El Niño and the structure of mutualistic and antagonistic bat food webs revealed by DNA barcoding

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Abstract

El Niño is a climatic event that can have large-scale impacts on global rainfall patterns, causing severe droughts in some regions and floods in others. The frequency of strong El Niño events is expected to increase in the future under scenarios of climate change. Despite this, the consequences of El Niño-induced droughts for ecological interactions are poorly understood. Here I applied DNA barcoding to assess the diets of frugivorous and insectivorous bats in the dry forest and rainforest of Costa Rica during one of the strongest El Niño on record (2015) and compare it with a non-El Niño year. My data indicated that the mutualistic network structure observed during the El Niño event was similar in both dry forest and rainforest, despite these habitats experiencing droughts and flooding, respectively. However, during the non-El Niño wet season in the dry forest, niche overlap was higher than the El Niño event. Antagonistic networks showed little change in the overall size and diversity of modules of interaction, but there were significant changes in modularity and the position of the nodes between the networks constructed during the El Niño year versus the normal year in dry forest. Additionally, I evaluated the relationship between wing morphology and diet specialization and differentiation of individuals. I observed that individuals of a common insectivorous bat species, *Pteronotus mesoamericanus*, showed differences in diet that correlated with wing morphology. To conclude, El Niño was associated with similar changes in the organisation of mutualistic networks in both dry and wet forests, as well as with modifications at the node level in antagonistic networks of dry forest. Such changes could have profound impacts for network resilience and the maintenance of interactions and species at both sites over time.

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Author's Declaration

I certify that the research presented in this thesis is the product of my own original work, except where due acknowledgement has been made in accordance with standard referencing practices of the biological sciences.

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The views expressed in this thesis, unless otherwise referenced, are my own and do not express the views of Queen Mary University of London.

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CHAPTER ONE

General Introduction

Origins of network theory

Network science is an old scientific field that was initially explored by Leonhard Euler in 1736 in an article called Seven Bridges of Königsberg (Euler, 1736). In this article, he described that it was impossible to cross a set of bridges in Russia in such a way that each bridge is only crossed once. Since the publication of this paper, there have been great advances in the field of network theory and analysis. However, most studies involving ecological networks can be grouped in three major categories: traditional food webs, host-parasitoid webs, and mutualistic webs (Ings *et al.* 2009). Traditional food webs and host-parasitoid webs have been explored for years (Ings *et al.* 1999), while the structure of mutualistic webs are of more recent interest and only became fairly common in the literature in the 2000s and 2010s (Bascompte *et al.* 2003; Jordano *et al.* 2003; Bascompte & Jordano 2007; Olesen *et al.* 2007; Dáttilo *et al.* 2016; Guimarães *et al.* 2017).

During the 1990s, important papers addressed the empirical and theoretical basis of network ecology, and others revealed wide patterns of network structure across different systems (Paine 1998; Polis 1991; Watts & Strogatz 1998; Barabási & Albert 1999). Watts and Strogatz (1998) verified that rather than being completely random or ordinated, network systems can have intermediate properties. Thus, they can be highly clustered, like regular graphs, but at the same time, similar to random graphs, have small path lengths. On the other hand, Barabási and Albert (1999) have showed that different network systems including genetic networks and even the World Wide Web have a complex network topology in which there is a power-law distribution for the vertex connectivities. However, when network theory was applied to ecological studies, some problems arose, such as: species diversity, individual variation, and interaction loops that were inadequately represented in the networks (Polis 1991).

Ecological networks for characterising trophic interactions

Network analysis offers a powerful approach for studying species interactions and an increasing number of publications are using networks for assessing ecosystem function (Ings *et al.* 2009). In recent years, different network metrics have been found to show specific trends across latitudinal gradients and across different environments (Cagnolo *et al.* 2011; Schleuning *et al.* 2012; Roslin *et al.* 2017). However, very few studies have addressed the importance of creating and analyzing networks at the individual level (e.g. Araújo *et al.* 2010; Guerra *et al.*

2017; Kuhnen *et al.* 2017), with some studies showing that the diets of specialist individuals are nested within the diets of generalists (Araújo *et al.* 2010; Pires *et al.* 2011).

Species interactions both at the individual- and community-level in ecological networks can be divided into multiple functional types, such as antagonistic, mutualistic, commensalist, competitive and parasitic), although such interactions are more simply classified as being either antagonistic or mutualistic. Mutualistic networks are composed of species that derive benefit from interacting, while in antagonistic networks one species benefits and the other loses from the interaction (Bascompte 2010). Mutualistic and antagonistic networks tend to differ in their structure. To describe and compare networks, researchers have developed several metrics that quantify general properties; these include nestedness and modularity (Tylianakis *et al.* 2010). Nestedness measures the degree to which the interactions of more specialized species are a subset of the interactions of the more generalist species in the community (Tylianakis *et al.* 2010). Modularity measures the degree to which species form clusters of interactions within which they have a higher density of interactions compared to their interactions with nodes outside the cluster (Rezende *et al.* 2009).

Multiple studies have suggested that nestedness and modularity are the metrics most likely to show changes across different ecological gradients (Burgos *et al.* 2007; James *et al.* 2012; Trøjelsgaard & Olesen 2013; Dalsgaard *et al.* 2013). Nestedness and modularity are both related to an increase in network stability and the minimization of perturbations from ecological disturbances, but they act in different ways (Memmott *et al.* 2004; Fortuna *et al.* 2010*;* Thébault & Fontaine 2010). Nestedness increases network stability through promoting high functional redundancy among species. Nestedness is also believed to be important in reducing interspecific competition (Bastolla *et al.* 2009). Modularity on the other hand is associated with clusters of individuals that interact more strongly with each other than with individuals in other clusters. High modularity is thought to reduce the chance that perturbations within a module are propagated to other parts of the network, and is also considered to promote network stability.

Many studies analysing variation in ecological networks and their key metrics across environmental gradients have relied on null models, both to interpret observed patterns as well as perform comparisons of networks (Pellissier *et al.* 2018). Some of the most commonly used null models in ecological network comparisons include the so-called Patefield algorithm, shuffle, swap, vaznull, and Erdõs-Rényi method (Erdõs & Rényi 1960; Patefield 1981; Vázquez *et al.* 2007; Dormann *et al.* 2008), which differ in the levels and ways of constraining the network matrices during the randomization procedures. For example, while the Patefield algorithm constrains the network by fixing the marginal total sums of the observed interaction matrix during the randomization, the shuffle algorithm constrains the network dimensions, the vaznull algorithm constrains the network's connectance and partially the total marginal sums, while the Erdõs-Rényi constrains the network's dimensions while creating a Poisson distribution of edges.

Mutualistic networks have been shown to be highly nested and modular (Bascompte & Jordano 2013), and while antagonistic networks are also more nested, the degree of modularity is more variable (Nuwagaba & Hui 2015). However, measurements of network structure can be influenced by multiple factors, such as sampling methods and sampling effort, as well as natural variation due to, for example, rainfall and resource availability (Nielsen & Bascompte 2007; Gibson *et al.* 2011; Laurindo *et al.* 2017). In the case of mutualistic networks, nestedness is significantly higher in dry conditions while modularity is higher during periods of increased rainfall (Rico-Gray *et al.* 2012; Dalsgaard *et al.* 2013; Trøjelsgaard & Olesen 2013; Schleuning *et al.* 2014). Thus both metrics are useful for evaluating the effect of shifts in precipitation in species interactions within different ecosystems. Nestedness and modularity can be measured using different algorithms such as NODF, wine, sort and binmatnest for nestedness (Rodríguez-Gironés *et al.* 2006; Almeida-Neto *et al.* 2008; Dormann *et al.* 2008; Galeano *et al.* 2009) and QuanBIMO, cluster walktrap, cluster optimal and fast greedy for modularity (Clauset *et al.* 2004; Csárdi & Nepusz 2006; Pons & Latapy 2006; Dormann & Strauss 2014) in ecological networks.

Table 1.1. Network measures and their ecological significance.

Network measure	Values	Measures	Ecological significance
Closeness centrality (CC)	0 (low) to 1 (high)	The position and importance of a node in the network. The higher the value, the more connected it is.	Prey species with high CC are under greater predatory pressure. Predator species with high CC are more generalist. Removal of prey species with high CC can lead to secondary extinctions.
Modularity	-1 (low) to 1 (high)	The partitioning of nodes and their connections inside the network into discrete clumps.	Species that segregate their niche are placed in different modules of interaction. Predator species in the same module have higher niche overlap. Prey in the same module are consumed by similar predators.
Nestedness	0 (low) to 1 (high)	The overlap between the diet of specialists and generalists.	Networks that are highly nested have most of the diet of the specialist species included as a subset of the diet of generalist species. Higher values of nestedness means that the network is more resilient to the loss of species, but at the species level suggests higher competition.
Number of compartments	0 to ∞	The isolated sub-sets of interactions that do not have any connections with the rest of the network.	Prey and predators present in one compartment have zero overlap or interaction with predators and prey present in other compartments
Robustness	0 (low) to 1 (high)	Measures the area below the curve of secondary extinction of one level when primary extinction of the other level is simulated.	If the robustness of one the levels is high, this level is very resilient to the extinction of species in the other level of the network.
Weighted connectance	0 (low) to 1 (high)	Linkage density divided by species richness in the network.	Higher values of weighted connectance means that each species in the network has a higher number of partners with whom they share links.
Average path length	0 (low) to ∞ (high)	The average number of links that is needed to go through any two nodes in the network.	If the average path length of a network is low, it means that all species are more tightly connected and competition for resources is high.
Niche overlap	0 (low) to 1 (high)	Measures the mean proportion of shared items in the diet of every species pair in the network.	Higher values of niche overlap between species suggests that competition is higher inside the network.

Traditional versus molecular approaches to build ecological networks

Any study of trophic interactions requires knowledge of species' or individuals' diets. However, accurate determination of the components of a species' diets is not straightforward (Deagle *et al.* 2005). Traditional approaches for measuring trophic interactions have typically relied on field observations, microscopic identification of prey items, and sometimes rearing specimens followed by morphological identification. All of these methods require knowledge of taxonomy, and are prone to limitations and biases in the resolution and accuracy of species identifications, and in sampling completeness (Evans *et al.* 2016). For example, these approaches tend to overlook cryptic and small species, which are usually hard to sample and detect, making hard to compare samples between researchers and systems (Symondson 2002; Lafferty *et al.* 2008).

During the mid 2000s there was a shift in the study of animal diets, away from more traditional approaches to the incorporation of molecular techniques such as DNA barcoding for diet characterization (Symondson 2002; Pompanon *et al.* 2012). DNA barcoding provides the means to identify species from trace material and thereby increase the resolution and quality of the characterization of trophic links. The use of such molecular methods such as DNA barcoding to describe biological communities in environmental samples, and trophic links in ecological networks, has allowed not only a better description of the species present in the community, but also the calculation of different values for some network metrics, such as: vulnerability, nestedness, and linkage density (Wirta *et al.* 2014; Derocles *et al.* 2015; Toju 2015; Decaëns *et al.* 2016; Evans *et al.* 2016; Roslin & Majaneva 2016). Yet despite this, only a few studies have used DNA barcodes to study species interactions within a network approach (García-Robledo *et al.* 2013; González-Varo *et al.* 2014; Wirta *et al.* 2014; Derocles *et al.* 2015; Erickson *et al.* 2017).

