002	-0.009	0.007	0.34
	Sample Shannon diversity	-0.002	-0.009	0.007	0.31
Birds - diurnal transects	Sample richness	-0.002	-0.006	0.006	0.48
	Sample abundance	-0.002	-0.006	0.006	0.42
	Sample Shannon diversity	-0.002	-0.006	0.006	0.42
Birds - mist-nets	Sample richness	-0.003	-0.008	0.007	0.48
	Sample abundance	-0.004	-0.008	0.007	0.54
	Sample Shannon diversity	-0.003	-0.008	0.007	0.50
Birds - nocturnal transects	Sample richness	-0.010	-0.014	0.012	0.72
	Sample abundance	-0.012	-0.014	0.011	0.87
	Sample Shannon diversity	-0.006	-0.014	0.012	0.47
Butterflies	Sample richness	-0.002	-0.009	0.012	0.53
	Sample abundance	-0.010	-0.009	0.012	0.96
	Sample Shannon diversity	-0.004	-0.009	0.012	0.66
Mammals	Sample richness	-0.007	-0.007	0.005	0.99
	Sample abundance	-0.001	-0.007	0.005	0.27
	Sample Shannon diversity	-0.008	-0.007	0.005	0.86

Appendix H - Box plots for each response variable across disturbance areas; for each of the main surevy groups.

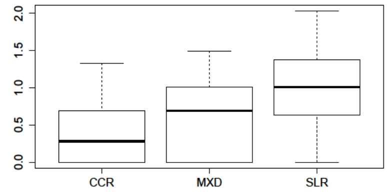
Amphibian sample abundance



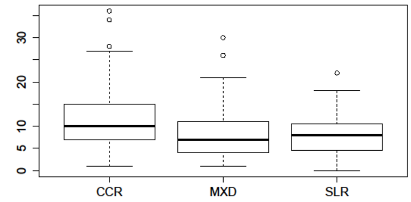
Amphibian sample richness



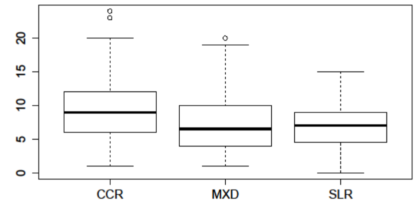
Amphibian sample Shannon diversity



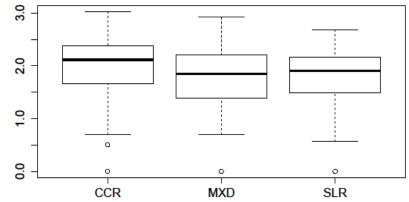
Bird sample abundance – diurnal transects



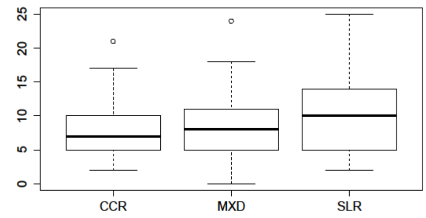
Bird sample richness – diurnal transects



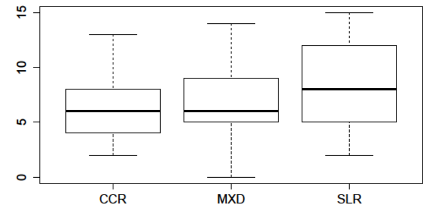
Bird sample Shannon diversity – diurnal transects



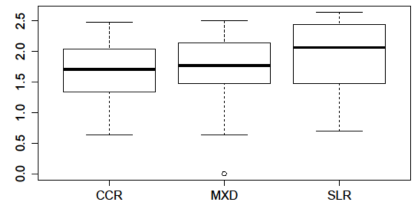
Bird sample abundance – mist-nets



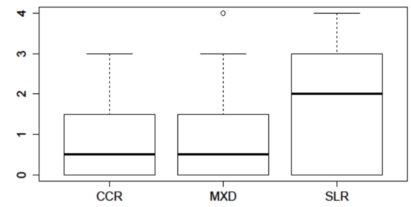
Bird sample richness – mist-nets



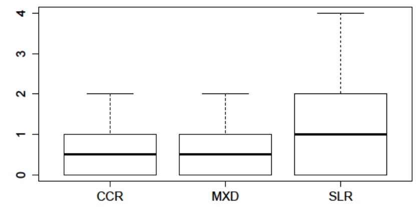
Bird sample Shannon diversity – mist-nets



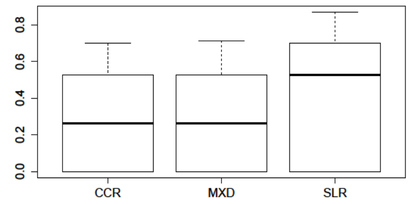
Nocturnal sample bird abundance



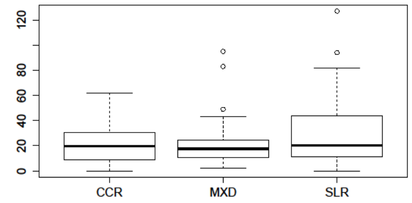
Nocturnal sample bird richness



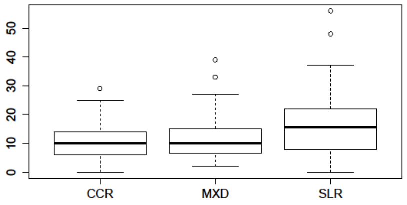
Nocturnal sample bird Shannon diversity



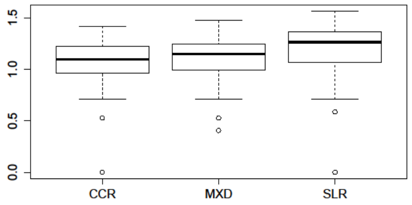
Butterfly sample abundance



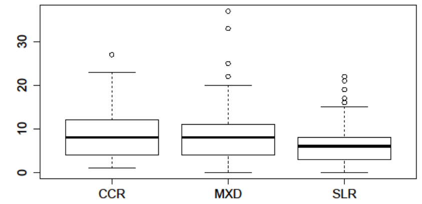
Butterfly sample richness



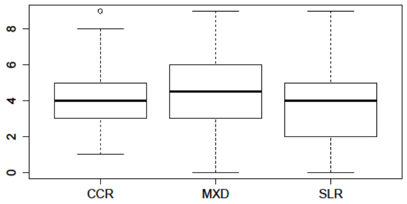
Butterfly sample Shannon diversity



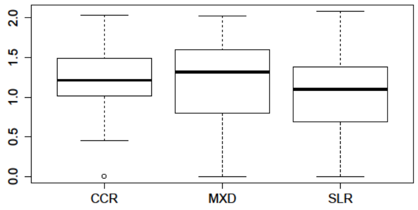
Mammal sample abundance



Mammal sample richness



Mammal sample Shannon diversity



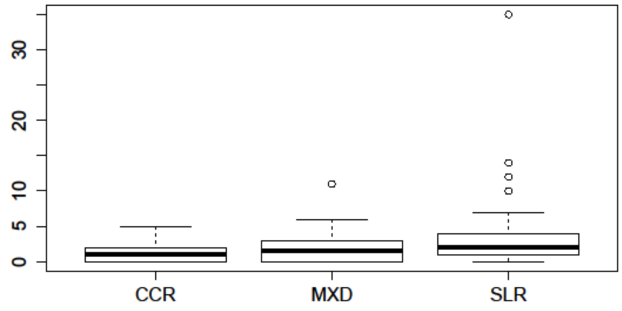
Appendix 2I – A summary of general linear mixed model (GLMM) outputs; response variables representing sample level species richness, abundance and species diversity. AICc values are shown for all sets of models; models are ranked by AICc values and the respective model weight; where the ΔAICc < 2 the most parsimonious model is the preferred model and indicated by * in the weight column.

Model selection tables	Intercept	Disturbance area	Distance to main river	Altitude (asl)	Slope extent	Offset (log(net-hours))	Bait type	df	logLik	AICc	delta AICc	weight
Amphibian sample species richness	0.2252	+			0.08515	na	na	5	-186.523	383.6	0	0.345*
	3.618	+		-0.005997		na	na	5	-186.833	384.2	0.62	0.253
	0.5845	+				na	na	4	-188.21	384.8	1.18	0.192
	0.6835	+	-0.000416			na	na	5	-187.264	385.1	1.48	0.165
	1.84	+	0.0001568	-0.003194	0.07627	na	na	7	-186.315	387.7	4.11	0.044
	null					na	na	2	-196.775	397.7	14.03	0
Amphibian sample abundance	0.7368	+				na	na	4	-252.735	513.9	0	0.412*
	0.4935	+			0.05922	na	na	5	-252.233	515	1.19	0.227
	2.331	+		-0.003144		na	na	5	-252.496	515.6	1.72	0.174
	0.7908	+	-0.0002199			na	na	5	-252.575	515.7	1.88	0.161
	0.8521	+	0.0002091	-0.0009433	0.07578	na	na	7	-252.174	519.5	5.6	0.025
	null					na	na	2	-279.019	562.2	48.3	0
Amphibian sample Shannon diversity	null					na	na	3	-86.968	180.2	0	0.776*
	0.431	+				na	na	5	-86.12	182.8	2.66	0.206
	0.2001	+			0.05456	na	na	6	-87.506	187.8	7.67	0.017
	2.209	+		-0.003516		na	na	6	-90.158	193.1	12.97	0.001
	0.4829	+	-0.0002259			na	na	6	-92.643	198.1	17.94	0
	1.148	+	0.0001193	-0.001904	0.05131	na	na	8	-97.546	212.5	32.37	0
Bird diurnal transects sample species richness	null					na	na	3	-453.339	912.8	0	0.547*
	2.201	+				na	na	5	-452.238	914.9	2.04	0.197
	1.069	+		0.002249		na	na	6	-451.82	916.2	3.37	0.102
	2.137	+			0.01468	na	na	6	-452.203	917	4.13	0.069
	2.19	+	3.67E-05			na	na	6	-452.226	917	4.18	0.068
	-0.08854	+	-2.40E-04	0.004413	0.0322	na	na	8	-451.393	919.8	6.92	0.017
Bird diurnal transects sample abundance	null					na	na	3	-486.364	978.9	0	0.439*
	2.409	+				na	na	5	-484.831	980.1	1.17	0.244
	1.184	+		0.002434		na	na	6	-484.468	981.5	2.61	0.119
	2.338	+			0.01636	na	na	6	-484.799	982.2	3.27	0.085
	2.423	+	-0.0000452			na	na	6	-484.818	982.2	3.31	0.084
	-0.658	+	-0.0004346	0.006048	0.03528	na	na	8	-483.681	984.3	5.45	0.029
Bird diurnal transects sample Shannon diversity	null					na	na	3	-168.737	343.6	0	0.929*
	1.984	+				na	na	5	-169.262	348.9	5.29	0.066
	1.909	+			0.01677	na	na	6	-170.924	354.4	10.78	0.004
	0.5027	+		0.002941		na	na	6	-173.664	359.9	16.26	0
	1.933	+	1.66E-04			na	na	6	-176.267	365.1	21.46	0
	-0.1787	+	-7.04E-05	0.003983	0.04039	na	na	8	-181.912	380.8	37.17	0
Bird mistnets sample species richness	null					+	na	3	-317.113	640.4	0	0.633*
	-1.413	+				+	na	5	-316.54	643.6	3.16	0.131

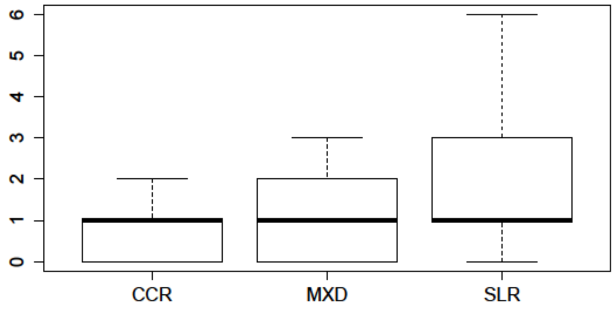
	-1.335	+	-0.0003192			+	na	6	-315.593	643.9	3.47	0.112
	-1.317	+			-0.02991	+	na	6	-316.27	645.2	4.82	0.057
	-1.56	+		0.000292		+	na	6	-316.536	645.8	5.36	0.044
	-3.771	+	-0.0005226	0.004937	0.002428	+	na	8	-314.875	647	6.56	0.024
Bird mistnets sample abundance	null					+	na	3	-363.527	733.3	0	0.64*
	-1.149	+				+	na	5	-363.104	736.7	3.46	0.114
	-1.059	+	-0.000376			+	na	6	-362.156	737	3.77	0.097
	-0.962	+			-0.05926	+	na	6	-362.299	737.3	4.05	0.084
	-1.498	+		0.0006966		+	na	6	-363.089	738.9	5.63	0.038
	-3.258	+	-0.0005425	0.004633	-0.02687	+	na	8	-361.21	739.7	6.4	0.026
Bird mistnets sample Shannon diversity	null					+	na	3	-86.746	179.7	0	0.971*
	-1.495	+				+	na	5	-88.157	186.8	7.12	0.028
	-1.415	+			-0.02442	+	na	6	-90.248	193.2	13.51	0.001
	-1.765	+		0.0005363		+	na	6	-92.649	198	18.32	0
	-1.42	+	-0.0002983			+	na	6	-94.861	202.4	22.74	0
	-4.232	+	-0.0005394	0.005662	0.009151	+	na	8	-100.733	218.7	39.01	0
Bird nocturnal transects sample species richness	-1.152	+			0.1334	na	na	5	-75.976	162.9	0	0.556*
	3.219	+	0.0007838	-0.009838	0.2093	na	na	7	-74.449	164.6	1.79	0.227
	null					na	na	2	-81.232	166.6	3.78	0.084
	-0.624	+				na	na	4	-79.151	166.9	4.04	0.074
	-0.7454	+	0.0003795			na	na	5	-78.86	168.6	5.77	0.031
	-1.851	+		0.002446		na	na	5	-78.986	168.9	6.02	0.027
Bird nocturnal transects sample abundance	6.047	+	0.0007667	-0.0144	0.1893	na	na	7	-98.315	212.4	0	0.539*
	null					na	na	2	-105.442	215.1	2.68	0.141
	-0.2399	+				na	na	4	-103.398	215.4	3.01	0.12
	-0.572	+			0.08575	na	na	5	-102.345	215.6	3.22	0.108
	1.531	+		-0.003491		na	na	5	-103.074	217.1	4.68	0.052
	-0.2947	+	0.0001587			na	na	5	-103.346	217.6	5.22	0.04
Bird nocturnal transects sample Shannon diversity	null					na	na	3	-40.275	86.9	0	0.925*
	0.3598	+				na	na	5	-40.834	92.6	5.67	0.054
	0.08971	+			0.06806	na	na	6	-40.625	94.5	7.64	0.02
	0.3373	+		4.47E-05		na	na	6	-45.972	105.2	18.33	0
	0.3147	+	0.0001467			na	na	6	-48.05	109.4	22.49	0
	2.041	+	0.0002875	-4.23E-03	0.09128	na	na	8	-51.473	121.2	34.33	0
Butterfly sample species richness	2.035	+				na	+	6	-364.725	742.3	0	0.951
	3.81	+	-7.58E-05	-0.003455	0.001347	na	+	9	-364.209	748.3	5.97	0.048
	2.349	+				na		5	-373.51	757.6	15.33	0
	4.507	+		-0.004244		na		6	-373.093	759	16.74	0
	2.406	+	-1.98E-04			na		6	-373.204	759.2	16.96	0
	2.247	+			0.02459	na		6	-373.389	759.6	17.33	0
	null					na		3	-378.226	762.7	20.4	0
Butterfly sample abundance	2.816	+				na	+	6	-448.424	909.7	0	0.774*
	7.23	+	4.20E-05	-0.008739	0.002173	na	+	9	-447.242	914.3	4.64	0.076
	3.054	+				na		5	-452.371	915.3	5.65	0.046
	7.113	+		-0.007985		na		6	-451.399	915.6	5.95	0.04
	null					na		3	-455.153	916.5	6.86	0.025
	3.118	+	-2.24E-04			na		6	-452.102	917	7.36	0.02
	2.958	+			0.02279	na		6	-452.296	917.4	7.74	0.016
	7.075	+	-6.08E-05	-0.007801	-0.008719	na		8	-451.378	920.2	10.53	0.004
Butterfly sample Shannon diversity	1.691	+				na	+	6	-119.175	251.2	0	0.942*
	null					na		3	-125.657	257.5	6.36	0.039
	1.938	+				na		5	-124.276	259.1	7.96	0.018
	1.961	+			-0.005851	na		6	-126.273	265.4	14.2	0.001
	2.849	+		-0.001792		na		6	-128.649	270.1	18.95	0
	1.934	+	1.32E-05			na		6	-131.519	275.9	24.69	0

	3.233	+	7.74E-05	-0.002956	-0.01506	na	+	9	-132.617	285.1	33.89	0
Mammal sample species richness	1.181	+			0.05389	na	na	5	-316.49	643.4	0	0.292
	null					na	na	2	-319.758	643.6	0.2	0.264*
	1.396	+				na	na	4	-318.036	644.3	0.95	0.181
	1.483	+	-0.000244			na	na	5	-317.305	645	1.63	0.129
	-0.2054	+	-0.0001779	0.002779	0.06013	na	na	7	-315.71	646.2	2.81	0.072
	1.261	+		0.0002637		na	na	5	-318.029	646.5	3.08	0.063
Mammal sample abundance	null					na	na	3	-464.464	935.1	0	0.515*
	2.16	+				na	na	5	-463.463	937.3	2.25	0.167
	1.782	+			0.09623	na	na	6	-462.486	937.6	2.46	0.15
	2.312	+	-0.0004219			na	na	6	-463.034	938.7	3.56	0.087
	3.279	+		-0.002183		na	na	6	-463.369	939.3	4.23	0.062
	1.216	+	-0.0002328	0.001319	0.08971	na	na	8	-462.35	941.7	6.62	0.019
Mammal sample Shannon diversity	null					na	na	3	-122.032	250.2	0	0.977*
	1.101	+				na	na	5	-123.789	258	7.76	0.02
	0.8651	+			0.05959	na	na	6	-124.663	261.9	11.68	0.003
	0.5457	+		0.001086		na	na	6	-128.74	270.1	19.84	0
	1.209	+	-0.0003066			na	na	6	-130.314	273.2	22.98	0
	-1.109	+	-0.0002553	0.003976	0.06675	na	na	8	-135.662	288.3	38.11	0

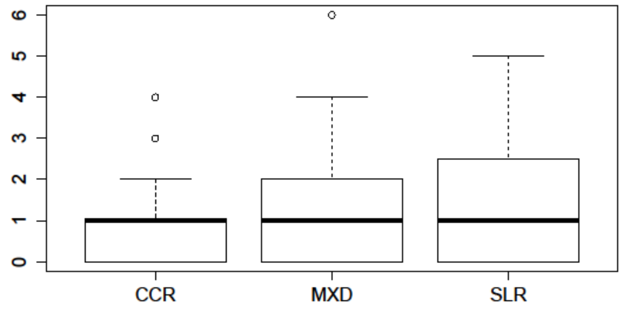
Appendix 2J - Box plots for each response variable across disturbance areas; for each of the indicator groups, as identified in chapter 1 of this thesis.



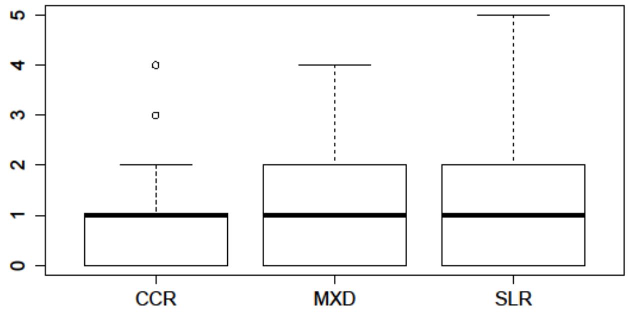
Indicator amphibian sample abundance



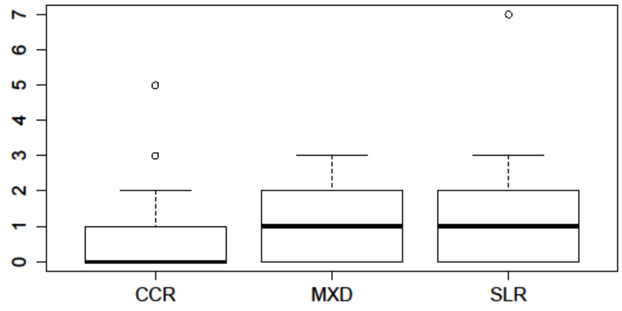
Indicator amphibian sample richness



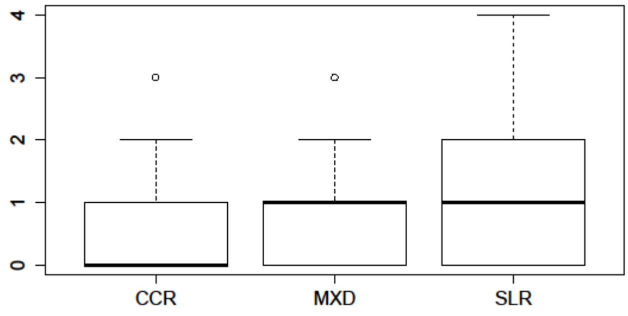
Indicator birds (transects) sample abundance



Indicator birds (transects) sample richness



Indicator birds (mistnets) sample abundance

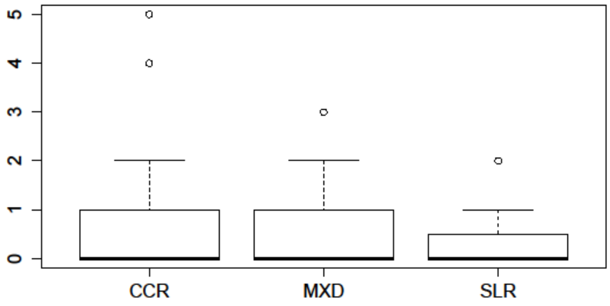


Indicator birds (mistnets) sample richness

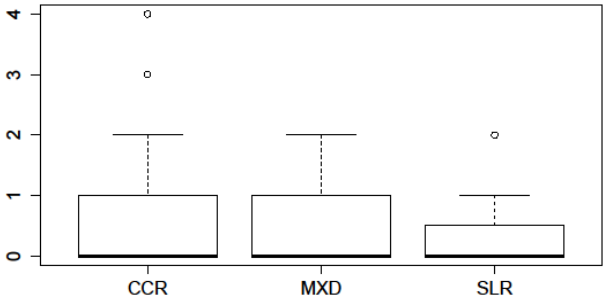
Appendix 2K – A summary of general linear mixed model (GLMM) outputs for indicators of primary forest habitat (as identified in Chapter 1 of this thesis); response variables representing sample level species richness and abundance. AICc values are shown for all sets of models; models are ranked by AICc values and the respective model weight; where the ΔAICc < 2, the most parsimonious model is the preferred model and indicated by * in the weight column.

Model selection tables	Intercept	Disturbance area	Distance to main river	Altitude (asl)	Slope extent	Offset (log(net-hours))	df	logLik	AICc	delta AICc	weight
Indicator amphibian sample abundance	-0.54	+			0.1793	na	6	-216.815	446.5	0	0.42
	0.2077	+				na	5	-218.703	448	1.53	0.195*
	5.18	+		-0.0098		na	6	-217.936	448.7	2.24	0.137
	0.3781	+	-0.000693			na	6	-218.105	449	2.58	0.116
	null					na	3	-221.774	449.8	3.32	0.08
	0.05737	+	0.000648	-0.002018	0.2441	na	8	-216.593	450.6	4.17	0.052
Indicator amphibian sample richness	-0.3195	+				na	5	-145.403	301.4	0	0.336
	-0.7444	+			0.1007	na	6	-144.715	302.3	0.87	0.218
	2.324	+		-0.005222		na	6	-145.103	303	1.64	0.148
	null					na	3	-148.41	303	1.66	0.146*
	-0.2619	+	-0.0002416			na	6	-145.316	303.5	2.07	0.119
	-0.04206	+	0.0008249	-0.002472	0.182	na	8	-144.293	306	4.64	0.033
Indicator bird sample abundance - diurnal transects	-0.2017	+				na	5	-231.117	472.6	0	0.243
	null					na	3	-233.248	472.7	0.02	0.241*
	-0.7448	+			0.1216	na	6	-230.291	473.1	0.51	0.188
	-2.257	+		0.004082		na	6	-230.623	473.8	1.17	0.135
	-0.3094	+	0.0003455			na	6	-230.759	474.1	1.45	0.118
	-3.698	+	0.000167	0.005311	0.1715	na	8	-229.008	475	2.36	0.075
Indicator bird sample richness - diurnal transects	null					na	3	-218.849	443.9	0	0.299*
	-0.2335	+				na	5	-216.938	444.3	0.42	0.242
	-0.3524	+	0.0003856			na	6	-216.396	445.4	1.5	0.141
	-0.6392	+			0.09087	na	6	-216.396	445.4	1.5	0.141
	-1.993	+		0.003494		na	6	-216.503	445.6	1.71	0.127
	-2.502	+	0.0003188	0.003121	0.1332	na	8	-215.233	447.4	3.58	0.05
Indicator bird sample abundance - mist nets	null					+	3	-168.508	343.2	0	0.551*
	-12.93	+	-0.0007869	0.01745	0.2497	+	8	-164.481	346.2	2.98	0.124
	-3.988	+			0.1355	+	6	-166.769	346.2	3.03	0.121
	-3.564	+				+	5	-167.874	346.2	3.04	0.121
	-4.598	+		0.002058		+	6	-167.833	348.4	5.16	0.042
	-3.528	+	-0.0001323			+	6	-167.84	348.4	5.17	0.041
Indicator bird sample richness - mist nets	null					+	3	-150.406	307	0	0.536*
	-12.18	+	-0.001042	0.01594	0.2285	+	8	-146.081	309.4	2.38	0.163
	-4.132	+			0.1327	+	6	-148.756	310.2	3.21	0.108
	-3.701	+				+	5	-149.915	310.3	3.32	0.102
	-3.567	+	-0.0004757			+	6	-149.399	311.5	4.5	0.057
	-3.186	+		-0.001024		+	6	-149.903	312.5	5.5	0.034

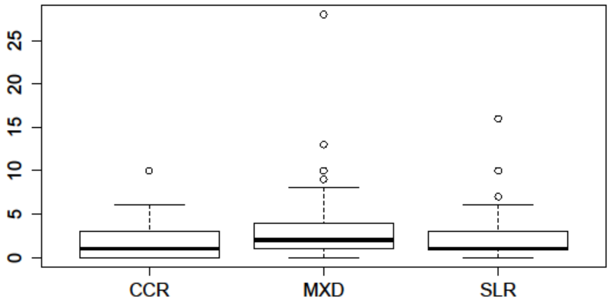
Appendix 2L - Box plots for each response variable across disturbance areas; for species of conservation concern, as identified in chapter 1 of this thesis.



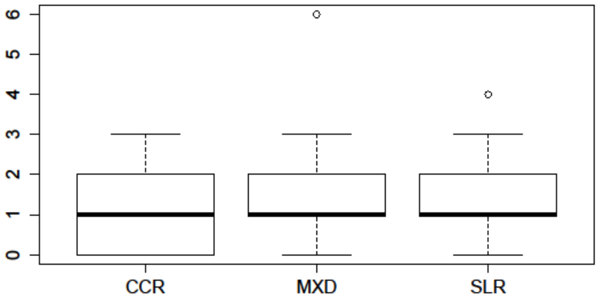
Sample abundance of birds of conservation concern – diurnal transects



Sample species richness of birds of conservation concern – diurnal transects



Sample abundance of mammals of conservation concern



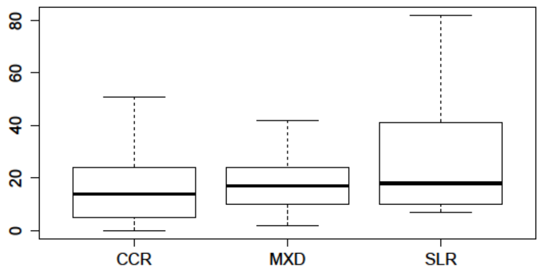
Sample species richness of mammals of conservation concern

Appendix 2M – A summary of general linear mixed model (GLMM) outputs for species of conservation concern (as identified in Chapter 1 of this thesis); response variables representing sample level species richness and abundance. AICc values are shown for all sets of models; models are ranked by AICc values and the respective model weight; where the ΔAICc < 2, the most parsimonious model is the preferred model and indicated by * in the weight column.

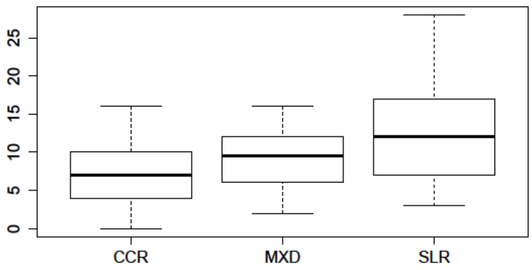
Model selection tables	**Intercept**	**Disturbance area**	**Distance to main river**	**Altitude (asl)**	**Slope extent**	**df**	**logLik**	**AICc**	**delta AICc**	**weight**
Conservation concern bird sample abundance - diurnal transects	null					3	-153.162	312.5	0	0.305*
	-0.4393	+				5	-151.129	312.7	0.17	0.28
	-3.424	+		0.005926		6	-150.594	313.7	1.27	0.162
	-0.5509	+	3.61E-04			6	-150.913	314.4	1.91	0.118
	-0.7138	+			0.06277	6	-150.994	314.5	2.07	0.109
	-5.081	+	-6.48E-05	0.008234	0.1168	8	-150.182	317.3	4.86	0.027
Conservation concern bird sample richness - diurnal transects	null					3	-143.267	292.7	0	0.339*
	-0.543	+				5	-141.318	293	0.34	0.285
	-0.9218	+			0.08657	6	-141.052	294.7	1.97	0.126
	-0.6409	+	0.00032			6	-141.146	294.9	2.16	0.115
	-2.224	+		0.003335		6	-141.153	294.9	2.18	0.114
	-3.223	+	0.000159	0.004166	0.1207	8	-140.667	298.3	5.62	0.02
Conservation concern mammal sample abundance	-0.04941	+			0.1352	6	-303.093	618.8	0	0.367
	0.4933	+				5	-304.706	619.8	1.06	0.217
	null					3	-307.238	620.6	1.87	0.144*
	3.654	+		-0.00614		6	-304.048	620.7	1.91	0.141
	0.5529	+	-0.00016			6	-304.649	621.9	3.11	0.077
	1.428	+	0.000305	-0.00312	0.1375	8	-302.812	622.6	3.87	0.053
Conservation concern mammal sample richness	-0.04167	+				5	-204.907	420.2	0	0.349
	null					3	-207.553	421.3	1.04	0.208*
	-0.2405	+			0.04971	6	-204.516	421.6	1.39	0.175
	-7.36E-03	+	-9.66E-05			6	-204.869	422.3	2.09	0.123
	-4.65E-01	+		0.000826		6	-204.884	422.4	2.12	0.121
	-2.043	+	1.07E-05	0.003308	0.07477	8	-204.236	425.5	5.26	0.025

Appendix 2N – Box plots for each response variable across disturbance areas; for banana feeding and carrion feeding butterflies.

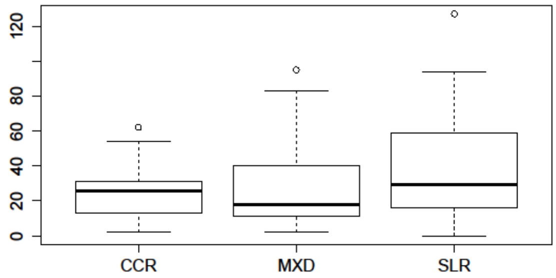
Fruit-baited butterfly traps – sample abundance



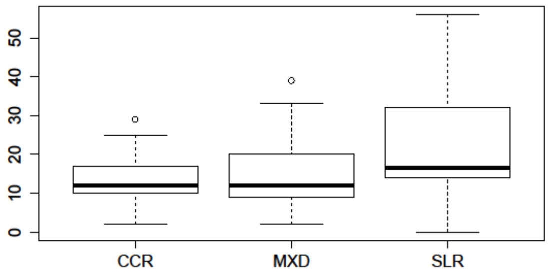
Fruit-baited butterfly traps – sample species richness



Carrion-baited butterfly traps - sample abundance

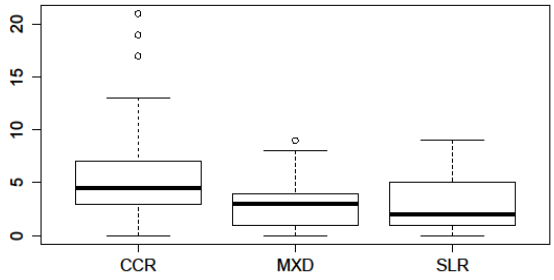


Carrion-baited butterfly traps – sample species richness

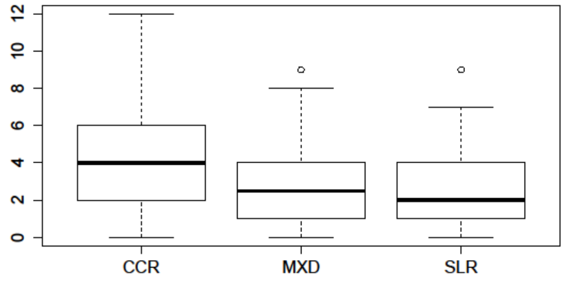


Appendix 2O- Box plots for each response variable across disturbance areas; for different feeding guilds of birds encountered on diurnal transects.

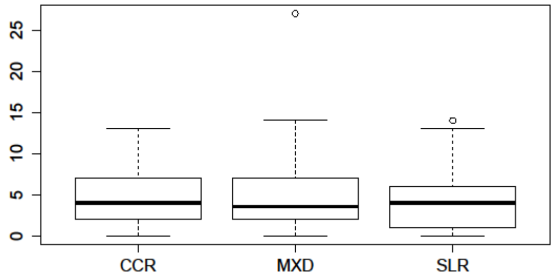
Frugivore bird sample abundance – transects



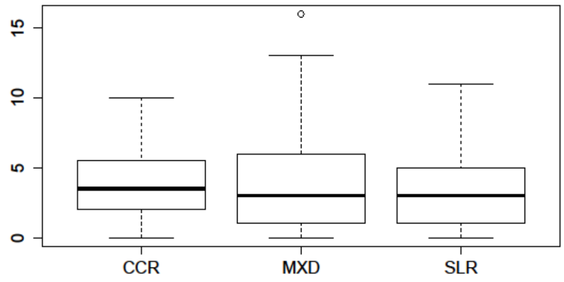
Frugivore bird sample species richness – transects



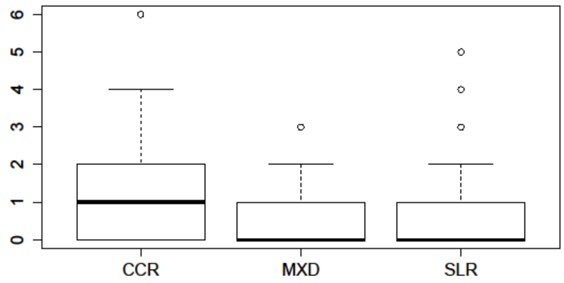
Insectivore bird sample abundance – transects



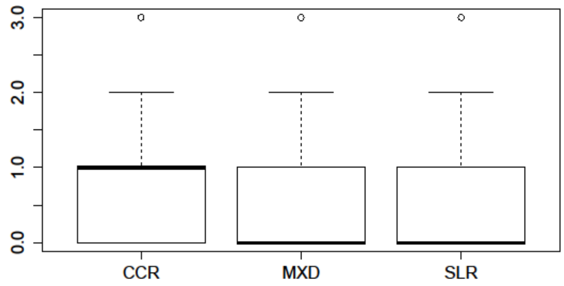
Insectivore bird sample species richness – transects



Omnivore bird sample abundance – transects

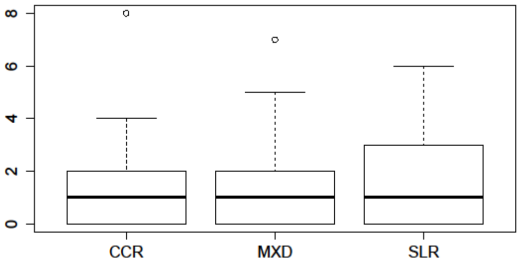


Omnivore bird sample species richness – transects

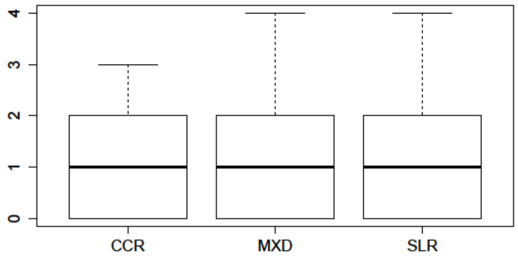


Appendix 2P - Box plots for each response variable across disturbance areas; for different feeding guilds of birds captured in mist-nets.

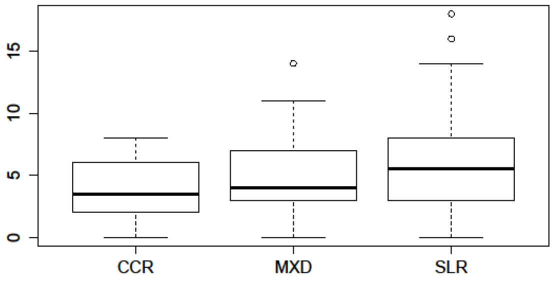
Frugivore bird sample abundance – mist-nets



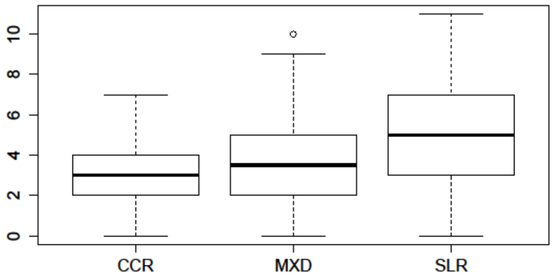
Frugivore bird sample species richness – mist-nets



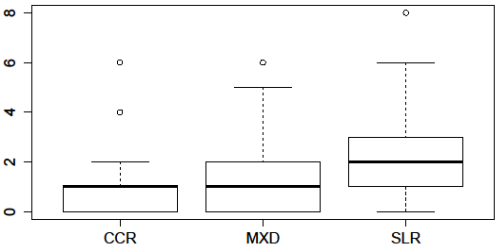
Insectivore bird sample abundance – mist-nets



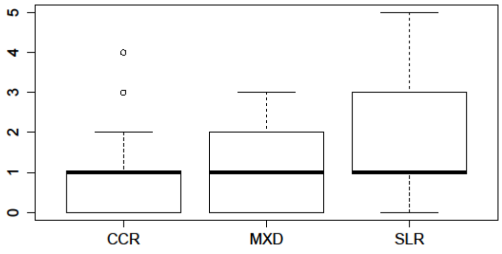
Insectivore bird sample species richness – mist-nets



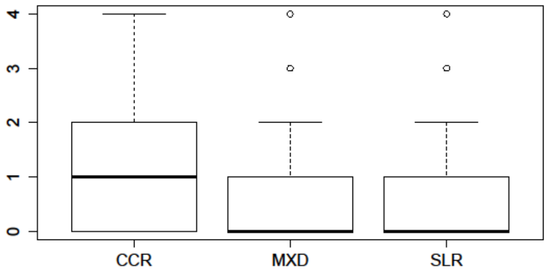
Nectivore bird sample abundance – mist-nets



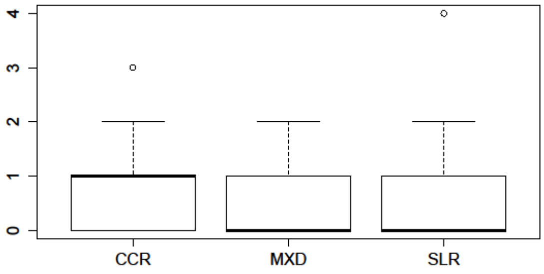
Nectivore bird sample species richness – mist-nets



Omnivore bird sample abundance – mist-nets

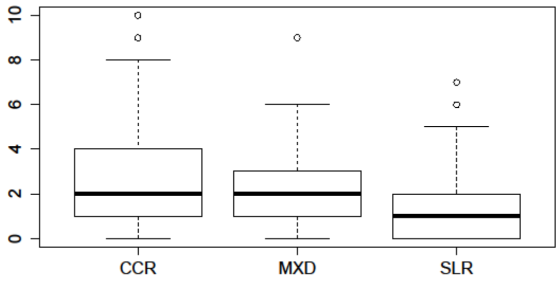


Omnivore bird sample species richness – mist-nets

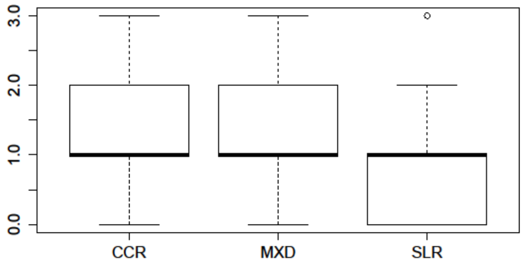


Appendix 2Q - Box plots for each response variable across disturbance areas; for different feeding guilds of medium-to-large terrestrial mammals.

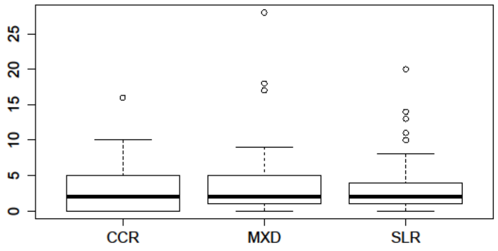
Carnivore sample mammal abundance



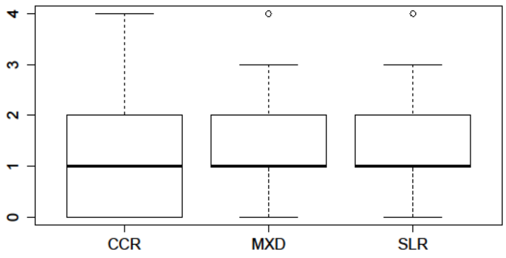
Carnivore sample mammal richness



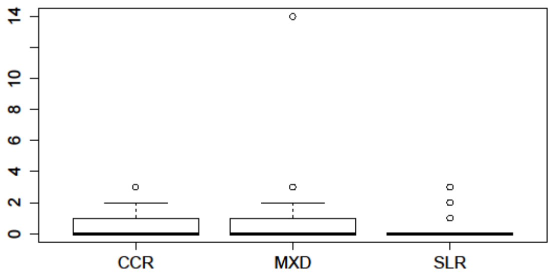
Herbivore sample mammal abundance



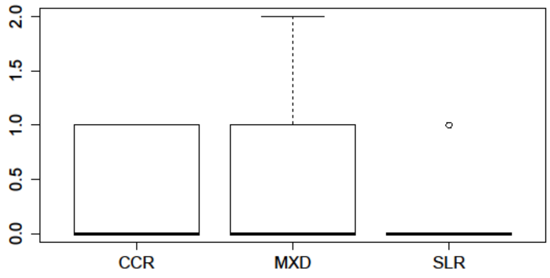
Herbivore sample mammal richness



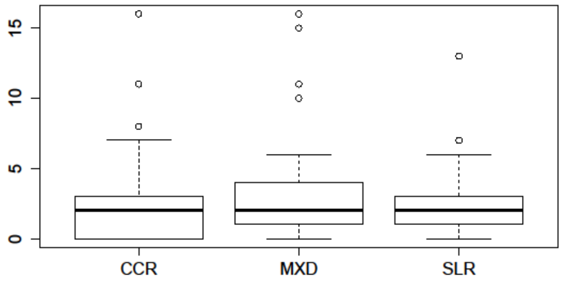
Insectivore sample mammal abundance



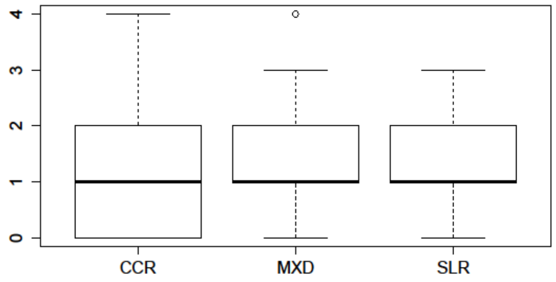
Insectivore sample mammal richness



Omnivore sample mammal abundance



Omnivore sample mammal richness



Appendix 2R – A summary of general linear mixed model (GLMM) outputs for different feeding guilds (as identified in Chapter 1 of this thesis); response variables representing sample level species richness and abundance. AICc values are shown for all sets of models; models are ranked by AICc values and the respective model weight; where the ΔAICc < 2, the most parsimonious model is the preferred model and indicated by * in the weight column.

Model selection tables	Intercept	Disturbance area	Distance to main river	Altitude (asl)	Slope extent	Offset (log(net-hours))	df	logLik	AICc	delta AICc	weight
Butterflies - fruit trap sample abundance	null					na	3	-212.662	431.8	0	0.509*
	2.849	+				na	5	-211.249	433.7	1.94	0.192
	8.422	+		-0.011		na	6	-210.151	434.1	2.29	0.162
	2.546	+			0.06873	na	6	-210.86	435.5	3.7	0.08
	2.813	+	0.00013			na	6	-211.196	436.2	4.38	0.057
	5.809	+	-0.0004527	-0.004357	-0.05629	na	8	-236.044	491.3	59.48	0
Butterflies - fruit trap sample richness	1.985	+				na	5	-164.979	341.2	0	0.481*
	3.915	+		-0.003798		na	6	-164.717	343.2	2.01	0.176
	1.991	+	-2.00E-05			na	6	-164.977	343.7	2.53	0.136
	1.993	+			-0.001892	na	6	-164.979	343.7	2.54	0.135
	null					na	3	-169.455	345.4	4.18	0.059
	4.712	+	1.00E-04	-0.005277	-0.0181	na	8	-164.62	348.4	7.23	0.013
Butterflies -fish trap sample abundance	null					na	3	-238.341	483.2	0	0.518*
	3.223	+				na	5	-236.794	484.8	1.68	0.224
	3.351	+	-0.0004359			na	6	-236.287	486.4	3.2	0.105
	6.262	+		-0.005961		na	6	-236.532	486.9	3.69	0.082
	3.255	+			-0.007805	na	6	-236.79	487.4	4.21	0.063
	5.809	+	-0.0004527	-0.004357	-0.05629	na	8	-236.044	491.3	8.13	0.009
Butterflies - fish trap sample richness	null					na	3	-200.644	407.8	0	0.373*
	2.615	+				na	5	-198.495	408.2	0.47	0.295
	2.703	+	-0.0003041			na	6	-198.122	410	2.26	0.12
	4.955	+		-0.004598		na	6	-198.252	410.3	2.52	0.106
	2.45	+			0.03987	na	6	-198.329	410.4	2.68	0.098
	3.747	+	-0.0002234	-0.002222	0.0155	na	8	-198.04	415.3	7.51	0.009
Bird transects - frugivore abundance	1.67	+				na	5	-364.63	739.7	0	0.401*
	1.753	+	-0.0002778			na	6	-364.275	741.1	1.45	0.194
	1.578	+			0.02108	na	6	-364.591	741.7	2.08	0.142
	1.296	+		0.0007422		na	6	-364.607	741.8	2.12	0.139
	null					na	3	-368.403	743	3.31	0.077
	-1.22	+	-0.0006618	0.005905	0.02712	na	8	-363.495	744	4.31	0.047
Bird transects - frugivore sample richness	1.389	+				na	5	-332.548	675.5	0	0.334
	null					na	3	-334.854	675.9	0.37	0.278*
	1.429	+	-0.0001332			na	6	-332.459	677.5	1.98	0.124
	0.8119	+		0.001146		na	6	-332.486	677.5	2.04	0.121
	1.284	+			0.02424	na	6	-332.491	677.5	2.05	0.12
	-0.9833	+	-0.0004249	0.004679	0.03374	na	8	-331.91	680.8	5.3	0.024
Bird transects - insectivore sample abundance	null					na	3	-396.404	799	0	0.744*
	1.455	+				na	5	-396.31	803	4.05	0.098
	-0.4739	+		0.003826		na	6	-395.557	803.7	4.71	0.071

	1.395	+	0.0001966			na	6	-396.114	804.8	5.82	0.04
	1.339	+			0.02651	na	6	-396.244	805	6.08	0.036
	-1.626	+	-0.0001558	0.005703	0.05806	na	8	-395.177	807.3	8.36	0.011
Bird transects - insectivore sample richness	null					na	3	-370.557	747.3	0	0.719*
	1.313	+				na	5	-370.3	751	3.73	0.112
	-0.2457	+		0.003092		na	6	-369.683	751.9	4.66	0.07
	1.252	+	0.0002019			na	6	-370.037	752.6	5.36	0.049
	1.212	+			0.02331	na	6	-370.236	753	5.76	0.04
	-0.8949	+	-0.0000348	0.003979	0.04893	na	8	-369.401	755.8	8.5	0.01
Bird transects - omnivore sample abundance	null					na	3	-193.817	393.8	0	0.446*
	0.02911	+				na	5	-192.326	395	1.26	0.238
	-2.214	+		0.004463		na	6	-191.864	396.3	2.5	0.128
	0.1165	+			-0.02041	na	6	-192.307	397.2	3.38	0.082
	0.03327	+	-1.41E-05			na	6	-192.326	397.2	3.42	0.081
	-4.676	+	-7.28E-04	0.009755	0.004101	na	8	-191.25	399.5	5.68	0.026
Bird transects - omnivore sample richness	-0.05836	+				na	5	-172.354	355.1	0	0.338
	null					na	3	-174.624	355.4	0.3	0.291*
	-2.102	+		0.004065		na	6	-171.756	356.1	0.97	0.208
	-0.08846	+	9.94E-05			na	6	-172.318	357.2	2.09	0.119
	-2.64	+	-3.88E-04	0.005993	-0.07094	na	8	-171.103	359.2	4.07	0.044
	0.1165	+			-0.02041	na	6	-192.307	397.2	42.07	0
Bird mistnets - frugivore sample abundance	null					+	3	-212.105	430.4	0	0.738*
	-2.812	+				+	5	-212.053	434.6	4.2	0.09
	-2.655	+	-0.000661			+	6	-211.33	435.4	4.96	0.062
	-2.501	+			-0.1004	+	6	-211.393	435.5	5.09	0.058
	-4.388	+		0.003142		+	6	-211.973	436.7	6.25	0.033
	-8.882	+	-0.001144	0.01279	-0.02238	+	8	-210.264	437.8	7.35	0.019
Bird mistnets - frugivore sample richness	null					+	3	-165.803	337.8	0	0.811*
	-3.212	+				+	5	-165.775	342.1	4.25	0.097
	-3.28	+			0.02139	+	6	-165.731	344.2	6.37	0.034
	-3.152	+		-0.0001201		+	6	-165.775	344.3	6.45	0.032
	-8.436	+	-0.0008891	0.01033	0.08416	+	8	-163.712	344.7	6.85	0.026
	-2.655	+	-0.000661			+	6	-211.33	435.4	97.56	0
Bird mistnets - nectivore sample abundance	-8.199	+		0.009881		+	6	-196.877	406.5	0	0.289
	null					+	3	-200.175	406.5	0.09	0.277*
	-3.261	+				+	5	-198.38	407.3	0.8	0.194
	-2.914	+			-0.1042	+	6	-197.697	408.1	1.64	0.127
	-3.311	+	0.0002106			+	6	-198.28	409.3	2.81	0.071
	-8.587	+	-0.0002997	0.01102	-0.0331	+	8	-196.545	410.3	3.86	0.042
Bird mistnets - sample nectivore richness	-7.937	+		0.009027		+	6	-165.361	343.4	0	0.312
	null					+	3	-169.007	344.2	0.78	0.211*
	-3.003	+			-0.1333	+	6	-165.773	344.3	0.82	0.207
	-3.423	+				+	5	-167.176	344.9	1.42	0.153
	-7.567	+	-0.0002764	0.008799	-0.06136	+	8	-164.739	346.7	3.28	0.061
	-3.465	+	0.0001796			+	6	-167.083	346.9	3.44	0.056
Bird mistnets - insectivore sample abundance	null					+	3	-299.605	605.4	0	0.514*
	-1.752	+	-0.000509			+	6	-297.354	607.4	2.01	0.188
	-1.874	+				+	5	-298.855	608.2	2.8	0.127
	-1.689	+			-0.05883	+	6	-298.305	609.3	3.91	0.073
	0.1206	+		-0.003974		+	6	-298.419	609.5	4.14	0.065
	-0.3717	+	-0.0004025	-0.002431	-0.05983	+	8	-296.834	610.9	5.49	0.033
Bird mistnets - insectivore sample richness	null					+	3	-265.844	537.9	0	0.602*
	-1.983	+	-0.0004428			+	6	-263.97	540.6	2.76	0.151

	-2.09	+				+	5	-265.281	541.1	3.18	0.123
	-0.247	+		-0.003672		+	6	-264.867	542.4	4.56	0.062
	-2.023	+			-0.02126	+	6	-265.196	543.1	5.21	0.044
	-1.588	+	-0.0004157	-0.0006823	-0.01887	+	8	-263.909	545	7.16	0.017
Bird mistnets - omnivore sample abundance	-2.984	+				+	5	-156.615	323.7	0	0.293*
	-6.839	+		0.007678		+	6	-155.834	324.4	0.64	0.212
	-12.4	+	-0.001202	0.01907	0.03993	+	8	-153.697	324.6	0.89	0.187
	-2.897	+	-0.0003616			+	6	-156.251	325.2	1.48	0.14
	-2.787	+			-0.06323	+	6	-156.274	325.3	1.52	0.137
	null					+	3	-160.99	328.2	4.45	0.032
Bird mistnets - sample omnivore richness	-3.247	+				+	5	-132.167	274.8	0	0.349*
	-6.434	+		0.006349		+	6	-131.567	275.8	1.01	0.211
	-3.075	+			-0.05558	+	6	-131.884	276.5	1.64	0.154
	-3.184	+	-0.0002665			+	6	-131.948	276.6	1.77	0.144
	-11.44	+	-0.001047	0.01659	0.03512	+	8	-129.965	277.2	2.33	0.109
	null					+	3	-136.697	279.6	4.76	0.032
Mammal - carnivore sample abundance	1.05	+				na	5	-277.759	565.9	0	0.396*
	0.7564	+			0.07256	na	6	-277.056	566.7	0.76	0.271
	1.133	+	-0.0002275			na	6	-277.628	567.8	1.91	0.153
	0.5859	+		0.0009042		na	6	-277.74	568.1	2.13	0.137
	-1.207	+	-0.0001754	0.003862	0.08399	na	8	-276.766	570.5	4.61	0.039
	null					na	3	-284.379	574.9	8.99	0.004
Mammal - carnivore sample richness	0.2671	+				na	5	-190.894	392.2	0	0.405*
	0.08356	+			0.04592	na	6	-190.58	393.7	1.54	0.188
	-0.823	+		0.002128		na	6	-190.776	394.1	1.93	0.154
	0.3079	+	-0.000115			na	6	-190.858	394.3	2.1	0.142
	null					na	3	-194.638	395.4	3.24	0.08
	-2.223	+	-0.0001163	0.004434	0.06445	na	8	-190.18	397.4	5.17	0.031
Mammal - herbivore sample abundance	null					na	3	-353.877	713.9	0	0.717*
	1.16	+				na	5	-353.774	718	4.04	0.095
	1.505	+	-0.000966			na	6	-352.838	718.3	4.34	0.082
	0.7261	+			0.1103	na	6	-353.303	719.2	5.27	0.051
	4.53	+		-0.006579		na	6	-353.441	719.5	5.55	0.045
	2.264	+	-0.0007624	-0.002011	0.05041	na	8	-352.678	722.4	8.45	0.01
Mammal - herbivore sample richness	null					na	3	-218.786	443.7	0	0.773*
	0.2978	+				na	5	-218.774	448	4.23	0.093
	0.4207	+	-0.0003464			na	6	-218.217	449	5.28	0.055
	0.1864	+			0.02801	na	6	-218.631	449.8	6.11	0.036
	-0.3128	+		0.001192		na	6	-218.722	450	6.29	0.033
	-1.446	+	-0.0003969	0.003504	0.02291	na	8	-217.873	452.8	9.02	0.008
Mammal - insectivore sample abundance	-3.181	+			0.4782	na	6	-121.002	254.6	0	0.714*
	-8.112	+	-0.0005729	0.009586	0.5246	na	8	-120.766	258.5	3.96	0.099
	null					na	3	-126.606	259.4	4.79	0.065
	-1.157	+				na	5	-124.647	259.7	5.12	0.055
	-0.4963	+	-0.001747			na	6	-123.769	260.1	5.53	0.045
	3.762	+		-0.009542		na	6	-124.454	261.5	6.9	0.023
Mammal - insectivore sample richness	-2.236	+			0.2181	na	6	-89.629	191.8	0	0.387
	-1.327	+				na	5	-91.1	192.6	0.77	0.263*
	-12.15	+	-0.0006614	0.01872	0.3359	na	8	-88.41	193.8	1.99	0.143
	-1.078	+	-0.0007034			na	6	-90.869	194.3	2.48	0.112
	-3.18	+		0.003618		na	6	-91.025	194.6	2.79	0.096
	null					na	3	-126.606	259.4	67.53	0
Mammal - omnivore sample abundance	null					na	3	-318.631	643.4	0	0.682*

	0.9122	+				na	5	-318.181	646.8	3.35	0.128
	0.7577	+	0.0004229			na	6	-317.722	648	4.6	0.068
	0.7292	+			0.04533	na	6	-317.967	648.5	5.09	0.053
	-0.5034	+		0.002758		na	6	-318.013	648.6	5.18	0.051
	-1.397	+	0.0005642	0.00332	0.1001	na	8	-316.881	650.8	7.35	0.017
Mammal - omnivore sample richness	null					na	3	-210.229	426.6	0	0.616*
	0.1155	+				na	5	-209.487	429.4	2.77	0.155
	-0.1188	+			0.05845	na	6	-208.915	430.4	3.79	0.093
	1.495	+		-0.002695		na	6	-209.241	431.1	4.44	0.067
	0.1755	+	-1.69E-04			na	6	-209.363	431.3	4.69	0.059
	0.4686	+	2.96E-06	-0.001094	0.05165	na	8	-208.884	434.8	8.16	0.01

Appendix 2S – A summary checklist of all species and an associated species code that relates to NMDS plots presented in Appendix 2T.

nMDS ID	Amphibians - nocturnal transects	Butterflies- baited traps	Birds - diurnal transects	Birds - mist-nets	Birds - nocturnal transects	Mammmals
sp1	Ameerega hahneli	Magneuptychia modesta	Amazonian Streaked-Antwren	Ash-throated Gnateater	Band-bellied Owl	Acouchy_G
sp2	Ameerega macero	Harjesia blanda	Ash-throated Gnateater	Band-tailed Manakin	Crested Owl	Agouti
sp3	Ameerega simulans	Taygetamorpha celia	Bamboo Antshrike	Barred Forest-Falcon	Great Potoo	Anteater_Giant
sp4	Bolitoglossa altamazonica	Erichthodes antonina	Band-tailed Manakin	Black Antbird	Long-tailed Potoo	Armadillo_Giant
sp5	Dendropsophus parviceps	Cissia terrestris	Barred Antshrike	Black-Banded Woodcreeper	Mottled Owl	Coati
sp6	Hypsiboas boans	Splendeuptychia itonis	Barred Forest-Falcon	Black-eared fairy	Ocellated Poorwill	Common_opposum
sp7	Hypsiboas geographicus	Harjesia obscura	Bartletts Tinamou	Black-faced Antbird	Pauraque	Deer_RB
sp8	Hypsiboas lanciformis	Hamadryas chloe	Bay-headed Tanager	Black-faced Antthrush	Spectacled Owl	Dog_Bush
sp9	Leptodactylus andreae	Memphis polycarmes	Black Antbird	Blackish Antbird	Tawny Bellied Screech Owl	Dog_SE
sp10	Leptodactylus lineatus	Memphis sp.	Black-banded Woodcreeper	Black-spotted Bare-eye		Jaguar
sp11	Leptodactylus rhodomystax	Memphis offa	Black-billed Thrush	Black-tailed Leaftosser		Jaguarundi
sp12	Leptodactylus sp1	Panacea prola	Black-capped Tinamou	Black-throated Brilliant		Margay
sp13	Noblella myrmecoides	Morpho achilles	Black-faced Antbird	Blue-black Grosbeak		Ocelot
sp14	Oreobates quixensis	Caligo superbus	Black-faced Antthrush	Blue-crowned Manakin		Paca
sp15	Osteocephalus castaneicola	Heliconius sara	Blackish Antbird	Blue-fronted Lancebill		Peccary_C
sp16	Osteocephalus germani	Heliconius (Laparus) doris	Black-tailed Trogon	Blue-tailed Emerald		Peccary_WL
sp17	Osteocephalus mimeticus	Caeruleuptychia lobelia	Black-throated Antbird	Bluish-fronted Jacamar		Puma
sp18	Phyllomedusa tomopterna	Archaeoprepona demophon	Black-fronted Nun bird	Bluish-slate Antshrike		Rabbit
sp19	Phyllomedusa vaillanti	Ostrinotes sospes	Blue Dacnis	Brownish-headed Antbird		Racoon
sp20	Pristimantis altamazonicus	Nessaea hewitsonii	Blue-and-yellow Macaw	Buff-rumped Warbler		Tamandua
sp21	Pristimantis buccinator	Memphis sp.	Blue-black Grosbeak	Buff-tailed Sicklebill		Tapir
sp22	Pristimantis carvalhoi	msp35	Blue-crowned Manakin	Buff-throated Foliage-gleaner		Tayra
sp23	Pristimantis ockendeni	Hypoleria lavinia	Blue-crowned Motmot	Buff-throated Saltator		Small_armadillo
sp24	Pristimantis olivaceus	Vila azeca azeca	Blue-crowned Trogon	Chestnut-bellied Seedeater		
sp25	Pristimantis reichlei	Heliconius numata timaeus	Blue-headed Macaw	Chestnut-bellied Seed-finch		
sp26	Pristimantis sp1	Heliconius hecale sysiphus	Blue-headed Parrot	Chestnut-crowned Foliage-gleaner		
sp27	Pristimantis sp3	Heliconius numata bicoloratus	Blue-necked Tanager	Chestnut-tailed Antbird		
sp28	Rhinella margaritifer	Heliconius elevatus	Blue-throated Piping-guan	Chestnut-winged Foliage-gleaner		
sp29	Rhinella marina	Heliconius pardalinus maeon	Bluish-fronted Jacamar	cinereous mourner		
sp30	Scinax funereus	Tithorea harmonia brunnea	Bluish-slate Antshrike	Dusky-throated Antshrike		
sp31	Scinax ruber	Tithorea harmonia assimilis	Bright-rumped Attila	Elegant Woodcreeper		
sp32	Teratohyla midas	Nessaea obrinus	Broad-billed Motmot	Emerald toucanet		
sp33	Teratohyla sp1	Tigridia acesta	Buckleys Forest-falcon	Euler's Flycatcher		
sp34		Baeotus aeilus	Buff-rumped Warbler	Fiery-capped		

sp35	Philaethria dido	Buff-throated Foliage-gleaner	Manakin Flammulated Pygmy-Tyrant
sp36	Hypna clytemnestra	Buff-throated Saltator	Foothill Antwren
sp37	Colobura dirce	Buff-throated Woodcreeper	Fork-tailed Woodnymph
sp38	Temenis laothoe	Casqued Oropendola	Goeldi's Antbird
sp39	Taygetis sylvia	Chestnut-backed Antshrike	Golden-bellied (Cusco) Warbler
sp40	Pareuptychia ocirrhoe interjecta	Chestnut-capped Puffbird	Golden-tailed Sapphire
sp41	Mesosemia sp	Chestnut-crowned Foliage-gleaner	Gould's Jewelfront
sp42	Fountainea halice	Chestnut-eared Aracari	Gray Antwren
sp43	msp68	Chestnut-fronted Macaw	Gray-breasted Sabrewing
sp44	Cissia proba	Chestnut-tailed Antbird	Gray-fronted Dove
sp45	Napeogenes juanjuiensis	Cinereous Tinamou	Great Antshrike
sp46	Oleria victorine	Collared Forest-falcon	Green Hermit
sp47	Amarynthis meneria	Collared Trogon	Green Honeycreeper
sp48	Hamadryas fornax	Crested Oropendola	Green Manakin
sp49	Callicore lyca aegina	Dot-winged Antwren	Green-and-gold Tanager
sp50	Fountainea halice	Dusky-capped Greenlet	Hairy-crested Antbird
sp51	Fountainea ryphea	Dusky-headed Parakeet	Half-collared Gnatwren
sp52	msp85	Dusky-tailed Flatbill	Hauxwell's Thrush
sp53	Batesia hypochlora	Dusky-throated Antshrike	Koepcke's Hermit
sp54	Narope sp	Eulers Flycatcher	Lined Forest-Falcon
sp55	Opsiphanes cassina	Fasciated Antshrike	Little Cuckoo
sp56	Ectima iona	Flammulated Pygmy-Tyrant	Long-tailed Hermit
sp57	Cissia sp	Goeldis Antbird	Long-tailed Woodcreeper
sp58	Panacea regina	Golden-collared Toucanet	Many-spotted Hummingbird
sp59	Memphis praxias	Golden-tailed Sapphire	McConnell's Flycatcher
sp60	Catoblepia xanthicles	Gray Antbird	Moustached Wren
sp61	Catoblepia berecynthia berecynthia	Gray Tinamou	Needle-billed Hermit
sp62	Taygetis virgilia	Gray-fronted Dove	Ocellated Woodcreeper
sp63	Taygetis elegia	Great Antshrike	Ochre-bellied flycatcher
sp64	Hypothris ninonia	Great Tinamou	Olivaceous Flatbill
sp65	Morpho helenor	Hairy-crested Antbird	Olive/Carmiol's Tanager
sp66	Heliconius numata lyrcaeus	House Wren	Olive-striped Flycatcher
sp67	Tithorea harmonia brunnea	King Fisher	Orange-bellied Euphonia
sp68	Memphis basilia drucei	King Vulture	Ornate Antwren
sp69	Myscelia capenas	Large-headed Flatbill	Pale-tailed Barbthroat
sp70	Adelpha boreas	Lemon-Chested Greenlet	Pectoral Sparrow
sp71	Chloreuptychia herseis	Lineated Woodpecker	Pink-throated Becard
sp72	Caeruleuptychia caerulea	Little Tinamou	Plain Antvireo
sp73	Temenis pulchra	Little Woodpecker	Plain Xenops
sp74	Adelpha iphiclus	Long-tailed Hermit	Plain-brown Woodcreeper
sp75	Chloreuptychia chlorimene	Long-winged Antwren	Plain-winged Antshrike

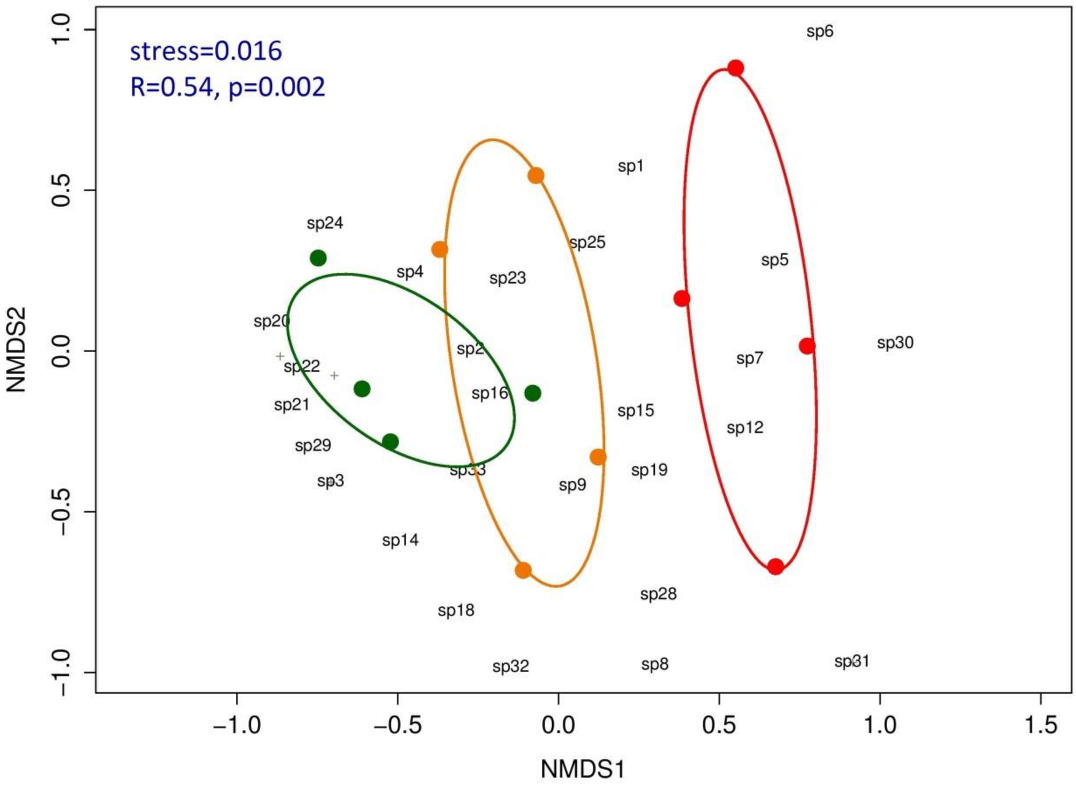
sp76	Opsiphanes invirae	Manu Antbird	Purple Honeycreeper
sp77	Contrafacia sp	McConnells Flycatcher	Red-crowned Ant-tanager
sp78	Agrias claudina	Mealy Parrot	Reddish Hermit
sp79	Satyrinae	Moustached Wren	Ringed Antpipit
sp80	Prepona laertes	Musician Wren	Round-tailed Manakin
sp81	Euselasia hahneli	Ocellated Poorwill	Ruddy Foliage-gleaner
sp82	Smyrna blomfildia	Olivaceous Woodcreeper	Ruddy Quail-dove
sp83	Narope cyllabarus	Olive Oropendola	Ruddy-tailed Flycatcher
sp84	Hamydras laodamia	Olive-backed Foliage-gleaner	Rufous Motmot
sp85	Harjesia obscura	Ornate Antwren	Rufous-breasted Piculet
sp86	Caligopsis seleucida	Pale-tailed Barbthroat	Rufous-tailed Antwren
sp87	Chloreuptychia arnaca	Pale-vented Pigeon	Russet-Backed Oropendola
sp88	Posttaygetis penelea	Pale-winged Trumpeter	Scaly-brested Wren
sp89	Memphis philomena	Palm Tanager	Semicollared Puffbird
sp90	Adelpha serpa	Paradise Tanager	Sepia-capped Flycatcher
sp91	Emesis sp	Pectoral Sparrow	Shrike-Like Cotinga/Elegant Mourner
sp92	Hamadryas amphinome	Picui Ground Dove	Silver-beaked Tanager
sp93	Taygetis sp	Pink-throated Becard	Slaty Thrush
sp94	Cithaerias pireta	Plain Xenops	Social Flycatcher
sp95	Haetera piera	Plain-brown Woodcreeper	Sooty Antbird
sp96	Taygetis inambari	plain-crowned spinetail	Speckled Spinetail
sp97	Historis acheronta	Plain-winged Antshrike	Spectacled Bristle-Tyrant
sp98	Pyrrhogyra crameri	Plumbeous Pigeon	Spot-backed Antbird
sp99	Caligo eurilochus	Purple-throated Fruitcrow	Spot-winged Antshrike
sp100	Memphis acaudata	Purplish Jay	Straight-billed Woodcreeper
sp101	Harjesia obscura	Pygmy Antwren	Streak-necked Flycatcher
sp102	Heliconius melpomeme aglaope	Razor-billed Curassow	Striped Woodhaunter
sp103	Adelpha boeotia	Red-and-green Macaw	Sulphur-bellied flycatcher
sp104	Heliconius xanthocles	Red-bellied Macaw	Swainson's Thrush
sp105	Siproeta stelenes	Red-billed Scythebill	Tawny-crowned greenlet
sp106	Ectima lirides	Red-throated Caracara	Tawny-throated Leaftosser
sp107	Adelpha jordani	Ringed Antpipit	Thrush-like Schiffornis
sp108	Heliconius leucadia	Round-tailed Manakin	Wedge-billed Woodcreeper
sp109	Hypoleria lavinia cajona	Ruddy Foliage-gleaner	White-bearded Hermit
sp110	Archaeoprepona demophoon	Ruddy Pigeon	White-bearded Manakin
sp111	Prepona dexamenus	Ruddy Quail-dove	White-browed Antbird
sp112	Eunica alpais	Rufous Motmot	White-browed Hermit
sp113	Ithomia arduinna	Rufous-bellied Euphonia	White-chinned Woodcreeper
sp114	Pierella lamia	Rufous-capped Antthrush	White-crested Spadebill

sp115	Chloreuptychia agatha	Rufous-fronted Antthrush	White-eyed Antwren
sp116	Hyposcada anchiala subsp	Russet-backed Oropendola	White-flanked Antwren
sp117	Oleria victorine	Rusty-belted Tapaculo	White-necked Jacobin
sp118	Heliconius erato emma	Scale-backed Antbird	White-shouldered Antshrike
sp119	Caligo cf. idomeneus	Scarlet Macaw	White-throated Antbird
sp120	Adelpha capucinus capucinus	Sclaters Antwren	White-winged Shrike-tanager
sp121	Thisbe irenia	Short-tailed Pygmy-Tyrant	Yellow-bellied Tanager
sp122	msp221	Silvered Antbird	Yellow-margined Flycatcher
sp123	Memphis phantes	Slate-colored Grosbeak	
sp124	Memphis xenocles	Slaty-capped Shrike-vireo	
sp125	Memphis acidalia	Slender footed tyrannulet	
sp126	Memphis polycarmes	Social Flycatcher	
sp127	Splendeuptychia sp	Sooty Antbird	
sp128	Lycorea halia	Southern Nightingale-Wren	
sp129	Adelpha cytherea	Speckled Chachalaca	
sp130	Adelpha pleasure	Spixs Guan	
sp131	Callicore hystaspes hystapses	Spot-backed Antbird	
sp132	Zischkaia ordinata	Spot-winged Antshrike	
sp133	Pierella hortona albofasciata	Squirrel Cuckoo	
sp134	Rareuptychia clio	Starred Wood-quail	
sp135	msp254	Straight-billed Woodcreeper	
sp136	Pierella lena	Striated Antbird	
sp137	Diaethria clymena	Stripe-chested Antwren	
sp138	Adelpha melona	Strong-billed Woodcreeper	
sp139	Prepona amydon	Swainsons Thrush	
sp140	Fountainea nessus	Swallow-tanager	
sp141	Hermeuptychia sp	Thrush-like Antpitta	
sp142	Ancyluris spp	Thrush-like Schiffornis	
sp143	Metamorpha elissa	Thrush-like Wren	
sp144	Memphis pithyusa	Turquoise Tanager	
sp145	Adelpha delinita	Undulated Tinamou	
sp146	Adelpha epione	Variegated Tinamou	
sp147	Memphis anna	Violaceous Jay	
sp148	Vila emilia	Warbling Antbird	
sp149	Adelpha erotica erotica	Wedge-billed Woodcreeper	
sp150	Eunica mygdonia mygdonia	White-browed Antbird	
sp151	msp448	White-browed Hermit	
sp152	Itaballia demophile	White-chinned Woodcreeper	
sp153	Astraptes fuglerator	White-eyed Parakeet	
sp154	Heliconius burneyi	White-flanked Antwren	
sp155	Baeotus japetus	White-lined Antbird	
sp156	Historis odius	White-necked Thrush	
sp157	Epiphile lampethusa	White-shouldered Antshrike	
sp158	Zaretis itys	White-tailed Trogon	
sp159	Taygetis mermeria	White-throated Antbird	
sp160	Yphthimoides renata	White-throated Toucan	
sp161	Taygetis thamyra	Yellow-breasted	