While molecular approaches can improve resolution in dietary studies, building entire ecological networks is labour intensive, and there are only a few well-resolved networks constructed to date (Vacher *et al.* 2016). Nevertheless, this method can speed up the process of taxonomic identification, particularly in species-rich communities (Ji *et al.* 2013; Evans *et al.* 2016) and the description of many different species' diets are now being characterised even with only minimal previous knowledge of the food items consumed (Boyer *et al.* 2013; Brown *et al.* 2012). Despite this, there are some problems and limitations in the application of DNA barcoding in ecological studies. In most cases DNA barcoding has been combined with high throughput sequencing (HTS) in an approach called metabarcoding, which is particularly useful for mixed templates (e.g. multiple dietary items). However, some taxa (e.g. Hymenoptera) do not amplify well during PCR procedures (Yu *et al.* 2012) particularly in mixed samples. Similarly, the taxonomic composition recovered may be biased by the use of a particular primer set, the number of PCRs carried out, the parameters used for sequence filtering, the threshold for the number of haplotypes retained (e.g. by copy numbers) and the operational taxonomic unit (OTU) clustering threshold applied (Flynn *et al.* 2015; Alberdi *et al.* 2018). In particular, the OTU thresholds rarely equate to traditional species boundaries, as the genetic variability for multiple individuals within one species may not match the thresholds set to define the OTUs for another species (Brown *et al.* 2015). Thus, individuals from one species can be represented across multiple OTUs or conversely multiple species may be clustered within a single OTU. As a consequence, networks constructed with OTUs are best thought of as containing nodes that represent equal pools of genetic diversity rather than a traditional taxonomic level.

Droughts and seasonality, and their potential impacts on network structure

Changes in precipitation and seasonality are known to have multiple effects on species interactions. These arise through either direct responses such as via changes in metabolic rates and/or physiological stress, or indirect responses such as through changes in the abundance of particular species (Woodward *et al.* 2010b). However, few studies have examined the impact of environmental change on species interactions. Exceptions to this trend have tended to analyse changes in network structure caused by climatic warming (Woodward *et al.* 2010a), and no such studies have included networks in which interactions were inferred using molecular data. The factors influencing network structure in response to environmental perturbation can be attributed to three main properties: immigration, speciation, and environmental filtering (Weiher & Kendy 1999). Depending on which species remain in the community following these processes, the network might undergo shifts in structure and stability (Romanuk *et al.* 2009).

A major perturbation in the context of precipitation and global warming is drought. Indeed, climate projections in the near future show that temperature increases in many parts of the world will lead to seasonal droughts (IPCC 2014). Drought stress is one of the main causes of plant death (Zeppel *et al.* 2013) and has been shown to lead to a rewiring of interactions in networks (Lu *et al.* 2016), and can also result in outbreaks of phytophagous insects (Mattson &

Haack 1987) as well as shifts in insect migration (Srygley *et al.* 2010). Different rainforests worldwide are predicted to experience not only a reduction in total annual rainfall, but also longer and more severe dry seasons (Borchert 1998). The effects of seasonal changes in precipitation on plants depend on soil water content (Zeppel *et al.* 2014), however, small reductions in rainfall across a small latitudinal gradient have been responsible for the transition from a rainforest to a savanna-like environment (Rawitscher 1948; Nepstad *et al.* 1994). Thus, the increase in the frequency of drought events associated with extreme seasonality are likely to have an impact on forest tree composition with cascading effects on ecosystem service dynamics and the survival of the species that are dependent on these resources.

El Niño and networks

The El Niño Southern Oscillation (ENSO) is marked by periodic oscillations in the temperatures of the sea-surface of the Pacific Ocean (Rasmusson & Carpenter 1982; Ropelewski & Halpert 1987; Trenberth & Hurrell 1994). This event occurs every three to six years and alters patterns of precipitation worldwide, leading to both severe droughts and floods (Holmgren *et al.* 2001). During El Niño events, there is an elevation of the ocean surface temperatures of the equatorial Pacific Ocean and a reduction in the emergence of cold-water in the Pacific Coast of South America (Ropelewski & Halpert 1987). The event lasts for one year, during which rainfall can increase tenfold in some areas (Holmgren *et al.* 2001). El Niño events are increasing in magnitude, with the frequency of strong events expected to increase in future years (Post 2013; Power *et al.* 2013; Cai *et al.* 2014).

In Central America, El Niño can provoke big changes in rainfall over relatively small distances (Waylen *et al.* 1998). For example, while the rainforests of the Caribbean coast of Costa Rica face floods in El Niño conditions, the dry forests of the Pacific coast experience severe droughts (Waylen *et al.* 1998). Extreme rainfall conditions can be categorized as events that fall below the 10th percentile, or above the 90th percentile, of the overall probability density function (Seneviratne *et al.* 2012). At the same time, events that are currently considered rare are likely to become more normal in the future, with unknown consequences. To date, few studies have monitored ecological interactions in extreme climatic conditions (Knapp *et al.* 2008). The El Niño of 2015-2016 was considered the strongest ever registered, and was associated with record water evaporation and temperature anomalies in the western Pacific Ocean (Avery *et al.* 2017). This event caused extremes in temperature and precipitation in different parts of the world, such as the

Amazon forest, Tasmania and China (Jiménez-Muñoz *et al.* 2016; Karoly *et al.* 2016; Wang *et al.* 2017) and only now the consequences have started to be assessed. Although some studies have assessed the impact of El Niño on species' diets (Putt & Prézelin 1985; DeLong *et al.* 1991; Grover *et al.* 2002; Salazar & Bustamante 2003), only one study to date has analysed such effects using a network approach (Stapp *et al.* 1999).

Precipitation changes associated with El Niño are known to lead to alterations in population dynamics and phenology of different animal and plant species (Lima *et al.* 1999; Sillet *et al.* 2000; Marshal *et al.* 2002; Garcia *et al.* 2003). As a consequence, El Niño events are correlated with changes in primary productivity, insect abundance and fruit availability (Jaksic *et al.* 1997; Holmgren *et al.* 2001; Ting *et al.* 2008), with potential knock-on effects for frugivorous and insectivorous animals (Butt *et al.* 2015), although such consequences have rarely been analysed using molecular methods. Impacts on flower and fruit production that arise from dramatic El Niño-induced fluctuations in precipitation, whether from drought or flood, can affect food resources for several months (Owens 1995; Gunarathne & Perera 2014). These changes can impact how species interact with their food sources (Wright *et al.* 1999; Meserve *et al.* 2009). Although there are well-documented relationships between precipitation and trophic interactions (Butt *et al.* 2015), relatively little is known about the potential consequences of these changes in mutualistic and antagonistic networks (Stapp *et al.* 1999; Lima *et al.* 2002). In one of the few cases where this was monitored, an extreme drought caused by an El Niño event led to the extinction of all wasp pollinators and the breakdown of mutualisms (Harrison 2000).

Bats (Mammalia: Chiroptera)

Bats are the second most speciose order of mammals with 1,300 species worldwide (Fenton & Simmons 2015). Phyllostomidae is one of the most diverse families of bats with approximately 160 species distributed in the Neotropical region (Simmons 2005; Fenton & Simmons 2015). This group shows unparalleled dietary diversification among mammals with a wide range of feeding habits ranging from insectivory to carnivory to hematophagy, although most species can be classified as predominantly insectivorous or frugivorous (Herrera *et al.* 2001; Muscarella & Fleming 2007; Fleming 2009; Clare *et al.* 2014). Most of the other bat families in the Neotropics contain insectivorous species only (Willig *et al.* 1993; Paglia *et al.* 2012; Oliveira *et al.* 2015; Emiliano *et al.* 2017). Although the diets of many bat species in the Neotropics have been described and analysed, there are very few studies involving molecular methods, and none have focused on frugivorous bat species.

Ecological networks remain a largely unexplored area for bat ecology, with only few studies in Latin America (Dumont *et al.* 2011; Mello *et al.* 2011a; Mello *et al.* 2011b; Laurindo *et al.* 2017; Zapata-Mesa *et al.* 2017). Thus, Neotropical bats, and particularly phyllostomids, present a unique opportunity to evaluate changes in antagonistic and mutualistic interactions within a group of related species. While few studies have examined bat-plant interaction networks, the findings from one paper report that these networks were robust to the removal of bats, and were also characterised by modular structures with an average of four modules identified (Mello *et al.* 2011b). The same study found that interactions inside modules showed a genus-to-genus pattern of association between bats and plants (*Artibeus* with *Ficus*, *Carollia* with *Piper* and *Sturnira* with *Solanum*) (Mello *et al.* 2011b). Bat-fruit networks have also been showed to have higher connectance and nestedness than bird-fruit networks with most of the differences explained by species richness (Mello *et al.* 2011a). Interestingly these networks appear to show strong effects of changes in fruit abundance, which can be caused by changes in temperature and rainfall patterns due to climate change (Laurindo *et al.* 2017). Additionally, the positions of bats within networks have been shown to relate to patterns of foraging behaviour, especially whether species are nomadic and can thus feed on fruiting trees distributed widely across the landscape, or whether they are less mobile and feed on plants that produce fruits across the whole year (Zapata-Mesa *et al.* 2017). In contrast, there are a number of studies that have described and analysed the diets of insectivorous bats (Agosta, 2002; Clare *et al.* 2011; Salinas-Ramos *et al.* 2015; Cravens *et al.* 2018), although none of them have analysed the interactions using a network approach.

Costa Rica

With an area of 51,100 km^2, Costa Rica is one of the smallest countries in the world, but contains around 6% of the world's biodiversity in terms of species (Mendoza & Jimenez 1995; Wendland & Bawa 1996; Sánchez-Azofeifa *et al.* 2001). Arthropods account for most of the biodiversity occurring in the tropics, with an estimated species richness of between 3.7 and 2.5 million species (Hamilton *et al.* 2010). Diversity can also be high at the landscape scale; patches of rainforest in Panama were estimated to have an arthropod species richness ranging from 18,000 to 30,000 species (Basset *et al.* 2012), and an estimated 15,000 Lepidoptera species are thought to inhabit the dry forests of Área de Conservación Guanacaste alone (Janzen & Hallwachs 2016).

Overall, there are 114 bat species described for Costa Rica (Rodríguez-Herrera *et al.* 2014), representing around ~9% of world's bat diversity (Fenton & Simmons 2015). Of this total, approximately 45% are strictly insectivorous (52 species), 25% are frugivorous (28 species), 9% are nectarivorous (10 species), 10% are primarily insectivores (12 species) that can include other food items in their diets, 8% are insectivorous/carnivorous species (9 species) and 3% are hematophagous (3 species). In this study, I have included 33 bat species in my mutualistic and antagonistic networks, of which 21 bat species were used to build the mutualistic networks as they included fruits in their diet, and 12 species were used to build antagonistic networks as they included insects in their diet.

Costa Rica is divided by a mountain range that extends from North to South (the Continental Divide) and marks an abrupt change in the precipitation patterns in the Caribbean region compared to the Pacific drainage basin (Sánchez-Murillo *et al.* 2016). Each side (Pacific and Caribbean) exhibits very different patterns of rainfall and seasonality (Muñoz *et al.* 2002). Costa Rica's precipitation shows variation across its range, from ~1500 mm in the Northwestern region to ~7000 mm in the Caribbean side (Sánchez-Murillo *et al.* 2016). Mean temperatures can also vary greatly from a mean of 27°C in the coastal lowlands to 10°C at the top of some mountains (Sánchez-Murillo *et al.* 2013).