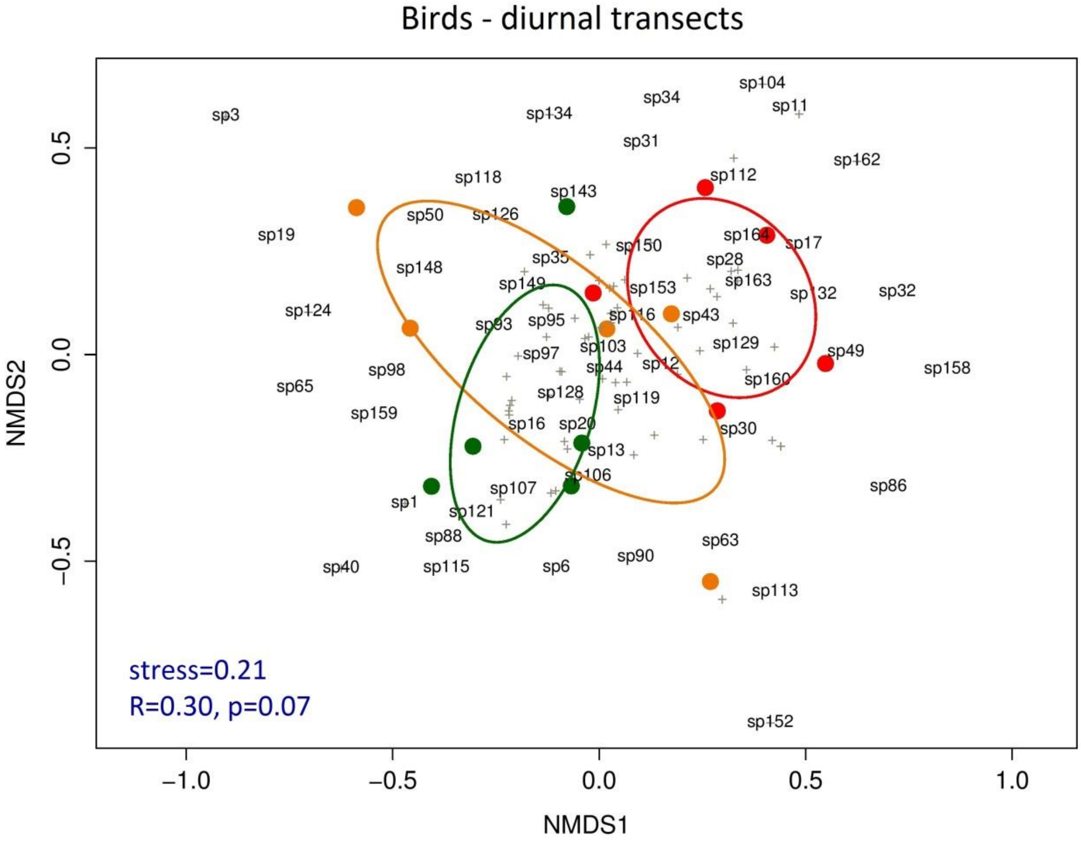
		Warbling Antbird
sp162	Adelpha zina irma	Yellow-browed Tody-flycatcher
sp163	Neruda aoede	Yellow-crowned Parrot
sp164	Adelpha mesentina	Yellow-rumped Cacique
sp165	Polygrapha xenocrates	Yellow-tufted Woodpecker
sp166	Catonephele acontius	
sp167	Catonephele numilia	
sp168	Pseudodebis valentina	
sp169	Pyrrhogyra otolais	
sp170	Bia actorion	
sp171	Consul fabius	
sp172	Splendeuptychia ashna	
sp173	Taygetis larua	

Appendix 2T – NMDS plots with species codes presented (species and related codes provided in Appenix 2S), for each study group.

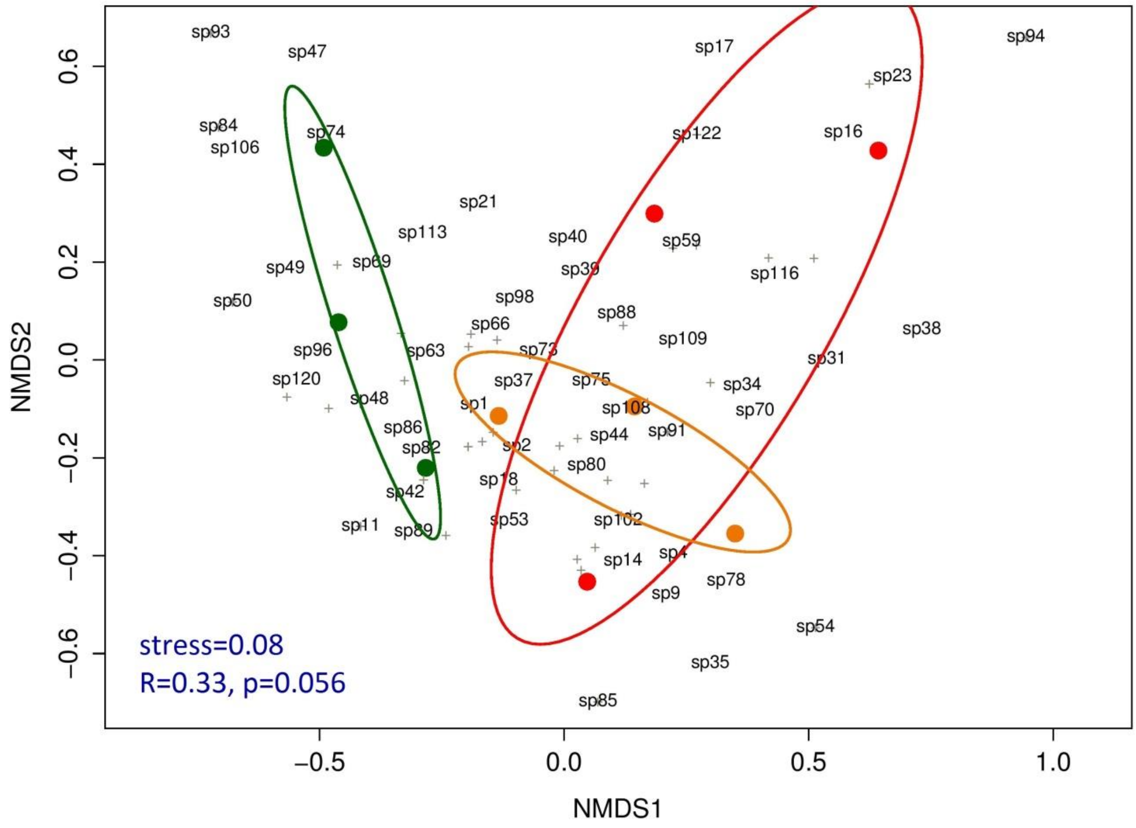
Amphibians - nocturnal transects



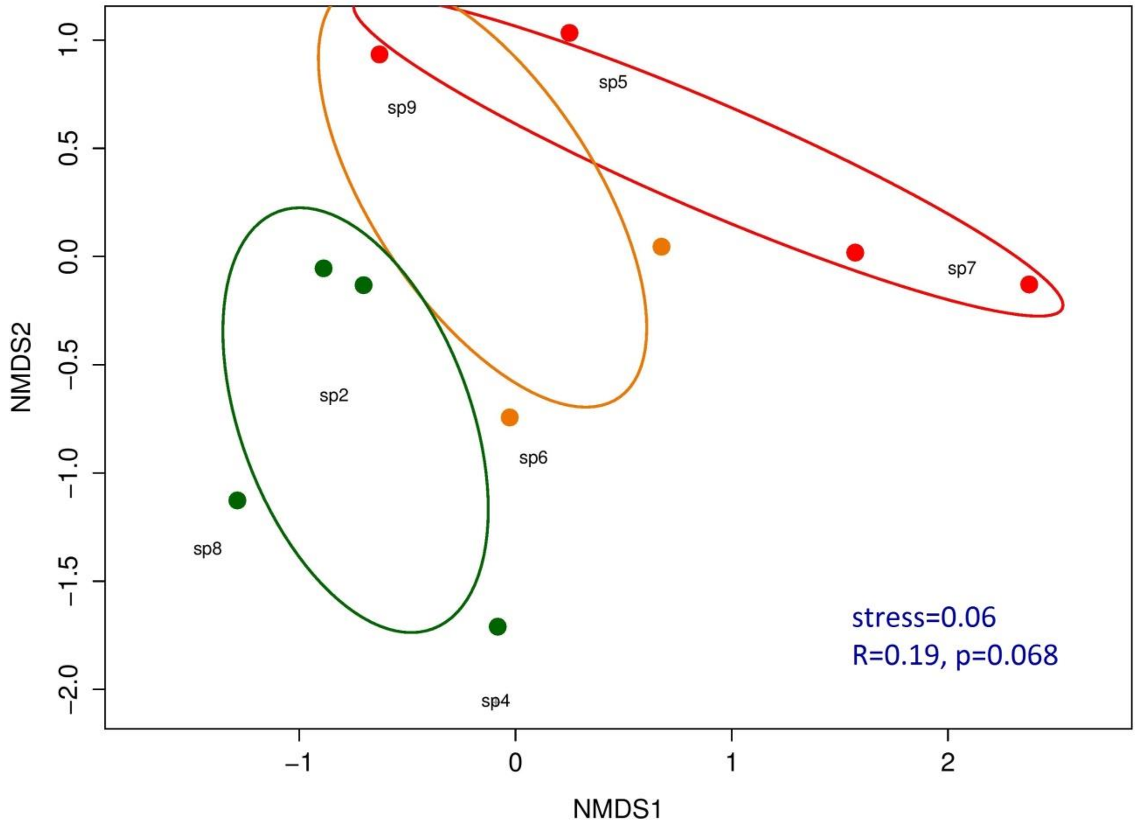
Birds - diurnal transects

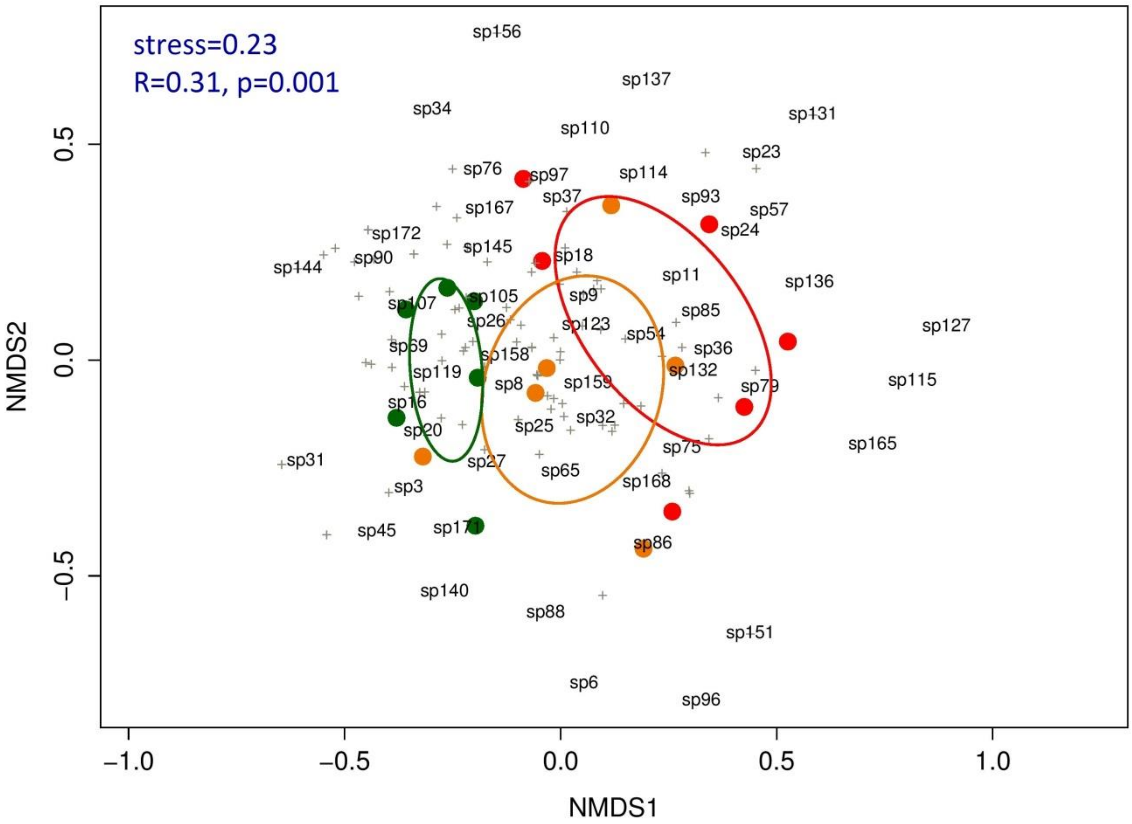


Birds - mist-nets

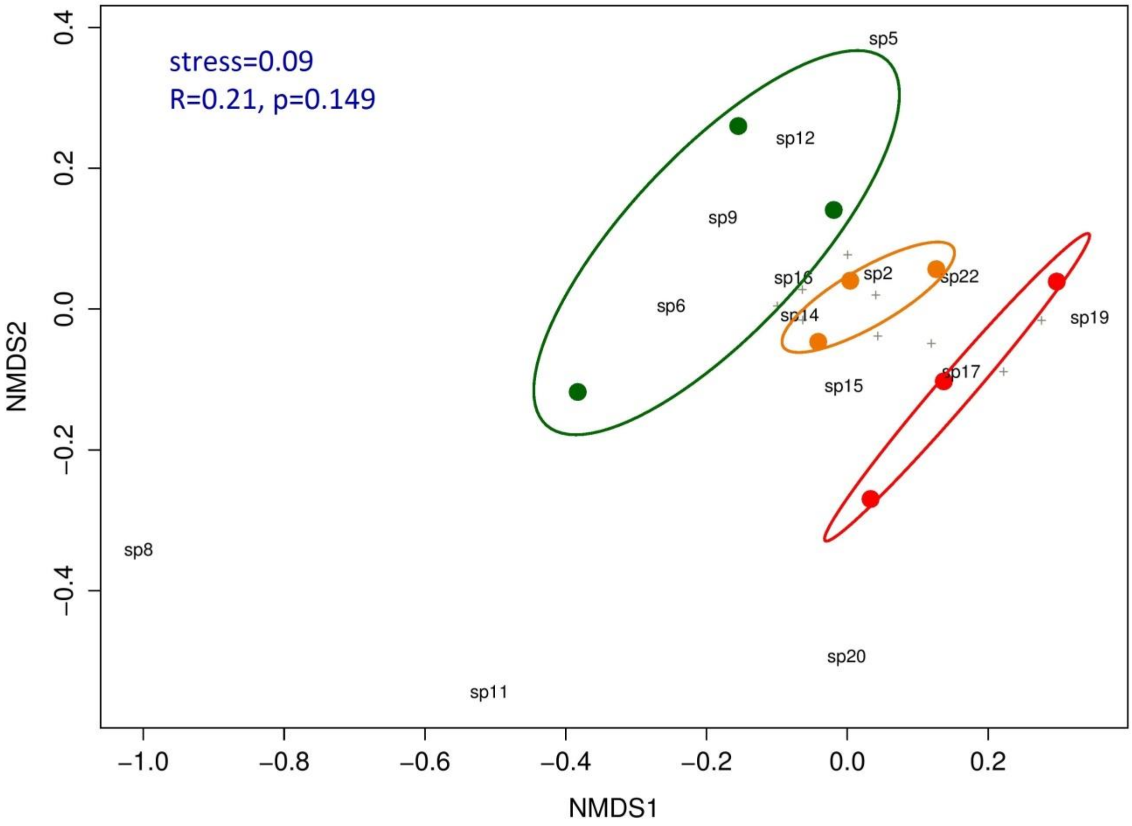


Birds - nocturnal transects



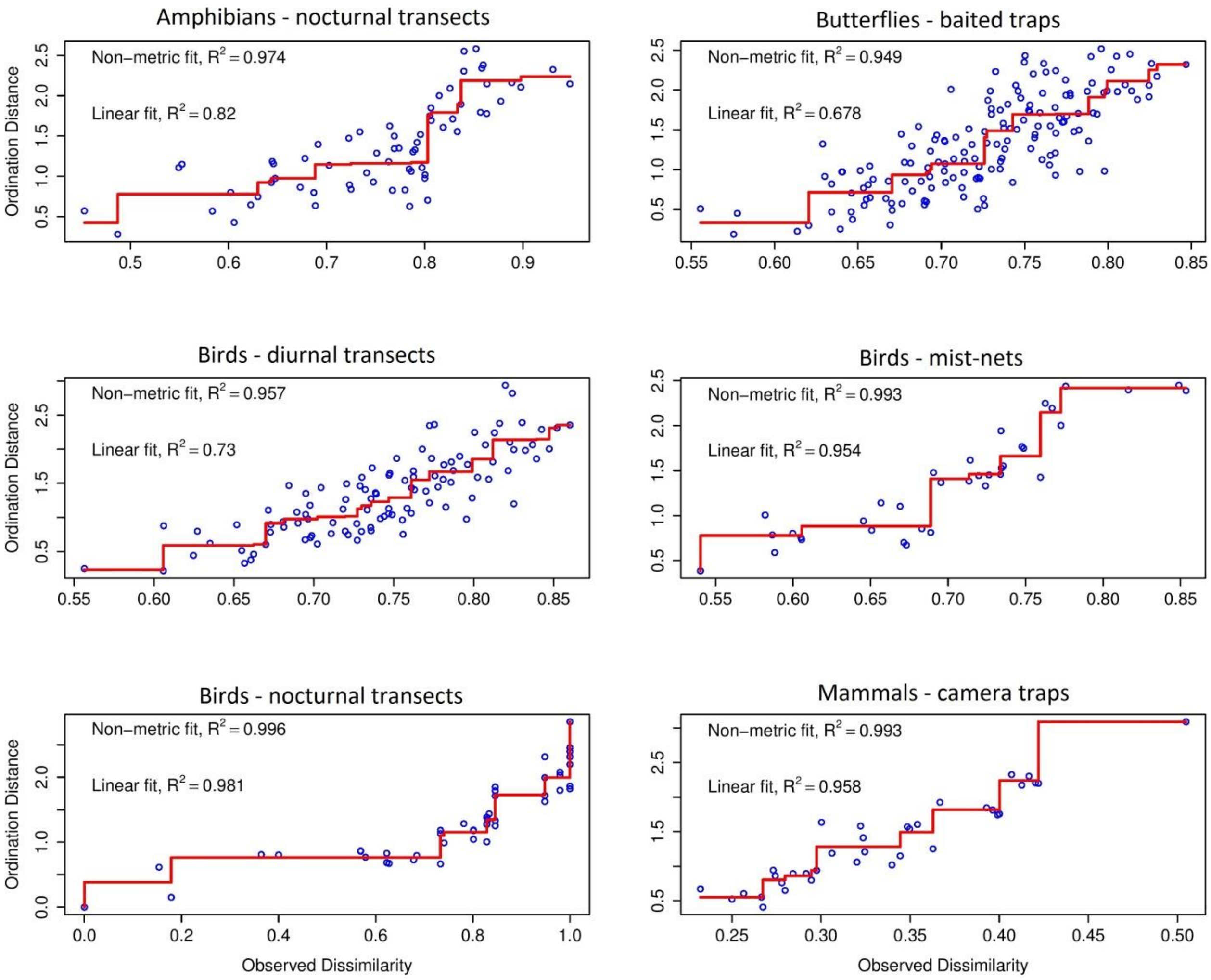


Butterflies - baited traps



Mammals - camera traps

Appendix 2U – Stress plots for each study group; in relation to NMDS plots presented in Figure 6 and Appendix 2T.



Chapter 3

Appendix 3A. Detailed field methodologies and sample design

Amphibians

In order to survey amphibians 16 nocturnal transects (500m x 6m) were established which ran directly from the road to 500m out into the forest (see Figure 1). Transect start points were randomly assigned as ‘road’ or ‘forest’ and walked from that point, and then the majority were repeated in the opposite direction on different night, in order to avoid temporal bias in species activity patterns. This resulted in 60 samples overall; 30 near and 30 far. Twelve transects were walked twice, one transect was walked three times and three transects were walked once. Nocturnal visual encounter transects (VES) were used due to their known effectiveness in sampling tropical herpetofauna (Beirne et al 2013; Bell & Donnelly 2006) and their higher yields per unit effort than other sampling methods in previous publications (Bell & Donnelly 2006; Rödel & Ernst 2004). All transects were surveyed at night, commencing at approximately 2000h and were surveyed by five searchers over a period of ~180 minutes and up to 2.5m in height. Search teams consisted of one experienced herpetologist and four trained international conservation volunteers. Diurnal VES transects were not performed as nocturnal VES sampling has previously been shown to be the most efficient method in herpetological inventorying and still allowed us to detect resting diurnal species (Doan 2003). Owing to the previous detection of a single case of chytridiomycosis within the study site (Global Vision International, Unpublished) codes of good practice were strictly adhered to. This was achieved by the systematic cleaning of tools and equipment. Sterile bags were used when handling amphibians and small reptiles.

Birds

Birds were sampled using mist netting (MN) and point count methodologies (PC). There are advantages and disadvantages of both sampling methods; MN has been found to be generally less efficient than PC (Barlow et al. 2006; Blake and Loiselle 2001; Whitman et al. 1997), however it offers a method less implicated by observer bias. MN is a useful and standardised technique to compare understory avifaunal communities composed of cryptic species but is restricted by missing canopy avifauna, unless nets are raised into higher strata of the forest (Barlow et al. 2006; Blake and Loiselle 2000). This study combines both methods so the advantages of one might counteract the weaknesses of the other (Rappole et al. 1998). A total of 16 MN sites were established with a minimum buffer distance of at least 500m to account for individual home range overlap (Duca et al. 2006; Hansbauer et al. 2008). Nine sites were located within 0–350m from the road (near sites) and seven sites were located within 350–700m from the road (far sites). Birds were captured using four mist nets (12m long and 2.5m high) placed in a random array and with 30m between each net. Capture sites were surveyed for four consecutive days (weather permitting) and on average for five hours per day. Nets were opened on very first light (around 0530-0600h) and closed around 1030h to provide for higher capture rates during high activity periods (Blake 1992) and nets were checked every 25 minutes.

PC surveys were conducted by one experienced staff member with a minimum of six months training and two to three international volunteers with a minimum of two weeks training. Points were located near to the road (1-350m) and far from the road (351-700m). In total 69 independent points were located (39 near and 30 far). The majority of points were repeated twice (median = 2, range = 1-4). Almost all counts were conducted beginning at 0600h and finishing mid-morning around 10:00h, to fit with periods of high avian vocal activity (Blake 1992; Mahood et al. 2012). Like mist netting, points were not surveyed in adverse weather when bird activity was heavily reduced; a small number of counts had to be conducted late afternoon due to cancellations due to bad weather conditions over a few mornings (Blake 1992). Counts began for half of the days near to the road and for the other half of the days far from the road, as to reduce temporal bias related to bird activity patterns (Blake 1992). For each survey session three to four points were surveyed for the duration of 10 minutes per point after allowing for a 3 minute settling period upon arrival at the count site. All birds heard and seen were recorded at each point. If uncertainty regarding an individual's identification existed, further efforts were made with sound recordings after the survey back at the field camp.

Butterflies

Six 400m transects were lightly cut to allow access to the trap locations. Five sites were located on each transect at distances of 50m, 100m, 200m, 300m and 400m from the road. Two traps were placed at each study site; comprising of one ground trap (1m above the ground) and one sub-canopy trap (10-15m from the ground) placed within 20m of each other. For data analysis sites that were considered 'near' sites were 50m, 100m and 200m from the road and 'far' sites were 300m and 400m away from the road. Traps were checked daily in the afternoons for nine consecutive days and bait replaced every 3 days to ensure effort in re-baiting traps was standardised without compromise of bait freshness (DeVries & Walla 2001; Hughes et al. 1998). Each transect was repeated twice. The number of individuals of each species for each distance was recorded. Individuals that were large enough and that did not possess transparent wings were marked using a non-toxic pen with a simple dot code on their upper wings to indicate trap height and distance where they were caught. Marking individuals allowed differentiation between recaptures and newly caught individuals in order to avoid pseudoreplication.

The study targeted fruit-feeding Nymphalidae butterflies. Fruit-feeding guilds in this family have been used by previous studies for effectiveness in detecting ecological change due to their sensitivity to forest disturbance (Hamer et al. 2003; Lewis 2001). They are relatively easy to capture and can represent between 40 to 55% of total Nymphalid species richness in tropical forests (DeVries & Walla 2001). Using rotting banana bait prepared following the methods by DeVries and Walla (1999), butterflies were caught using Van Someren-Rydon traps measuring 1m in length and 0.25m in diameter as described by Austin and Riley (1995) and Hughes et al. (1998). This simple fruit-baited trap has been used successfully by previous studies on Nymphalidae butterflies in the tropics (Armstrong 2010; DeVries & Walla 2001; Hill et al. 2001; Hughes et al. 1998).

Recording of data

All amphibians encountered were identified in the field where possible (see Beirne & Whitworth (2011) for full indentification criteria and a complete list of identification resources used throughout the project). Unidentifiable individuals were anaesthetised with Lidocaine and fixed with 10% formalin then subsequently identified and stored at the Ecuadorian Natural Science Museum (MECN) in Quito. Butterflies were identified in the field where possible, using an unpublished set of field guides developed by GVI staff members since 2005. Birds were identified during MN sessions using Ridgely and Greenfield (2001) and Schulenberg (2000) in addition to this to assist with PC sound recordings.

Appendix 3A References

Armstrong, C., 2010. The effect of forest edges on the community structure of tropical fruit-feeding butterflies. The Plymouth Student Scientist. 3(2), 3-17.

Austin, G.T., Riley, T.J., 1995. Portable bait traps for the study of butterflies. Tropical Lepidoptera, 6(1), 5-9.

Barlow, L. A., Mestre, M., Gardner, T.A., Peres, C.A., 2006. The value of primary, secondary and plantation forests for Amazonian birds. Biological Conservation 136(2), 212-223.

Beirne, C., Whitworth, A., 2011. Frogs of the Yachana Reserve.

Beirne, C., Burdekin, O., Whitworth, A., 2013. Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in Eastern Ecuador. The Herpetological Journal, 23(4), 209-209.

Bell, K. E., Donnelly, M. A., 2006. Influence of forest fragmentation on community structure of frogs and lizards in northeastern Costa Rica. Conservation Biology, 20(6), 1750-1760.

Blake, J.G., 1992. Temporal variation in point counts of birds in a lowland wet forest in Costa Rica. The Condor, 94:265-275.

Blake, J.G., Loiselle, B.A., 2000. Diversity of birds along an elevational gradient in the Cordillera Central, Costa Rica. The Auk, (3), 663-686.

Blake, J.G., Loiselle, B.A., 2001. Bird assemblages in second-growth and old-growth forests, Costa Rica: Perspectives from mist nets and point counts. The Auk. 118(2), 304-326

DeVries, P.J., Walla, T.R., 1999. Species diversity in spatial and temporal dimensions of fruit-feeding butterflies from two Ecuadorian rainforests. Biological Journal of the Linnean Society, 68:333-353.

DeVries, P.J., Walla, T.R., 2001. Species diversity and community structure in neotropical fruit-feeding butterflies. Biological Journal of the Linnean Society, 74, 1-15.

Doan, T. M., 2003. Which methods are most effective for surveying rain forest herpetofauna?. Journal of herpetology, 37(1), 72-81.

Duca, C., Guerra, T.J., Marini, M. Â., 2006. Territory size of three Antbirds (Aves, Passeriformes) in an Atlantic Forest fragment in southeastern Brazil. Revista Bras. Zoo. 23(3), 692-698.

Hamer, K.C., Hill, J.K., Benedick, S., Mustaffa, N., Sherratti, T.N., Maryati, M., Chey, V.K., 2003. Ecology of butterflies in natural and selectively logged forests of northern Borneo: the importance of habitat heterogeneity. Journal of Applied Ecology. 40, 150-162.

Hansbauer, M.M., Storch, I., Leu, S., Nieto-Holguin, J., Pimentel, R.G., Knauer, F., Metzger, J.W., 2008. Movements of neotropical understory passerines affected by anthropogenic forest edges in the Brazilian Atlantic rainforest. Biological Conservation 141, 782-791.

Hill, J.K., Hamer, K.C., Tangah, J., Dawood, M., 2001. Ecology of tropical butterflies in rainforest gaps. Oecologia, 128, 294–302.

Hughes, J.B., Daily, G.C., Ehrlich, P.R., 1998. Use of fruit bait traps for monitoring of butterflies (Lepidoptera: Nymphalidae). Revista de Biología Tropical

Lewis, O.T., 2001. Effect of experimental selective logging on tropical butterflies. Conservation Biology, 15(2), 389-400.

Mahood, S.P., Lees, A.C., Peres, C.A., 2012. Amazonian countryside habitats provide limited avian conservation value. Biodiversity Conservation, 21, 385-405

Rappole, J. H., Winker, K., Powell, G. V., 1998. Migratory Bird Habitat Use in Southern Mexico: Mist Nets versus Point Counts (Hábitat Utilizado por las Aves Migratorias en México: Redes vs. Conteos de Punto). Journal of Field Ornithology, 635-643.

Ridgely, R. S., Greenfield, P. J., 2001. The Birds ofEcuador. Vol. II, field guide. Cornell University Press, Ithaca, NY.

Rödel, M. O., Ernst, R., 2004. Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. Ecotropica, 10, 1-14.

Schulenberg, T. S., Marantz, C. A., English, P. H., 2000. Voices of Amazonian Birds: Birds of the Rainforest of Southern Peru and Northern Bolivia. Cornell Laboratory of Ornithology.

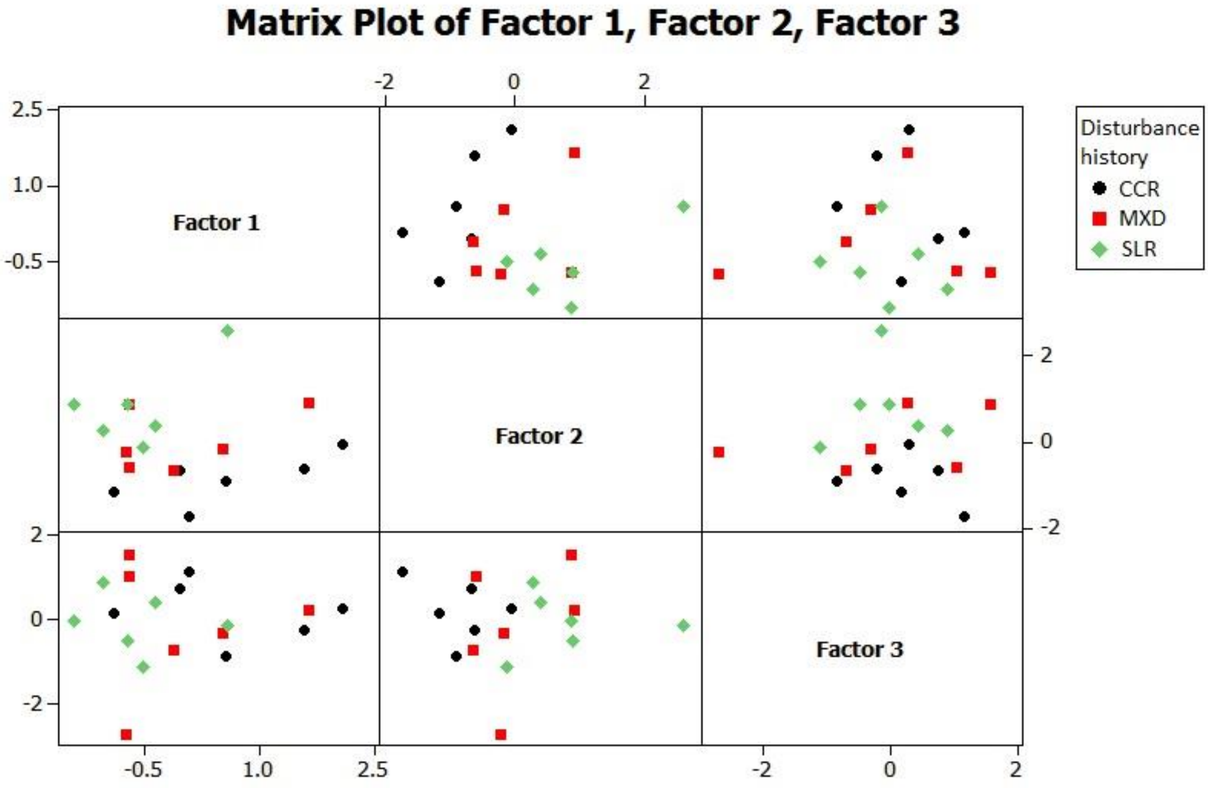
Whitman, A. A., Hagan III, J. M., & Brokaw, N. V., 1997. A comparison of two bird survey techniques used in a subtropical forest. Condor, 955-965.

Chapter 4

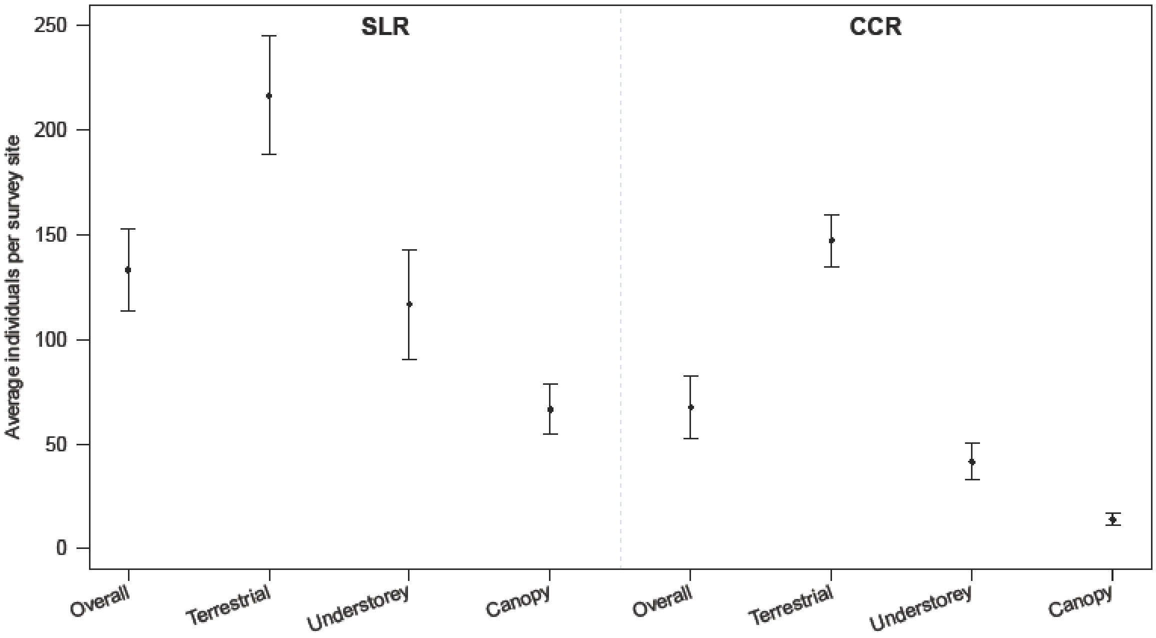
Appendix 4A. Factor analysis outputs of the vegetation mapping data across butterfly survey sites.

```
Rotated Factor Loadings and Communalities
Quartimax Rotation

Variable                Factor1  Factor2  Factor3  Communality
Leaf Litter               0.556   -0.535    0.273        0.669
Canopy Height             0.022    0.817   -0.138        0.688
Canopy Coverage           0.174    0.685    0.213        0.544
Herb Layer               -0.905   -0.261   -0.064        0.891
Shrub Layer              -0.764   -0.125    0.060        0.602
Epiphyte_number          -0.719    0.472    0.248        0.802
Trees >10cm dbh/100m2     0.060   -0.015   -0.943        0.893

Variance                 2.2626   1.7298   1.0968       5.0892
% Var                     0.323    0.247    0.157        0.727


Sorted Rotated Factor Loadings and Communalities

Variable                Factor1  Factor2  Factor3  Communality
Herb Layer               -0.905    0.000    0.000        0.891
Shrub Layer              -0.764    0.000    0.000        0.602
Epiphyte_number          -0.719    0.472    0.000        0.802
Leaf Litter               0.556   -0.535    0.000        0.669
Canopy Height             0.000    0.817    0.000        0.688
Canopy Coverage           0.000    0.685    0.000        0.544
Trees >10cm dbh/100m2     0.000    0.000   -0.943        0.893

Variance                 2.2626   1.7298   1.0968       5.0892
% Var                     0.323    0.247    0.157        0.727
```

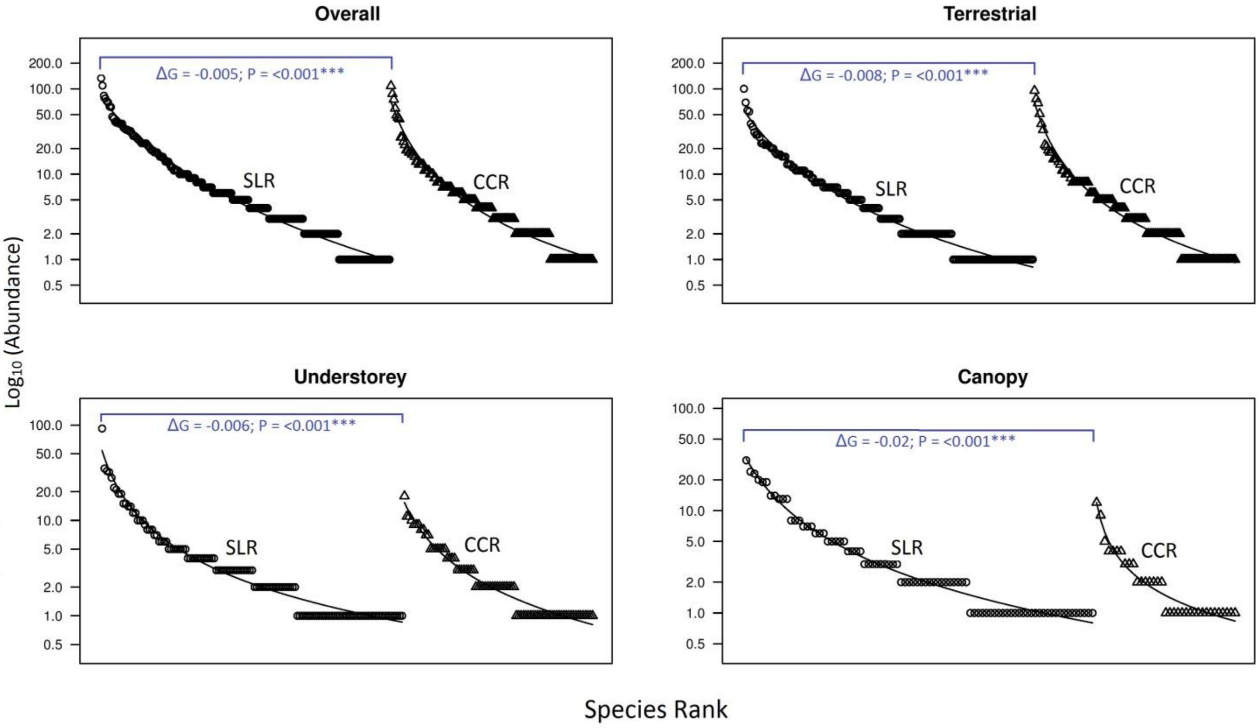
Appendix 4B. Correlation matrix of factor scores.



Appendix 4C. Butterfly abundance between vertical strata and between SLR and CCR disturbance histories.



Appendix 4D. Dominance-diversity plots between SLR and CCR disturbance habitats; and across vertical strata.



Appendix 4E. Top model averaged coefficients (with shrinkage)

```
Estimated species richness
            Estimate Std. Error Adjusted SE z value Pr(>|z|)    
(Intercept)  76.7481    76.1388     77.1644   0.995  0.31993    
habitatpcr   16.4471     8.3290      8.5038   1.934  0.05310 .  
habitatslr   33.1465    12.4326     12.6416   2.622  0.00874 ** 
stratalow    48.8606     6.5864      6.7545   7.234  < 2e-16 ***
stratamid    34.0645     6.5864      6.7545   5.043    5e-07 ***
alt          -0.0818     0.1492      0.1512   0.541  0.58856    
```

```
Shannon diversity
                       Estimate Std. Error Adjusted SE z value Pr(>|z|)    
(Intercept)           2.207e+00  1.049e-01   1.076e-01  20.501  < 2e-16 ***
habitatpcr            9.267e-01  1.488e-01   1.527e-01   6.069  < 2e-16 ***
habitatslr            1.044e+00  1.635e-01   1.674e-01   6.236  < 2e-16 ***
stratalow             1.207e+00  1.406e-01   1.445e-01   8.351  < 2e-16 ***
stratamid             8.617e-01  1.406e-01   1.445e-01   5.963  < 2e-16 ***
habitatpcr:stratalow -9.267e-01  1.988e-01   2.044e-01   4.535  5.8e-06 ***
habitatslr:stratalow -5.650e-01  1.988e-01   2.044e-01   2.765  0.00570 ** 
habitatpcr:stratamid -5.283e-01  1.988e-01   2.044e-01   2.585  0.00973 ** 
habitatslr:stratamid -6.000e-01  1.988e-01   2.044e-01   2.936  0.00332 ** 
river                -6.213e-05  1.134e-04   1.149e-04   0.541  0.58868    
```

```
Abundance
             Estimate Std. Error Adjusted SE z value Pr(>|z|)    
(Intercept) 179.57824  186.89962   189.11173   0.950 0.342322    
alt          -0.29211    0.37325     0.37764   0.774 0.439218    
habitatpcr   56.77455   15.92814    16.28846   3.486 0.000491 ***
habitatslr  129.53913   25.24087    25.71572   5.037    5e-07 ***
river        -0.05857    0.02149     0.02197   2.666 0.007672 ** 
stratalow   123.00000   11.38562    11.68329  10.528  < 2e-16 ***
stratamid    38.27778   11.38562    11.68329   3.276 0.001052 ** 
```

Appendix 4F. Moran's index test results for spatio-autocorrelation; carried out on model residuals from the selected model for each response variable tested.

Estimated species richness

```
$observed
[1] -0.03870772
$expected
[1] -0.01886792
$sd
[1] 0.02465298
$p.value
[1] 0.4209568
```

Shannon diversity

```
> Moran.I(butts$diversity, butts.dists.inv)
$observed
[1] -0.01657358
$expected
[1] -0.01886792
$sd
[1] 0.02449041
$p.value
[1] 0.9253608
```

Abundance

```
> Moran.I(butts$abundance, butts.dists.inv)
$observed
[1] -0.03659737
$expected
[1] -0.01886792
$sd
[1] 0.02421746
$p.value
[1] 0.4641115
```

Appendix 4G. List of species detected within this study.

MSP number	Identification
7	*Magneuptychia modesta*
9	*Harjesia blanda*
11	*Taygetamorpha celia*
12	*Erichthodes antonina*
13	*Cissia terrestris*
15	*Splendeuptychia itonis*
16	*Harjesia obscura*
18	*Hamadryas chloe*
22	*Memphis polycarmes*
23	*Memphis sp.*
24	*Memphis offa*
25	*Panacea prola*
26	*Morpho achilles*
27	*Caligo superbus*
28	*Heliconius sara*
29	*Heliconius (Laparus) doris*
30	*Caeruleuptychia lobelia*
31	*Archaeoprepona demophon*
32	*Ostrinotes sospes*
33	*Nessaea hewitsonii*
34	*Memphis sp.*
35	UID
36	*Hypoleria lavinia*
37	*Vila azeca azeca*
39	*Heliconius numata timaeus*
40	*Heliconius hecale sysiphus*
41	*Heliconius numata bicoloratus*
43	*Heliconius elevatus*
45	*Heliconius pardalinus maeon*
46	*Tithorea harmonia brunnea*
47	*Tithorea harmonia assimilis*
49	*Nessaea obrinus*
51	*Tigridia acesta*
53	*Baeotus aeilus*
55	*Philaethria dido*
57	*Hypna clytemnestra*
58	*Colobura dirce*
60	*Dynamine ines*
61	*Temenis laothoe*
62	*Taygetis sylvia*
63	*Pareuptychia ocirrhoe interjecta*
66	*Mesosemia sp*
67	*Fountainea halice*
68	UID
69	*Cissia proba*
70	*Napeogenes juanjuiensis*
73	*Oleria victorine*
76	*Amarynthis meneria*
77	*Hamadryas fornax*
78	*Callicore excelsior*
79	*Callicore lyca aegina*
80	*Fountainea halice*
81	*Eunica sophonisba*
83	*Fountainea ryphea*
85	UID
87	*Batesia hypochlora*
89	*Narope sp*
90	*Opsiphanes cassina*
91	*Ectima iona*
92	*Hamadryas feronia*
93	*Cissia sp.*
94	*Splendeuptychia latia*
96	*Panacea regina*
97	*Memphis praxias*
98	*Catoblepia xanthicles*
99	*Catoblepia berecynthia berecynthia*
102	*Taygetis elegia*
103	*Hypothris ninonia*
104	*Morpho helenor*
106	*Heliconius numata lyrcaeus*
107	*Tithorea harmonia brunnea*
108	*Memphis basilia drucei*
111	*Myscelia capenas*
113	*Adelpha boreas*
114	*Chloreuptychia herseis*
115, 318	*Caeruleuptychia caerulea*
116, 325	*Temenis pulchra*
120, 290	*Adelpha iphiclus*
122	*Chloreuptychia chlorimene*
123	*Opsiphanes invirae*
126	*Contrafacia sp.*
127	*Agrias claudina*
129	*Satyrinae*
130	*Prepona laertes*
131	*Euselasia hahneli*
132	*Smyrna blomfildia*
142	*Narope cyllabarus*
143, 310	*Hamydras laodamia*

8, 145	*Harjesia obscura*
150	*Caligopsis seleucida*
151	*Chloreuptychia arnaca*
154	*Posttaygetis penelea*
110, 155	*Memphis philomena*
156	*Adelpha serpa*
159	*Emesis sp.*
160	*Hamadryas amphinome*
162	*Taygetis sp.*
164	*Cithaerias pireta*
165	*Haetera piera*
166	*Taygetis inambari*
167	*Historis acheronta*
168, 326	*Callicore cynosura*
170	*Pyrrhogyra crameri*
95, 171	*Caligo eurilochus*
173	*Godyris zavaleta*
174	*Harjesia obscura*
175	*Heliconius melpomeme aglaope*
176	*Adelpha boeotia*
177	*Heliconius xanthocles*
178	*Tithorea harmonia spp*
179	*Siproeta stelenes*
181, 265	*Ectima lirides*
182	*Adelpha jordani*
183	*Heliconius leucadia*
184	*Doxocopa lavinia*
185	*Hypoleria lavinia cajona*
186	*Archaeoprepona demophoon*
187	*Prepona dexamenus*
188	*Heliconius numata bicoloratus*
189	*UID*
190	*Memphis sp.*
191	*Eunica alpais*
192	*Callicore pygas cyllene*
193	*Morpho menelaus*
194	*Heliconius erato*
195	*Eunica sydonia*
196	*Ithomia arduinna*
197	*Pierella lamia*
198	*Chloreuptychia agatha*
199	*Siderone syntyche*
200	*Biblis hyperia*
201	*Eunica orphise*
202	*Eunica sp*
203	*Ceratinia tutia*
204	*Hyposcada anchiala subsp*
205	*Oleria victorine*
206	*Hyposcada illinissa*
207	*Heliconius erato emma*
208	*Caligo cf. idomeneus*
210	*Eueides libitina*
214	*Adelpha capucinus capucinus*
215	*Melinaea menophilus hicetas*
216	*Baeotus beotus*
217	*Baeotus deucalion*
219	*Eunica sp.*
220	*Thisbe irenia*
221	UID
21, 189, 222	*Memphis phantes*
224, 307	*Memphis acidalia*
20, 225	*Memphis polycarmes*
226	*Heliconius numata lyrcaeus*
227	*Splendeuptychia sp.*
82, 101, 229	*Taygetis virgilia*
230	*Lycorea halia*
231	*Adelpha cytherea*
232	*Adelpha pleasure*
233	*Callicore hystaspes hystapses*
237	*Taygetis thamyra*
238	*Zischkaia ordinata*
239	*Pierella hortona albofasciata*
240	*Eurybia cyclopia*
242	*Splendeuptychia aurigera*
244	*Rareuptychia clio*
247	*Heliconius doris*
248	*Eueides lampeto acacetes*
249	*Ancyluris sp.*
250	*Eunica sp.*
251	*Dynamine giselia*
252	*Eunica sp.*
254	UID
262	*Diaethria clymena*
263	*Pierella lena*
264	*Adelpha melona*
266	*Prepona amydon*
267	*Fountainea nessus*
270	*Hermeuptychia sp.*
272	*Ancyluris spp*
274	*Metamorpha elissa*
275	*Memphis pithyusa*
276	*Rhetus periander*
281	*Parides neophilus*
223, 288	*Memphis xenocles*
295	*Calycopis sp.*
296	*Asterope degandii*

297	*Adelpha epione*	341	*Mesosemia eumene*
298	UID	342	*Adelpha attica attica*
54, 299	*Adelpha delinita*	343	*Manataria sp.*
300	*Memphis anna*	448	UID
157, 172, 301	*Memphis acaudata*	483	*Itaballia demophile*
304	*Eurybia patrona promota*	565	*Astraptes fuglerator*
312	*Mithras orobia*	105, 124	*Heliconius burneyi*
313	*Vila emilia*	109, 125, 134	*Doxocopa agathina*
315	*Adelpha erotica erotica*	112, 121	*Baeotus japetus*
319	*Eunica mygdonia mygdonia*	119, 146	*Historis odius*
320	*Euselasia telectus*	135, 148	*Manataria hercyna*
321	*Panaropsis semiota*	138, 149	*Epiphile lampethusa*
322	*Dynamine chryseis*	144, 88	*Zaretis itys*
323	*Protographium agesilaus*	10, 19, 86	*Taygetis mermeria*
324	*Doxocopa linda*	2, 4, 117	*Yphthimoides renata*
325	*Temenis pulchra*	56, 136	*Adelpha zina irma*
327	*Archaeoprepona meander*	44, 118	*Neruda aoede*
328	*Castilia perilla*	257	*Eurybia molochina*
329	UID	128, 133	*Adelpha mesentina*
330	*Memphis polyxo*	158, 161, 311	*Polygrapha xenocrates*
331	*Heliconius sp.*	48, 42	*Catonephele acontius*
332	*Adelpha lycorias lara*	50, 52, 141	*Catonephele numilia*
333	UID	5, 65	*Pseudodebis valentina*
334	*Eurybia halimede/dardus*	71, 140	*Pyrrhogyra otolais*
335	UID	75, 100, 139	*Bia actorion*
336	*Forbestra olivencia*	84, 147, 137	*Consul fabius*
337	*Eresia clio*	14, 228	*Splendeuptychia ashna*
338	*Marpesia chiron*		
339	UID	6, 17	*Taygetis larua*
340	UID		

Appendix 4H. GLMM outputs for bait type as a predictor of observed species richness and abundance.

Observed Species Richness

```
> summary(a1)
Call:
glmmadmb(formula = rich ~ bait + (1 | siteID), data =
mist, family = "nbinom",
    link = "log")
AIC: 2614.3
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   1.6505     0.0866   19.06  < 2e-16 ***
baitFish      0.5805     0.0902    6.43  1.2e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’
0.1 ‘ ’ 1
Number of observations: total=432, siteID=18
Random effect variance(s):
Group=siteID
            Variance StdDev
(Intercept)  0.05859  0.242
Negative binomial dispersion parameter: 1.3999 (std.
err.: 0.12332)
Log-likelihood: -1303.15
> a2 <- glmmadmb(formula = rich ~
(1|siteID)  , data = mist,
+ family = "nbinom", link = "log")
> summary(a2)
Call:
glmmadmb(formula = rich ~ (1 | siteID), data = mist,
family = "nbinom",
    link = "log")
AIC: 2651.5
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   1.9808     0.0781    25.4   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’
0.1 ‘ ’ 1
Number of observations: total=432, siteID=18
Random effect variance(s):
Group=siteID
            Variance StdDev
(Intercept)  0.06958 0.2638
Negative binomial dispersion parameter: 1.2464 (std.
err.: 0.10533)
Log-likelihood: -1322.74
> AIC(a1,a2)
   df     AIC
a1  4 2614.30
a2  3 2651.48
> anova(a1,a2)
Analysis of Deviance Table
Model 1: rich ~ 1
Model 2: rich ~ bait
  NoPar  LogLik Df Deviance  Pr(>Chi)
1     3 -1322.7
2     4 -1303.2  1    39.18 3.865e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’
0.1 ‘ ’ 1
```

Observed Abundance

```
> summary(a1)
Call:
glmmadmb(formula = abund ~ bait + (1 | siteID),
mist,
    family = "nbinom", link = "log")
AIC: 3008.2
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   2.1231     0.0946   22.45  < 2e-16
baitFish      0.5837     0.1086    5.38  7.6e-08
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05
0.1 ‘ ’ 1
Number of observations: total=432, siteID=18
Random effect variance(s):
Group=siteID
            Variance StdDev
(Intercept)  0.05454 0.2335
Negative binomial dispersion parameter: 0.87581
err.: 0.065253)
Log-likelihood: -1500.12
> a2 <- glmmadmb(formula = abund ~
(1|siteID)  , data = mist,
+ family = "nbinom", link = "log")
> summary(a2)
Call:
glmmadmb(formula = abund ~ (1 | siteID), data =
family = "nbinom",
    link = "log")
AIC: 3034.1
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   2.4527     0.0844    29.1   <2e-16
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05
0.1 ‘ ’ 1
Number of observations: total=432, siteID=18
Random effect variance(s):
Group=siteID
            Variance StdDev
(Intercept)   0.0723 0.2689
Negative binomial dispersion parameter: 0.82246
err.: 0.060225)
Log-likelihood: -1514.03
> AIC(a1,a2)
   df     AIC
a1  4 3008.24
a2  3 3034.06
> anova(a1,a2)
Analysis of Deviance Table
Model 1: abund ~ 1
Model 2: abund ~ bait
  NoPar  LogLik Df Deviance  Pr(>Chi)
1     3 -1514.0
2     4 -1500.1  1    27.82 1.331e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05
0.1 ‘ ’ 1
```

Chapter 5

Appendix 5A. Species detections by methodology, across the two study sites.

Where: Site total = total number of observations across all methodologies; ACT = Arboreal Camera Trapping, DT = Diurnal Transects; NT = Nocturnal Transects; INC = Incidentals.

Common name	**Species name**	**Manu Learning Centre**					**Shipetiari N. C.**				**Study total**
		Site total	**ACT**	**DT**	**NT**	**INC**	**Site total**	**ACT**	**DT**	**INC**	
Allen's olingo	*Bassaricyon alleni*	1	1				0				1
Bicolour-spined porcupine	*Coendou bicolor*	8	6		1	1	0				8
Black-eared common opossum	*Didelphis marsupialis*	0					2	2			2
Black-faced spider monkey	*Ateles chamek*	56	3			53	1	1			57
Bolivian bamboo rat	*Dactylomys boliviensis*	1			1		0				1
Bolivian red howler monkey	*Alouatta sara*	61	3	1		57	5	3	2		66
Bolivian squirrel monkey	*Saimiri boliviensis*	160	4			156	13	6	3	4	173
Brown titi monkey	*Callicebus brunneus*	171		12		159	15		9	6	186
Brown-eared woolly opossum	*Caluromys lanatus*	41	41				2	2			43
Four-eyed opossums (Brown/Gray)	*Metachirus nudicaudatus / Philander opossum*	18	18				0				18
Gray monk saki monkey	*Pithecia irrorata*	0					2	2			2
Hoffman's two-toed sloth	*Choloepus hoffmanni*	5	3		1	1	0				5
Kinkajou	*Potos flavus*	29	18		6	5	38	38			67
Large-headed capuchin	*Sapajus macrocephalus*	177	2	3		172	15	12	1	2	192
Margay	*Leopardus wiedii*	1				1	0				1
Peruvian night monkey	*Aotus nigriceps*	55	22		8	25	112	109		3	167
Peruvian woolly monkey	*Lagothrix cana*	132	30	2		100	0				132
Saddleback tamarin	*Saguinus fuscicollis*	44				44	12	5	2	5	56
Short-furred woolly mouse opossum	*Micoureus regina*	3			2	1	0				3
Silky pygmy anteater	*Cyclopes didactylus*	2	2				0				2
Southern Amazonian red squirrel	*Sciurus spadiceus*	5				5	3		1	2	8
Tamandua	*Tamandua tetradactyla*	3				3	2	2			5
White-fronted capuchin	*Cebus albifrons*	0					5	4		1	5
White-bellied slender mouse opossum	*Marmosops noctivagus*	1				1	0				1

Appendix 5B. Model outputs related to the number of detections between mid and upper canopy situated cameras.

```
Models:
m2: MAMMAL.DETECT ~ 1 + (1 | TREE)
m1: MAMMAL.DETECT ~ HEIGHT + (1 | TREE)
   Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
m2  3 256.73 260.73 -125.37   250.73
m1  4 251.59 256.92 -121.80   243.59 7.1425      1   0.007528 **
```

i.e.the model with height in it has strong support.

```
> summary(m1)
Linear mixed model fit by REML ['lmerMod']
Formula: MAMMAL.DETECT ~ HEIGHT + (1 | TREE)
REML criterion at convergence: 233.4

Scaled residuals:
Min      1Q  Median     3Q     Max
-1.0773 -0.4502 -0.0384 -0.0384  3.8211

Random effects:
Groups   Name       Variance Std.Dev.
TREE     (Intercept)   0.0     0.00
Residual           378.4    19.45
Number of obs: 28, groups:  TREE, 15

Fixed effects:
Estimate Std. Error t value
(Intercept)   20.955      5.199   4.031
HEIGHTM      -20.209      7.352  -2.749
Correlation of Fixed Effects:
HEIGHTM -0.707
```

i.e. for every 100 trap days you get 21 observations in the upper canopy and just 0.8 in the mid canopy.

Appendix 5C. Total identifiable arboreal vertebrate detections stratified by vertical camera location.

Common name	Species name	Mid-canopy			Upper-canopy		
		Observed	Frequency / 100 trap nights	Frequency / camera	Observed	Frequency / 100 trap nights	Frequency / camera
Allen's olingo	*Bassaricyon alleni*	0	0	NA	1	0.07	0.07
Bicolour-spined porcupine	*Coendou bicolor*	0	0	NA	6	0.42	0.07
Black-banded woodcreeper	*Dendrocolaptes picumnus*	0	0	NA	1	0.07	0.07
Black-eared common oppossum	*Didelphis marsupialis*	0	0	NA	2	0.14	0.07
Black-faced spider monkey	*Ateles chamek*	0	0	NA	4	0.28	0.21
Bolivian red howler monkey	*Alouatta sara*	0	0	NA	6	0.42	0.21
Bolivian squirrel monkey	*Saimiri boliviensis*	2	0.13	0.14	8	0.56	0.29
Brown-eared woolly opossum	*Caluromys lanatus*	0	0	NA	43	3	0.29
Double-toothed kite	*Harpagus bidentatus*	0	0	NA	1	0.07	0.07
Four-eyed opossums (Brown/Gray)	*Metachirus nudicaudatus / Philander opossum*	0	0	NA	18	1.26	0.07
Gray monk saki monkey	*Pithecia irrorata*	0	0	NA	2	0.14	0.07
Hoffman's two-toed sloth	*Choloepus hoffmanni*	0	0	NA	3	0.21	0.07
Kinkajou	*Potos flavus*	2	0.13	0.14	54	3.77	0.71
Large-headed capuchin	*Sapajus macrocephalus*	1	0.07	0.07	13	0.91	0.21
Olive oropendola	*Psarocolius bifasciatus*	1	0.07	0.07	5	0.35	0.29
Pale-winged trumpeter	*Psophia leucoptera*	0	0	NA	1	0.07	0.07
Paradise tanager	*Tangara chilensis*	0	0	NA	1	0.07	0.07
Peruvian night monkey	*Aotus nigriceps*	0	0	NA	131	9.14	0.64
Peruvian woolly monkey	*Lagothrix cana*	3	0.2	0.07	27	1.88	0.21
Razor-billed curassow	*Mitu tuberosum*	0	0	NA	1	0.07	0.07
Russte-backed oropendola	*Psarocolius angustifrons*	0	0	NA	1	0.07	0.07
Saddleback tamarin	*Saguinus fuscicollis*	3	0.2	0.07	2	0.14	0.07
Silky pygmy anteater	*Cyclopes didactylus*	0	0	NA	2	0.14	0.14
Spix's guan	*Penelope jacquacu*	0	0	NA	9	0.63	0.36
Tamandua	*Tamandua tetradactyla*	0	0	NA	2	0.14	0.14
Unidentified nightjar	NA	0	0	NA	1	0.07	0.07
Unidentified woodcreeper	NA	0	0	NA	3	0.21	0.14
Violaceus jay	*Cyanocorax violaceus*	0	0	NA	1	0.07	0.07
White hawk	*Pseudastur albicollis*	0	0	NA	2	0.14	0.07
White-fronted capuchin	*Cebus albifrons*	1	0.07	0.07	3	0.21	0.07
Observed mammal species richness		**6**			**18**		
Observed total species richness		**7**			**30**		

Chapter 6

Appendix 6A – The mean relative abundances for amphibians at each site within the three disturbance areas. Nocturnal transect relative abundances (RA) represent the number of individuals encountered per 100 survey location nights (20ha) surveyed per night; pitfall trap relative abundances (RA) represent the number of individuals encountered per 200 trapping days at a given site; n = number of encounters; species strongly associated with either wetland or large river habitat which were excluded from analysis are marked by an * next to the species name.

Species	Nocturnal transects							Pitfall traps							Family	Strata	Species identifier code in Fig. 4	Species identifier code in Fig. 5
	CCR		MXD		SLR		Total	CCR		MXD		SLR		Total				
	n	RA	n	RA	n	RA	n	n	RA	n	RA	n	RA	n				
Adenomera andreae	6	14	7	14	2	4	15	61	90	51	76	24	36	136	L	T	Aa	Sp1
Adenomera sp1	1	2	1	2	1	2	3	15	22	10	15	4	6	29	L	T	Ap	Sp2
Allobates conspicuus	-	-	-	-	-	-	0	1	1	0	0	0	0	1	A	T		Sp34
Allobates femoralis	-	-	-	-	-	-	0	0	0	0	0	1	1	1	A	T		Sp37
Allobates trilineatus	-	-	-	-	-	-	0	14	21	3	4	0	0	17	A	T	At	Sp35
Ameerega hahneli	3	7	2	4	0	0	5	1	1	1	1	0	0	2	D	T	Ah	Sp3
Ameerega macero	2	5	24	49	41	87	67	1	1	13	19	17	25	31	D	T	Am	Sp4
Ameerega sp1	0	0	0	0	4	9	4	0	0	0	0	6	9	6	D	T	As	Sp5
Bolitoglossa cf. altamazonica	2	5	31	63	61	130	94	-	-	-	-	-	-	0	P	S	Ba	Sp6
Cochranella nola	0	0	3	6	3	6	6	-	-	-	-	-	-	0	C	A		Sp7
* *Dendropsophus parviceps*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
Dendropsophus rhodopeplus	1	2	2	4	0	0	3	-	-	-	-	-	-	0	H	A		Sp8
Dendropsophus sarayacuensis	0	0	1	2	0	0	1	-	-	-	-	-	-	0	H	A		Sp32
* *Engystomops freibergi*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	T		
* *Hypsiboas boans*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
* *Hypsiboas geographicus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
* *Hypsiboas lanciformis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
* *Hypsiboas maculateralis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
* *Hypsiboas punctatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
Leptodactylus pentadactylus	0	0	0	0	1	2	1	2	3	0	0	1	1	3	L	T		Sp9
Leptodactylus rhodomystax	1	2	1	2	0	0	2	8	12	1	1	0	0	9	L	T		Sp10
Leptodactylus rhodonotus	-	-	-	-	-	-	0	1	1	0	0	0	0	1	L	T		Sp36
Lithodytes lineatus	0	0	1	2	2	4	3	2	3	9	13	3	4	14	L	T		Sp11
Noblella myrmecoides	0	0	0	0	2	4	2	-	-	-	-	-	-	0	C	T		Sp12
Oreobates quixensis	0	0	1	2	1	2	2	-	-	-	-	-	-	0	C	T		Sp14
Osteocephalus castaneicola	7	16	13	27	5	11	25	2	3	0	0	0	0	2	H	A	Oc	Sp15
Osteocephalus helenae	0	0	0	0	3	6	3	-	-	-	-	-	-	0	H	A		Sp16
Phyllomedusa tomopterna	0	0	2	4	2	4	4	-	-	-	-	-	-	0	H	A		Sp18
* *Phyllomedusa vaillanti*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
Pristimantis altamazonicus	0	0	1	2	5	11	6	0	0	1	1	0	0	1	C	S		Sp19
Pristimantis buccinator	0	0	1	2	0	0	1	-	-	-	-	-	-	0	C	S		Sp20
Pristimantis carvalhoi	0	0	5	10	16	34	21	0	0	0	0	3	4	3	C	S	Pc	Sp21
Pristimantis ockendeni	25	57	61	124	59	126	145	2	3	0	0	1	1	3	C	S	Po	Sp22
Pristimantis olivaceus	0	0	2	4	3	6	5	-	-	-	-	-	-	0	C	S		Sp23

Pristimantis reichlei	21	48	24	49	34	72	79	3	4	1	1	0	0	4	C	S	Pr	Sp24
Pristimantis sp1	1	2	3	6	8	17	12	0	0	2	3	0	0	2	C	S		Sp26
Pristimantis sp3	0	0	4	8	18	38	22	0	0	2	3	1	1	3	C	S		Sp27
Rhinella margaritifera	0	0	2	4	11	23	13	9	13	14	21	17	25	40	B	T	Rt	Sp28
Rhinella marina	0	0	0	0	2	4	2	42	62	33	49	34	50	109	B	T	Rm	Sp29
Scinax funereus	2	5	0	0	0	0	2	-	-	-	-	-	-	0	H	A		Sp30
** Scinax garbei*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A		
Scinax ictericus	2	5	0	0	0	0	2	-	-	-	-	-	-	0	H	A		Sp31
Teratohyla midas	0	0	1	2	0	0	1	-	-	-	-	-	-	0	C	A		Sp33

Appendix manuscript 1 - Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in Eastern Ecuador.

Authors and contributions

Christopher Beirne[1,2], Oliver Burdekin[1,3] and Andrew Whitworth[1,3,4]

[1] Global Vision International, 3rd Floor, The Senate, Exeter, EX1 1UG, UK.

[2] Centre for Ecology & Conservation, School of Biosciences, University Exeter, Cornwall Campus, Cornwall, TR10 9EZ, UK

[3] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.

[4] Institute of Biodiversity, Animal Health and Comparative Medicine, Room 306 - Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK.

Designed the study: CB AW

Collected the data: CB OB AW

Performed the analysis: CB AW

Contributed to writing of the manuscript: CB OB AW

Citation:

Beirne, C. Burdekin, O. Whitworth, A. (2013) Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in Eastern Ecuador. Herpetological Journal. 23, 209–219.

NB: To access all relevant appendices please access the manuscript online.

Abstract

One of the key drivers of worldwide species loss is habitat change, defined as habitat deforestation, fragmentation and deterioration. We studied the effects of structural habitat change on herpetological richness and diversity in the Yachana Reserve, Amazonian Equador, using pitfall traps and visual encounter surveys between 2009 an 2010, recording 1551 amphibians of 37 different species and 234 reptiles of 27 species. Estimated species richness and diversity was less in pasture land and plantation habitats. Abandoned plantations supported relatively high abundances of individuals, but were markedly depauperate in species richness and diversity. Abandoned pastureland showed the opposite trend, retaining higher species richness and diversity than abandoned plantation sites, but in significantly lower relative abundances. We emphasise the importance of small reserves with a matrix of anthropogenic disturbance in preserving areas of primary habitat and providing areas of secondary regeneration. Such reserves can aid in the identification of the factors that underlie inter-specific variation in responses to habitat change at the species level.

Introduction

One of the key drivers of worldwide species loss is habitat change, defined as habitat deforestation, fragmentation and deterioration (Urbina-Cardona et al. 2006; Urbina-Cardona 2008; Gardner 2010). The rapid rate of forest conversion in the neotropics has presented a large-scale expansion of secondary forest, plantation and pastureland (Wright 2005; Gardner et al. 2007c). Despite the increasingly dominant role of these degraded habitats within tropical landscapes, there is little consensus within the scientific community about the extent of their conservation value for herpetofaunal communities (Faria et al. 2007; Ficetola et al. 2007; Gardner et al. 2007c). Wright et al. (2006) predicted that the future loss of primary forest will be counterbalanced by regenerating secondary forest and that the predicted loss of species due to habitat change may not occur. Several studies have acknowledged that richness values are often unaltered on some occasions slightly increased within secondary forest and (Fredericksen & Fredericksen 2002, 2004), whilst Gillespie et al. (2012) highlight the potential disaster in converting secondary forests to plantations. The study of habitat change is of major importance and deserves more attention, particularly within plantations and when regenerating secondary habitats. Amphibians and reptiles are important primary, mid-level and top consumers in neotropical ecosystems; therefore, it is important to understand the specific responses of these organisms to structural habitat change (Bell & Donnelly 2006). Amphibians and reptiles are also considered to be the most threatened groups of terrestrial vertebrates (Gardner et al. 2007c; Böhm et al. 2013). This is especially true in the neotropics which, despite an estimated 89% of threatened species being affected by habitat loss, have been the subject of just 10% of the world's herpetological studies (Gardner et al. 2007a). There is a general consensus amongst herpetologists that information about the effect of structural habitat change on determining amphibian and reptile distributions is limited and should be addressed in current research (Pearman 1997; Krishnamurthy 2003; Urbina-Cardona et al. 2006).

Leaf-litter and low strata herpetofauna lend themselves well to biological conservation studies as they are abundant in neotropical forests and are easy to sample (albeit requiring more survey effort than temperate regions). The structural habitat changes associated with secondary and plantation forests cause microhabitat changes through both environmental factors (i.e. incident light, temperature, and relative humidity), and interspecific interactions (i.e. predation, parasitism and competition). To date, loss of reproductive sites, loss of genetic diversity, changes in home ranges, population isolation due to the incapacity to cross anthropogenic matrix habitats, changes in individual growth rates and activity patterns, and changes in microhabitat use have been documented (Gibbons et al. 2000; Gardner et al. 2007a; Urbina-Cardona 2008; Dixo et al. 2009). Despite these alterations, two recent studies report that the variety of microhabitats provided by shaded plantations and degraded forest edges are sufficient to maintain up to 80% of primary forest leaf-litter herpetofauna diversity (Faria et al. 2007; Dixo & Martins 2008). However, active plantations appear to be more detrimental to lizard richness than abandoned ex-plantation sites (Glor et al. 2001). Other research suggests that the management of for example cacao agroforestry will enhance the richness and abundance of disturbance-tolerant species but native forests remain vital for rare, more specialised species (Wanger et al. 2009). Recent work has demonstrated that species-specific responses to these environmental and inter-specific factors can vary (Oldekop et al. 2012). Despite the fact that some researchers find stable or increasing richness values following structural changes (Fredericksen & Fredericksen 2002, 2004), community structure will frequently be disrupted and distinct from that of original forest, usually containing a large abundance of generalist species and a loss of primary forest specialised species in altered landscapes (Heinen 1992; Furlani et al. 2009). Widespread, abundant, habitat generalists might dominate similarity analyses even when relatively rare specialists are present. Additional species-level analyses of habitat specialization will be needed before the conservation value of tropical secondary regenerating forest is fully understood (Dent & Wright 2009).

In this study we focused on an Ecuadorian lowland rainforest and aimed to answer the following questions: (i) What are the effects of structural habitat change of tropical lowland rainforest on herpetological richness and diversity? (ii) How does structural habitat change influence community composition? (iii) Are there species-specific variations in responses to habitat modification?