Study sites

- *La Selva Biological Station*

The rainforests of La Selva Biological Station are located on the Caribbean slope of northeastern Costa Rica (10°26'N, 83°59' W) (Sigel *et al.* 2006). This site is composed of 1,611 hectares of lowland wet tropical forest, of which most is old growth lowland rain forest (73%) but a great diversity of habitats resides inside it, including cleared pastures, secondary forest and abandoned plantations (Sigel *et al.* 2006). The mean annual precipitation is 3,962 mm with mean temperatures ranging between 24.7° to 27.1° during the months of January and August, respectively (McDade & Hartshorn 1994; Sanford *et al.* 1994). The dry season in La Selva lasts from January to May, and the wet season from May to September. However, there are two short periods from October to January when rainfall can be unpredictably low or high (Newstrom *et al.* 1994).

- *Sector Santa Rosa (of ACG)*

Área de Conservación Guanacaste comprises an area of roughly 1,200 km^2, representing 2% of the whole territory of Costa Rica, and contains mangroves and dry forests, rainforests and cloud forests. Most of this area has been subject to severe habitat modification through activities such as logging, burning, ranching, and hunting (Fernández-Triana *et al.* 2014). Within the Área de Conservación Guanacaste is the dry forest of Sector Santa Rosa (of ACG), which is located in northwestern Costa Rica and covers 10,800 hectares (Janzen 1983). This park was used as a cattle ranch for a minimum of 200 years, during which approximately 30% of the original forest was cleared for plantation and the rest was cut and allowed to grow, returning to a secondary woody succession (Janzen 1983). It has a mean annual temperature of 25.8° C, a mean annual precipitation of 1,575 mm ranging from 880 to 3030 mm (Powers *et al.* 2009) and a dry season going from mid-December to late May (Chapman 1998).

Aims

This thesis is centred on three main objectives. First, to construct mutualistic networks of bat-plant interactions in the rainforests and dry forests of Costa Rica during one of the strongest El Niño events on record (2015), and to use null models to determine whether network structures deviate from expected patterns. Second, to construct antagonistic networks of bat-insect interactions for the dry forests of Costa Rica for a normal (2009) and an El Niño year (2015), and to assess whether differences are consistent with predictions based on the known effects of drought on ecological interactions. Third, to estimate variation in wing shape among individuals of the bat *Pteronotus mesoamericanus* and to examine the impact of this variation on diet and position within the network (generality and differentiation between individuals). These objectives were addressed using DNA barcoding to characterize the diets of insectivorous and frugivorous bats and geometric morphometrics to estimate the wing shape of bats from the species *Pteronotus mesoamericanus.*

Thesis organisation

The thesis is structured into five chapters, and each of the three data chapters addresses one of the stated key objectives as follows:

In Chapter 2, I analyse the interactions between frugivorous bats and plants using DNA barcoding to build the observed links in the network for the rainforests and dry forests of Costa Rica. Network metrics were compared to null models to estimate deviations from expected structure, and to assess whether these are consistent with network rewiring due to droughts and floods during the El Niño. I discuss the possible impact of the changes in network structure for the stability and persistence of the interactions.

In Chapter 3, I analyse the interactions between insectivorous bats and the insects present in their diet using DNA metabarcoding to build the observed links in the network for the dry forests of Costa Rica. I examined species interactions associated with changes in rainfall by comparing antagonistic networks from a normal year versus an El Niño year. I discuss how changes in network modularity as well as module number and size may impact species interactions through network stability and persistence.

In Chapter 4, I analyse how variation in wing shape among individuals of the bat species *Pteronotus mesoamericanus* is related to dietary breadth and specialisation. I analyse the shape of bat wings using geometric morphometrics and obtain information on individual diets through DNA metabarcoding of faeces. I discuss how the number of food items included in the diet and the differentiation between individuals might be related to differences in the efficiency of individuals in capturing different types of prey or differences in habitat use.

Each data chapter is organized as a paper that comprises an Abstract, Introduction, Methods, Results and Discussion section. Chapter 5 is a general discussion where the main patterns found across the chapters are discussed.

CHAPTER TWO

Nestedness and modularity of tropical seed dispersal networks during an extreme El Niño event

Abstract

Ecological networks offer a useful analytical approach for studying interactions among taxa, and the impacts of abiotic factors on these interactions. Climate change is expected to increase the frequency of extreme El Niño events, with the cycle of 2015-2016 registered as one of the strongest in history. El Niño is one of the main drivers of fluctuations in precipitation and fruiting production in the tropics, which can have consequent cascading effects on frugivores. As places get wetter (higher rainfall), mutualistic interactions between frugivores and the plants they eat tend to be more modular and less nested. Here I constructed mutualistic networks comprising frugivorous bats and plants for the wet season of the wet forest during the non-El Niño year of 2009 and for both wet and dry forests during the El Niño cycle of 2015-2016. These forests experienced extreme flooding and drought respectively during the El Niño cycle, while rainfall was considered normal during 2009. This provided the opportunity to assess whether patterns and inferred changes of nestedness and modularity are consistent with the known consequences of anomalous precipitation and the network structure during the non-El Niño year. I determined mutualistic links by DNA barcoding bat faeces, and tested whether observed values of nestedness and modularity deviated from random using null models. I expected that during the wet season of the non-El Niño year, the network of the dry forest would not show any difference regarding nestedness and modularity in relation to the null models. I also expected that it would be more modular and less nested than the wet season during the El Niño year. I expected that during flooding, networks in the rainforest would be more modular and less nested than the null model, whereas in the dry forest during the severe drought, networks would be more nested and less modular than the null model. I also expected that the dry forest would be more nested and less modular than the rainforest in relation to the null model predictions. Similar expectations were made regarding rainfall changes in relation to seasonality, where wetter seasons would be more modular and less nested and drier seasons would be more nested and less modular. However, despite the contrasting effects of droughts and floods in the dry forest and rainforest, I observed similar patterns in nestedness and modularity for both forests in relation to the null models. I found higher values of modularity, but lower of nestedness for most networks in comparison to the null models. Over all the comparisons between networks (forest and season wide) against the null models, I found higher nestedness in the dry forest than the rainforest and a lower difference between dry forest in the wet versus dry season than expected by null models. The network of the wet season of the non-El Niño year was less nested than expected by the null models and had a

higher niche overlap than the network of the wet season during the El Niño year. A lower nestedness might decrease the amount of species supported by the habitat as well as increase species competition. However, this might be compensated during El Niño years by the lower niche overlap. Although the increase in modularity might reduce the number of coexisting species in the environment, higher compartmentalization of the networks leads to greater stability, slower spread of disturbance and smaller chances of having trophic cascades. Therefore, changes in network structure seen in El Niño conditions are likely to have dual effects on networks with some effects leading to greater stability while others to increasing competition.

Introduction

The construction of ecological networks is a very useful analytical approach that has become increasingly prevalent in recent years for studying interactions among taxa across ecosystems, as well as the impacts of abiotic factors on these interactions (Ings *et al.* 2009). Studies from diverse taxa have suggested that mutualistic networks, such as those containing plants and frugivorous animals, commonly show similar properties. In particular, these networks appear to be highly nested, in which interactions involving specialist taxa represent a subset of those involving generalists, and also highly modular, with multiple weakly linked clusters of densely connected taxa (Bascompte *et al.* 2003; Olesen *et al.* 2007; Fortuna *et al.* 2010; Donatti *et al.* 2011; Krasnov *et al.* 2012). Such nestedness and modularity both increase network stability, and resilience (robustness) to the loss of species from ecosystems, while minimizing perturbations (Memmott *et al.* 2004; Fortuna *et al.* 2010; Thébault & Fontaine 2010). Nestedness is also thought to reduce interspecific competition, thereby allowing more species to coexist (Bastolla *et al.* 2009).

A major challenge of constructing mutualistic networks is characterizing the links between plants and animals. Many vertebrate frugivores may feed on just fruit pulp, egesting no identifiable seeds for morphological examination. For these reasons, DNA barcoding, which can be applied to traces of nucleic acids, provides a powerful means of inferring ecological interactions (Clare 2014; Evans *et al.* 2016; Roslin & Majaneva 2016). Such molecular approaches have resolved previously unknown links in already well-studied food webs, revealing metrics such as connectance and nestedness to differ by orders of magnitude from earlier estimates derived from traditional approaches (Wirta *et al.* 2014). On the other hand, most studies using molecular tools to analyze animal diets have focused on predation (Jedlicka *et al.* 2013; Brown *et al.* 2014; Clare *et al.* 2014; Kruger *et al.* 2014; Chanin *et al.* 2015) with fewer studies using DNA barcoding to understand plant-mammal mutualisms, though this is rapidly changing (Bradley *et al.* 2007; Quéméré *et al.* 2013; Kartzinel *et al.* 2015; Galimberti *et al.* 2016).

Here I focus on mutualistic interactions between frugivorous bats and plants in Costa Rica, applying a DNA barcoding approach. Bats number over 1,300 species worldwide, of which ~20% feed on nectar or fruit (Kunz *et al.* 2011; Rojas *et al.* 2012; Fenton & Simmons 2015). In the Neotropics, phyllostomid bats are widespread and critically important pollinators and seed dispersers, and, together with frugivorous birds, account for over 80% of the seed dispersal activity (Galindo-González *et al.* 2000). Previous work suggests bat-plant mutualistic networks in the Neotropics are highly connected, nested and robust to plant extinctions, but with low modularity (Mello *et al.* 2011). Such network structures imply considerable behavioral flexibility that might confer resilience to changes in the environment, yet it is not known how extreme climatic events may affect the structure and robustness of these networks.

The El Niño Southern Oscillation (ENSO) is an irregular climatic event that is associated with extremes in rainfall, and is expected to increase in frequency with climate change (Cai *et al.* 2014). El Niño is one of the main drivers of precipitation fluctuations globally and is responsible for increasing seasonality in the tropics (Wright *et al.* 1999; Holmgren 2001; Malhi & Wright 2004). Such responses, however, differ widely among regions (Holmgren 2001); for example, in parts of Central America, El Niño causes floods in the rainforests of the Caribbean coast, but droughts in the Pacific dry forests (Waylen *et al.* 1998). These contrasting effects are critically important as rainfall is a principal factor influencing plant phenology and thus primary productivity. Changes in weather due to El Niño, including both droughts and floods, have been directly linked to fluctuations in fruit production (Wright *et al.* 1999) although responses can be complex (Gunarathne & Perera 2014; Butt *et al.* 2015), with cascading effects for wild animal and plant populations (Wright *et al.* 1999; Harrison 2000; Butt *et al.* 2015). Such impacts of El Niño might be especially important in the humid tropics, where nectarivorous and frugivorous vertebrates perform much of the pollination and seed dispersal; however, these consequences have been little-studied and remain poorly understood (Wright *et al.* 1999; Fredriksson *et al.* 2006; Wolf *et al.* 2015).