Methods

Study Site

The research was conducted in the Yachana Reserve between April 2009 and December 2010 (Figure 1). The reserve is situated within the Napo province in the Amazonian region of Ecuador (77°13'43.9"W, 0°50'45.281"S; 300-350m altitude). The Yachana Reserve is a legally-designated Bosque Protector (Protected Forest), consisting of approximately 1000 ha of predominantly primary lowland rainforest, as well as abandoned regenerating plantations (generally cacao, *Theobroma cacao*), small abandoned pastureland patches, riparian forest and a road. The reserve is surrounded by large areas of pasture land, small active cacao farms and forest. The abandoned pastureland and plantation sites within the study are generally <3 ha in size and are interspersed within patches of forest on the south side of the road. The largest part of contiguous forest is found on the northern side of the road: the majority of forest sites were located here. The abandoned pastureland and plantations had been abandoned by farmers and their cattle for ~10 years at the time of sampling (information obtained through the Yachana Foundation and local landowners). The regenerating plantation sites contained a mix of native shrubs and trees, now beginning to succeed the plantation trees, some of which still remained, whilst the pastureland was still heavily dominated by grass, with little succession from other plants.

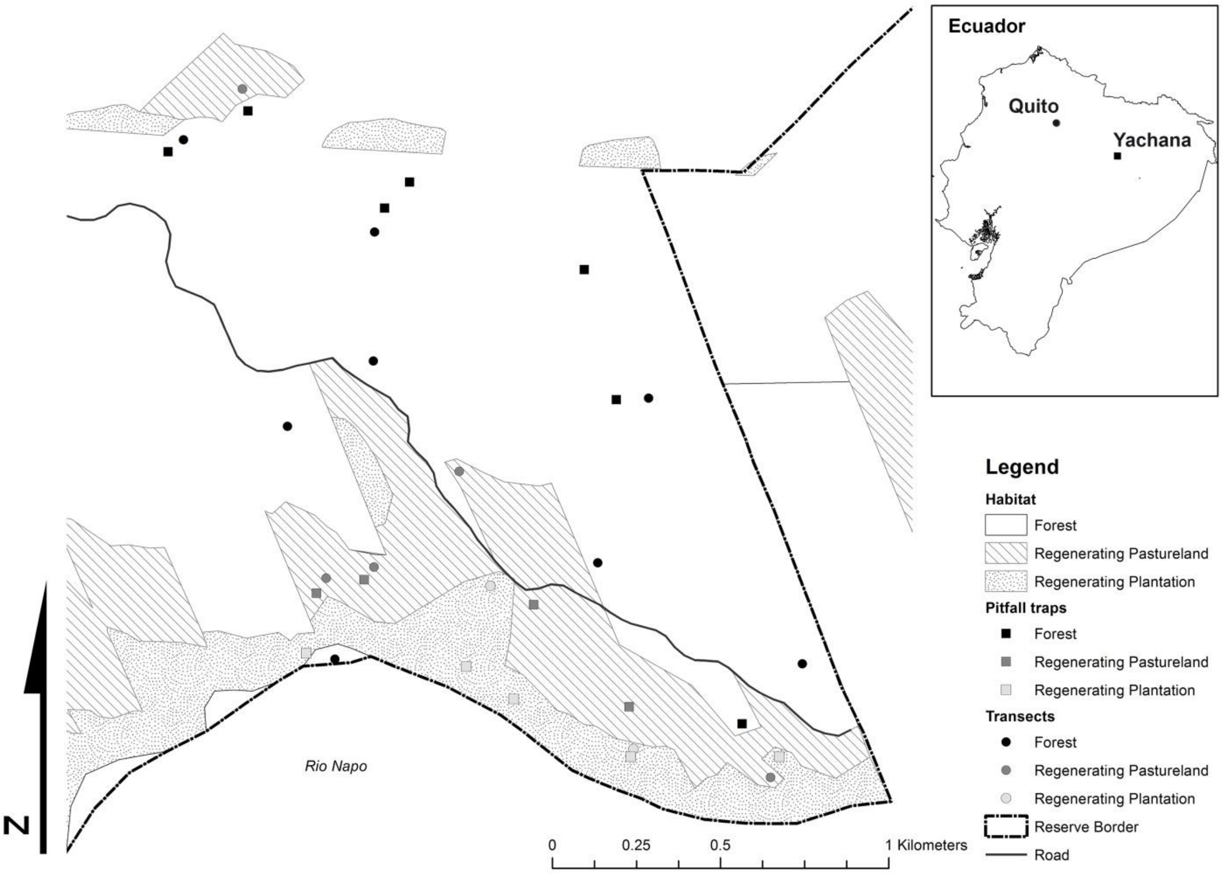


Figure 1. Shows the situation of the Yachana Reserve within Amazonian Ecuador and the location of the survey sites across different habitats. Each survey site was also habitat feature mapped.

Site locations and sampling methodology

Surveying was conducted through both wet and dry seasons (November to March is generally considered the wet season and April to October the drier season), in order to obtain an annual representation of community structures. Pitfall traps (PFT) and nocturnal visual encounter surveys (VES) were conducted simultaneously in order to avoid any bias in capturing a temporally different fauna due to the trapping method used at any particular time. Where possible, sites were placed within a given habitat at least 70 m from a clear habitat edge or stream/water body to reduce the influence of edge effects following

Demaynadier & Hunter (1998). Due to the steep and locally dense nature of the terrain, sample sites were placed in areas that were accessible yet away from existing trails within the reserve. Locating sampling sites off the trails avoided known detection biases (Von May & Donnelly 2009).

Fifteen PFT arrays were established throughout the reserve: seven within primary forest, four within abandoned pastureland and four within regenerating plantation. The 25 m long arrays consisted of four 25-litre buckets connected by 8 m lengths of drift fence that were 40 cm in height. Pitfall traps were opened for a period of 10 days in each trapping session. Seven trapping sessions were conducted throughout the study period resulting in 70 days of pitfall trapping per site. Open PFT's were checked once daily between 0630h and 1300h. Lids were placed 10 cm above the buckets to prevent flooding during prolonged periods of rain during the trapping periods and then closed tight between sessions.

VES were used to complement the pitfall data due to their known effectiveness in sampling tropical herpetofauna (Bell & Donnelly 2006) and their higher yields per unit effort than other sampling methods (Rödel & Ernst 2004; Bell & Donnelly 2006). Fifteen 75x6 m (450 m^2) VES transects were established throughout the reserve: eight within primary forest, five within abandoned pastureland and two within regenerating plantation. All transects were surveyed at night, commencing at 2015 h±15 mins. Transects were surveyed by five searchers over a period of 90 mins up to 2 m in height. Search teams consisted of one experienced herpetologist and four trained international conservation volunteers. Each transect was searched every three months for the duration of the study period (n = 10), with the exception of five transects which were added during the study period and were thus searched on fewer occasions. We ensured that these five additional transects were studied throughout both dry and wet seasons, as with all other transect sites. The order in which transects were searched within each of the three month periods was randomised to avoid systematic sampling bias. Diurnal VES transects were not performed, as nocturnal VES sampling has previously been shown to be the most efficient method in herpetological inventorying and still allows to detect resting diurnal species (Doan 2003).

All amphibians and reptiles encountered were identified in the field where possible (see Beirne & Whitworth (2011) and Whitworth & Beirne (2011) for full indentification criteria and a complete list of identification resources used throughout the project). For PFT, individuals were released approximately 40 m away from the trap site to reduce the probability of recapture. Individuals captured during VES were released behind the searchers, so that the same individual could not be encountered twice within a survey. Unidentifiable individuals were anaesthetised with Lidocaine and fixed with 10% formalin then subsequently identified and stored at the Ecuadorian Natural Science Museum (MECN) in Quito.

Owing to the previous detection of a single case of chytridiomycosis within the study site (Global Vision International, unpublished) codes of good practice were strictly adhered to. This was achieved by the systematic cleaning of tools and equipment. Sterile bags were used when handling amphibians and small reptiles.

Habitat Classification

In order to confirm that each sampling site was appropriately assigned as forest, plantation or pasture habitat, each visual encounter survey and pitfall site was subjected to vegetation mapping on one occasion following the guidelines outlined by the Ecuadorian Natural Science Museum (MECN), Quito. All sites were mapped between June and December 2009. The following parameters were estimated: Upper canopy and mid canopy cover (% estimate only, conducted by two trained observers to the nearest 5%); height of both upper and mid canopy using clinometers to measure base height of the canopy (m); shrub and herb coverage using a modified Braun-Blanquet scale (Hurst and Allen, 2007); vine, palm, epiphyte, fern, grass and plantation coverage by using the DAFOR scale (5 = dominant, 4 = abundant, 3 = frequent, 2 = occasional, and 1 = rare); and leaf litter depth measured to the nearest 0.5 cm, using a marked dowel to the top of the leaf litter.

PFTs had three vegetation mapping plot points consisting of a 10x10 m grid, one situated at the middle point of the pitfall array and one at both of the ends. VES had the same grid plots conducted along the transect line, one at each end and then two further plots along the transect line. The data gathered from the plots were then averaged to provide a representative set of values for each survey site. In order to compare structural features between habitat classifications, average values for each structural habitat parameter were calculated per site

(Electronic Appendix 1). A factor analysis was then performed using Minitab analysis software (v14.12) in order to detect the separation of sites by their specific habitat variables. Factor scores were sorted and rotated with an Equamax rotation in order to provide the most logical representation of the data visually.

The influence of structural habitat change on species richness and diversity

In order to determine the influence of structural habitat change on herpetofaunal assemblage richness and diversity we first determined the effectiveness of the sampling techniques. Captures from both PFT and VES were then combined in order to provide as near to "true" representation of herpetofaunal assemblages as possible (Gardner et al. 2007c). Reptiles and amphibians were analysed separately to reflect differences in life histories (Gardner et al. 2007b). In order to control for differences in sampling efforts, species accumulation curves were calculated using the Rich package (Rossi 2011) and plotted using R (R Core Team 2012). Species richness was defined as the mean of two non-parametric richness estimators – Chao 1 and jack 1. Species diversity was defined using the Shannon diversity index. Repeating the analyses using Fisher's Alpha and Simpson diversity indices did not change the results and as such are not presented. All richness and diversity estimators were calculated in Estimate S (Colwell 2006).

In order confirm the association between structural habitat parameters and site level species richness and diversity, a series of general linear models were applied (Minitab V14.12). The three site specific habitat structure factor scores generated from the factor analysis that had eigenvalues greater than one were used as explanatory variables to determine their influence on estimated richness and Shannon diversity index as dependent variables.

The influence of structural habitat change on community composition

Community compositions and structures were compared by producing dominance-diversity (Whittaker) plots using the vegan package (Oksanen et al. 2011) in program R (R Core Team 2012). Such plots compare the evenness of a community, whereby shallow curves represent a community of many species of similar abundance whereas steep curves represent a skewed assemblage with one or more species in substantially higher relative abundance than others. Significant differences in slope, and therefore significant differences in community evenness, were assessed through the use of a linear model with log relative abundance as the response term and an interaction between species rank and habitat type as continuous and categorical fixed effects, respectively. Results are reported as ΔG which corresponds to absolute change in gradient between forest and the modified habitats, whereby more negative values denote steeper curves and thus less even assemblages.

The influence of habitat change on species specific relative abundance

In order to determine if herpetofaunal responses to structural habitat change were species specific, we determined the relationship between habitat structure parameters and species specific relative abundances using a series of general linear models (Minitab V14.12). Relative abundance values were calculated for both VES and PFT methods. VES-based abundances were calculated as numbers of individuals encountered within 450 m^2 of the transect area, and PFT abundances were defined as the number of individuals encountered at an individual trap array based on 70 nights of trapping. Where a sufficient number of individuals had been encountered (n>10), significant differences in relative abundances across habitats were determined using the Kruskall-Wallis test (also conducted on overall relative abundance levels for amphibians and reptiles).

Results

Habitat classifications

The factor analysis resulted in the original 13 habitat structure variables producing three factors with an eigenvalue greater than one. These three factors represent 65.8% of variation in the original data set (Factors 1, 2 and 3 contained 31%, 19.3% and 15.5% of the variation respectively). Factor 1 loaded positively with increasing upper canopy height, upper canopy coverage and fern, epiphyte and vine coverage and negatively with the presence of grass (Figure 2). Factor 2 loaded positively with increasing plantation plant coverage, mid canopy coverage and shrub layer and negatively with mid canopy height. Factor 3 loaded with increasing mid canopy height, shrub coverage and the palms abundance (Electronic Appendix 2). The first factor separates sites by the structure of the higher canopy and presence of primary forest features. The second factor represents features related to the mid-forest structure. Factors 1 and 2 were plotted against each other in order to demonstrate the validity of our habitat classifications (Figure 2). The pastureland and forest sites clearly separated along factor 1 (primary forest features vs. grass) whilst the plantation areas separated from both forest and pastureland sites on factor 2 (mid canopy features).

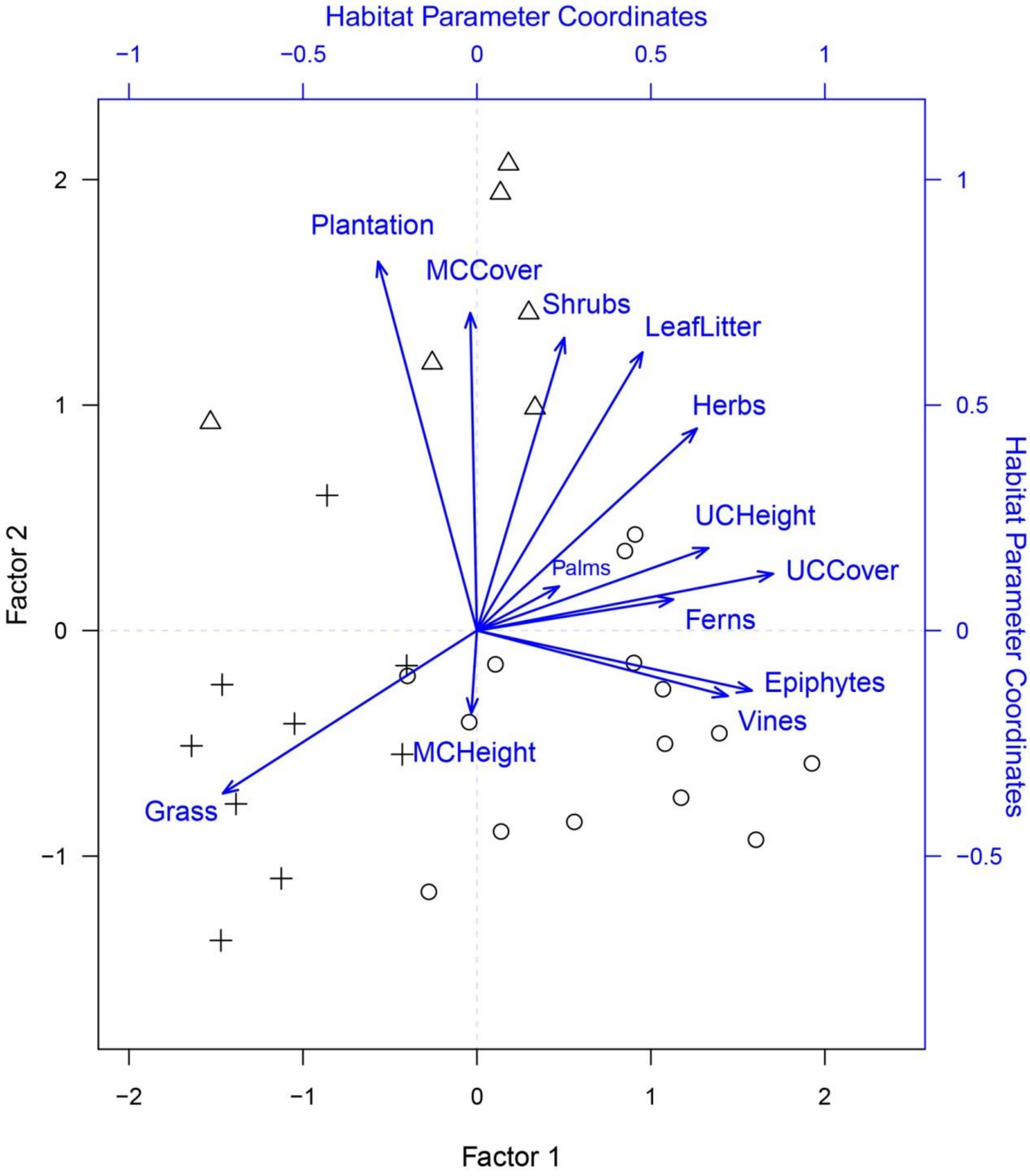


Figure 2. Shows the habitat variable loadings for factor 1 vs. factor 2 (Equamax rotation). The arrows demonstrate the direction and strength of each variable and C stands for canopy. Site specific scores plotted against one another for factor 1 (primary forest vs. grass) and factor 2 (mid canopy structure). O = forest, + = pasture, and Δ = plantation.

The influence of structural habitat change on species richness and diversity

In total, 1551 amphibians of 37 different species and 234 reptiles of 27 species were recorded (Table 1). Survey coverage across all habitats was over 75%, with the exception of reptiles within pastureland habitat with coverage of just 55.7% of estimated species richness. Forest harboured the highest frequency of exclusive species (18) and the highest proportions of total species for both amphibians and reptiles (97.3% and 88.9% respectively).

Table 1. Capture frequency, actual and estimated species richness and sample completeness per habitat classification. Where: [a] Number of individuals encountered, [b] Number of species observed, [c] Mean estimated species richness (Chao 1 and jack 1) *'s denote bias corrected Chao1 estimates, [d] Sampling coverage defined as: $^{b}/^{c}*100$, [e] Number of species found exclusively within the given habitat, [f] Number of species observed as a percentage of combined species across all habitats.

	Habitat class	**n[a]**	**Species[b]**	**Estimated Richness[c]**	**Coverage (%)[d]**	**Exclusive Species[e]**	**Completeness (%)[f]**
Amphibians	Forest	1028	36	42.6	84.5	18	97.30
	Plantation	355	14	17.4*	80.3	0	37.84
	Pasture	168	13	16.9*	76.7	1	35.14
	Total	1551	37	-	-		-
Reptiles	Forest	137	24	29.0	82.8	10	88.88
	Plantation	63	14	17.0	82.4	2	51.85
	Pasture	34	12	21.5*	55.7	1	44.44
	Total	234	27	-	-	-	-

Examination of the individual rarefaction curves 95% confidence intervals suggests that, for amphibians, forest habitat supports more species than abandoned plantation and pasture (Figure 3).

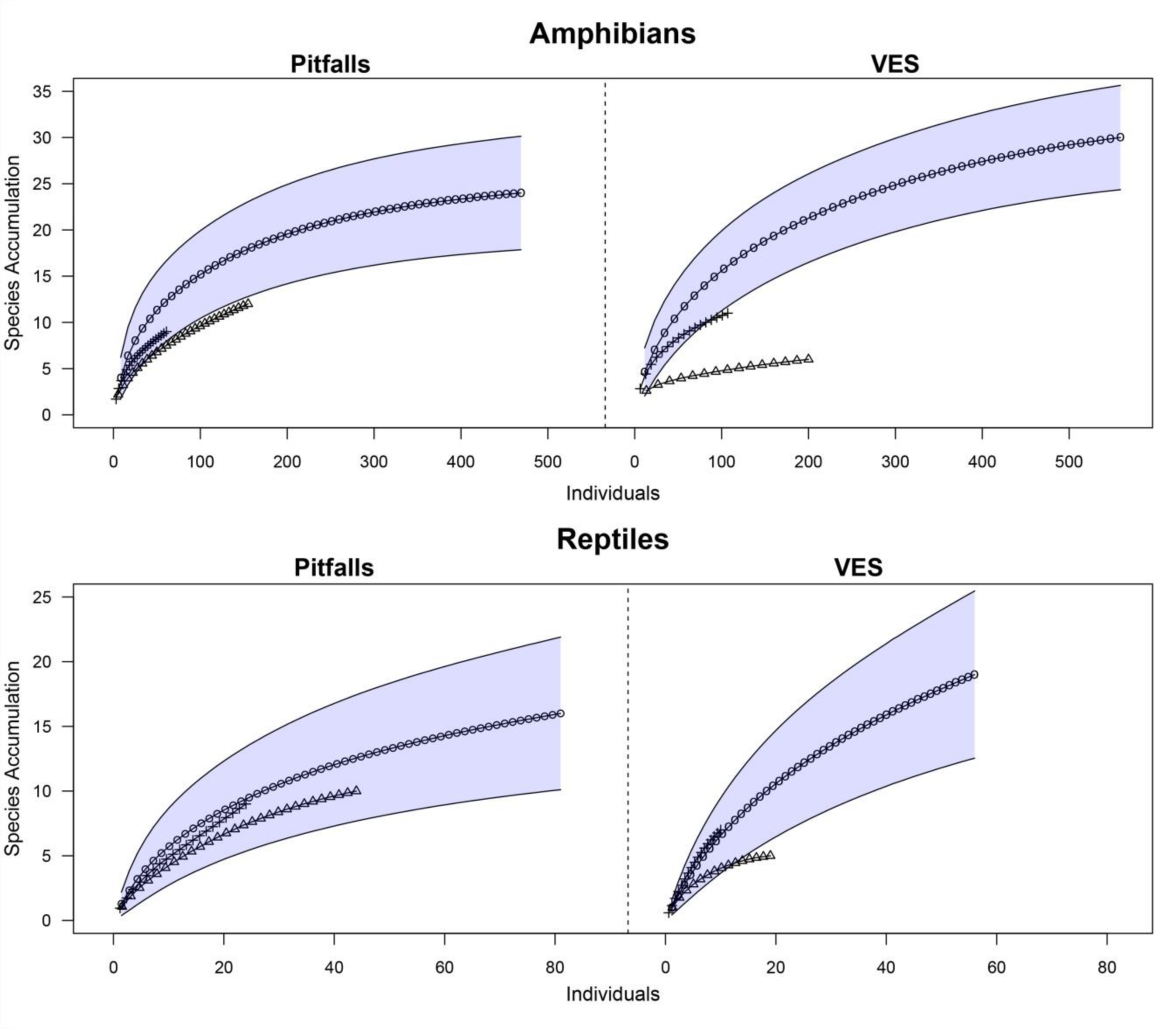


Figure 3. Individual rarefaction curves for both amphibians and reptiles between the three habitats for both pitfall trapping and visual encounter surveys. The gray areas represent the 95% confidence intervals for primary habitat. Mean species accumulation lines falling outside of this envelope are deemed statistically significant. O = forest, + = pasture, and Δ = plantation.

Reptiles show a less defined pattern, with only the number of species recorded in plantations through VES being lower than in the forest. The forest habitat was the most diverse, followed by pasture and plantation for both amphibians and reptiles (Shannon estimates, Figure 4).

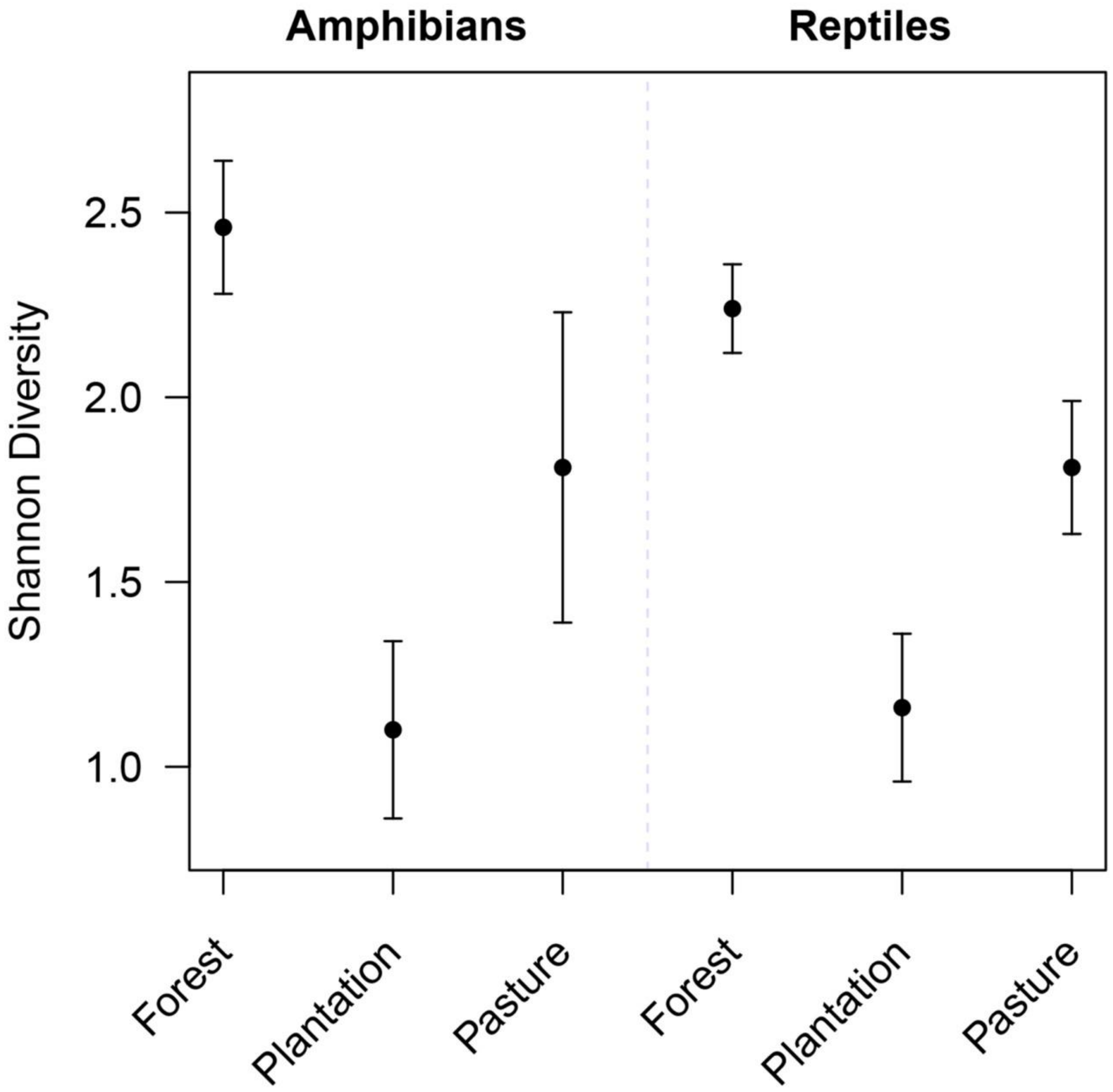


Figure 4. Shannon species diversity estimates with 95% confidence intervals for amphibians and reptiles between the three habitat types.

The same trend was found for other diversity estimators: Simpson and Fisher's alpha (data not shown).We found strong evidence that anthropogenic habitat change, particularly the introduction of grassland and plantation, increasing density of the mid canopy and shrub layers with a concurrent reduction in mid-canopy height, were detrimental to overall amphibian richness and diversity. Increasing upper canopy height, upper canopy coverage and fern, epiphyte and vine coverage and the decrease of grass coverage (factor 1 loading parameters) were associated with increased estimated richness and Shannon diversity of amphibian assemblages (GLM: p=0.01 for estimated richness and p=<0.01 for Shannon diversity), whereas increasing plantation coverage, mid canopy coverage and shrub layer and decreasing mid-canopy height (habitat parameters loaded onto factor 2) are associated with decreases (GLM: p=0.01 for estimated richness and p=<0.01 for Shannon diversity). Factor 3 showed no significant association with amphibian richness or diversity (p=0.46 for estimated richness and p=0.42 for Shannon diversity). No evidence was found for associations between habitat parameters and reptile estimated richness or diversity (Electronic Appendix 5).

The influence of structural habitat change on community composition

Dominance-diversity plots demonstrate that, for both amphibians and reptiles, forest habitat supports a significantly more even assemblage (regular intervals between species) and more rare species (increased tail length) than both plantation and pasture (Figure 5). All plantation and pasture assemblage comparisons to the primary forest were significantly more skewed at the 95% level, except for reptiles in plantation habitat using the pitfall methodology which was marginal (0.058). For amphibians, the plantation habitat assemblage is particularly skewed, with Pristimantis kichwarum (Ra) and Ameerega bilinguis (A) being substantially more abundant than accompanying species. For reptiles, the plots highlight differences in detectability between the two methods employed (VES and PFT): Leposoma parietale (N) dominating PFT sites across all habitats and for VES sites Anolis trachyderma (Af), Anolis fuscaratus (A) and Anolis nitens scypheus (Ac) dominating forest, plantation and pasture habitats respectively.

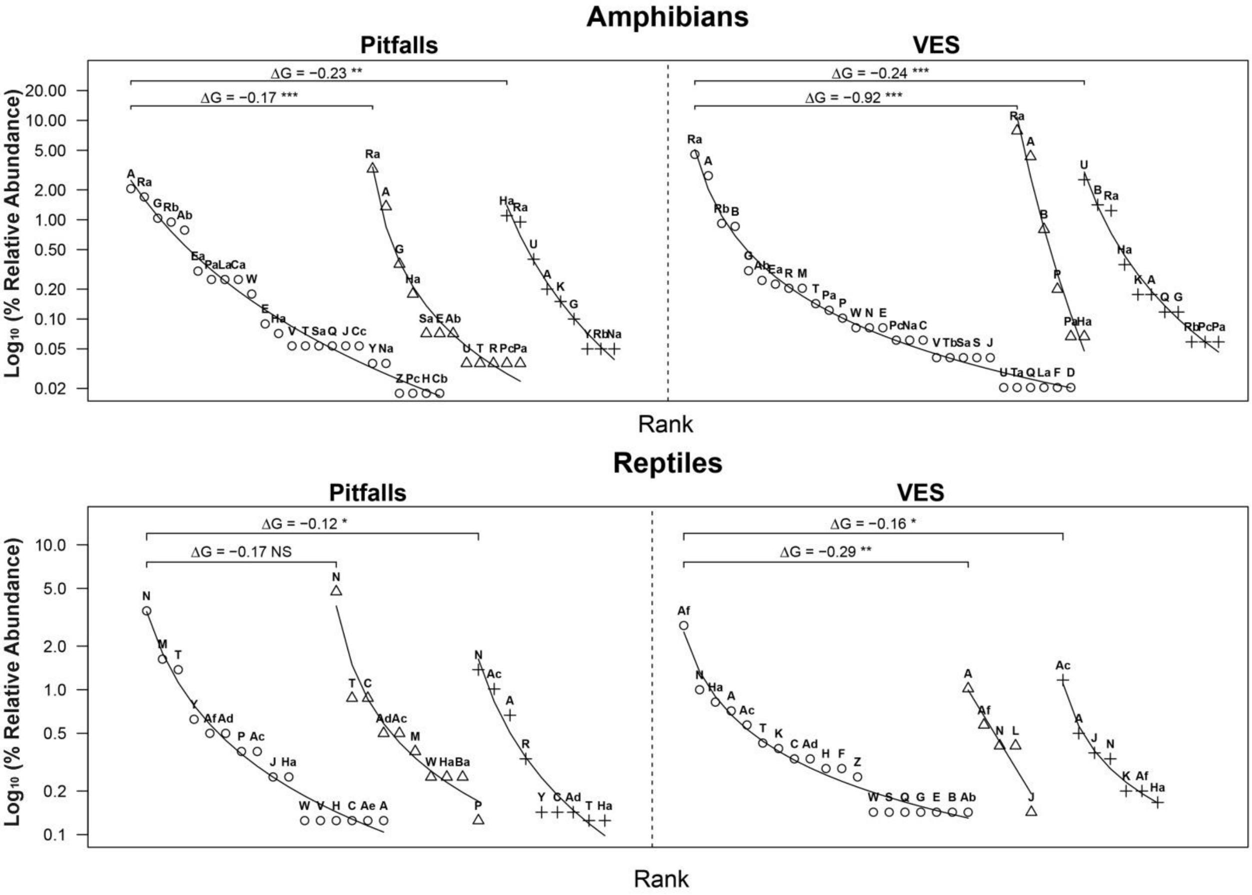


Figure 5. Dominance-diversity (Whittaker) plots for amphibians and reptiles. Each set displays plots for both PFT and VES. Species represented by points are labelled with a code provided in Tables 2 and 3 for amphibians and Tables 4 and 5 for reptiles. For each habitat the relative abundance of each species (ni/N) was plotted on a logarithmic scale against the species rank ordered from most to least abundant. O = forest, + = pasture, and Δ = plantation. Linear models were used to determine if the slopes of plantation and grassland were significantly different to forest where ΔG denotes to absolute change in gradient from the forest habitats predicted line and the symbol denote the level of significance of the deviation where *** = 0.001, ** = 0.01,* = 0.05 and NS = not significant.

The influence of habitat change on species specific relative abundance

When including only species observed in all three habitats, strong evidence of species-specific affinities for different habitat types, regardless of the genus to which amphibians belong was found (Table 2). Three species (Allobates zaparo, Engystomops petersi and Pristimantis lanthanites) were more abundant in forest and one species (P. kichwarum) was more abundant in plantation habitat across both methodologies. A further two species were found to be more abundant in forest habitat (Hypodactylus nigrovittatus and P. altamazonicus), one species was more abundant in plantation habitat (Ameerega bilinguis) and one species was more abundant in pasture habitat (Leptodactylus andreae) in one PFT or VES each. There was no clear trend for overall amphibian abundance regardless of species. The relative abundance of individuals was higher in forest habitat using PFT and in plantation habitat using VES. The lack of overall trend in total abundance is likely driven by species specific variation in detectability dependant on the sampling methodology employed.

Table 2. Details the mean relative abundances for amphibians at each site within the three habitat types. Where: F = Forest, Pl = Plantation, and Pa = Pasture. PFT relative abundances represent the number of individuals encountered per 70 trapping days at a given site; VES relative abundances represent the number of individuals encountered within 450m^2 of transect. N = frequency of individuals encountered across all habitats; P = p-value for Kruskall-Wallis analysis of variance (only conducted in species with an N>10). Codes given next to species name relate to those given in Figure 5. Survey effort is given in trapping nights for PFT and observer hours for VES. For complete tables see Electronic online Appendices 3A and 3B.

Species	Pitfalls					VES				
	F	Pl	Pa	N	P	F	Pl	Pa	N	P
A. zaparo (Ab)	5.5	0.5	0	46	**0.01**	0.2	0	0	12	**0.01**
A. bilinguis (A)	14.4	9.5	1.2	157	0.11	2.8	4.3	0.1	204	**0.01**
B. peruviana (B)	-	-	-	-	-	0.9	0.8	1.1	78	0.55
C. insperatus (Ca)	1.8	0	0	14	0.25	-	-	-	-	-
E. petersi (Ea)	2.1	0	0	17	**0.02**	0.2	0	0	11	**0.02**
H. nigrovittatus (G)	7.3	2.5	0.6	70	**0.03**	0.3	0	0.1	17	0.12
L. andreae (Ha)	0.5	1.3	6.4	31	**0.01**	-	-	-	-	-
L. rhodomystax (La)	1.8	0	0	14	0.53	-	-	-	-	-
P. altamazonicus (Pa)	1.8	0.3	0	15	**0.01**	-	-	-	-	-
P. kichwarum (Ra)	11.9	22.8	5.5	205	**0.03**	4.6	7.9	1	362	**0.01**
P. lanthanites (Rb)	6.6	0	0.3	54	**0.01**	0.9	0	0.1	46	**0.01**
P. varabilis (U)	-	-	-	-	-	0	0	2.1	44	0.09
All Species	58.6	38.8	17.8	685	**0.03**	11.4	13.3	5.1	866	**0.04**
Survey effort	560	280	280			490	150	170		

No evidence was found for individual-specific habitat affinities (Table 3) in reptiles. Considering overall abundance of reptiles, only species in plantations recorded with VES were significantly more abundant than in other habitats. The lack of significant associations could reflect reptiles as being robust to anthropogenic habitat change within small reserves, or could be due to lower detection probability and therefore decreased sample size.

Table 3. Details the mean relative abundances for reptiles at each site within the three habitat types. Where: F = Forest, Pl = Plantation, and Pa = Pasture. PFT relative abundances represent the number of individuals encountered per 70 trapping days at a given site; VES relative abundances represent the number of individuals encountered within 450m^2 of transect. N = frequency of individuals encountered across all habitats; P = p-value for Kruskall-Wallis analysis of variance (only conducted in species with an N>10). Codes given next to species name relate to those given in Figure 5. Survey effort is given in trapping nights for PFT and observer hours for VES. For full table output see Electonic online Appendices 4A and 4B.

Species	**Pitfalls**					**VES**				
	F	Pl	Pa	N	P	F	Pl	Pa	N	P
A. fuscoauratus (A)	-	-	-	-	-	0.2	1.2	0.2	15	0.21
A. nitens scypheus (Ac)	0.5	0.7	2.5	11	0.13	-	-	-	-	-
A. trachyderma (Af)	-	-	-	-	-	0.7	0.6	0.1	21	0.14
K. pelviceps (M)	2.3	0.7	0.0	15	0.06	-	-	-	-	-
L. parietale (N)	5.0	8.6	4.2	62	0.15	0.3	0.4	0.1	11	0.41
P. guianensis (T)	2.0	1.4	0.4	16	0.39	-	-	-	-	-
All Species	14.5	15.7	10.0	149	0.51	2.5	**2.8**	1.1	85	**0.03**
Survey effort	560	280	280			490	150	170		

Discussion

The analyses presented here highlight the same general trend: anthropogenic disturbance, in the form of pasture land and plantation, was detrimental to herpetofauna communities even after 10 years of regeneration. In general, abandoned plantation supported comparable relative abundances of individuals to forest, but was depauperate in species diversity. Abandoned pastureland supported higher species diversity estimates than plantation sites, with lower relative abundances. Species-specific analyses demonstrated that such trends were driven by idiosyncratic responses to disturbance. Whilst the majority of species declined in abundance, some species increased. The degree to which the herpetofauna is affected by disturbance and our ability to detect responses is dependent on the nature of the disturbance, the sum of the species specific responses to habitat disturbance within each study site, and the methodology employed.

Amphibians

Despite encouraging estimates of richness and diversity elsewhere (Ewers & Didham 2006; Faria et al. 2007), our comparison of estimated species richness, individual based rarefaction analysis and diversity indices demonstrate that regenerating pastureland and regenerating plantation did not support comparable levels of amphibian species richness and diversity to forest habitat. In real terms, regenerating pasture and plantation areas were characterised by as much as 60% decrease in estimated species richness. Although relative abundance was maintained in plantation habitat, it decreased between 30-45% within regenerating pastureland areas in comparison to forest habitat. The habitat structure analysis indicates that the patterns in richness and abundance are driven by the physical structure and abundance of vegetation. Primary forest characteristics, such as a high and dense upper canopy and increasing abundances of ferns, epiphytes and vines were correlated with increased richness and diversity. Disturbed forest characteristics such as the presence of grassland, plantation trees and increasing mid canopy density were found to be detrimental.

In agreement with recent multiple taxon assessments regarding the impact of habitat change (Dent & Wright 2009; Pardini et al. 2009) we found species-specific responses to anthropogenic disturbance. For example, A. bilinguis and A. zaparo are sympatric fossorial amphibians with similar life histories and size. Despite this, no statistically significant decrease in A. bilinguis relative abundance was detected outside of primary forest, whereas the abundance of A. zaparo was found to be reduced by as much as 90%.

Of the eight species-specific responses detected, only one and two species were found in significantly increased relative abundance in pasture and plantation habitats, respectively. The high abundance of amphibians within plantation habitat was driven almost exclusively by the increased abundance of the generalist species P. kichwarum. Such generalists have broad habitat and dietary requirements which can render them either insensitive to or benefiters from structural habitat change (Dent & Wright 2009). This is supported by the fact that the relative abundance of P. kichwarum correlates positively with disturbed habitat characteristics across both methodologies. Species specific associations with habitat parameters such as canopy height, plantation presence and epiphyte abundance on the relative abundances were detected for seven further species. Oldekop et al. (2012) have also demonstrated that distribution patterns of leaf-litter frogs were correlated with habitat characteristics (epiphytic ferns) across environmental gradients. Such species-specific associations with habitat features highlight the potential driving factors behind community level changes, and may inform future management strategies. Failure to detect species which were not influenced by habitat structure suggests the influence of factors not measured here such as, food availability, underlying physiology, predation and inter-specific competition.

Reptiles

Diversity indices, estimated richness and individual-based rarefaction curves suggest that forest habitat sustains higher reptile richness, diversity and a more even species composition. However, the responses are not as clear as for amphibians. No overall differences in relative abundance between habitat types were detected using PFT. However, a significant increase in relative abundance in plantation habitat was detected using VES. The overall richness, diversity and abundance trends observed were not associated with structural habitat features. Despite reptile assemblages in the anthropogenically modified habitats being less even than in forest, no species-specific habitat affinities or associations with structural habitat characteristics were detected. These results may suggest that reptiles are generally more resilient to habitat disturbance than amphibians; however, the sample size for reptiles was considerably lower than for amphibians. These results highlight the difficulty of understanding reptile distributions specifically, as they are generally wider ranging and often less frequently encountered.

Spatial caveat

Our findings concur with those found by Gardner et al. (2007a) who report the value of primary forest and the substantially lower estimates of neighbouring regenerating plantation forests with regards to diversity and abundances. The restricted spatial extent precludes discerning the permanent presence of a species from transient movement out of more suitable primary habitat. Such individuals could falsely bias the estimates of species richness and diversity, especially since the methods employed (PFT and VES without marking of individuals) cannot distinguish between an individual temporarily occupying an unsuitable habitat from one which permanently occupies it. This is not an issue within studies utilising spatially independent study sites, and consequently the importance of defining the spatial scale of degraded forests cannot be overestimated. This study nevertheless robustly demonstrates that at local scales within small heterogeneous forest reserves, regenerating plantation and pastureland generally support lower herpetofaunal richness and diversity than forest habitats and that idiosyncratic species specific responses to structural habitat features underpin such differences.

The Yachana Reserve

Over six years of research at the Yachana Reserve (2005-2010) a species list was compiled by field staff from Global Vision International, which consists of 71 amphibian and 72 reptile species. These numbers are considerably higher than the figures stated within this study as we only used two main methods focussed towards terrestrial leaf-litter herpetofauna, avoiding habitats such as swamps, streams and high canopy. Vigle (2008) found similar numbers at the Biological Research station of Jatun Sacha, also based in the lowlands of Ecuador. De la Torre & Reck (2003) however, working from the Tiputini research station in the Biosphere Reserve of Yasuni, also situated in lowland Ecuador, used six survey methods over four years and produced a species list containing 105 amphibians and 80 reptiles. This suggests that their large areas of contiguous primary forest contain up to ~30% more amphibian species but not a great deal more reptiles. This is likely due to the higher sensitivity of amphibians to disturbance regimes. What these inventorying figures show is that despite utilising different survey methods and effort, a small private reserve in areas of past disturbance history can sustain relatively high levels of herpetological diversity and are most certainly worth protecting for future land management plans to assist in providing areas of regeneration and connectivity between protected areas.

Conclusions

We find that structural habitat change, in this case cacao plantation and pastureland are generally detrimental to herpetofaunal richness, diversity and relative abundance in comparison to forest habitat despite 10 years of regeneration. Where relative abundance of amphibians is increased, the responses are driven by a small number of generalist species responding positively to disturbance, skewing community assemblages through their dominance. Habitat characteristics were found to correlate with diversity, richness and species specific abundances, elucidating potential drivers of the observed trends. Further species specific investigations are recommended in order to elucidate why particular species display different responses to habitat change. Such information will be critical in determining the potential of different types of regenerating forest to sustain natural levels of diversity. Understanding such variation in responses can also aid in the conservation of future herpetological communities as agricultural practices increase, causing further habitat change in tropical forests.

We emphasise the value of small reserves with a matrix of anthropogenic disturbance, such as the Yachana Reserve, in preserving areas of forest habitat and encouraging secondary regeneration. Such reserves are well suited to the identification of the factors that underlie inter-specific variation in responses to habitat change at the species level. Such research is vital for the production of sustainable management guidelines for future agricultural land use changes in tropical ecosystems. We firmly support that herpetofaunal conservation priorities and land management strategies should focus on the preservation of primary forest as advocated by Gardner et al. (2008) and further suggest that by expanding reserves by protecting surrounding secondary areas and providing a timescale of regeneration, it may be possible to partially retain primary forest richness and diversity levels.

References

Beirne, C. & Whitworth, A. (2011). Frogs of the Yachana Reserve.

Bell, K.E. & Donnelly, M.A. (2006). Influence of forest fragmentation on community structure of frogs and lizards in northeastern Costa Rica. Conservation Biology, 20, 1750–60.

Böhm, M., Collen, B., Baillie, J.E.M., Chanson, J., Cox, N., Hammerson, G., Hoffmann, M., Livingstone, S.R. & Ram, M. (2013). The Conservation Status of the World's Reptiles. Biological Conservation, 157, 372–385.

Colwell, R.K. (2006). Estimate S: Statistical estimation of species richness and shared species from samples.

Demaynadier, P. & Hunter, M. (1998). Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. Conservation Biology, 12, 340–352.

Dent, D.H. & Wright, J.S. (2009). The future of tropical species in secondary forests: A quantitative review. Biological Conservation, 142, 2833–2843.

Dixo, M. & Martins, M. (2008). Are leaf-litter frogs and lizards affected by edge effects due to forest fragmentation in Brazilian Atlantic forest? Journal of Tropical Ecology, 24, 551-554.

Dixo, M., Metzger, J.P., Morgante, J.S. & Zamudio, K.R. (2009). Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. Biological Conservation, 142, 1560–1569.

Doan, T. (2003). Which methods are most effective for surveying rain forest herpetofauna? Journal of Herpetology, 37, 72–81.

Ewers, R.M. & Didham, R.K. (2006). Confounding factors in the detection of species responses to habitat fragmentation. Biological reviews of the Cambridge Philosophical Society, 81, 117–142.

Faria, D., Paciencia, M.L.B., Dixo, M., Laps, R.R. & Baumgarten, J. (2007). Ferns, frogs, lizards, birds and bats in forest fragments and shade cacao plantations in two contrasting landscapes in the Atlantic forest, Brazil. Biodiversity and Conservation, 16, 2335–2357.

Ficetola, G.F., Furlani, D., Colombo, G. & Bernardi, F. (2007). Assessing the value of secondary forest for amphibians: Eleutherodactylus frogs in a gradient of forest alteration. Biodiversity and Conservation, 17, 2185–2195.

Fredericksen, N. & Fredericksen, T. (2002). Terrestrial wildlife responses to logging and fire in a Bolivian tropical humid forest. Biodiversity and Conservation, 11, 27–38.

Fredericksen, N.J. & Fredericksen, T.S. (2004). Impacts of selective logging on amphibians in a Bolivian tropical humid forest. Forest Ecology and Management, 191, 275–282.

Furlani, D., Ficetola, G.F., Colombo, G., Ugurlucan, M. & De Bernardi, F. (2009). Deforestation and the structure of frog communities in the Humedale Terraba-Sierpe, Costa Rica. Zoological Science, 26, 197–202.

Gardner, T.A. (2010). Monitoring forest biodiversity: improving conservation through ecologically-responsible management. Routledge.

Gardner, T.A., Barlow, J. & Peres, C.A. (2007a). Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. Biological Conservation, 138, 166–179.

Gardner, T.A., Fitzherbert, E.B., Drewes, R.C., Howell, K.M. & Caro, T. (2007b). Spatial and temporal patterns of abundance and diversity of an east African leaf litter amphibian fauna. Biotropica, 39, 105–113.

Gardner, T.A., Hernández, M.I.M., Barlow, J. & Peres, C.A. (2008). Understanding the biodiversity consequences of habitat change: the value of secondary and plantation forests for neotropical dung beetles. Journal of Applied Ecology, 45, 883–893.

Gardner, T.A., Ribeiro-Junior, M.A., Barlow, J., Avila-Pires, T.C.S., Hoogmoed, M.S. & Peres, C.A. (2007c). The value of primary, secondary, and plantation forests for a neotropical herpetofauna. Conservation Biology, 21, 775–787.

Gibbons, J., Scott, D., Ryan, T. & Buhlmann, K. (2000). The global decline of reptiles, deja vu amphibians. BioScience, 50, 653–666.

Gillespie, G.R., Ahmad, E., Elahan, B., Evans, A., Ancrenaz, M., Goossens, B. & Scroggie, M.P. (2012). Conservation of amphibians in Borneo: Relative value of secondary tropical forest and non-forest habitats. Biological Conservation, 152, 136–144.

Glor, R., Flecker, A., Benard, M. & Power, A. (2001). Lizard diversity and agricultural disturbance in a Caribbean forest landscape. Biodiversity and Conservation, 10, 711–723.

Heinen, J. (1992). Comparisons of the leaf litter herpetofauna in abandoned cacao plantations and primary rain forest in Costa Rica: some implications for faunal restoration. Biotropica, 24, 431–439.

Krishnamurthy, S. (2003). Amphibian assemblages in undisturbed and disturbed areas of Kudremukh National Park, central Western Ghats, India. Environmental Conservation, 30, 274–282.

De la Torre, S. & Reck, G. (2003). Ecología y Ambiente en el Ecuador: Memorias del I Congreso de Ecología y Ambiente, Ecuador país megadiverso. Universidad San Francisco de Quito., Quito.

Von May, R. & Donnelly, M. (2009). Do trails affect relative abundance estimates of rainforest frogs and lizards? Austral Ecology, 34, 613–620.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2011). vegan: Community Ecology Package.

Oldekop, J. a, Bebbington, A.J., Truelove, N.K., Tysklind, N., Villamarín, S. & Preziosi, R.F. (2012). Co-occurrence patterns of common and rare leaf-litter frogs, epiphytic ferns and dung beetles across a gradient of human disturbance. PloS one, 7, e38922.

Pardini, R., Faria, D., Accacio, G.M., Laps, R.R., Mariano-Neto, E., Paciencia, M.L.B., Dixo, M. & Baumgarten, J. (2009). The challenge of maintaining Atlantic forest biodiversity: A multi-taxa conservation assessment of specialist and generalist species in an agro-forestry mosaic in southern Bahia. Biological Conservation, 142, 1178–1190.

Pearman, P.B. (1997). Correlates of amphibian diversity in an altered landscape of Amazonian Ecuador. Conservation Biology, 11, 1211–1225.

R Core Team. (2012). R: A Language and Environment for Statistical Computing. Vienna Austria R Foundation for Statistical Computing.

Rossi, J.-P. (2011). rich: An R Package to Analyse Species Richness. Diversity, 3, 112–120.

Rödel, M.O. & Ernst, R. (2004). Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. Ecotropica, 10, 1–14.

Urbina-Cardona, J.N. (2008). Conservation of Neotropical herpetofauna: research trends and challenges. Tropical Conservation Science, 1, 359–375.

Urbina-Cardona, J.N., Olivares-Pérez, M. & Reynoso, V.H. (2006). Herpetofauna diversity and microenvironment correlates across a pasture-edge-interior ecotone in tropical rainforest fragments in the Los Tuxtlas Biosphere Reserve of Veracruz, Mexico. Biological Conservation, 132, 61–75.

Vigle, G. (2008). The amphibians and reptiles of the Estación Biológica Jatun Sacha in the lowland rainforest of Amazonian Ecuador: A 20-year record. Breviora, 514, 1–30.

Wanger, T.C., Saro, A., Iskandar, D.T., Brook, B.W., Sodhi, N.S., Clough, Y. & Tscharntke, T. (2009). Conservation value of cacao agroforestry for amphibians and reptiles in South-East Asia: combining correlative models with follow-up field experiments. Journal of Applied Ecology, 46, 823–832.

Whitworth, A. & Beirne, C. (2011). Reptiles of the Yachana Reserve.

Wright, S.J. (2005). Tropical forests in a changing environment. Trends in Ecology and Evolution, 20, 553–560.

Wright, S.J., Muller-Landau, H.C., Wright, Joseph, S. & Muller-Landau, Helene, C. (2006). The future of tropical forest species. Biotropica, 38, 287–301.

Appendix manuscript 2 – A first test of the thread bobbin tracking technique as a method for studying the ecology of herpetofauna in a tropical rainforest.

Authors and contributions

Emily Waddell[1,2], Andrew Whitworth[1,2] and Ross MacLeod[2]

[1] The Crees Foundation, Urb. Mariscal Gamarra B-5, Zona 1, Cusco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, Room 306 - Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK.

Designed the study: AW EW RM

Collected the data: EW AW

Performed the analysis: EW

Contributed to writing of the manuscript: EW AW RM

Abstract

The lack of information known about amphibians and reptiles in highly threatened tropical rainforest habitats has led to a need for innovative methods that can rapidly generate data on ecological behavior. The thread bobbin technique has proven successful on gathering ecological information in a range of habitats but has not yet been utilized in tropical rainforests. Here we test the method for the first time in a humid tropical forest habitat on 14 herpetofaunal species. The thread bobbin was effective for large anurans (one leptodactylid and one bufonid), medium-large terrestrial snakes (one boid, three colubrids and one viperid), and testudines (one chelid) but was largely unsuccessful for arboreal snakes (one boid and one colubrid), small and slender snakes (two colubrids), and small anurans (one strabomantid). We tracked 18 individuals for 1.2–15 d (mean 4.6 d) and for a distance of 5.5–469.3 m (mean 159.2 m). The thread trail revealed the exact movements of the tracked animal, providing detailed information on activity and microhabitat use that many alternative tracking methods cannot provide. Conservation projects rely heavily upon understanding the life history of species and without this prior knowledge conservation efforts can fail, wasting funds and resources. We show that this method is a cost-effective technique that could be used widely to rapidly gather detailed ecological information on the life history of relatively unknown rainforest reptiles and amphibians.

Introduction

Amphibians and reptiles are key components of their ecosystems (Heyer et al. 1994; Beaupre & Douglas 2009; Hillman et al. 2009; Foster et al. 2012), yet both groups are threatened worldwide. Declines are steepest in the most diverse regions of the world such as tropical rainforests (Duellman 1999), due to an amalgamation of factors including habitat destruction, invasive species, exploitation, climate change, and disease (Lips 1998; Gibbons et al. 2000; Collins & Storfer 2003; Stuart et al. 2004).

These threats are likely underestimated due to the lack of basic ecological knowledge of rainforest amphibians and reptiles. As a result, true distributions and population trends remain undetermined; for example, 25% of evaluated amphibians and 18.3% of reptiles are classified as Data Deficient by the IUCN Red List (IUCN. 2015. The IUCN Red List of Threatened Species. Version 2015.2. Available from http://www.iucnredlist.org [Accessed 17 October 15]). This is emphasized by the low numbers of reptiles that have been evaluated by the IUCN; just 43% of known species compared with almost all known species of birds and mammals and 86.1% of amphibians (IUCN 2015). The lack of ecological information on rainforest herpetofauna may be partially attributed to the challenges of surveying in this dense habitat and often difficult terrain. Thus there is a need for innovative survey methods that can be used to help gather ecological data on herpetofaunal groups (Böhm et al. 2013).

The most basic and frequently utilized method to study the ecology and habitat preferences of tropical rainforest species consists of descriptive data from simple field surveys (Heyer et al. 1994; Duellman 2005; McDiarmid et al. 2012; Beirne et al. 2013). This method can contribute important ecological knowledge but are generally limited to providing single data points for individuals. In contrast, tracking methods can generate large amounts of detailed ecological data by the repeat location of target individuals over several days (Heyer et al. 1994) and can be used to investigate home ranges, dispersal, activity patterns, habitat preferences, and microhabitat utilization. External and internal radio transmitters are the primary method for animal tracking and have been successfully used on a wide variety of herpetofauna in range of habitats including tropical rainforest (Eggert 2002; Kay 2004; Rowley & Alford 2007; Wasko & Sasa 2009). More recently automated telemetry has also been used to track a range of rainforest species (Kays et al. 2011), overcoming many of the shortfalls of traditional radio-tracking in this habitat. A less conventional method is the use of radioactive isotopes, in which a device with small amounts of radioactive material is implanted inside an animal (Ashton 1994). Radioactive isotopes have been successfully used on both amphibians and lizards (Munger 1984; Thompson 1993), though it is no longer widely used due to welfare concerns and difficulties with licences (Beausoleil et al. 2004; Mellor et al. 2004). The smaller the device for both methods, the lower the detectability (Munger 1984; Mellor et al. 2004), which is decreased further in dense vegetation and can, therefore, be a major limitation within tropical rainforest habitat (Cresswell 2005). The biggest disadvantage of these methods is that they only allow data to be gathered when an individual is relocated, thus distances are measured along the straight line between relocations and habitat preference information is limited to relocation site only. Furthermore, some of these methods are expensive and require high levels of expertise for internal implants, which are also highly intrusive.

Novel or less conventional techniques have also been developed to provide detailed information on movement patterns and microhabitat preference that aren't possible using conventional methods, resulting in the ability to collect more ecological data over a shorter period of time (Tozetti & Martins 2007). Fluorescent powders involve covering an animal's ventral surface with UV powder so that UV traces are left on the substrate as the individual moves, which is then possible to follow using a black-light (Plummer & Ferner 2012). This method has been successfully used to study a range of herpetofauna in a variety of habitats (Blankenship et al. 1990; Stark & Fox 2000; Eggert 2002; Stark et al. 2005; Rittenhouse et al. 2006; Lindquist et al. 2007; Furman et al. 2011). Another technique involves the external attachment of a thread bobbin via an adhesive so that the thread is pulled out as the animal moves allowing the exact track of the animal to be recorded (Heyer et al. 1994). This technique has been successful for several herpetofaunal species (Stickel 1950; Dole 1965; Díaz-Paniagua et al. 1995; Tozetti & Toledo 2005; Tozetti & Martins 2007). These methods are both relatively easy to use and cost effective (Mellor et al. 2004). However, fluorescent powders have limited success in tropical rainforest habitat providing a maximum total tracking distance of just 16.65 m for amphibians (Lindquist et al. 2007) and 60 m for small mammals (Nicolas & Colyn 2007). The thread bobbin method has yet to be tested in rainforest habitat but tracking distances of up to 300 m in semi-humid tropical grass and shrublands (Tozetti & Toldeo 2005; Tozetti & Martins 2007) indicate that the thread bobbin method has the potential to be successfully used in tropical rainforest habitat to gather information over a greater distance than that of UV powders.

This study tests for the first time the thread bobbin method on a variety of herpetofaunal species in a tropical rainforest to find out which reptile and amphibian species/groups can successfully be equipped with a thread bobbin device. More specifically, we evaluated the longevity of bobbins as tracking devices and report on the distances we were able to track different reptiles and amphibians. A final objective of our research was to compare the thread bobbin technique to other tracking methods in terms of cost, effort, and the type of information collected.

Methods

Study site

We conducted field research between 2 July and 4 September 2012 at the Manu Learning Center (MLC), in the Manu Biosphere Reserve, South-east Peru. The MLC is a research station within the Fundo Mascoitania reserve (12°47'21.9"S, 071°23'30.5"W), operated and managed by the Crees Foundation. The reserve is located in regenerating tropical lowland rainforest in the Amazon basin to the east of the Andean foothills with an altitude ranging between 450–740 m.

Attachment methods

We captured all animals opportunistically or during visual encounter surveys as part of the Crees Foundation's research and monitoring program. We brought back each individual to the MLC to accurately measure the body mass and length; only attaching the bobbin to individuals with a body mass of 70 g or more so that the device represented no more than 10% of an animals' overall body mass, as recommended by Richards et al. (1994) for short term attachment. However, in most cases it was well below 10%. The bobbin was a nylon thread cocoon bobbin (Danfield Ltd., Leigh, UK; Figure 1a), which unwound from the inside out and came in two strengths: normal and double strength. Each bobbin was 39 mm in length, 14 mm at the widest part and tapered towards each end. The weight was 4.5 g per full bobbin and the thread was a total length of 500 m for normal strength and 250 m for double strength. We used half bobbins on individuals close to the 70 g weight minimum or particularly slender snake species. We created these by manually extracting thread until the weight of the bobbin was halved. Before attachment, we enclosed the bobbin in plastic wrapping (cling film) with a small hole at one end to allow the thread to unwind. This ensured that none of the thread was stuck to the adhesive and the animal would be left unattached to the thread once it finished. For snakes we attached bobbins to the dorsal lateral region at the posterior third of the body using duct tape (Gorilla Tape ®; Figure 1b). The amount of tape used depended on the size of the individual, though the tape was always attached half-way around the girth of the body avoiding the ventral scales so that the device would not restrict internal functions. The corners were rounded to decrease the chances of the tape peeling loose when the animal moved through the substrate. We attached the bobbin via a black elastic harness around the waist/carapace in turtles and anurans (Figure 1c and 1d). Each individual had its waist/carapace measured and the elastic (6 mm wide) cut to this measurement. The ends of the elastic were secured with two small pieces of duct tape across and around the join. The bobbin was covered in duct tape and attached to the harness by a thin strip of duct tape secured by a further two smaller strips of tape. We used only normal strength bobbins for this attachment.

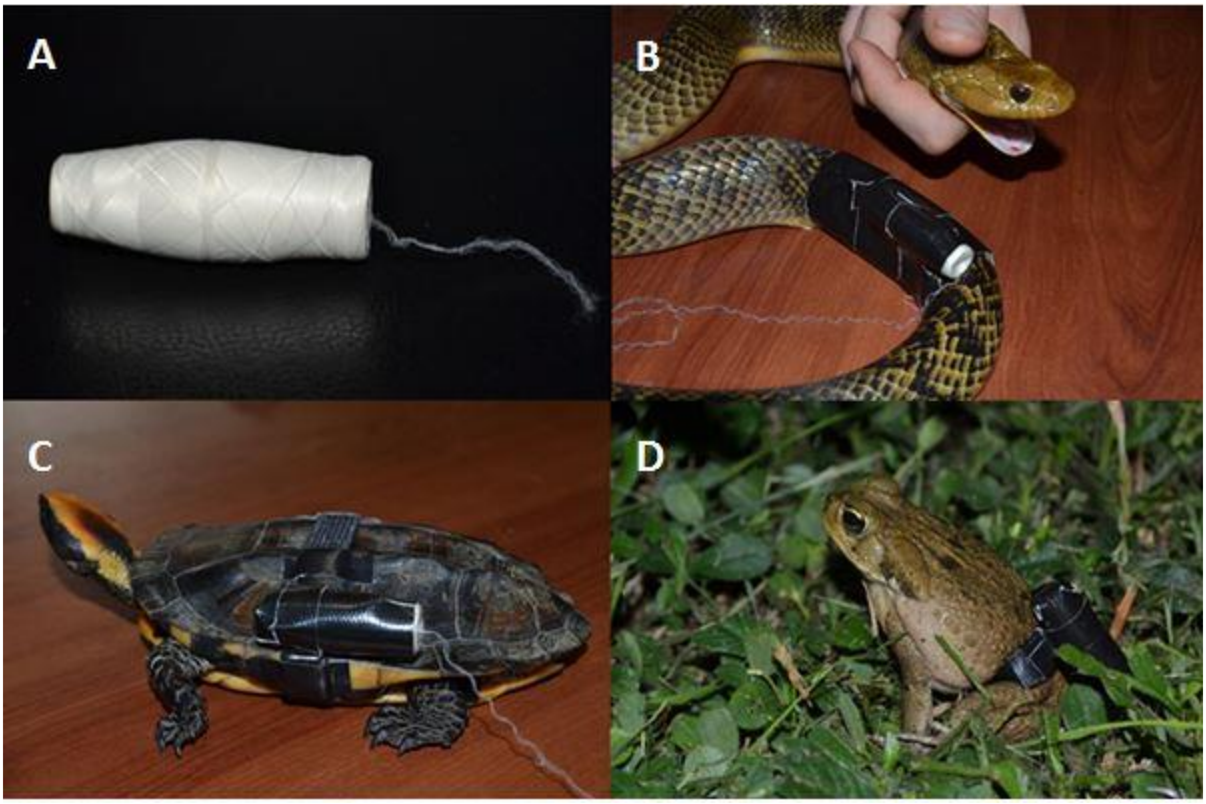


Figure 1: Photographs showing nylon cocoon bobbin (A) and attachment for snake (B), turtle (C) and anuran (D).

We tested the tracking potential of thread for smaller medium-bodied anuran species (weight <70 g) and very slender snake species by exploring a thread-end attachment strategy. We secured the bobbin to the habitat and attached the thread end to the animal; via a small harness for anurans or directly using super glue and a small piece of duct tape for snakes. We released these individuals within a controlled area of the MLC gardens and observed how they moved. We used both strengths of bobbin when testing thread-end attachment.

Release and tracking

We released animals at their capture site (marked with a Garmin eTrex H GPS to an accuracy of 8 m) within 2 h of attachment and within 48 h of capture. The loose end of the thread was tied to something stationary within the habitat (e.g., the trunk of a tree or a branch) and the position marked with a yellow flag. We relocated each individual each evening (1600–2000) and morning (0600–1000) by following the thread from the last relocation site. At each relocation site we marked the animal's position with a yellow flag and recorded the GPS coordinates. We measured the length of the thread (equal to the effective distance moved; EDM) by laying a tape measure on top of the thread, starting approximately 2 m from the animal (exact position marked and measured once animal had moved) making sure not to disturb the animal. We measured the straight line distance (SLD) between relocation sites by hand with a tape measure if the two locations were within sight of one another. When the relocations were too far apart to measure by hand we calculated the SLD from the GPS points of the two relocations using Google Earth. At each relocation, we recorded the animal's activity and its current microhabitat; split into five categories: (1) hidden in substrate; (2) exposed on substrate; (3) hidden in water; (4) exposed in water; and (5) in refuge. Also noted were features along the thread trail: different substrates or microhabitats travelled through, minimum distance moved in water (to prevent overestimating the distance due to the potential of thread drag caused by water flow), distance spent off ground, and the maximum height. We tracked anurans for 3–5 d, depending on how delicate their skin was and based on recommendations by Dole (1965), and reptiles for as long as the method was successful for (i.e., bobbin started to come off or thread ran out) which was up to 15 d. A second bobbin was attached to one anuran individual that had moved a large proportion of the thread length after 1 d. Where possible, we recaptured animals at the end of their tracking period (i.e., when the animals hadn't escaped due to the bobbin detaching or the thread running out) and removed the bobbin attachment, with care taken to remove duct tape from snakes so not to damage scales, through soaking in warm water.

Analysis

We assigned each individual to one of three distinct categories: Category 1 — relocated more than twice and therefore deemed as having been successfully tracked; Category 2 — tracking data collected considered to be inadequate but had the bobbin successfully attached, and Category 3 — method was completely unsuccessful due to the method failing before relocation or the method could not be tested on the species as there was no way for the bobbin to be attached safely and ethically. We only used data from Category 1 tracking attempts to test the effectiveness of the method at gathering ecological data as adequate tracking data was needed for each individual to allow for sufficient comparisons to be made. This was done by comparing 'relocation only data' and 'relocation plus thread trail data' to quantify how much additional information the method provided.

Statistical analysis

All statistical analyses were carried out in R 2.15.3 (R Core Team 2013). We tested all data for normality using a Shapiro-Wilk normality test and data was then analyzed using a non-parametric Wilcoxon signed rank test. We compared the straight line distance (SLD) with effective distance moved (EDM) and 'relocation alone data' with 'relocation plus thread trail data' for the different substrates utilized and maximum height from ground.

Comparison of methods

We compared the thread bobbin tracking for use in tropical forests to other tracking methods by categorizing specific variables into qualitative low, medium, and high categories. We compiled this using information primarily from method descriptions in Heyer et al. (1994a), Beausoleil et al. (2004), and McDiarmid et al. (2012) as well as observations and conclusions from data collected within this study. We based ‘equipment costs’ on prices of commonly used sources of equipment necessary to track one individual for the specific method and did not incorporate travel or labor costs. We categorized ‘longevity’ as the range of time one individual can be tracked for; our categories were low: <3 mo (no seasonal dynamics captured), medium: 3 - 6 mo (some seasonal dynamics captured) and high: >6 mo (seasonal to annual dynamics captured). Specific explanations for each category placement for both ‘detail of data on activity’ and ‘detail of data on microhabitat use’ (i.e., what part of the forest structure an animal moves through and use of refugia) are included in the table. The categorization of these variables takes into account whether or not data can be collected in between relocations and how accurate the relocation data is. We categorized ‘suitability over large distances’ as how suitable and practical the method is to track herpetofauna over a large area in a tropical rainforest; our categories were low if the method is not suitable, medium if the method can be suitable but data is limited, and high if the method is highly suitable for such studies. The ‘potential impact’ includes the impact on the tracked animal other than being handled and ranges from low where the animal is subjected to the presence of the researcher during relocations to high where there is an invasive procedure as part of method. Specific explanations are included in the table for each method. We recognize that other methods allow the measurement of more specialized variables, such as body temperature; however, we focused on the variables presented here as they are useful for carrying out basic ecological studies on poorly known species.

Results

Test of bobbin method

Overall, we tested the bobbin tracking method on 33 individuals of 14 species (Table 1). We collected detailed ecological data on 18 individuals (Table 2) of eight species that we successfully tracked (Category 1) with the bobbin attached either directly or via a harness. The method was tested on a further ten individuals (from five species) but data gathered was considered inadequate (Category 2), and for a further five individuals (from four species) the method was deemed unsuitable (Category 3) (Table 1).

The thread bobbin method worked well for large anurans (*Rhinella marina* and *Leptodactylus rhodomystax*), medium-large terrestrial snakes (*Epicrates cenchria*, *Oxyrhopus melanogenys, Oxyrhopus petolarius, Xenodon severus* and *Lachesis muta*) and a testudine (*Platemys platycephala*) but was largely unsuccessful for arboreal snakes (*Corallus hortulanus* and *Pseustes sulphureus*), small slender snakes (*Leptodeira annulata* and *Siphlophis compressus*) and small anurans (*Oreobates quixensis*). Thread end attachment was deemed as unsuccessful after the thread readily snapped (standard thread) or restricted the animals' movement (stronger thread). The bobbin was either removed by the researcher or it fell off at the end of tracking (once the thread snapped and once it finished) and no skin abrasions were recorded for any of the harness wearing individuals.

Table 1. Species on which the thread bobbin method was tested and the outcomes. Abbreviations are To = Toads, F = Frogs, B = Boids, C = Colubrids, V = Vipers, Te = Testudines; B = bobbin only method; T = thread-end only method; and BH = both bobbin and thread-end method. Categories: 1 — relocated more than twice with adequate tracking data; 2 — tracking data collected inadequate but bobbin successfully attached; and 3 — method unsuccessful by failing before relocation or bobbin could not be attached.

				No. of individuals		**Tracking outcome**							
	Latin name	**Method**	**Category**	*Tested (successful)*	*half bobbin used*	*bobbin taken off*	*bobbin fell off*	*thread snapped*	*thread finished*	*movement restricted*	*lost*	*escaped harness*	*predated*
Amphibians:													
To	*Rhinella marina*	B	1,2	14 (9)	2	10	0	1	2	0	0	0	1
F	*Leptodactylus rhodomystax*	B	1,2	4 (2)	2	2	0	0	0	0	0	2	0
	Oreobates quixensis	T	3	1 (0)	-	0	0	1	0	0	0	0	0
Reptiles:													
B	*Corallus hortulanus*	B	2	1 (0)	0	0	0	0	0	0	1	0	0
	Epicrates cenchria	B	1	1 (1)	0	0	1	0	0	0	0	0	0
C	*Helicops angulatus*	B	3	1 (0)	1	0	1	0	0	0	0	0	0
	Leptodeira annulata	T	3	2 (0)	-	0	0	1	0	1	0	0	0
	Oxyrhopus melanogenys	B	1	1 (1)	0	0	1	0	0	0	0	0	0
	Oxyrhopus petolarius	B	1	2 (2)	1	1	1	0	0	0	0	0	0
	Pseustes sulphureus	B	2	1 (0)	0	0	0	0	0	0	1	0	0
	Siphlophis compressus	BH	3	1 (0)	1	0	0	1	0	1	0	0	0
	Xenodon severus	B	1	1 (1)	0	0	0	1	0	0	0	0	0
V	*Lachesis muta*	B	1,2	2 (1)	0	0	2	0	0	0	0	0	0
Te	*Platemys platycephala*	B	1	1 (1)	0	0	0	0	1	0	0	0	0
			Total	**33 (18)**	**7**	**13**	**6**	**5**	**3**	**2**	**2**	**2**	**1**

Effectiveness of thread bobbin method at gathering ecological data

Eighteen individuals (11 amphibians and 7 reptiles) were relocated a total of 167 times, with 97% of these observing the animal resting. The effective distance moved (EDM), as indicated by the length of thread unwound between each relocation, was more than twice the straight line distance (SLD) between relocations (Table 2; For encounters between which animal moved EDM: median = 12.23 m, Inter-quartile range (IQR) = 48.9 m, n = 76; SLD: median = 4.5 m, IQR = 16.98 m, n = 76). This difference was statistically significant (Wilcoxon signed rank test on all relocations: $V = 3044.5$, df = 333, $P = < 0.001$), indicating that the bobbin tracking method provided much more accurate information on movement distances than would be possible using alternative methods that rely only on SLD relocation data. There is the possibility of error in those SLD calculated using GPS coordinates on Google Earth due to both GPS inaccuracy and Google Earth software errors. However, we used this approach only five times (out of 76 relocations were the animal moved) and when analyses were re-run to include the average GPS error (± 16 m) the results were still significant ($P = < 0.001$).

The number of different substrates utilized and the maximum height from the ground were compared in 'relocation alone data' (R hereafter) and 'relocation plus thread trail' (T hereafter) data (Table 2). These differences were significant for both; different substrates (Wilcoxon signed rank test: $V = 0$, df = 35, $P = 0.001$) and maximum height (Wilcoxon signed rank test: $V = 0$, df = 35, $P = 0.036$). Seven of the nine individuals that moved through water at least once in their tracking were never relocated in water (Table 2); including one individual (*X. severus*) which moved nearly 40% of its total EDM in water.

There was a wide variation in movement distances observed between species (Table 2), with some individuals moving almost the full 500 m within 3.5–4.5 d and others moving < 65 m over 11–15 d. On average, the study demonstrated that the bobbin method can collect detailed ecological data over 4.6 d (range 1.25–15 d) and record movement distances of 159 m (range 5.5–469.3 m).