The ENSO cycle of 2015-2016 is one of the strongest on record (Jacox *et al.* 2016). In Costa Rica, this event led to extremely wet rainy season in the wet forests, with rainfall levels exceeding those of the previous 47 years. The opposite trend was observed in Costa Rica's coastal dry forests, where rainfall levels were lower in the rainy season than those of the previous 31 years. Thus, both types of forest experienced extreme climatic conditions associated with El Niño (Seneviratne *et al.* 2012). ENSO events are expected to have important consequences for ecological interactions on the basis of earlier work that has indicated network structure is strongly influenced by precipitation (Trøjelsgaard & Olesen 2013), including historical climate change (Dalsgaard *et al.* 2013). In general, higher rainfall and seasonality are correlated with more modular networks (Dalsgaard *et al.* 2013; Trøjelsgaard & Olesen 2013; Schleuning *et al.* 2014), and lower rainfall with greater nestedness (Rico-Gray *et al.* 2012), consequences that are likely to result from changes in resource availability.

To determine how opposite extremes in rainfall (unusually wet and dry conditions) induced by El Niño might influence mutualistic interactions among plants and frugivorous bats, I analysed and compared networks of mutualistic interactions across the wet and dry seasons in both wet forest and dry forest in Costa Rica during the ENSO event of 2016. For the dry forest, I also constructed a network of bat-plant interactions for a non-El Niño year using available in 2009 by E. Clare. In addition to comparisons across seasons, forests, and years, I used null models to estimate the magnitude of the change of the observed network metrics in relation to randomized matrices. The use of null models has become an important statistical approach in network ecology for assessing the extent to which metrics can deviate from expected values, especially given that building networks can be extremely labor-intensive (Evans *et al.* 2016) and, for this reason, sample sizes of whole networks typically preclude normal statistical tests (Elmas *et al.* 2018). Comparisons of null distributions also provide the means to test for significant differences between networks while accounting for variation within each network.

Based on known responses of network structure to precipitation, in which low precipitation is associated with higher nestedness and lower modularity, I expected that, for the dry forest, lower-than-average rainfall in the failed wet season in the El Niño year would result in a network with higher nestedness and lower modularity than a network for the same site and season in the non-El Niño year, where precipitation was higher. Within years, I expected that networks would show higher modularity and lower nestedness in the wet forest than in the dry forest based on null distributions. Similarly, within each forest type, I predicted that wetter-than-average seasons would lead to higher network modularity and lower nestedness than expected from the null models, while drier-than-average seasons would result in higher nestedness and lower modularity than the null models (Table 2.1). Additionally, I looked for changes in other network metrics to evaluate the magnitude of the changes in the structure of species interactions in relation with values from null models

Methods

Study sites

Fieldwork was conducted at two forest sites in Costa Rica that show contrasting seasonality and precipitation: an Atlantic rainforest at La Selva Biological Station (10°25′19” N, 84°00′54” W) and at a Pacific dry forest at Sector Santa Rosa (10°48’53” N, 85˚36’54” W) in the Área de Conservación Guanacaste (ACG) Costa Rica (Figure 2.1). La Selva Biological Station covers 1,611 ha of lowland wet tropical forest between 35 to 137 m on the Caribbean slope of the Cordillera Central mountain range. It has a mean annual temperature of 25˚ C with a mean annual precipitation of 3,962 mm (Sigel *et al.* 2006). Sector Santa Rosa (of ACG) covers >38,000 ha of tropical dry forest ranging from 0 m to 300 m, and is part of Área de Conservación Guanacaste (Asensio *et al.* 2015). Sector Santa Rosa (of ACG) has a mean annual temperature of 25˚ C with a mean annual precipitation of 1,575 mm. Seasonality is more pronounced in the former site (range 2,809-6,164 mm) than the latter (range 880 - 3,030 mm, six-month dry season) (Gillespie *et al.* 2000; Powers *et al.* 2009).

Bat sampling

I captured bats using four to six mist nets (6m - 12m) opened along trails and near watercourses in the study area from 18h - 22h. In addition, a canopy net and harp trap were used in 2009 but these had low capture rates and so were not used in 2015. Sampling took place in the dry season during Jan-Feb (Santa Rosa of ACG) and Mar-Apr (La Selva) (2015), in the wet season May-July (Santa Rosa of ACG) (2009), and in the wet season July-Aug (Santa Rosa of ACG) and September-October (La Selva) (2015). Sampling and bat identification during the non-El Niño year was conducted by E. Clare. Sampling effort was equal to approximately 2,250 m^2 hours during each of the seasons during the El Niño year, and approximately the same during the non-El Niño year. I marked the bats with wing punches to avoid recaptures, measured the forearm length with callipers (0.1 mm precision) and identified species following Reid (1997), Timm & Laval (1998) and Laval & Rodríguez-Herrera (2002). Bats were held in cloth bags for a maximum of 2 hours for the collection of faecal samples. All samples were frozen after collection (-20° C).

DNA extraction, PCR and sequencing

For this study I focused on nectar and fruit eating species, which produced faecal samples consisting of either seeds or digested fruit pulp. For the DNA extraction, PCR and sequencing of the samples from the El Niño year, I followed standard protocols for plants and was conducted by the Canadian Centre for DNA barcoding (CCDB) (Ivanova *et al.* 2011). In brief, dried plant material from faeces (fruit pulp or seed) was placed in a sterile strip-tube with a pre-aliquoted sterile stainless steel bead and the tissue was ground using a Tissue Lyser (Qiagen, USA). The ground material was incubated with 2x CTAB buffer at 65°C for 1 hour and DNA extraction was performed using a semi-automated glass fiber filtration methods (Ivanova *et al.* 2008; Fazekas *et al.* 2012). Following established methods, I amplified a 552 bp fragment of the 5' end of the large subunit of RuBisCO (*rbc*L) and a ~350 bp fragment of the second nuclear encoded internal transcribed spacer (ITS2), and performed Sanger sequencing using a ABI 3730xl capillary sequencer (Ivanova *et al.* 2005; Ivanova & Grainger 2006; Kuzmina & Ivanova 2011a; Kuzmina & Ivanova 2011b; Fazekas *et al*. 2012). Although plant DNA barcoding yields lower species resolution compared to fungi and animals (Hollingsworth *et al.* 2011), it provides robust results for identification of vascular plants at the genus level (Kress *et al.* 2009; Parmentier *et al.* 2013; Braukmann *et al.* 2017).

Samples from the non-El Niño year were processed by Clare *et al.* (2018). Briefly DNA was isolated from three to five intact seeds per sample using the NucleoSpin 96 Plant II DNA isolation kit (Macherey-Nagel). Seeds present in individual fecal samples were usually identical in appearance and were assumed to be from the same piece of ingested fruit. In six cases seeds with differing morphology were observed; in these instances, morphological distinguishable seed types were separated. For samples in which seeds were not observed, 10mg of dried guano was used for DNA extraction. Extraction followed the manufacturer's protocol with the exception of an extended lysis stage of one hour. PCR was used to amplify two plant barcode regions (*rbcL* and *matK*) and the non-coding plastid region (*trnH-psbA*) (CBOL 2009). The *rbcL* and *trnH-psbA* regions were amplified using primers rbcLa_F and rbcLajf_634R, and *trnH* and psbA (Kress *et al.* 2005; Fazekas *et al.* 2012). For *matK* the primers 1R_KIM and 3F_KIM (Fazekas *et al.* 2012) were used. PCR amplification with these *matK* primers was weak or absent for a number of samples that did amplify for the other regions. For these samples PCR was repeated using an alternate combination of primers: XF and 3F_KIM (Ford *et al.* 2009; Fazekas *et al.* 2012). PCR amplification reactions occurred in 10µL reaction volumes containing 2µL of 5X Phire reaction buffer (Finnzymes), 0.05μ L of 10mM dNTPs, 0.1μ L of each 10μ M primer, and 0.125μ L of Phire Hot Start II polymerase (Finnzymes). PCRs were performed on a Veriti gradient thermal cycler (ABI) using the following protocol: initial denaturation at 98°C for 90s, thirty-five cycles of 98°C for 5s, 55–66°C for 10s (depending on primer set), 72°C for 7–10s (depending on region), followed by a final extension at 72°C for 60s and hold at 4°C. Amplicons were sequenced bi-directionally with the same primers as used for amplification. Cycle sequencing reactions occurred in 11μL reaction volumes containing 0.5 μL of BigDye terminator mix (ABI), 2μ L of 5X sequencing buffer, 1μ L 10uM primer, and 0.5μ L of PCR product. These reactions were run on the PCR thermal cycler using the following protocol: initial denaturation at 96°C for 2min, 30 cycles of 96°C for 30s, 55°C for 15s, 60°C for 4min, followed by a hold at 4°C. I assembled contigs and edited all sequences using Sequencher 4.8 (Gene Codes Corp, Ann Arbor, MI).

Identification of plant DNA sequences from bat faecal samples

I initially filtered all sequences for quality and excluded low quality sequences where the PHRED score was <30 as indexed on the Barcode of Life Data Management System (BOLD) (Ratnasingham & Hebert 2007). I compared the obtained *rbc*L and ITS2 sequences with the reference libraries of GenBank and BOLD using the BLAST algorithm with default search parameters (Altschul *et al.* 1990) and the combined BLAST and Hidden Markov Model methods implemented by the BOLD server (Ratnasingham & Hebert 2007). For each reference database (BOLD, GenBank), I assigned query sequences to taxon based on highest percentage similarity, and considered a threshold of ≥97% to be a reliable assignment (Lamb *et al.* 2016). When there was agreement between species-level matches for both markers (*rbc*L and ITS2) in both databases, with at least one match >97%, I assigned to the level of species. In cases where the query matched with equal similarity to multiple taxa of the same genus, I assigned the taxon to the level of the genus only, and similarly I used the same approach to assign query sequences to the level of the family. Where *rbc*L and ITS2 sequences matched different species from different genera, both at >97%, I concluded that two taxa were present in the sample and therefore assigned to both genera. Query sequences that did not show significant similarity to a reference were excluded from the analysis.

To corroborate my species assignments, for each candidate genus match, I reconstructed a gene phylogeny in which I included my query sequences together with all available reference sequences from species of the same genus present in BOLD that are also known to occur in Costa Rica. Sequences from *rbc*L and ITS2 of each plant genus were aligned with ClustalW (Larkin *et al.* 2007) in BioEdit v7.2.5 (Hall 1999). For each alignment I ran a model selection test to check which would be the best method to build the phylogenetic tree based on the lowest BIC value. I ran model selection and built the phylogenetic trees using MEGA 6.06 (Tamura *et al.* 2013). These phylogenies (not shown) recovered paraphyletic groupings for some species, perhaps through a lack of reference material, and therefore such species assignments were considered unreliable. To address this issue, I took a conservative approach and reduced all data to genus-level designations and repeated my analyses to check for consistency of results (see Supplementary material).