Table 2. Comparison of ecological data gathered on activity, substrate utilization and habitat use using thread bobbin method based on individual movement patterns recorded in tropical forest habitat. EDM = total effective distance moved, SLD = total straight line distance between relocations, and % of EDM = the percentage the SLD is of the EDM. R = data recorded solely at relocations; T = relocation data plus thread trail data; except for movement in water where R = number of relocations in water, and T = the percentage of the EDM that was in water. *Individual had second bobbin attached during tracking.

Individual	**Tracked days** ***(No. of relocations)***	**Total EDM**	**Total SLD**	**% of EDM**	**Different substrates**		**Max. height (m)**		**Movement in water**	
					R	T	R	T	R	T
R. marina 1	4.5 *(9)*	465.6	154.6	33	3	4	0	0	0	5.60%
R. marina 2	5 *(10)*	367.45	178	48	2	3	1	1	0	1.60%
R. marina 3	5 *(10)*	128.5	55.55	43	2	2	0	0	0	0
*R. marina 5**	3.6 *(6)*	469.3	248.3	53	2	4	0	0	1	23.70%
R. marina 8	4 *(8)*	162	46.9	29	2	3	0	0	0	3.40%
R. marina 10	4 *(8)*	103.2	40.7	39	2	3	0	0	0	0
R. marina 11	4 *(8)*	206.8	135.1	65	2	3	0	0.8	0	11.20%
R. marina 12	4 *(8)*	56.5	32.35	57	2	4	0	0	0	0.90%
R. marina 14	3 *(6)*	208.8	48.6	22	1	1	0	0	0	0
L. rhodomystax	3 *(6)*	59.7	38.7	65	2	2	0	0	0	0
L. rhodomystax 2	3 *(6)*	14	5.3	38	2	3	0	0	0	14.30%
O. melanogenys	15 *(30)*	62.42	26.91	43	4	4	0	0.3	0	0
O. petolarius	4 *(8)*	67.8	12.9	19	2	4	0	7	0	0
O. petolarius 2	1.25 *(3)*	57	16.4	29	2	3	0	5	0	0
X. severus	5 *(10)*	209	42.5	20	1	3	0	0.3	0	39.20%
L. muta	11 *(22)*	50.7	26.35	52	1	1	0	0	0	0
E. cenchria	1.2 *(3)*	5.5	5	91	2	3	0	0	0	0
P. platycephala	3 *(6)*	170.8	51.4	30	4	5	0	0.2	4	47.20%
Average	4.6 *(9.3)*	159.2	64.8	43.1	2.1	3.1	0.1	0.8	-	-

Comparative assessment of method

The cost of one bobbin (Danfield Ltd, Leigh, UK) is just under £0.20 (minimum order of 200 purchased twice for this study), resulting in £80 of costs. Further equipment amounted to ~ £90, making a total of £170. If external radio-transmitters were used then 29 transmitters would have been needed at £92/transmitter (weight range: 2.0-3.8 g, durability: up to 6 months, Holohil Systems Ltd., Ontario, Canada). With the extra costs of a portable receiver (£466; TR-4, Telonic Inc., Arizona, US) with antenna (approximately £100) the total would be approximately £3,234. Furthermore, calculated based on above costs, the external transmitters cost approximately £0.25/relocation (6 months = 364 relocations) and thread bobbins cost approximately £0.021/relocation, based on the average number of relocations (9.3) in this study.

Discussion

Overall, the results of this study have demonstrated that the thread bobbin method is suitable for use as a rapid ecological survey method in tropical rainforests with successful tracking data being collected for a range of different types of herpetofauna. The results also highlighted six species the method is currently not suitable for, as well as issues encountered with the method during the tracking of individuals from three species we did successfully track during the study. Our results also show that this tracking technique provides greater detail on true movement patterns and habitat utilization than relocation only methods. Furthermore, we show that this method is inexpensive and simple to use compared to more conventional techniques such as radio-tracking.

Test of bobbin method

The results show the method to be successful for medium-large terrestrial species that may occasionally utilize aquatic and semi-arboreal habitats. The range of different snake species tracked (highly muscular to long and slender, having smooth to keeled scales) shows the methods versatility within this key group in which ecological information is particularly sparse. The numbers of successfully tracked anurans shows the success for two species of large-bodied amphibians (*Rhinella marina* in particular) and furthermore, the method was successful with a semi-aquatic testudine. Smaller, lighter bobbins would facilitate the attachment of smaller species, and when contacted, Danfield Ltd manufactures said it was possible to bespoke 1 g (65 m) and 1.5 g (100 m) bobbins (£65/kg, minimum of 3 kg). The results also highlighted limitations associated with the method within this habitat specifically regarding species suitability and the attachment of the device on these individuals. The duct tape lost effectiveness in persistently wet conditions; therefore, other adhesives, such as superglues may better facilitate adhesion (Madrid-Sotelo & Garcia-

Aguayo 2008). There is the possibility that the presence of the device may be detrimental to those individuals that escaped; however, it is likely that they will escape due to duct tape losing its effectiveness with no long term impact upon the animal (Richards et al. 1994). The presence of the device may increase predation risk (Blomquist & Hunter 2007), which was recorded once in this study; however, considering that this was a unique occurrence this could be due to chance predation and not necessarily attributable to the tracking attachment. The presence of the researcher during relocation may influence animal behavior (Ward et al. 2013) and thus could bias results, however all tracking methods with the exception of automated radio-telemetry requires regular relocation therefore this isn't exclusively a disadvantage of this method. Steps were taken to reduce disturbance by keeping at least 2 m from the animal during relocations. The length of the thread limits the distance an animal can be tracked for. The replacement of the thread bobbin as the thread neared the end (as demonstrated here for one individual) could extend the length of tracking; however, this approach would also increase the potential impact on the animal due to increased handling.

Effectiveness of thread bobbin method at gathering ecological data

Measuring the distance along the thread (EDM) was found to be a truer representation of an animals' activity than measuring simple straight line distances (SLD). This is especially true for active individuals that occasionally utilize small areas perhaps looking for an appropriate retreat site or leaving a retreat site to feed and then return. This was observed multiple times within this study and increased the EDM but made little or no difference to the SLD. Recording details along the thread trail allowed for data to be gathered on how animals utilized their habitat when active. As almost all tracked individuals in this study were resting when relocated, this is important information that would otherwise be left unknown but may be of crucial importance when considering specific management and conservation plans of such species. Useful ecological information recorded using thread bobbins in this study included detailed information on arboreal movements, substrate utilization, and aquatic movements, with our results showing habitat preferences of specific species (e.g., arboreal and aquatic movements in *O. petolarius* and *X. severus* respectively) that were clearly recorded along the thread trail but would likely be undetected with traditional tracking methods.

Comparative assessment of method

The use of thread bobbins is a cost effective tracking method that can gather detailed ecological data over a short term study; ideal for rapidly surveying a tropical rainforest habitat (Table 3). Alternative tracking methods, such as radio-tracking are expensive in comparison and do not provide the same depth of ecological information over the short term (Key & Woods, 1996). Assumptions on the path of movement may be made, however our results have shown that ecological conclusions drawn from looking at relocation-only data could be inaccurate. The utilization of semi-arboreal and aquatic habitats by *O. petolarius* and *X. severus* are examples from this study of the ecological information you can gain from using this method over others. Such ecological information is necessary to know in order to identify key life history traits before considering future conservation plans in order to maximum success (Griffith et al. 1989). Fluorescent powders may provide similar depth of ecological information; however, they are limited in their longevity with maximum recorded distances of only 17 m and 60 m in a tropical rainforest habitat for an amphibian and a mammal species respectively (cf. a maximum of 469.3 m in this study). Furthermore, habitat complexity, humidity and frequent rain within rainforest limit the suitability of fluorescent powders (Nicolas & Colyn 2007). No expertise is necessary with the thread bobbin method, unlike radioactive isotopes or internal radio-transmitters, and as it requires basic equipment, the method would not be restricted by any issues encountered when using electronics in humid and dense tropical rainforests. In comparison, the ease of the thread bobbin method along with the ecological information it provided and its very low costs, means this technique should be considered as a useful tool when studying the ecology of rainforest species.

Table 3. Comparison of ecological survey methods used for herpetofauna in tropical rainforests. Compiled primarily based on information in Heyer et al. (1994a), Beausoleil et al (2004) and McDiarmid et al. (2012) and studies using the method with herpetofauna and/or in tropical rainforest, as well as observations and conclusions from data collected within this study. The categories low, medium, and high are qualitative scores explained specifically after each category placement and in the methods separately for each variable.

	Radio-transmitters		*Automated radio-telemetry*	*Radioactive isotopes*	*Fluorescent powders*	*Thread bobbins*
	Internal	*External*				
Equipment costs	Medium–High - <£1,000[1,2]		High - >£1,000[3,4]	Medium–High - <£1,000[5]	Low - <£100[6,7]	Low - <£100[8,9]
Longevity	High - months-years[1]		High - months-years[3]	High - months-years[10]	Low - days[7,11]	Low - days to weeks[9,12]
Detail of data on microhabitat use	Medium - exact relocation site repeatedly recorded[13,9]		Medium - relocation to within 30-142 m[3,4]	Medium - exact relocation site repeatedly recorded[5]	High - exact movements recorded[7,14]	High - exact movements recorded[9,12]
Detail of data on activity	Low–Medium - exact relocation site repeatedly recorded[13,9]		High - almost real-time activity data[3,4]	Low–Medium - exact relocation site repeatedly recorded[5]	Medium - activities and behaviors recorded at and potentially between relocations[7,15]	Medium - activities and behaviors recorded at and potentially between relocations[9]
Suitability over large distances	Medium–High - movements over km, through increased effort, dense vegetation decreases signal[3,16]		High - movements over km automatically recorded[3,4]	Low - difficult to locate over wide area[5]	Low - less than 100m[11,15]	Low–Medium - movements up to 500m
Potential impact	High - surgery/force-feeding and relocation[4,13,17]	Medium - device attachment and carrying and relocation[4,5,17]	Low–Medium - device attachment and carrying[3,4]	High - implantation/ injection, radioactive material and relocation[18]	Low – relocation[7,19]	Medium - device attachment and carrying and relocation[20]
Size minimum of animal (g)	4g[3]		4g[3]	Very small[5]	No minimum[7]	60g

Conclusions

Tropical rainforests have a highly complex three-dimensional structure in which microhabitat utilization of burrows and logs, as well as arboreal and aquatic environments are pivotal aspects in the ecology of many rainforest species. Therefore, the ability of this tracking method to provide information about the finely detailed movements of species through these features makes it a highly relevant tool when studying the ecology of tropical rainforest species. This study has displayed the suitability of the thread bobbin method for a range of species; however, there is great potential for its use on other rainforest herpetofauna species (e.g., medium–large lizards and tortoises), as well as potentially being highly applicable for a wide range of tropical rainforest taxa. Mammals and invertebrates have successfully been tracked using this method in different environments (Key & Woods, 1996; Cunha & Vieira 2002; Steinwald et al. 2006; Schlacher & Lucrezi 2010; Meyer & Cowie 2011) and studies investigating the ecology of suitably sized rainforest taxa might also consider this method as a way to provide greater in-depth information. Given the low cost, it would be worthwhile having the necessary materials readily available to use on focal species when the opportunity arises, thus maximizing the amount of ecological data that can be collected when there is a natural scarcity of encounters within short field seasons and difficulty of sampling in the tropics. Developing methods that allow for rapid collection of ecological data on tropical rainforest taxa will provide valuable information on species, leading to more detailed and informative assessment of populations over time and better evaluations to predict whether species are in need of management or conservation actions. Basic ecological information provides a starting point to understanding the species' life history traits necessary for management and conservation strategies. In this study, five out of the eight tracked species have not yet been evaluated by the IUCN, demonstrating the severe lack of basic knowledge of tropical herpetofauna populations.

References

Ashton Jr, R.E. 1975. A study of movement, home range, and winter behavior of Desmognathus fuscus (Rafinesque). Journal of Herpetology 9:85–91.

Ashton Jr, R.E. 1994. Tracking with radioactive tags. Pp. 158–163 In Measuring and monitoring biological diversity: standard methods for amphibians. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster. Smithsonian Institution Press, Washington and London.

Beaupre, S.J. and L.E. Douglas. 2009. Chapter 9: Snakes as Indicators and Monitors of Ecosystem Properties. Pp. 244–261 In Snakes Ecology and Conservation. Mullen, S. J. and R.A. Seigel. Cornell University Press, New York, USA.

Beausoleil, N.J., D.J. Mellor and K.J. Stafford 2004. Methods for marking New Zealand wildlife: amphibians, reptiles and marine mammals. Department of Conservation, Wellington, New Zealand.

Beirne, C., O. Burdekin, and A. Whitworth. 2013. Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in Eastern Ecuador. The Herpetological Journal 23:209–219.

Blankenship, E.L., T.W. Bryan and S.P. Jacobsen. 1990. A method for tracking tortoises using fluorescent powder. Herpetological Review 21:88–89.

Blomquist, S.M. and M.L. Hunter. 2007. Externally attached radio-transmitters have limited effects on the antipredator behaviour and vagility of Rana pipiens and Rana sylvatica. Journal of Herpetology 41:430–438.

Böhm, M., B. Collen, J.E.M. Baillie, P. Bowles, J. Chanson, N. Cox, G. Hammerson, M. Hoffmann, S.R. Livingstone, M. Ram, et al. 2013. The conservation status of the world’s reptiles. Biological Conservation 157: 372–385.

Collins, J.P. and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and distributions 9:89–98.

Cresswell, B. 2005. Practical radio-tracking. Biotrack Ltd., Wareham, Dorset, U.K.

Cunha, A.A., and M.V. Vieira. 2002. Support diameter, incline, and vertical movements of four didelphid marsupials in the Atlantic forest of Brazil. Journal of Zoology 258:419–426.

Díaz-Paniagua, C., C. Keller, and A.C. Andrew. 1995. Annual variation of activity and daily distances moved in adult spur-thighed tortoises, Testudo graeca, in Southwestern Spain. Herpetologica 51: 225–233.

Dole, J.W. 1965. Summer movements of adult Leopard frogs, Rana pipiens (Schreber), in northern Michigan. Ecology 46:236–255.

Dodd, C.K., Jr. 1992. Fluorescent powder is only partially successful in tracking movements of the Six-lined Racerunner (Cnemidophorus sexlineatus). Florida Field Naturalist 20:8–14.

Duellman, W.E. 1999. Patterns of distribution of amphibians: a global perspective. The Johns Hopkins University Press, Baltimore, USA.

Duellman, W.E. 2005. Cusco Amazonico: The lives of amphibians and reptiles in an Amazonian rainforest. Comstock Publishing Associates, Cornell University Press, Ithaca and London.

Eggert, C. 2002. Use of fluorescent pigments and implantable transmitters to track a fossorial toad (Pelobates fuscus). Herpetological Journal 12:69–74.

Foster, M.S., R.W. McDiarmid, and N. Chernoff. 2012. Chapter 1: Studying Reptile Diversity. Pp. 3-5 In Reptile Biodiversity: standard methods for inventory and monitoring. McDiarmid, R. W., M.S. Foster, C. Guyer, J.W. Gibbons, N. Chernoff. University of California Press, Berkeley and Los Angeles, USA, London, England

Furman, B.L.S., B.R. Scheffers, and C.A. Paszkowski. 2011. The use of fluorescent powdered pigments as a tracking technique for snakes. Herpetological Conservation and Biology 6:473–478.

Gibbons, J.W., D.E. Scott, T.J. Ryan, K.A. Buhlmann, T.D. Tuberville, B.S. Metts, J.L. Greene, T. Mills, Y. Leiden, S. Poppy and C.T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. Bioscience 50:653–666.

Griffith, B., J.M. Scott, J.W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science (Washington) 245(4917):477-480.

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster. 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press, Washington and London.

Hillman, S., P. Withers, R. Drewes, and S. Hillyard. 2009. Ecological and Environmental Physiology of Amphibians. Oxford University Press Inc., New York, USA.

Kay, W.R. 2004. Movements and home ranges of radio-tracked Crocodylus porosus in the Cambridge Gulf region of Western Australia. Wildlife Research 31:495–508

Kays, R., S. Tilak, M. Crofoot, T. Fountain, D. Obando, A. Ortega, F. Kuemmeth, J.M. Mandel, G. Swenson, T. Lambert, et al. 2011. Tracking animal location and activity with an automated radio telemetry system in a tropical rainforest. The Computer Journal, bxr072.

Key, G.E., and R.D. Woods 1996. Spool-and-line studies on the behavioural ecology of rats (Rattus spp.) in the Galapagos Islands. Canadian Journal of Zoology 74:733-737.

Lindquist, E.D., S.A. Sapoznick, E.J.G. Rodriguez, P.B. Johantgen, and J.M. Criswell. 2007. Nocturnal position in the Panamanian Golden frog, Atelopus zeteki (Anura, Bufonidae), with notes on fluorescent pigment tracking. Phyllomedusa 6:37–44.

Lips K.R. 1998. Decline of a tropical montane amphibian fauna. Conservation Biology 12:703–707.

Madrid-Sotelo, C.A., and A. Garcia-Aguayo. 2008. A simple method for externally attaching radio-transmitters to snakes. North-Western Journal of Zoology 4:335–338.

McDiarmid, R. W., M.S. Foster, C. Guyer, J.W. Gibbons, N. Chernoff. 2012. Reptile Biodiversity: standard methods for inventory and monitoring. University of California Press, Berkeley and Los Angeles, USA, London, England

Mellor, D.J., N.J. Beausoleil, and K.J. Stafford. 2004. Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand. Department of Conservation, Wellington, New Zealand.

Meyer, W.M., and R.H. Cowie. 2011. Distribution, movement, and microhabitat use of the introduced predatory snail Euglandina rosea in Hawaii: implications for management. Invertebrate Biology 130:325–333.

Munger, J.C. 1984. Home ranges of horned lizards (Phrynosoma): Circumscribed and exclusive? Oecologia 62:351–360.

Nicolas, V., and M. Colyn. 2007. Efficiency of fluorescent powder tracking for studying use of space by small mammals in an African rainforest. African Journal of Ecology 45:577–580.

Plummer, M.V. and J.W. Ferner. 2012. Chapter 9: Marking Reptiles. Pp. 143–150 In Reptile Biodiversity: standard methods for inventory and monitoring. McDiarmid, R. W., M.S. Foster, C. Guyer, J.W. Gibbons, N. Chernoff. University of California Press, Berkeley and Los Angeles, USA, London, England.

Richards, S.J., U. Sinsch, and R.A. Alford. 1994. Radio tracking. Pp. 155–158 In Measuring and monitoring biological diversity: standard methods for amphibians. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster. Smithsonian Institution Press, Washington and London.

Rittenhouse, T.A.G., T.T. Altnether, and R.D. Semlitsch. 2006. Fluorescent powder pigments as a harmless tracking method for Ambystomatids and Ranids. Herpetological Review 37:188–191.

Rowley, J.J., and R.A. Alford 2007. Movement patterns and habitat use of rainforest stream frogs in northern Queensland, Australia: implications for extinction vulnerability. Wildlife Research 34:371-378.

Schlacher, T.A., and S. Lucrezi. 2010. Compression of home ranges in ghost crabs on sandy beaches impacted by vehicle traffic. Marine biology 157:2467–2474.

Stark, R.C., and S.F. Fox. 2000. Use of fluorescent powder to track horned lizards. Herpetological Review 31:230–231.

Stark, R.C., S.F. Fox, and D.M. Leslie Jr. 2005. Male Texas horned lizards increase daily movements and area covered in spring: A mate searching strategy? Journal of Herpetology 39:169–173.

Steinwald, M.C., B.J. Swanson, and P.M. Waser. 2006. Effects of spool-and-line tracking on small desert mammals. The Southwestern Naturalist 51:71–78.

Stickel, L.F. 1950. Populations and home range relationships of the box turtle, Terrapene c. carolina (Linnaeus). Ecological Monographs 20:351-378.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Thompson, G. 1993. Daily movement patterns and habitat preferences of Varanus caudolineatus (Reptilia: Varanidae). Wildlife Research 20:227-231.

Tozetti, A.M., and M. Martins. 2007. A technique for external radio-transmitter attachment and the use of thread-bobbins for studying snake movements. South American Journal of Herpetology 2:184–190.

Tozetti, A.M., and L.F. Toledo. 2005. Short-term movement and retreat sites of Leptodactylus labyrinthicus (Anura: Leptodactylidae) during the breeding season: A spool-and-line tracking study. Journal of Herpetology 39:640–644.

Ward, M.P., J.H. Sperry, and P.J. Weatherhead 2013. Evaluation of automated radio telemetry for quantifying movements and home ranges of snakes. Journal of Herpetology 47:337–345.

Wasko, D.K., and M. Sasa 2009. Activity Patterns of a Neotropical Ambush Predator: Spatial Ecology of the Fer-de-lance (Bothrops asper, Serpentes: Viperidae) in Costa Rica. Biotropica 41:241–249.

Appendix manuscript 3 - Bamboo traps as refugia for Pristimantis olivaceus (Anura: Craugastoridae) and as breeding site for Osteocephalus castaneicola (Anura: Hylidae)

Authors and contributions

Lawrence Whittaker[1], Andrew Whitworth[1,2], Alex Fowler[1], Marcus Brent-Smith[1], Christopher Beirne[1,3] and Jaime Villacampa[1]

[1] The Crees Foundation, Mariscal Gamarra, B-5, Zona-1, 2da Etapa, Cuzco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, G12 8QQ, Glasgow, UK. E-mail: andy@crees-manu.org.

[3] Centre for Ecology & Conservation, School of Biosciences, University Exeter, Cornwall Campus, Cornwall, TR10 9EZ, UK.

Designed the study: AW AF

Collected the data: AW AF MBS

Performed the analysis: LW JV AW

Contributed to writing of the manuscript: AW LW CB JV

Citation:

Whittaker, L. Whitworth, A. Fowler, A. Brent-Smith, M. Beirne, C and Villacampa, J. (2015) Bamboo traps as refugia for *Pristimantis olivaceus* (Anura: Craugastoridae) and as breeding site for *Osteocephalus castaneicola* (Anura: Hylidae). Phyllomedusa. 14(2).

Short communication

Ecological and reproductive information on tropical amphibians remains sparse; particularly with respect to the use of phytotelm breeding sites (von May *et al.* 2009). Phytotelmatas, such as leaf axils, bromeliads, brazil-nut husks, tree cavities, and bamboo internodes are important breeding sites for several amphibians throughout the tropics (Caldwell 1993, Moravec *et al.* 2009). Bamboo internodes are one of the least documented of these, known only to be utilized in the Neotropics by *Ranitomeya sirensis, Fritziana ohausi, Nyctimantis rugiceps,* and *Aparasphenodon pomba* (Duellman and Grey 1983, Lehtinen *et al.* 2004, Waldram 2008, Assis *et al.* 2013). Here we provide newNew information about the use of bamboo internodes by *Pristimantis olivaceus* (Köhler, Morales, Lötters, Reichle, and Aparicio, 1998) and *Osteocephalus castaneicola* (Moravec, Aparicio, Guerrero-Reinhard, Calderón, Jungfer, and Gvoždík, 2009). *Osteocephalus castaneicola* is a hylid treefrog classified as "Least Concern" by the International Union for Conservation of Nature (IUCN) Red List (IUCN 2013); little is known about its ecology (Moravec *et al.* 2009, AmphibiaWeb 2015). However, some congeners have been recorded utilising phytotelmata breeding sites (e.g., Ferreira *et al.* 2012). *Pristimantis olivaceus* is an arboreal member of Craugastoridae; currently, it is considered "Data Deficient" (von May *et al.* 2008, IUCN 2015) and information about its ecology is scarce (Duellman and Lehr 2009).

The Manu Learning Centre (MLC) is a research facility owned and operated by The Crees Foundation (www.crees-manu.org) on a reserve known locally as Fundo Mascoitania. The reserve is located on the Alto Madre de Díos River, situated in the Manu Biosphere Reserve in southeastern Peru (12°47'21.849" S 71°23'28.06" W, 460 m asl, ~ 643 ha). From 2011–2014, the average seasonal rainfall in the wet season (October–March) was 3098 mm but just 1557 mm in the dry season (April–September; weather data collected from the MLC). The reserve contains a patch of bamboo (*Guadua* sp.)-dominated forest of approximately 18.2 ha. The hollow, segmented internodes of the bamboo naturally collect rain water and are an ideal reproductive site for amphibians that are able to enter them through a breakage event in the bamboo or a hole made by another animal (Waldram 2008).

We initiated a pilot study from July–August 2012 to determine whether crafted pieces of bamboo attached to trees and filled with water, would attract amphibians. Traps were cut from cross sections of bamboo internodes and fashioned into lengths of 50 cm. We discovered two instances of tadpoles of *Osteocephalus castaneicola* in the traps, as well as three adult *Pristimantis olivaceus*. The latter were brought back to the MLC to be photographed and euthanized (Figure 1).

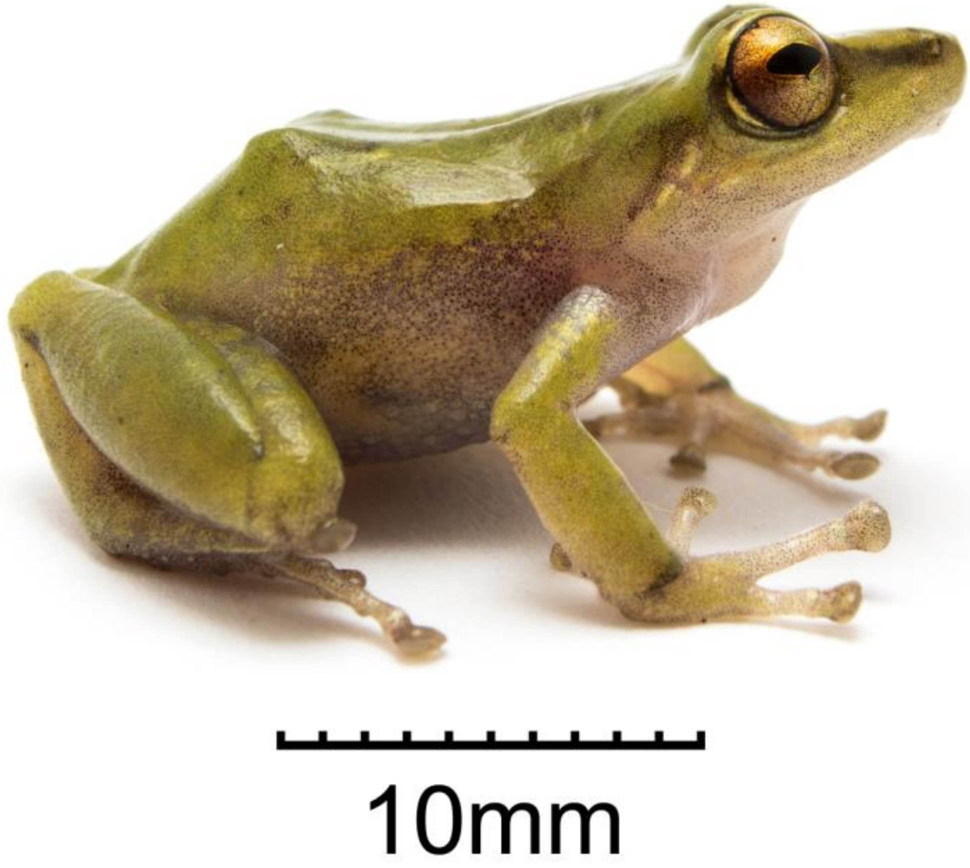


Figure 1. Individual of *Pristimantis olivaceus* collected at the MLC study area (SVL = 24.1mm).

We continued the study with a structured sampling design for a year beginning in mid-October 2012. The traps were designed with two different types of openings: (1) a large circular opening in the top of the bamboo made by removing the top, and (2) a 2 × 5-cm rectangular window cut in the side of the bamboo near the closed top. There were 14 sampling locations, each of which contained a total of four traps, as follow: a high open-top trap, a high closed-top trap, a low open-top trap and a low closed-top trap. Low traps were 60 cm and high traps 170 cm from the ground.

Traps were checked once every two weeks to ensure that a liter of water was present. If necessary, water was replenished from a nearby stream, because if the volume of water is too low, it is more difficult to determine the presence of frogs, tadpoles, or eggs (von May *et al*. 2009). In every check we recorded the presence of frogs, tadpoles or eggs. Tadpoles of *Osteocephalus castaneicola* were brought back to the MLC research station and raised to verify their identification and photograph developmental stages (Figure 2).

Figure 2. Stages of development of *O. castaneicola* in the bamboo refugia from the MLC study area (average adult SVL = 52.5mm).

A Mann Whitney-U test was conducted to determine if *Osteocephalus castaneicola* showed any preference for the height of the breeding site or the kind of opening (Hollander and Wolfe 1973). Thirty-two reproductive observations (froglets, tadpoles, or eggs) of *O. castaneicola* were made during the structured study period (total of 34 including occurrences from the pilot period). Seventeen observations were found within both closed and open-top traps, showing no preference between trap openings (w = 112, p = 0.436). Although more anurans were observed in the high traps than the low (21 and 13, respectively), the difference was not significant (w = 88, p = 0.584). *Osteocephalus castaneicola* used the traps from mid-July through November; however, no observations were made from December–June (Figure 3).

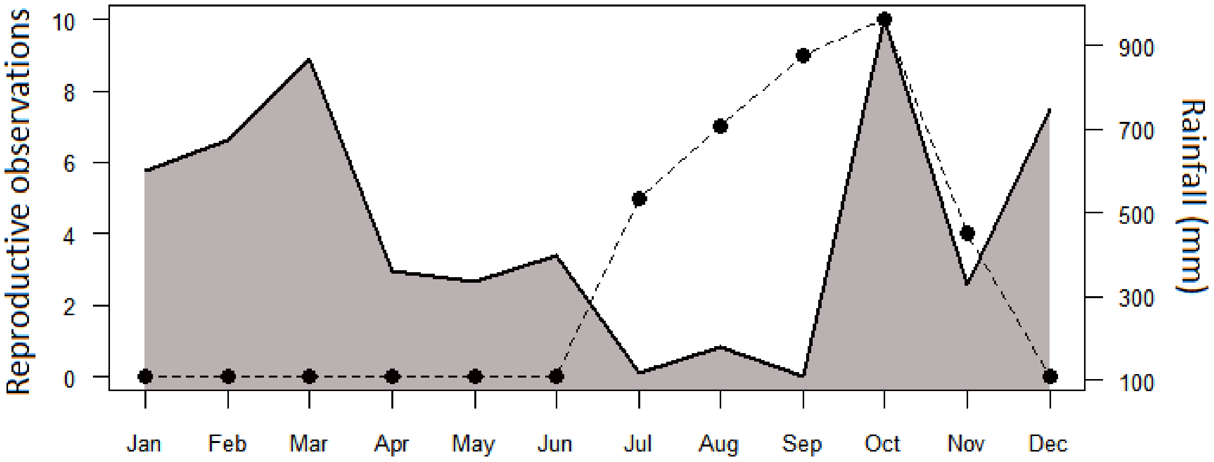


Figure 3. Presence of *Osteocephalus castaneicola* eggs, tadpoles or froglets each month (black dots) throughout the study period alongside total monthly rainfall (solid line).

Reproductive activity (recorded as the presence of froglets, tadpoles, or eggs) in *O. castaneicola* seems to begin in the middle of the dry season (July) and reach a peak by the onset of the wet season (October). Subsequently, records drastically fall off as the wet season continues through to November, after which no activity is recorded. The onset of egg deposition in July may be correlated with the presence standing water from the previous wet season; such water may be characterized by a stable high nutrient content and abundance of potential food resources such as mosquito larvae (von May *et al*. 2009). The onset of the heavy wet season may disturb this stable high nutrient environment and limit the available resources for tadpoles of *O. castaneicola* to feed and develop. *Osteocephalus castaneicola* has been documented to breed in water-filled husks of Brazil nuts (Moravec *et al*. 2009) and to avoid appropriate reproductive sites that had already been used by other species of anurans or by predacious insect larvae (Lehtinen *et al*. 2004, von May *et al*. 2009). At the MLC reserve, we also found *O. castaneicola*to breed in pitfall-traps partially filled with water. These occurrences, along with the lack of preference for open or closed-top bamboo traps, indicate that *O. castaneicola* opportunistically selects standing water sources for breeding. However, *O. castaneicola* does appear to use specific cues associated with seasonal rainfall patterns to initiate reproductive events (Figure 3).

Pristimantis olivaceus only used bamboo internodes as refugia during the dry season to protect itself from increased heat and sun in a moist, secure environment (Glorioso and Waddle 2014). We also have observed a few *P. olivaceus* in bromeliads at the MLC reserve but firm conclusions cannot be made from these data about breeding sites. Nevertheless, both bamboo internodes and bromeliads likely are important microhabitats and refugia for this species. Other studies utilizing PVC traps have reported amphibians to use the traps as refugia, rather than as water-containing breeding sites (Glorioso and Waddle 2014, Trimble and Aarde 2014).

The use of naturally occurring bamboo internodes is a convenient, cost effective way to study phytotelmata breeding/dwelling species, especially because the traps can be made from bamboo naturally present within the study area. Von May *et al.* (2009) suggested that there is no significant difference between the use of naturally occurring bamboo and artificial PVC traps.

Although originally considered to be "uncharacteristic" of anurans, phytotelmata-breeding species now are thought to comprise of 154 species worldwide (Lannoo *et al.* 1987, Lehtinen *et al.* 2004, Moravec *et al.* 2009). In terms of future progress, phytotelmata provide unique opportunities to study frog development, tadpole behavior, and predator/prey relationships of anuran species. These microhabitats are natural laboratories in which to control environmental factors such as water level, prey/predator presence, and size; factors that cannot be controlled in larger bodies of water and fast-flowing streams (Lehtinen *et al.* 2004).

References

AmphibiaWeb. 2015. Information on amphibian biology and conservation: An online reference. (11 July 2015). Berkeley, California: AmphibiaWeb. Electronic Database accessible at: http://amphibiaweb.org/. Captured on 11 July 2015.

Assis, C. L., D. J. Santana, F. A. Silva, F. M. Quintela, and R. N. Feio. 2013. A new and possibly critically endangered species of casque-headed tree frog *Aparasphenodon* Miranda-Ribeiro, 1920 (Anura, Hylidae) from southeastern Brazil. *Zootaxa 3716:* 583–591.

Caldwell, J. P. 1993. Brazil nut fruit capsules as phytotelmata: interactions among anuran and insect larvae. *Canadian Journal of Zoology 71:* 1193–1201.

Duellman, W. E. and P. Gray. 1983. Developmental biology and systematics of the egg-brooding hylid frogs, genera *Flectonotus* and *Fritziana*. *Herpetologica 39:* 333–359.

Duellman, W. E. and E. Lehr. 2009. *Terrestrial Breeding Frogs (Strabomantidae) in Peru*. Münster. Nature und Tier Verlag. 382 pp.

Ferreira, E., R. G. Rocha, A. Malvasio, and C. Fonseca. 2012. Pipe refuge occupancy by herpetofauna in the Amazonia/Cerrado ecotone. *The Herpetological Journal 22:* 59–62.

Glorioso, B. M and J. H. Waddle. 2014. A review of pipe and bamboo artificial refugia as sampling tools in anuran studies. *Herpetological Conservation and Biology 9:* 609–625.

Hollander M. and D. A. Wolfe. 1999. **Nonparametric Statistical Methods.** 2nd edition. New York. John Wiley & Sons. 787 pp.

IUCN. 2013. *Osteocephalus castaneicola*. The IUCN Red List of Threatened Species. Version 2014.3. Eletronic Database accessible at http://www.iucnredlist.org. Captured on 4 January 2015.

IUCN. 2015. *Pristimantis olivaceus*. The IUCN Red List of Threatened Species. Version 2015.2. Eletronic Database accessible at http://www.iucnredlist.org. Captured on 11 July 2015.

Lannoo, M. J., D. S. Townsend, and R. J. Wassersug. 1987. Larval life in the leaves: arboreal tadpole types, with special attention to the morphology, ecology, and behavior of the oophagous *Osteopilus brunneus* (Hylidae) larva. *Fieldiana Zoology 38:* 1–31.

Lehtinen, R. M., M. J. Lannoo, and R. J. Wassersug, 2004. Phytotelm-breeding anurans: past, present and future research. *Miscellaneous Publications, Museum of Zoology, University of Michigan 193:* 1–9.

Moravec, J., J. Aparicio, M. Guerrero-Reinhard, G. Calderón, K. H. Jungfer, and V. Gvoždík. 2009. A new species of *Osteocephalus* (Anura: Hylidae) from Amazonian Bolivia: first evidence of tree frog breeding in fruit capsules of the Brazil nut tree. *Zootaxa 2215:* 37–54.

Pounds, A. J. and M. L. Crump. 1987. Harlequin frogs along a tropical montane stream, Aggregation and the risk of predation by frog-eating flies. *Biotropica 19:* 306–309.