For samples from the non-El Niño year, plant DNA sequences from bat faecal samples were identified in Clare *et al.* (2018). Briefly, all recovered sequences were compared to GenBank and BOLD, with the exception of the *trn*H-*psb*A region which is not currently searchable within BOLD. In assigning a taxonomic name to a sequence, a threshold of 1% identity was used rather than highest BLAST score, which is determined in part by the length of overlap between the query sequence and the reference sequence. The different plant barcode regions provide resolution at different taxonomic depths in different taxa. It has been demonstrated that *rbc*L provides generic level resolution (CBOL 2009) in almost all cases (occasionally to species level), whereas the matK and trnH-psbA regions can provide resolution to species in ~60-90% of cases (depending on the taxa and geographic scope) (e.g., Lahaye *et al.* 2008; Burgess *et al.* 2011). Due to taxonomic incompleteness of the plant reference databases, the sequence comparison results for many sequences did not match with 100% identity to the reference database. In these cases sequences were assigned to family, genus, or species depending on the region and the percent identity using the following conservative criteria. For *rbc*L, sequence matches with 99.75-100% identity were assigned to genus level, and matches with 99-99.75% identity to family level. For *mat*K, matches with 100% identity were assigned to putative species, or species cluster if there was more than one match with 100% identity; matches between 99- 100% identity were assigned to genus rank, and matches with 98-99% identity to family. For the *trn*H-*psb*A region, no sequence matches with 100% identity were observed. Most matched sequences ranged from 98-99% identity to the queried sequence. The variable length of the trnH-psbA region, the presence of repeated sequence motifs and a lack of reference sequences complicated the interpretation of *trn*H-*psb*A BLAST analysis with the GenBank dataset. Therefore, most assignments were made to genus rank for this region. For two genera, however, the *trn*H-*psb*A data corroborated the designation based on *mat*K and also provided an increased level of resolution. Unique sequences for these samples were therefore designated with a number (in addition to genus) and treated as putative species. Species level designation was also accepted in one case for sequences that matched a genus with only a single known species, and in a second case where sequences matched a genus of three species, two of which occur well outside the study area.

Network matrices

I compiled the inferred interactions into interaction incidence matrices where each cell value represented the number of observed interactions between each bat-plant taxon pair. I considered one realized interaction when the DNA of a plant taxon was detected in the faeces of one individual bat. I constructed matrices for (1) each forest site in which I pooled data from both seasons during the El Niño year ('La Selva' and 'Santa Rosa'). Then, (2) I separated the data collected by season (wet and dry), by site (rainforest and dry forest), and by year (El Niño and non-El Niño). Each of the networks generated after this procedure was compared against null models.

Descriptors of network structure

To determine network structure and resilience from each habitat during a whole year, and for each habitat during each season, I assessed network structure by measuring six key metrics. First, I quantified nestedness, which measures the extent to which the interactions of one species are a sub-set of the interactions of another species when the matrix of interaction is organized by decreasing number of links (Dormann *et al.* 2009). I calculated nestedness using the weighted NODF approach, which is a measure of nestedness that uses overlap and decreasing fill in the weighted matrix, that has been shown to outperform other methods for estimating nestedness in binary networks (Almeida-Neto & Ulrich 2011). Second, I quantified modularity, characterized as more interactions within a module than between modules (Dormann & Strauss 2014), using the QuanBiMo algorithm that is based on simulated annealing and is more specifically designed for weighted bipartite networks (Dormann & Strauss 2014). Third, I calculated weighted connectance by dividing linkage density by the number of species in the network (Tylianakis *et al.* 2007), which reveals the number of links in the network in relation to the total number of links (Altena *et al.* 2016). Fourth, I measured the number of compartments, which are defined as isolated sub-sets of nodes interacting with each other that do not have any connections with another compartment in the network (Dormann *et al.* 2009). Fifth, robustness was calculated as the area below the curve of secondary extinction of bats when primary extinction of plant species was simulated according to three methods: random extinction of plant species (random), extinction of most connected to least connected plant species (degree) and extinction from the least connected to most connected plant species (abundance). And finally, niche overlap among bat species was calculated using the Morisita-Horn index (Horn 1966). Apart from robustness, all metrics chosen have little or no biases to sampling completeness and network size (Fründ *et al.* 2015).

I used the function networklevel from the Bipartite package (Dormann *et al.* 2008) to determine network structure and resilience from each habitat during a whole year and for each habitat during each season and calculated the following network metrics: nestedness, weighted nestedness, weighted NODF, modularity (QuanBiMo), number of compartments and robustness.

Statistical analysis

To test whether in each case the network metrics deviated from expected values, and to compare null distributions of metrics between habitats, and between seasons within each habitat and years, I used the swap algorithm (Dormann *et al.* 2014). The swap algorithm initially randomizes the network matrix using the Patefield algorithm (Patefield 1981), then swaps the interactions while constraining for connectance. Thus, it produces network matrices with the same connectance and marginal totals as the original matrix, but produces networks that are more specialized than those generated under other randomization algorithms (Artzy-Randrup & Stone, 2005; Dormann *et al.* 2008). I followed Gotelli & Ulrich (2011) for the choice of the swap algorithm for the randomization as more constrained null models are better to to avoid type I error. Thus, it is also more parsimonious and conservative for hypothesis testing (Gotelli & Ulrich 2012).

I generated 1,000 random matrices with the same total marginal sum and connectance as the observed networks, and I used the Monte Carlo procedure (α= 5%) to check if the observed network metric value was higher or lower than expected by chance. To assess whether network structure differed between between seasons within each forest, and also between forest types across the whole year, for each comparison I calculated the difference in the observed metric values, and compared this to a null distribution of 1,000 differences obtained by Monte Carlo procedure. Due to the high calculation intensity of modularity QuanBiMo, I generated only 100 random matrices using the swap algorithm to calculate its significance.

To understand the effect of habitat and seasonality on the composition and interactions of networks during the ENSO event, I compared networks constructed for the dry and wet season within each of the two forest types. I used the R package betalink (Poisot *et al.* 2012) and calculated the dissimilarity of interaction matrices between habitats and between seasons within each habitat. The values for network dissimilarities were calculated based on the dissimilarity in the species composition of communities in the networks (β_S), based on the differences in the interactions observed between species common to both networks (β_{OS}), based only on differences in the interactions between both networks (β_{WN}) and based on the dissimilarity of the interaction structure that was induced by the dissimilarity in species composition (β_{ST}) (Poisot *et al.* 2012). To determine the sampling completeness of my networks and the proportion of the total plant species richness present in bat diets that were sampled, I used the Chao 1 index according to the method proposed by Macgregor *et al.* (2017) for the networks, and individual based rarefaction curves for the estimation of each bat species diet. All statistical analysis and network drawings were performed using R, version 3.3.2 (R Development Core Team 2015).

Results

In 130 sampling nights I captured 1,041 bats from 42 species, and collected guano samples from 435 individuals from 21 bat species. Analyses of faecal material from these 21 bat species recovered a total of 47 plant taxa, representing a total of 374 observed interactions (Figure 2.2-2.5). Of these 47 taxa, 26 plant taxa were resolved to species, 16 to genus, five to family and one to order (though see also the supplementary material for analysis on genera).

Network structure in dry forest versus rainforest in relation to null models

Contrary to the initial predictions (Table 2.1), I found that networks constructed for both the dry forest and rainforest in the El Niño year (pooling seasons) showed similar deviations from expectations based on null models. Specifically, compared to their respective null models, the network of each forest was more compartmentalized (Rainforest – mean=1.38; SD=0.59; p-value< 0.05; Dry forest – mean=1.14; SD=0.36; p-value<0.01), less nested (weighted NODF) (Rainforest – mean=20.92; SD=0.08; p-value<0.01; Dry forest – mean= 22.37; SD=3.61; p-value=0.01), less connected (weighted connectance) (Rainforest – mean= 0.12; SD=0.01; p-value<0.01; Dry forest – mean= 0.14; SD=0.01; p-value=0.01), more modular (Rainforest – mean= 0.35; SD=0.07; p-value=0.01; Dry forest – mean=0.41; SD= 0.02; p-value= 0.00) (Table 2.4).

Network structure in wet versus dry seasons within each forest during the El Niño year in relation to null models

For the dry forest, I found that most of the network metrics deviated from the null distribution for the dry season, whereas there were no significant differences for the wet season. During the dry season, the network was more compartmentalized (mean= 1.24; SD=0.45; p-value=0.00), less nested (weighted NODF) (mean=15.29; SD=2.95; p-value=0.00), less connected (weighted connectance) (mean= 0.12; SD=0.01; p-value=0.05), and more modular than expected from the null models (mean= 0.50; SD=0.03; p-value=0.01) (Table 2.4).

Similar to the dry forest, I found that network metrics in the rainforest also only deviated from expected in the dry season. The network was less nested (weighted NODF) (mean=12.73; SD=3.03; p-value=0.03) and more modular (mean=0.37; SD=0.04; p-value=0.00) than expected by the null models. With regard to differences between seasons, I detected that the difference in robustness of bat species to plant extinctions was lower than expected by chance (p-value=0.03) between seasons in the rainforest.

Network structure in dry forest during the El Niño versus non-El Niño wet season in relation to null models

In contrast to the wet season during the El Niño year (for which no deviation from the null models for any metric was found) the non-El Niño year showed lower nestedness (weighted NODF - mean=22.80; SD=3.45; p-value=0.01), connectance (weighted connectance - mean=0.14; SD=0.01; p-value=0.00) and niche overlap (mean=0.38; SD=0.06; p-value=0.001) in relation to the null models (Table 2.4). The network during the wet season of the non-El Niño year also showed a higher value of niche overlap than during the El Niño year.

Sampling completeness across forests and seasons

Values of sampling completeness ranged from 78.85% for the wet season of the non-El Niño year to 95.65% (dry forest) for the wet season of the El Niño (rainforest) (Table 2.2). However, none of the rarefaction curves built for any bat species present in my networks during either season reached a plateau (Figures 2.6 - 2.12).

Network dissimilarity across forests and seasons

All networks showed a high level of interaction dissimilarity between seasons and habitats ($\beta_{WN} > 0.800$) with only a small to intermediate portion of the dissimilarity in the structure of the interactions explained by turnover in species assemblages ($0.230 < \beta_{ST} < 0.420$). Dissimilarity in species composition between assemblages was intermediate ($0.410 < \beta_{S} < 0.882$). Interaction dissimilarity established between species common to both networks (β_{OS}) was also intermediate with values ranging from 0.460 to 0.590 (Table 2.3).

Discussion

Network structure in dry forest versus rainforest in relation to null models

Mutualistic networks are expected to be more modular and less nested under wet conditions, and less modular and more nested under dry conditions. Despite this, I observed similar deviations in network metrics from null expectations for the rainforest and dry forest in the El Niño year (pooling data across seasons), despite the fact that during this period these forest experienced flooding and drought, respectively. In both forests, the observed network structure showed lower nestedness than expected compared to their respective null distributions, with potentially contrasting consequences for network resilience. For example, nested mutualistic networks are thought to contribute to an increase in the maximum amount of biodiversity supported in the environment (Bastolla *et al.* 2009), with low nestedness related to increased effective competition (Bastolla *et al.* 2009) driving niche separation. This is important because nestedness helps to buffer against secondary extinctions and temporal fluctuations (Tylianakis *et al.* 2010). Similarly, the observed low connectance in relation to the null models might also have consequences, since this network metric is thought to contribute to ecosystem function stability during fluctuating environmental conditions (Tylianakis *et al.* 2010).

Most observations of robustness suggest stability of communities, likely as a result of low connectance and nestedness in the networks (Thébault & Faontaine 2010). These effects are particularly important as connectance and nestedness are thought to show little temporal variation within and between years (Dupont *et al.* 2009; Vázquez *et al.* 2009). In habitats such as forest and savannah, recovery to the conditions before disturbances such as floods and droughts is slow (Maron *et al.* 2015). These findings imply that the network structure observed during the present El Niño event might take some time to recover. Given that the frequency of strong El Niño events is expected to increase, with wet areas set to become wetter and dry areas to become drier (Chou *et al.* 2013; Cai *et al.* 2014), the lag in recovery of network structure could lead to an overall reduction in the biodiversity supported in these forest ecosystems.

Along with low nestedness I observed higher values of modularity than those expected under null models for both forests, suggesting that the interacting species are showing a higher niche partitioning than expected. Modularity was not only significantly higher than expected by chance, but values for both forests were also higher than the calculated ones using a similar algorithm for previously observed mutualistic networks of phyllostomid bats in other regions of

South America during normal conditions (Mello *et al.* 2011). Similarly, the higher than expected compartmentalization in both habitats might reduce the number of coexisting species (Bastolla *et al.* 2009), and has also been linked to greater stability, slower spread of disturbance, and reduced likelihood of trophic cascades in networks (Tylianakis *et al.* 2010).

Fluctuations in rainfall have an impact in different groups of herbivorous mammal populations through alterations in the amount and quality of food resources (Mandujano 2006; White 2008) with severe droughts in some Pacific areas provoked by El Niño reducing the production of the entire plant community (Wright & Calderon 2006) while in rainforests heavy rain can be a trigger for flowering (Wright 1991). Despite the contrasting consequences of droughts and floods for the plant community, similar responses to opposite water stress in two very dissimilar species communities suggests a generalized response to stress that may become more prevalent as extreme weather cycles increase in frequency (also see Butt *et al.* 2015).

Network structure in wet versus dry seasons within each forest in relation to null models

In the dry forest, I found that while the wet season during the El Niño year showed no changes in network structure in relation to the null models, the network in the dry season showed several metrics that deviated from expectations, and that these occurred in different directions. Explanations for these observations are likely to reflect changes in fruit availability. Notably, in the dry forest, most of the consumption of fleshy fruits occurs at the beginning of the wet season (Vieira & Scariot 2006). Although, there was a big decrease in the rainfall in the wet season during this study, the second month of the wet season (June) had rainfall within the historical standard deviation which may have restored some of the normal conditions of the forest in terms of fruit availability. This may explain why nestedness (weighted NODF) was low during the dry season and why the network did not show any significant deviation in structure in the wet season.

In the rainforest, the changes across the seasons were also not consistent, which might also reflect changes in food availability across the year. The lower nestedness and higher modularity of the network during the dry season might reflect bat niche specialization during this season due to a reduction in food availability. On the other hand, the absence of any significant difference in relation to the null models during the wet season might result from an increase in fruit production, with bats exploiting more common resources.

Network structure in dry forest during the El Niño versus non-El Niño wet season in relation to null models

In contrast to the results from the network for the El Niño wet season (dry forest) in which metrics did not deviate from null expectations, the network constructed for the non-El Niño wet season for the same site showed significant deviations. Specicially, for the latter case, I found lower than expected values of nestedness, connectance and niche overlap. This result indicates that bats during the non-El Niño wet season seem to be more specialized in their diet (lower connectance and nestedness), which might have led also to lower values of niche overlap. Comparing the null distributions of networks metrics for non-El Niño versus El Niño revealed higher niche overlap during the non-El Niño wet season, implying lower diet specialization. However, since we do not have data for multiple years in the comparison, and therefore the extent of interannual variability is not understood.

Sampling completeness across forests and seasons

I found that only two networks (rainforest dry season and dry forest wet season during the El Niño year had a higher value of sampling completeness in relation to the minimum value proposed by Macgregor, Evans, and Pocock (2017) (90%). Therefore, more interactions are expected would be expected to be included with additional sampling and part of my results my remain speculative. The addition of new interactions to these networks due to additional sampling might result in changes on the observed metric values in both directions (higher or lower). However, our focus on key metrics that have low effect of network size on their variation would help to balance possible biases resulting from undersampling.

Network dissimilarity across forests and seasons

I observed high interaction dissimilarity between seasons within forest types, between forests and between the El Niño and non-El Niño year (Table 2.3). Similar observations have been made for other mutualistic networks over time (years) where the percentage of retained interactions was low, ranging from 5% to 31% (Petanidou *et al.* 2008; Alarcón *et al.* 2008; Vázquez *et al.* 2009). It is likely that most variation in interactions between seasons in the dry forest in my study can be explained by differences in fruit availability, which tends to show high temporal variation (Kushwaha *et al.* 2011). The dissimilarity in species composition between the rainforest and dry forest in my observed networks is in accordance with what has been observed for the species turnover between lowland dry forests and rainforests in Costa Rica, where 50%-100% of the flora and fauna were common to both forests (Janzen 1986). Mello (2009) suggested that patterns of temporal species turnover in phyllostomid bats are related to the abundance of preferred food items with understory and canopy frugivores positively correlated with the rainfall in the Atlantic rainforest. However, species that forage in the canopy tend to be more specialized on tree species that produce big numbers of fruits for short periods of time, while understory bats feed mainly on plants that produce few fruits over many months of the year (Mello 2009). Therefore, the impact of El Niño is unlikely to be equally distributed across all bat species.

Although El Niño causes floods in some regions of the world, the overall trend is to promote droughts in tropical rainforests (Holmgren *et al.* 2001) with occurrences of high annual rainfall and low seasonality considered unlikely (Borchert 1998). This trend is usually reversed during the year after El Niño due to La Niña (Holmgren *et al.* 2001). However, most studies evaluate the effects of droughts, but not floods, in rainforests (Wright 1999; Harrison 2000). Dunham *et al.* (2011) worked in Madagascar, and reported one of the few studies that examined the impact of heavy rains caused by an ENSO event on a rainforest. Here the authors observed a disruption in the cycle of the primary fruit food sources, with a consequent increase in infant mortality in lemurs. Working in semi-deciduous forest in Panama during the strong ENSO event of 1982-1983, Leigh *et al.* (1990) reported a drought that led to tree mortality that was 5 times higher than usual, yet resulted in no clear serious negative consequences for animal populations. The authors suggested that the forest might have adapted to surviving droughts provoked by ENSO events without too much damage (Leigh *et al.* 1990).

Conclusions

Based on comparisons with null models, I found that the networks in boths forests showed similar deviations from expectations during the El Niño, which might promote biodiversity on one hand through increasing compartmentalization, but which might deplete it on the other by increasing competition and decreasing stability via lower nestedness and connectance. However, the comparisons of the network structure for the wet season in the dry forest between an El Niño and a non-El Niño year indicates only a difference in niche overlap, which was lower during the El Niño year, when species would be more specialized in their diets. Thus, more studies across multiple years including data from El Niño and non-El Niño years are necessary to better understand the effect of associated changes in rainfall and interannual variability on the changes provoked by this climatic event, and help to validate results coming from null model estimations and comparisons. More studies are also necessary to see if there are similar responses in mutualistic networks to different stressors such as habitat fragmentation or other scenarios which potentially boost the effects of extreme climatic events (Butt *et al.* 2015).

Tables

Table 2.1. Expected changes of food resources and different metrics of mutualistic networks of frugivorous bats and the plants they eat in the dry forest of Sector Santa Rosa (of ACG) and rainforest of La Selva Biological Station in Costa Rica during an extreme El Niño event (2015) in relation to null models (network metrics) and historical patterns (food resources).

Habitat	**Network metrics**	**Rainfall (obs.)**	**Food resources**
Dry forest	Lower modularity Higher nestedness and other metrics	Drought	Lower fruit availability
Seasonality (wet vs. dry)	Decreased pairwise differences in network metrics between wet and dry season	Decreased seasonality	Lower difference of fruit availability between dry and wet season
Rainforest	Higher modularity Lower nestedness and other metrics	Flood	Higher fruit availability
Seasonality (wet vs. dry)	Increased pairwise differences in network metrics between wet and dry season	Increased seasonality	Higher difference of fruit availability between dry and wet season

2.2. Sampling completeness of mutualistic networks of frugivorous bats in the dry forest of Sector Santa Rosa (of ACG) (wet and dry season) and rainforest of La Selva Biological Station (wet and dry season) of Costa Rica during a non-El Niño year (2009) and an extreme El Niño event (2015).

Type of forest (year – season)	**Sampling completeness (%)**
Dry forest (El Niño – whole year)	86.44
Dry forest (non-El Niño year – wet season)	78.85
Dry forest (El Niño – dry season)	86.49
Dry forest (El Niño – wet season)	94.74
Rainforest (El Niño – whole year)	79.71
Rainforest (El Niño – dry season)	95.65
Rainforest (El Niño – wet season)	83.17

Table 2.3. Values of β dissimilarities between mutualistic networks of frugivorous bats in the dry forest of Sector Santa Rosa (of ACG) (wet and dry season) and rainforest of La Selva Biological Station (wet and dry season) of Costa Rica during a non-El Niño year (2009) and an extreme El Niño event (2015).

Dissimilarities	**Dry forest - Rainforest**	**Dry forest Wet - Dry**	**Rainforest Wet - Dry**	**Dry forest El Niño wet season – Non-El Niño wet season**
Dissimilarity in the species composition of the communities in the networks (β_S)	0.632	0.417	0.536	0.882
Dissimilarity of interaction established between species common to both networks (β_{OS})	0.462	0.583	0.520	-
Dissimilarity of interactions (β_{WN})	0.868	0.821	0.812	1.000
Dissimilarity of interactions based due to species turnover between both networks (β_{ST})	0.406	0.238	0.292	-

Table 2.4. Network metrics of frugivorous bat mutualistic networks in the wet and dry season of the dry forest of Santa Rosa National Park and rainforest of La Selva Biological Station in Costa Rica during a non-El Niño year (2009) and an extreme El Niño year (2015). Significance tested in relation to 1,000 random generated networks for each season according to the swap algorithm using the Monte Carlo procedure.

	Dry forest				Rainforest		
Network metric	Whole year	Dry	Wet	Non-El Niño Wet	Whole year	Dry	Wet
Number of compartments	▲ 2.00**	▲ 3.00**	1.00	1.00	▲ 2.00*	2.00	2.00
Nestedness	21.65	23.72	21.75	26.76	12.54	33.57	21.75
Weighted nestedness	0.41	0.24	0.48	0.41	0.52	0.19	0.48
Weighted NODF	▼ 13.94*	▼ 6.57**	18.23	▼ 15.37*	▼ 13.72**	▼ 6.92*	18.24
Weighted connectance	▼ 0.12*	▼ 0.11*	0.14	▼ 0.10**	▼ 0.09**	0.11	0.14
Modularity (QuanBiMo)	▲ 0.53**	▲ 0.57*	0.50	0.52	▲ 0.46*	▲ 0.55**	0.48
Niche overlap (bats)	0.18	0.18	0.20	▼ 0.22**	0.18	0.14	0.20
Robustness (bats - random)	0.66	0.61	0.20	0.61	0.61	0.56	0.65
Robustness (bats - degree)	0.43	0.43	0.38	0.42	0.30	0.39	0.38
Robustness (bats- abund.)	0.82	0.80	0.82	0.78	0.80	0.68	0.82

* Indicates a p-value < 0.05; ** Indicates a p-value < 0.01

Table 2.5. Mean, standard deviation and significance values for the 1,000 random generated networks using the swap algorithm tested for the rainforest of La Selva Biological Station and dry forest of Santa Rosa National Park mutualistic networks of frugivorous bats of Costa Rica during an El Niño year (2015).