Trimble, M. J. and R. J. van Aarde. 2014. A note on polyvinyl chloride (PVC) pipe traps for sampling vegetation-dwelling frogs in South Africa. *African Journal of Ecology 52:* 381–383.

von May, R., K. Siu-Ting, J. M. Jacobs, M. Medina-Müller, G. Gagliardi, L. O. Rodríguez, and M. A. Donnelly. 2008. Species diversity and conservation status of amphibians in Madre De Dios, Southern Peru. *Herpetological Conservation and Biology 4:* 14–29.

von May, R., M. Medina-Müller., M. A. Donnelly, and K. Summers. 2009. Breeding-site selection by the poison frog *Ranitomeya biolat* in Amazonian bamboo forests: an experimental approach. *Canadian Journal of Zoology 87:* 453–463.

Waldram, M. 2008. Breeding biology of *Ranitomeya* biolat in the Tambopata Region of Amazonian Peru. *Journal of Herpetology 42*: 232–237.

Appendix manuscript 4 - *Osteocephalus mimeticus* (Melin, 1941) (Amphibia: Anura: Hylidae): New locality, range extension and notes on distribution.

Authors and contributions

Jaime Villacampa[1], Andrew Whitworth[1,2] and Oliver Burdekin[1]

[1] The Crees Foundation, Mariscal Gamarra, B-5, Zona-1, 2da Etapa, Cuzco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, G12 8QQ, Glasgow, UK.

Designed the study: AW JV OB

Collected the data: AW JV OB

Performed the analysis: OB

Contributed to writing of the manuscript: AW JV OB

Citation:

Ortega, J. V. Whitworth, A. & Burdekin, O. (2013). *Osteocephalus mimeticus* (Melin, 1941)(Amphibia: Anura: Hylidae): New locality, range extension and notes on distribution. CheckList, 9(5).

Abstract

We report a new locality for *Osteocephalus mimeticus* from southeast Peru which is the first record for the Madre de Dios region and a first record for Manu Biosphere Reserve. Combined with data from Jungfer *et al.* (2013) it also supposes a range extension of ~210 km to the southeast. We provide notes related to the environment in which this species has been found, along with photos of different individuals. We have produced a potential range map for the species, derived from known confirmed localities in which *O. mimeticus* has been previously found, combined with environmental and climatic data.

Main text

Osteocephalus mimeticus is a tree frog species that ranges in the Andean eastern foothills of north and central Peru and in the adjacent lowlands. This species is catalogued as Least Concern in the IUCN Red List (Angulo *et al.* 2004). We report a new locality record for this species from southeast Peru that is the first record for the Madre de Dios region and a first record for Manu Biosphere Reserve. Along with information from Jungfer *et al.* (2013) we provide a map which supposes a range extension of ~210 km to the southeast, in southern Peru.

Between 3rd March - 23rd October 2012, ten adult individuals of *O. mimeticus* were found at the side of a fast flowing stream in the Mascoitania land (71°24'15.394"W; 12°48'4.291"S, 518 m asl), on the western side of the Alto Madre de Dios basin, Madre de Dios Region (Figure 6). Two of the individuals were female (SVL = 78-83.1 mm) and eight were male (SVL = 55-57 mm).

The locality Mascoitania holds a research station and lodge; the Manu Learning Centre (71°23'28.06"W; 12°47'21.849"S, 460 m asl) that is situated next to the Alto Madre de Dios River in the buffer area of Manu National Park, on the eastern Andean foothills; between Manu National Park and the Amarakaeri Communal Reserve (Figure 1). It is a 643 ha private reserve owned by the Crees Foundation, that hosts tourism, research and volunteering activities. It contains areas of primary terra firme, regenerating logged, regenerating clear-felled and bamboo forest with an altitudinal gradient of approximately 460-700 m asl.

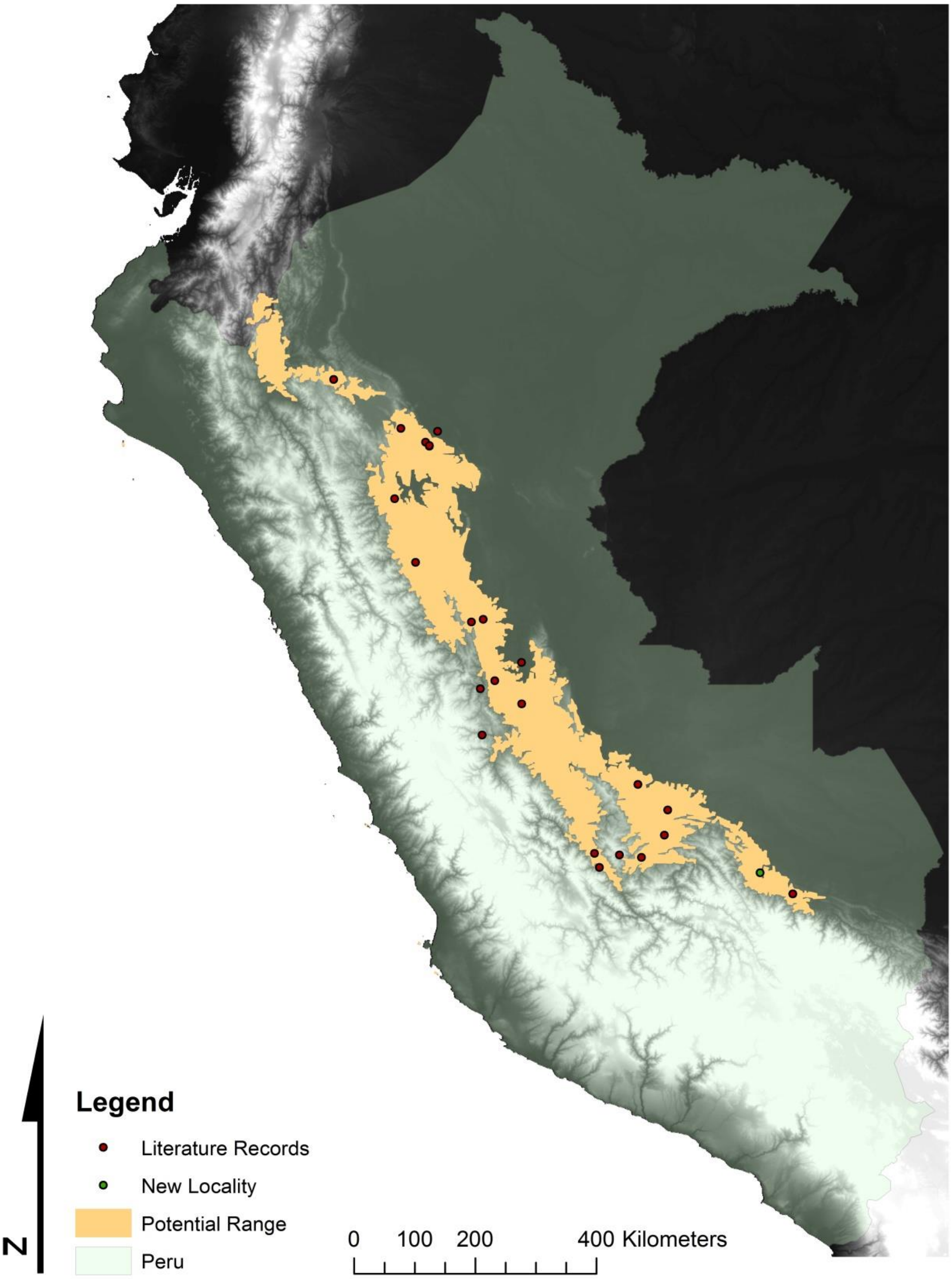


Figure 1 - Map showing locality records of *Osteocephalus mimeticus* and a potential predicted range using MaxEnt. Based on locality records reported in Chávez and Vásquez (2012), Henle (1981) and Jungfer (2010; 2013). Also showing the situation of the Manu Learning Center and the Mascoitania land (new locality).

At the Manu Learning Centre 58 confirmed species of amphibians have been recorded to date, including another three species of the genus *Osteocephalus*: *O. helenae* (also found in the same stream but usually found near small standing pools of water at the sides of the main river or within the forest), *O. taurinus* (breeding associated with seasonal temporary ponds) and *O. castaneicola* (associated with breeding in bamboo and bromeliads; arboreal water sources).

The map shown in Figure 1 was created using confirmed sightings. Environmental factors were obtained from WorldClim (version 1.4; http://www.worldclim.org/; Hijmans *et al.* 2005) and used to predict the potential distribution map of *O. mimeticus* with the modelling program MaxEnt (version 3.3.3e; http://www.cs.princeton.edu/~schapire/maxent/; Phillips *et al.* 2004; 2006). MaxEnt has been shown to perform well at low sample sizes (Hernandez *et al.* 2006; Raxworthy *et al.* 2007), as in the case of *O. mimeticus*.

Five pilot models were constructed using the WorldClim data each focussing on a different environmental variable (e.g. precipitation). Variables with the highest percent contribution to each pilot model were used to construct a further model (Baldwin and Bender 2008). This model was jackknifed to assess variable importance and to point out highly correlated variables (Baldwin 2009). Variables for the final model were chosen (see online cersion - Appendix A) and the model bootstrapped (100 replicates) to create a continuous logistic output of probability distribution. The average training area under curve (AUC) value for the replicate runs in the receiver operator characteristic (ROC) output was 0.984 showing a very good model fit.

To create a binary presence / absence map the output was processed using ArcGIS 10 using the 10 percentile training presence logisitic threshold (Cao *et al.* 2013). Land-cover classification was not used in the model due to the temporal scale of the occurrence records (Anderson and Martínez-Meyer 2004). This may have led to some overprediction and it should be noted the map shows potential distribution and not realized distribution (Phillips *et al.* 2006). Further work is required in both sampling effort and identification of potentially uninhabited areas. We suggest that this species only persists where a suitable niche and breeding site is found.

All the individuals of *O. mimeticus* were found at the side of the Mascoitania Stream (Figure 6), one of the main streams running through the reserve, into the Alto Madre de Dios River, sourcing from the Piñi-Piñi range to the west. Mascoitania Stream is a rocky fast-moving stream where individuals were found in low branches and leaves, but two males were found on the ground on a sandy stream bank. It appears that *O. mimeticus* chooses an area of these fast flowing rocky streams, that has a slower moving current, and still/slow moving pools at the side to breed in (April-September), following the higher water levels of the wet season (November-February). Shortly after finding many of the adults, various juveniles were encountered sitting on low lying vegetation along the sandy banks.

Individuals fit the description by Henle (1981; 1992) .They show strong sexual dimorphism in size and skin texture. Males have a strongly tuberculated dorsum and all of those encountered were found in this condition (Figures 2 and 3). Females had a smooth skin and two specimens that were taken contained eggs, more than 900 in MUSM #31349 (Figures 4 and 5). Both sexes have a dark brown iris with small golden blotches or lines in the centre area/around the pupil. Juveniles have a golden ground coloured dorsum with black mottling in the centre, the limbs are coloured in a similar way but with bolder mottling and the flanks are completely black. The iris is bright orange/red in colour (Figure 7).

Four voucher specimens (two males; MUSM #31350 & #31660 and two females; MUSM #31349 & #31661) were deposited at the Universidad Nacional Mayor de San Marcos **in** Lima, Peru (Permit provided by the Ministerio de Agricultura of Peru; Permit Number ‘Codigo de Tramite’: 25397; Authorisation Number ‘Autorización No.’ 2904-2012-AG-DGFFS-DGEFFS).

Figures 2-7 - (2) MUSM #31350, male, SVL = 35.3 mm (3) MUSM #31349, female, SVL = 78 mm (4) Ventral surface of male; MUSM #31660, SVL = 55 mm. (5) Ventral surface of female; MUSM #31661, SVL = 83.1 mm. (6) Mascoitania stream, where the adults, meta-morphs and tadpoles were found. (7) Juvenile, not collected.

References

Anderson, R. P. and E. Martínez-Meyer. 2004. Modeling species' geographic distributions for preliminary conservation assessments: an implementation with the spiny pocket mice (Heteromys) of Ecuador. Biological Conservation 116: 167-179.

Ángulo, A., K-H. Jungfer, R. Reynolds and J. Icochea. 2004. Osteocephalus elkejungingerae. IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2.. Electronic Database accessible at http://www.iucnredlist.org/. Captured on 24 November 2012.

Baldwin, R. A. 2009. Use of maximum entropy modeling in wildlife research. Entropy 11: 854-866.

Baldwin, R. A. and L. C. Bender. 2008. Den-site characteristics of black bears in Rocky Mountain National Park, Colorado. Journal of Wildlife Management 72(8): 1717-1724.Cao, Y., R.E DeWalt, J. L. Robinson, T. Tweddale, L. Hinz and M. Passino. 2013. Using Maxent to model the historic distributions of stonefly species in Illinois streams: The effects of regularization and threshold selections. Ecological Modelling 259: 30-39.

Chávez, G. and D. Vásquez. 2012. A new species of Andean semiaquatic lizard of the genus Potamites (Sauria, Gymnophtalmidae) from southern Peru. ZooKeys 168: 31-43.

Henle, K. 1981. Hyla elkejungingerae, ein neuer Hylide aus dem peruanischen Regenwald (Amphibia: Salientia: Hylidae). Amphibia-Reptilia 2: 123-132.

Henle, K. 1992. Zur Amphibienfauna Perus nebst Beschreibung eines neuen Eleutherodactylus (Leptodactylidae). Bonner Zoologische Beiträge 43: 79-129.

Hernandez, P. A., Graham, C. H., Master, L. L., & Albert, D. L. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography 29 (5): 773-785.

Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones and A. Jarvis, 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

Jungfer, K.-H. 2010. The taxonomic status of some spiny-backed treefrogs, genus Osteocephalus (Amphibia: Anura: Hylidae). Zootaxa 2407: 28-50.

Jungfer, K.-H., J. Faivovich, J.M. Padial, S. Castroviejo-Fisher, M.L. Lyra, B.V.M. Berneck, P.P. Iglesias, P.J.R. Kok, R.D. MacCuclloch, M.T. Rodrigues, V.K. Verdade, C.P. Torres Gastello, J.C. Chaparro, P.H. Valdujo, S. Reichle, J. Moravec, V. Gvoždík, G. Gagliardi-Urrutia, R. Ernst, I. De la Riva, D.B. Means, A.P. Lima, J.C. Señaris, W.C. Wheeler and C.F.B. Haddad. 2013. Systematics of spiny-backed treefrogs (Hylidae: Osteocephalus): an Amazonian puzzle. Zoologica Scripta, 00, 000–000.

Phillips, S. J., M. Dudik and R.E. Schapire. 2004. A maximum entropy approach to species distribution modelling; p. 655-662 in Proceedings of the 21st International Conference on Machine Learning. ACM Press, New York.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231-259.

Raxworthy, C. J., Ingram, C. O. M., Rabibisoa, N. I., & Pearson, R. G. 2007. Applications of Ecological Niche Modeling for Species Delimitation: A Review and Empirical Evaluation Using Day Geckos (Phelsuma) from Madagascar. Systematic Biology 56(6): 907-923.

Appendix manuscript 5 - *Doryfera johannae* (Bourcier, 1847) (Aves: Apodiformes: Trochilidae): New locality, range extension and notes on distribution.

Authors and contributions

Alice Brown[1], Andrew Whitworth[1,2], Alex Fowler[1], Marcus Brent-Smith[1] and Oliver Burdekin[1]

[1] The Crees Foundation, Mariscal Gamarra, B-5, Zona-1, 2da Etapa, Cuzco, Peru.

[2] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, G12 8QQ, Glasgow, UK.

Designed the study: AW OB AB

Collected the data: AW AF MBS OB

Performed the analysis: OB

Contributed to writing of the manuscript: AB AW OB

Citation:

Brown, A. G. E. Whitworth, A. W. Fowler, A. Brent-Smith, M. & Burdekin, O. (2015). *Doryfera johannae* (Bourcier, 1847)(Aves: Apodiformes: Trochilidae): new locality, range extension and notes on distribution. Check List, 11(5), 1749.

Abstract

We present a new distribution map, including new locality records for the Blue-fronted Lancebill (*Doryfera johannae*) from southeast Peru. One of these records is the first physical capture record for the Madre de Dios region and supposes a range extension of ca. 470 km to the southeast. We provide notes related to the environment in which this individual was found, along with photos of the captured female from the Manu Learning Centre in the buffer zone of Manu Biosphere Reserve.

Main text

The Blue-fronted Lancebill (*Doryfera johannae)* is a hummingbird found in Brazil, Colombia, Ecuador, Guyana, Peru, and Venezuela (Avibase 2013; Stotz et al. 1996; Schuchmann 1999). Its natural habitats comprise subtropical or tropical moist lowland forests and subtropical or tropical moist montane forests, mostly along the eastern slopes of the Andes (IUCN 2013). Two subspecies are currently recognised: *D. johannae johannae* (east slope of Andes from central-eastern Colombia to central Peru) and *D. johannae guianensis* (southern Guyana, southern Venezuela and *tepuis* of adjacent northern Brazil) (Avibase 2013; Figure 1). Schulenberg et al. (2007) report its elevational range as between 500–1,400 m above sea level (a.s.l.) and its abundance in Peru as uncommon.
Within Peru, maps of distribution currently show its existence only in northern and central Peru (BirdLife International and NatureServe 2012; InfoNatura 2007; IUCN 2013; Schulenberg et al. 2007; Clements and Shany 2001), with the exception of a visual sighting and audio recording at Amazonia Lodge (71°22'32.934" W, 012°52'12.0138" S; 500–1050 m a.s.l.) in southeastrn Peru (Walter et al. 2006; Xeno canto 2013).
Based on a model of Amazonian deforestation, over three generations (12 years) the Blue-fronted Lancebill is expected to lose 16.2–17% of suitable habitat within its distribution (Soares-Filho et al. 2006). Based on these figures the species is predicted to decline by <25% during this time (IUCN 2013), therefore understanding more about its basic ecology and present distribution is critical for future potential conservation efforts.

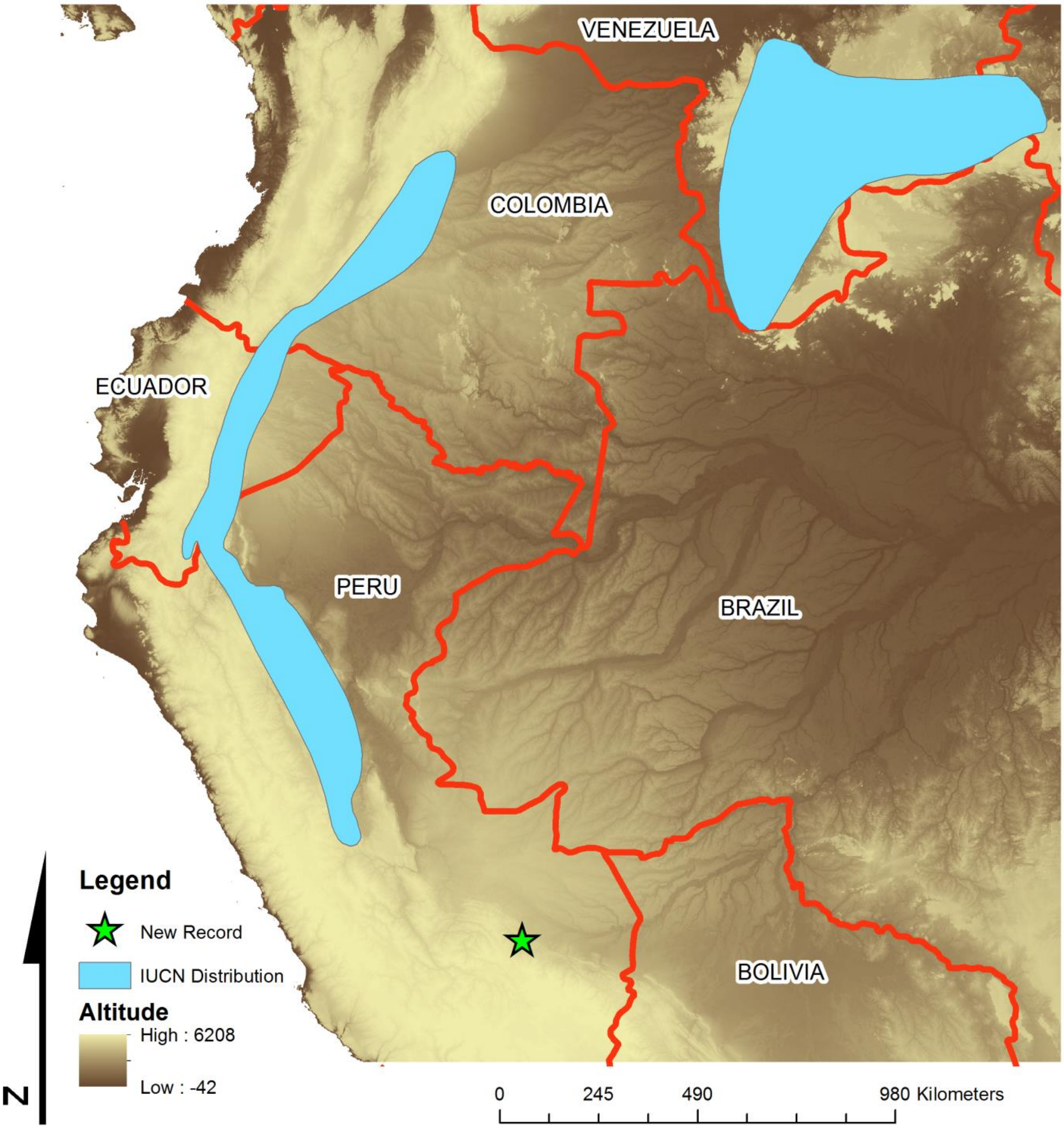


Figure 1. Map of the known distribution of *D. johannae* shaded in blue and the new locality record for *D. johannae johannae* in Peru, ca. 470 km to the southeast (shown by green star). Distribution shapefile taken from BirdLife International and NatureServe (2012).

The locality Mascoitania holds a research station and lodge; the Manu Learning Centre (71°23'28.06" W, 012°47'21.849" S, 460 m a.s.l.). The lodge is situated next to the Alto Madre de Dios River in the buffer area of Manu National Biosphere Reserve, on the eastern Andean foothills; between Manu National Park and the Amarakaeri Communal Reserve (Figure 1). Mascoitania is a 643 ha private reserve owned by The Crees Foundation, hosting tourism, research and volunteering activities. It contains areas of primary terra firme, regenerating logged, regenerating clear-felled and bamboo forest with an altitudinal gradient of approximately 460–700 m a.s.l. Over 485 species of birds have been recorded to date at the Manu Learning Centre, including 28 species from the family Trochilidae (species list available from research@crees-manu.org). All mist netting at the Manu Learning Centre was conducted under permit provided by the Ministry of Agriculture (Ministerio de Agricultura) of Peru; Permit Number (Codigo de Tramite): 25397; Authorisation Number (Autorización No.) 2904-2012-AG-DGFFS-DGEFFS.

A map was created using presence locations of *D. johannae* as recorded in the literature (Appendix 1). Environmental factors were taken from WorldClim (version 1.4; http://www.worldclim.org; Hijmans et al. 2005) and added to the modelling program MaxEnt (version 3.3.3e; http://www.cs.princeton.edu/~schapire/maxent/; Phillips et al. 2004, 2006). No land use variables were included due to the historical nature of the presence locations. MaxEnt has been shown to perform well at low sample sizes (Hernandez et al. 2006, Raxworthy et al. 2007), such as the sample size for *D. johannae*. Five pilot models were constructed using the WorldClim data, four of which focussed on a different environmental variable (e.g., precipitation), with the fifth including all environmental variables. Variables with the highest permutation importance to each pilot model were used to construct a further model (Baldwin and Bender 2008). This model was jackknifed to assess variable importance and to identify highly correlated variables (Baldwin 2009). The final model was bootstrapped (100 replicates) to create a continuous logistic output of probability distribution.

In order to address sampling bias all models were run with a mask to limit the area used for background points by MaxEnt (Merow et al. 2013). The mask was determined by splitting the samples into two distinct groups (sample A and sample B, Figure 3). A minimum convex hull was drawn around each sample group, buffered by the observed mean distance for each group determined by running the Average Nearest Neighbor tool in ArcGIS 10 Spatial Analyst. Results from the model were then projected onto the previously masked area using MaxEnt. To create a binary suitable/unsuitable habitat map the output was processed in ArcGIS 10 using the maximum training sensitivity plus specificity logistic threshold (Cao et al. 2013).

At 7:25h on 2 August 2013, a female *D. johannae* was captured during a mist-netting session on the Mascoitania land (71°24'25.886" W, 012°48'33.475" S, 700 m a.s.l.) on the western side of the Alto Madre de Dios basin, Madre de Dios Region. It was captured in a 10 m mist-net setup heading east to west along a ridge line trail within primary forest. The bird was measured (Table 1), photographed (Figure 2) and then released.

Table 1 - Physical measurements (in g and mm) from the captured female

Weight	Primaries	Tail length	Total length	Culmen length	Culmen width	Culmen depth
8.5 g	52 mm	29 mm	95 mm	32.5 mm	3.1 mm	2.5 mm

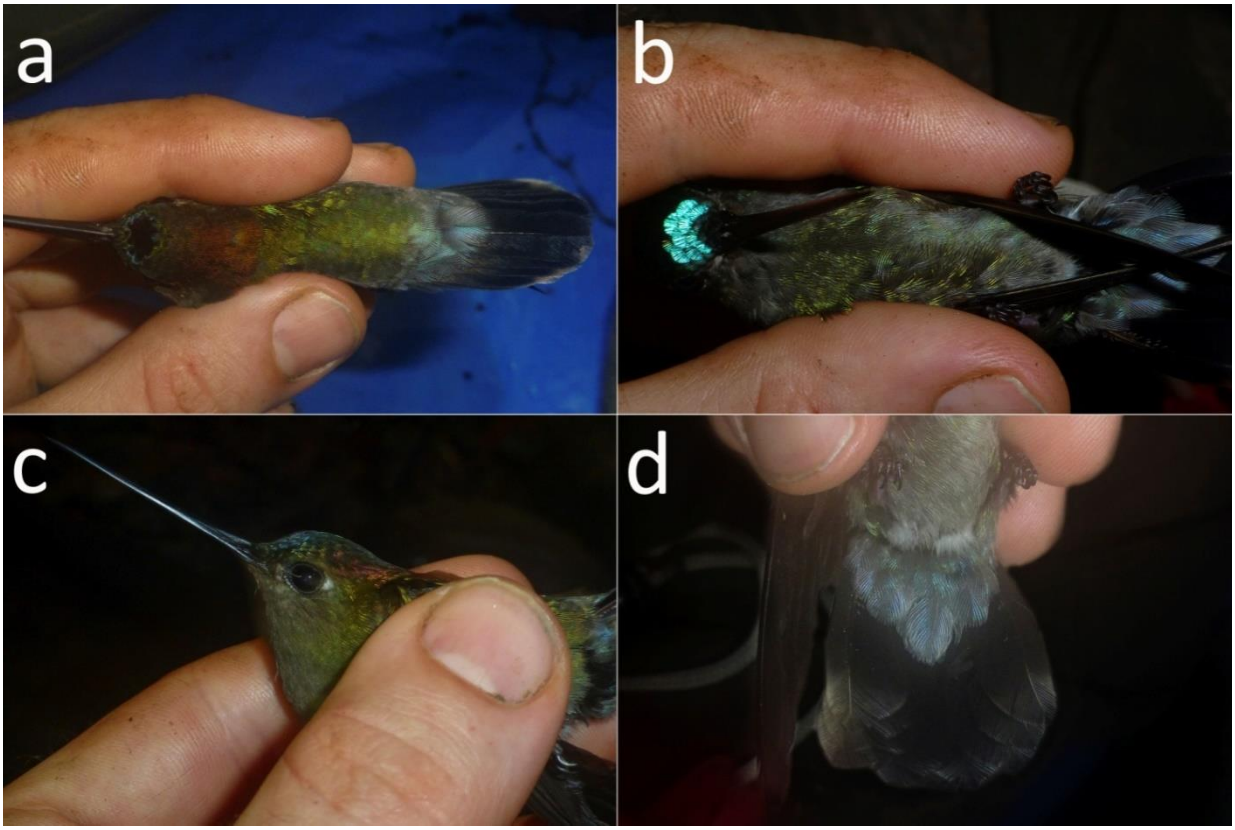


Figure 2 - Photographs of the captured female individual.

The map in Figure 3 shows the presence locations of *D. johannae* as recorded in the literature along with the predicted distribution map created in this study. The average training area under curve (AUC) value for the replicate runs in the receiver operator characteristic (ROC) output was 0.92 in the final model, showing a very good model fit. However, there is evidence of some overprediction and it should be noted that the map shows only potential and not realised distribution (Phillips et al. 2006).

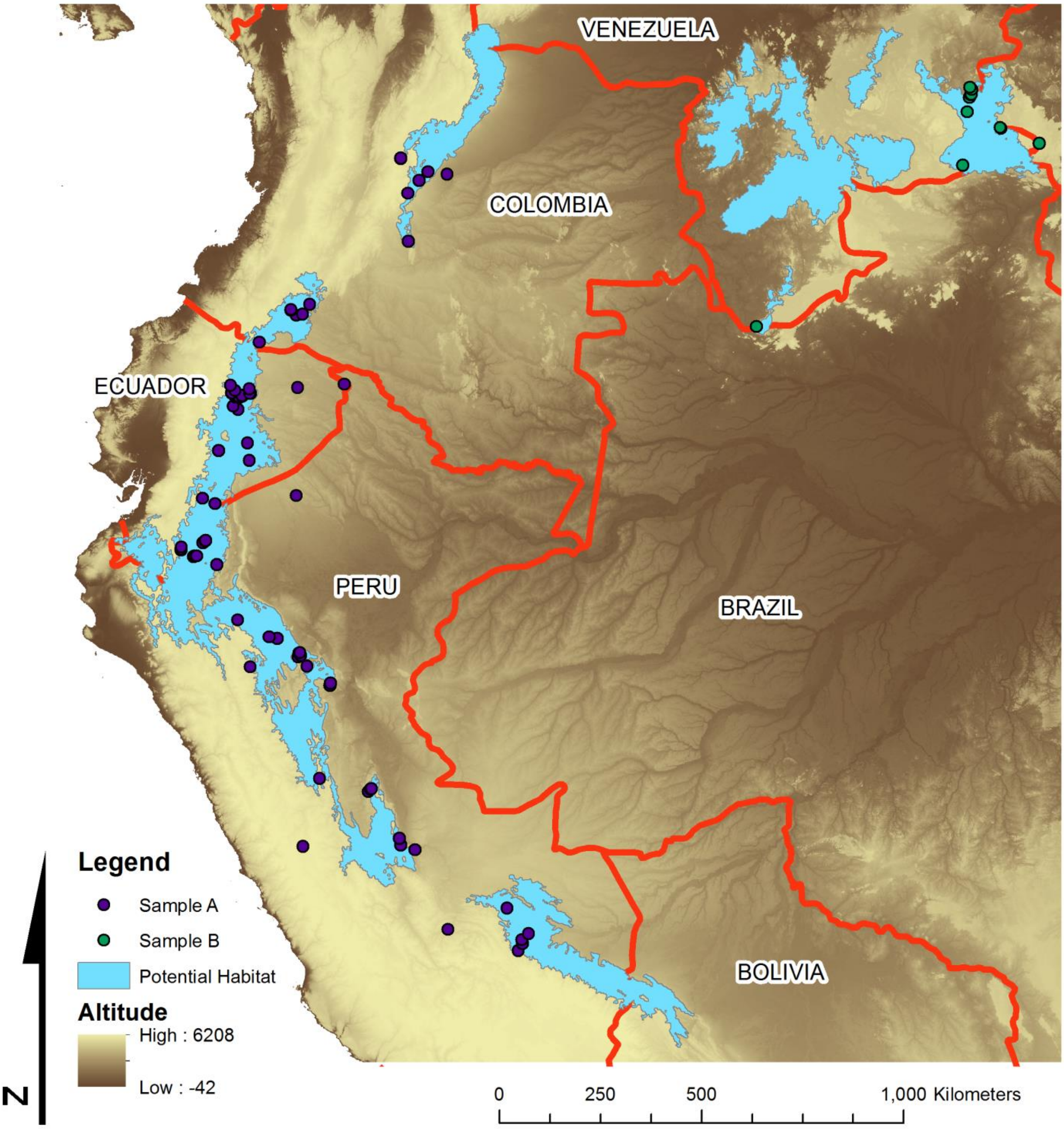


Figure 3. Known locality records of both *D. johannae johannae* (Sample A records) and *D. johannae guianensis* (Sample B records) and a map of the potential distribution; with new records from southeast Peru projecting a range extension for the species of approximately 470 km to the southeast, from the Pasco region of central Peru.

Previous maps of the distribution of *D. johannae* show the species to be present in northern and central Peru. This mist-net capture from the Mascoitania land is the first physical capture record for the Madre de Dios region and the Manu Biosphere Reserve. Along with other audio and visual records from southeast Peru our findings represent a range extension for the species from the Pasco region of central Peru to ca. 470 km to the southeast. Our map projection indicates that this species may exist even further to the southeast of Peru and into the northwest region of Bolivia. However, as there is evidence that the map may show some degree of over prediction (Phillips et al. 2006), physical verification of this predicted presence in Bolivia is needed.

References

Avibase (2013). Avibase – Bird checklists of the world. Accessed at http://avibase.bsc-eoc.org/checklist.jsp?lang=EN, 26 October 2013.

Baldwin, R.A. (2009). Use of maximum entropy modeling in wildlife research. Entropy 11: 854 866. doi: http://dx.doi.org/10.3390/e11040854

Baldwin, R.A. and Bender, L.C. (2008). Den-site characteristics of Black Bears in Rocky Mountain National Park, Colorado. Journal of Wildlife Management 72(8): 1717–1724. doi: http://dx.doi.org/10.2193/2007-393

BirdLife International and NatureServe (2012). Bird species distribution maps of the world. BirdLife International, Cambridge, UK and NatureServe, Arlington, USA.

Cao, Y., DeWalt, R.E., Robinson, J.L., Tweddale, T., Hinz, L. and Passino, M. (2013). Using Maxent to model the historic distributions of stonefly species in Illinois streams: The effects of regularization and threshold selections. Ecological Modelling 259: 30–39. doi: http://dx.doi.org/10.1016/j.ecolmodel.2013.03.012

Clements, J.F. and Shany, N. (2001). A field guide to the birds of Peru. Temecula, CA: Ibis Publishing Company.

Hernandez, P.A., Graham, C.H., Master, L.L., and Albert, D.L. (2006). The effect of sample size and species characteristics on performance of different species distribution modelling methods. Ecography 29(5): 773–785. doi: http://dx.doi.org/10.1.1.307.3273

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978. doi: http://dx.doi.org/10.1002/joc.1276

InfoNatura (2007). Animals and Ecosystems of Latin America [web application]. Version 5.0 . Arlington, Virginia (USA): NatureServe. Accessed at http://www.natureserve.org/infonatura, 26 October 2013.

IUCN. 2013. The IUCN Red List of threatened species. Version 2013.1. Accessed at http://www.iucnredlist.org/, 26 October 2013.

Merow, C., Smith, M.J., and Silander Jr, J.A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography 36: 1058–1069. doi: http://dx.doi.org/10.1111/j.1600-0587.2013.07872.x

Phillips, S.J., Dudik, M. and Schapire, R.E. (2004). A maximum entropy approach to species distribution modelling; pp. 655–662, in: Proceedings of the 21st International Conference on Machine Learning. New York: ACM Press. http://www.cs.princeton.edu/~mdudik/PhillipsDuSc04.pdf

Phillips, S.J., Anderson, R.P. and Schapire, R.E. (2006). Maximum entropy modelling of species geographic distributions. Ecological Modelling 190: 231–259. doi: http://dx.doi.org/10.1016/j.ecolmodel.2005.03.026

Raxworthy, C., Ingram, C., Rabibisoa, N. and Pearson, R. (2007). Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (Phelsuma) from Madagascar. Systematic Biology 56(6): 907–923. doi: http://dx.doi.org/10.1080/10635150701775111

Schuchmann, K. L. (1999). Family Trochilidae (Hummingbirds); pp. 468–680, in: J. del Hoyo, A. Elliott and J. Sargatal (eds.). Handbook of the birds of the world, volume 5: barn owls to hummingbirds. Barcelona: Lynx Edicions.

Schulenberg, T. S., Stotz, D. F., Lane, D. F., O'Neill, J. P. & Parker, T. A. (2007). Birds of Peru. Princeton, NJ: Princeton University Press.

Soares-Filho, B. S., Nepstad, D.C., Curran, L.M., Cerqueira, G. C., Garcia, R.A., Ramos, C.A., Voll, E., McDonald, A., Lefebvre, P., Schlesinger, P. (2006). Modelling conservation in the Amazon basin. Nature 440(7083): 520–523. doi: http://dx.doi.org/10.1038/nature04389

Stotz, D. F., Fitzpatrick, J. W., Parker, T. A., and Moskovits, D. K. (1996). Neotropical birds: ecology and conservation. University of Chicago Press, Chicago.

Walker, B., Stotz, D. F., Pequeño, T. and Fitzpatrick, J. W. (2006). Birds of the Manu Biosphere Reserve, pp. 23–49; in B.D. Patterson, D. F. Stotz, and S. Solari (eds.). Mammals and birds of the Manu Biosphere Reserve, Peru. Fieldiana, Zoology (n.s.) 110.

Xeno canto (2013). Xeno canto – Sharing bird sounds from around the world. Accessed at http://www.xeno-canto.org, 26 October, 2013.