Network metrics	Rainforest				Dry forest			
	Obs.	**Mean**	**SD**	**p-value**	**Obs.**	**Mean**	**SD**	**p-value**
Number of compartments	2.00	1.38	0.59	0.048	2.00	1.14	0.36	0.003
Nestedness	12.54	11.61	1.89	0.28	21.65	18.98	3.91	0.22
Weighted nestedness	0.52	0.60	0.08	0.18	0.41	0.47	0.08	0.21
Weighted NODF	13.72	20.92	2.64	0.001	13.94	22.37	3.61	0.01
Weighted connectance	0.09	0.12	0.01	0.001	0.12	0.14	0.01	0.01
Modularity (QuanBiMo)	0.46	0.35	0.07	0.01	0.53	0.41	0.02	0.00
Niche overlap (bats)	0.18	0.24	0.04	0.05	0.18	0.19	0.03	0.30
Robustness (bats – random)	0.61	0.59	0.01	0.07	0.66	0.65	0.01	0.21
Robustness (bats- degree)	0.30	0.30	0.03	0.41	0.43	0.40	0.04	0.29
Robustness (bats – abundance)	0.80	0.84	0.03	0.06	0.82	0.86	0.03	0.11

Table 2.6. Mean, standard deviation and significance values for the 1,000 random generated networks using the swap algorithm tested for the dry forest of Santa Rosa National Park mutualistic networks of frugivorous bats of Costa Rica during a non-El Niño year and an extreme El Niño year (2015).

Network metrics	**Dry season**				**Wet season (El Niño)**				**Wet season (non-El Niño year)**			
	Obs.	**Mean**	**SD**	**p-value**	**Obs.**	**Mean**	**SD**	**p-value**	**Obs.**	**Mean**	**SD**	**p-value**
Number of compartments	3.00	1.24	0.45	0.00	1.00	1.13	0.34	0.14	1.00	1.12	0.32	0.11
Nestedness	23.72	22.11	4.83	0.32	21.75	23.31	4.89	0.44	26.76	19.53	4.12	0.06
Weighted nestedness	0.24	0.40	0.10	0.07	0.48	0.45	0.11	0.45	0.41	0.54	0.09	0.14
Weighted NODF	6.57	15.29	2.95	0.00	18.23	22.05	4.59	0.22	15.37	22.80	3.45	0.01
Weighted connectance	0.11	0.12	0.01	0.05	0.14	0.15	0.01	0.14	0.10	0.14	0.01	0.00
Modularity (QuanBiMo)	0.57	0.50	0.03	0.01	0.50	0.46	0.04	0.12	0.52	0.46	0.04	0.08
Niche overlap (bats)	0.18	0.21	0.04	0.17	0.20	0.21	0.03	0.40	0.22	0.38	0.06	0.001
Robustness (bats - random)	0.61	0.62	0.01	0.21	0.63	0.63	0.01	0.48	0.61	0.62	0.01	0.26
Robustness (bats – degree)	0.43	0.40	0.04	0.28	0.38	0.40	0.05	0.33	0.42	0.38	0.04	0.14
Robustness (bats – abund.)	0.80	0.77	0.04	0.24	0.82	0.83	0.05	0.38	0.78	0.81	0.04	0.23

Table 2.7. Mean, standard deviation and significance values for the 1,000 random generated networks using the swap algorithm tested for the rainforest of La Selva Biological Station mutualistic networks of frugivorous bats of Costa Rica during an El Niño year (2015).

Network metrics	**Dry season**				**Wet forest**			
	Obs.	**Mean**	**SD**	**p-value**	**Obs.**	**Mean**	**SD**	**p-value**
Number of compartments	2.00	1.98	0.62	0.17	2.00	1.67	0.73	0.14
Nestedness	33.57	28.05	4.83	0.15	21.75	23.06	5.22	0.47
Weighted nestedness	0.19	0.36	0.12	0.09	0.48	0.46	0.11	0.43
Weighted NODF	6.92	12.73	3.03	0.03	18.24	21.88	4.61	0.21
Weighted connectance	0.11	0.12	0.01	0.10	0.14	0.15	0.01	0.15
Modularity (QuanBiMo)	0.55	0.37	0.04	0.00	0.48	0.42	0.03	0.07
Niche overlap (bats)	0.14	0.17	0.05	0.24	0.20	0.21	0.03	0.36
Robustness (bats – random)	0.56	0.56	0.01	0.41	0.65	0.63	0.01	0.09
Robustness (bats- degree)	0.39	0.35	0.04	0.16	0.38	0.40	0.05	0.35
Robustness (bats – abund.)	0.68	0.74	0.04	0.10	0.82	0.83	0.05	0.37

Table 2.8. Significance values of the difference between observed mutualistic networks of frugivorous bats during a non-El Niño year (2009) and an extreme El Niño year (2015) in dry forest of Santa Rosa National Park and rainforest of La Selva Biological Station in the wet and dry season in relation to the difference of 1,000 random generated networks using the swap algorithm for each habitat and season.

Network metric	Diff. Rainforest – Dry forest	Diff. Dry forest Dry - Wet	Diff. Dry forest El Niño wet – non-El Niño wet	Diff. Rainforest Dry - Wet
Number of compartments	ns. (p=0.52)	ns. (p=1.00)	ns. (p=0.11)	ns. (p=0.35)
Nestedness	ns. (p=0.88)	ns. (p=0.09)	ns. (p=0.24)	ns. (p=0.32)
Weighted nestedness	ns. (p=0.88)	ns. (p=0.18)	ns. (p=0.29)	ns. (p=0.09)
Weighted NODF	ns. (p=0.11)	ns. (p=0.47)	ns. (p=0.31)	ns. (p=0.24)
Weighted connectance	ns. (p=0.28)	ns. (p=0.14)	ns. (p=0.31)	ns. (p=0.22)
Modularity (QuanBiMo)	---	---	---	---
Niche overlap (bats)	ns. (p=0.13)	ns. (p=0.75)	H (p=0.027)	ns. (p=0.43)
Robustness (bats – random)	ns. (p=0.31)	ns. (p=0.28)	ns. (p=0.38)	ns. (p=0.23)
Robustness (bats – degree)	ns. (p=0.63)	ns. (p=0.56)	ns. (p=0.49)	L (p=0.03)
Robustness (bats – abund.)	ns. (p=0.33)	ns. (p=0.63)	ns. (p=0.40)	ns. (p=0.39)

H indicates higher; L indicates lower; ns. indicates not statistically significant.

Figures

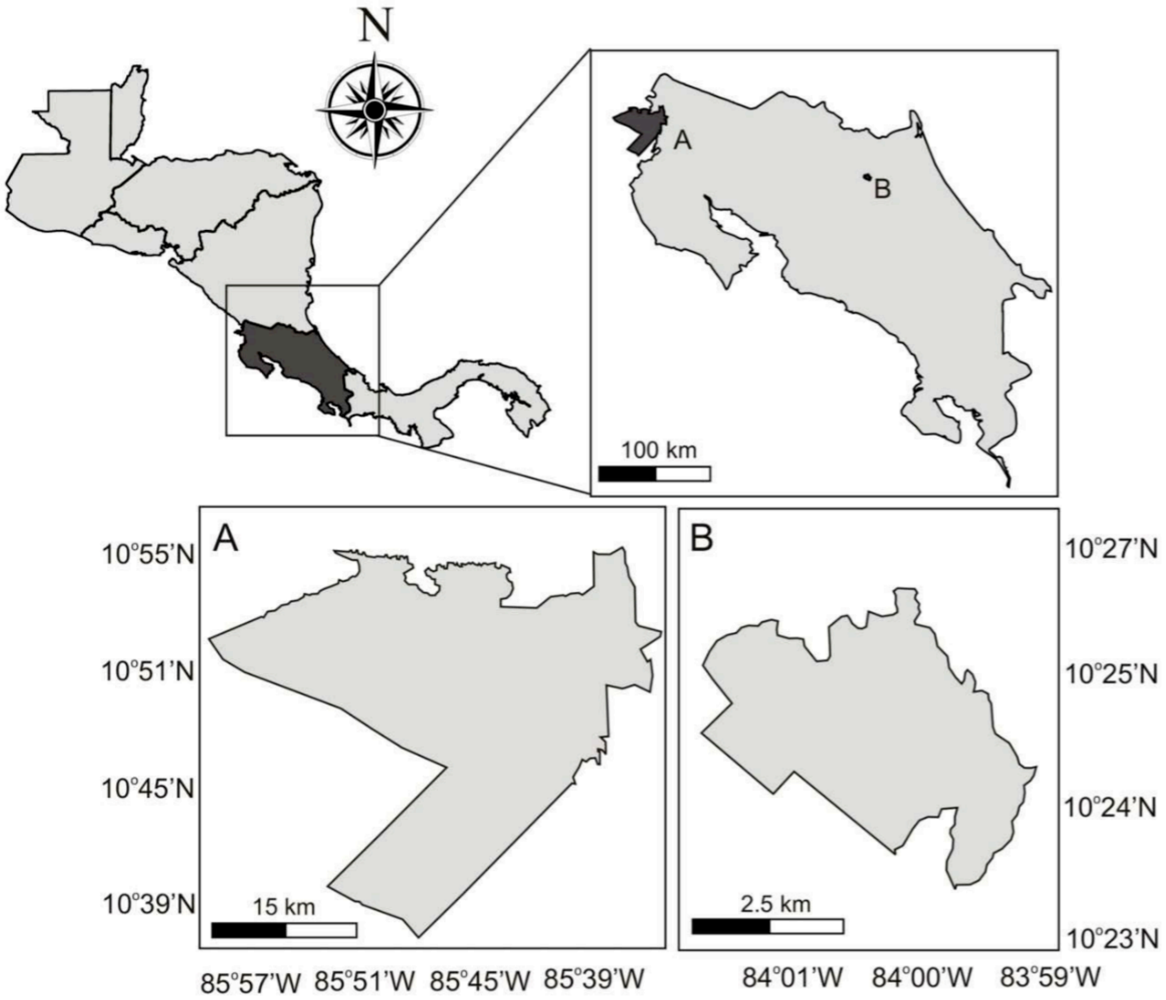


Figure 2.1. Map of Central America with Costa Rica and the field sites of the present study highlighted. A= Sector Santa Rosa (of ACG); B= La Selva Biological Station.

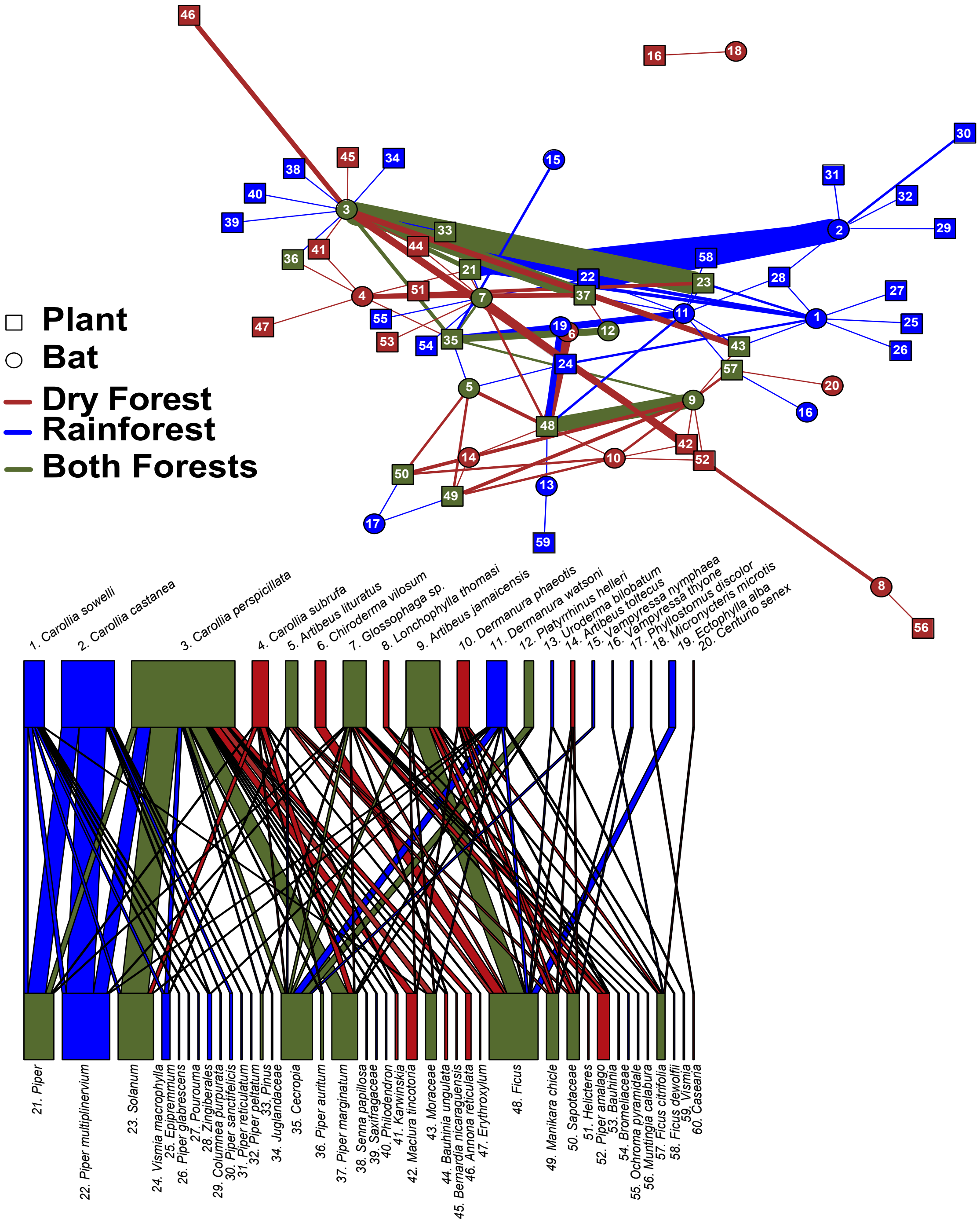


Figure 2.2. Mutualistic networks showing interactions of frugivorous bats and the plants they eat in the wet and dry seasons of the dry forest of Sector Santa Rosa (of ACG) (Costa Rica) during an extreme El Niño event (2015). Bats are represented by the boxes at the top of the bipartite plot while plants are represented by boxes at the lower level of the plot width is proportional to frequency of detection.

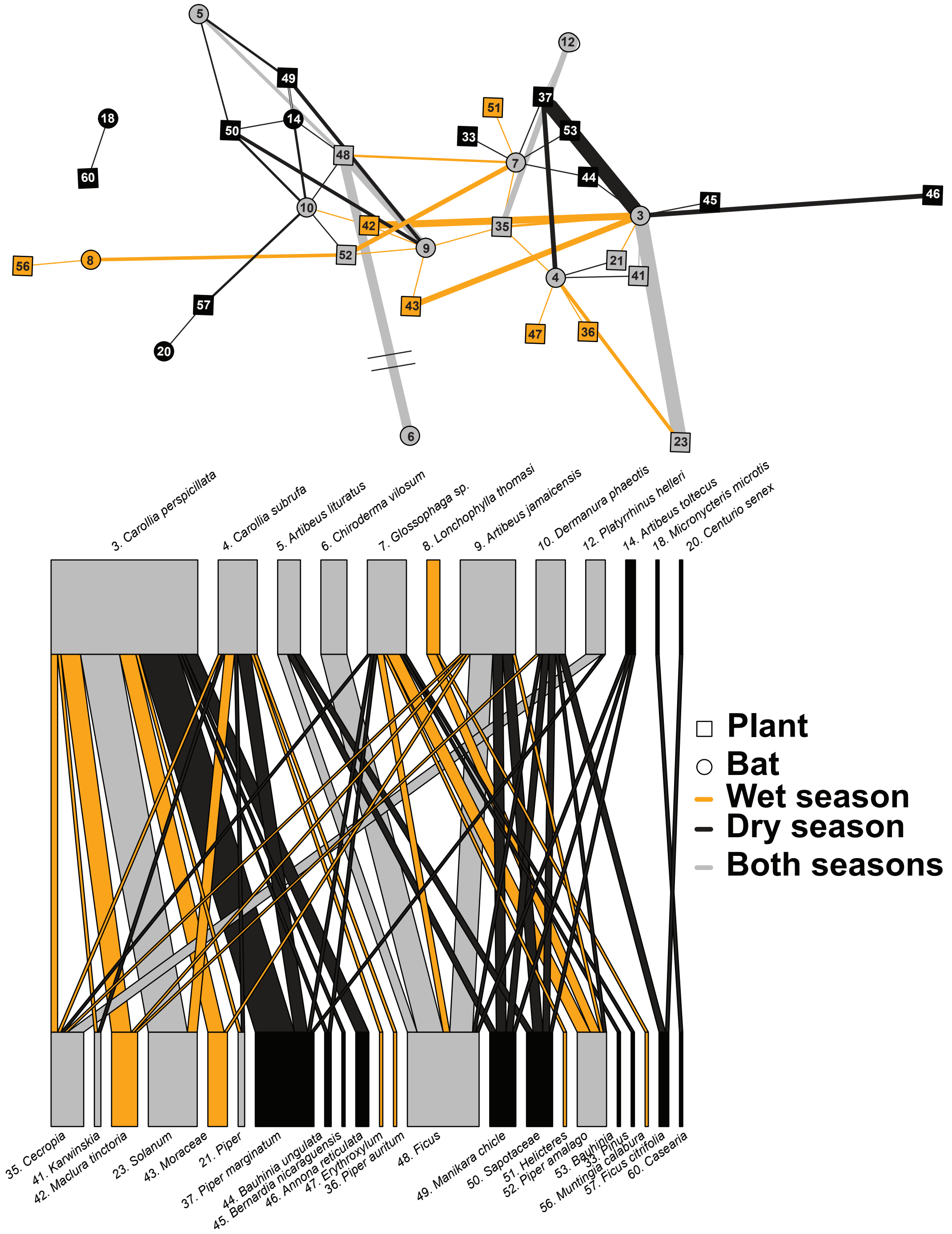


Figure 2.3. Mutualistic networks showing interactions of frugivorous bats and the plants they eat in the wet and dry seasons of the dry forest of Sector Santa Rosa (of ACG) (Costa Rica) during an extreme El Niño event (2015). Bats are represented by the boxes at the top of the bipartite plot while plants are represented by boxes at the lower level of the plot width is proportional to frequency of detection.

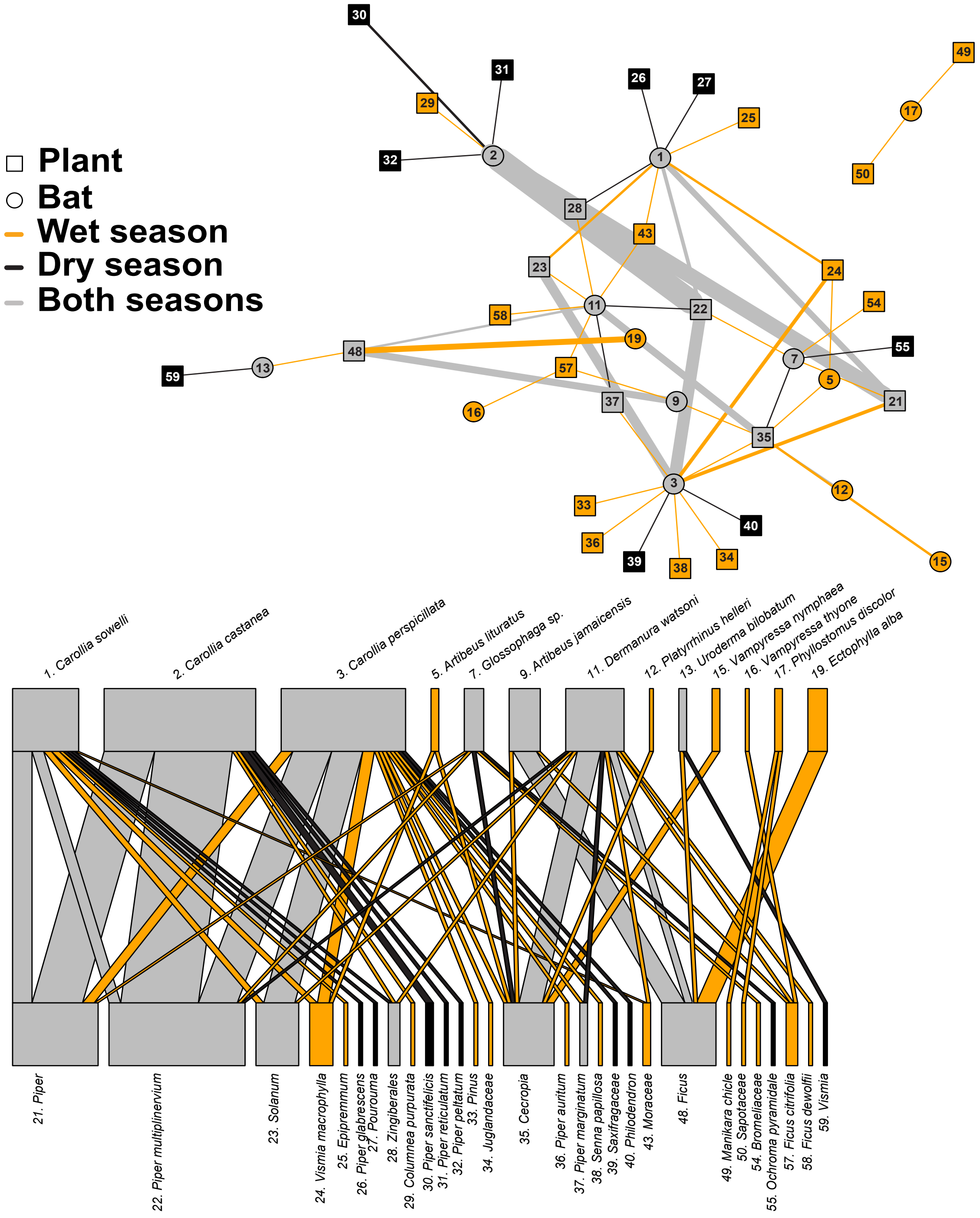


Figure 2.4. Mutualistic networks showing interactions of frugivorous bats and the plants they eat in the wet and dry seasons of the rainforest of La Selva Biological Station (Costa Rica) during an extreme El Niño event (2015). Bats are represented by the boxes at the top of the bipartite plot while plants are represented by boxes at the lower level of the plot width is proportional to frequency of detection.

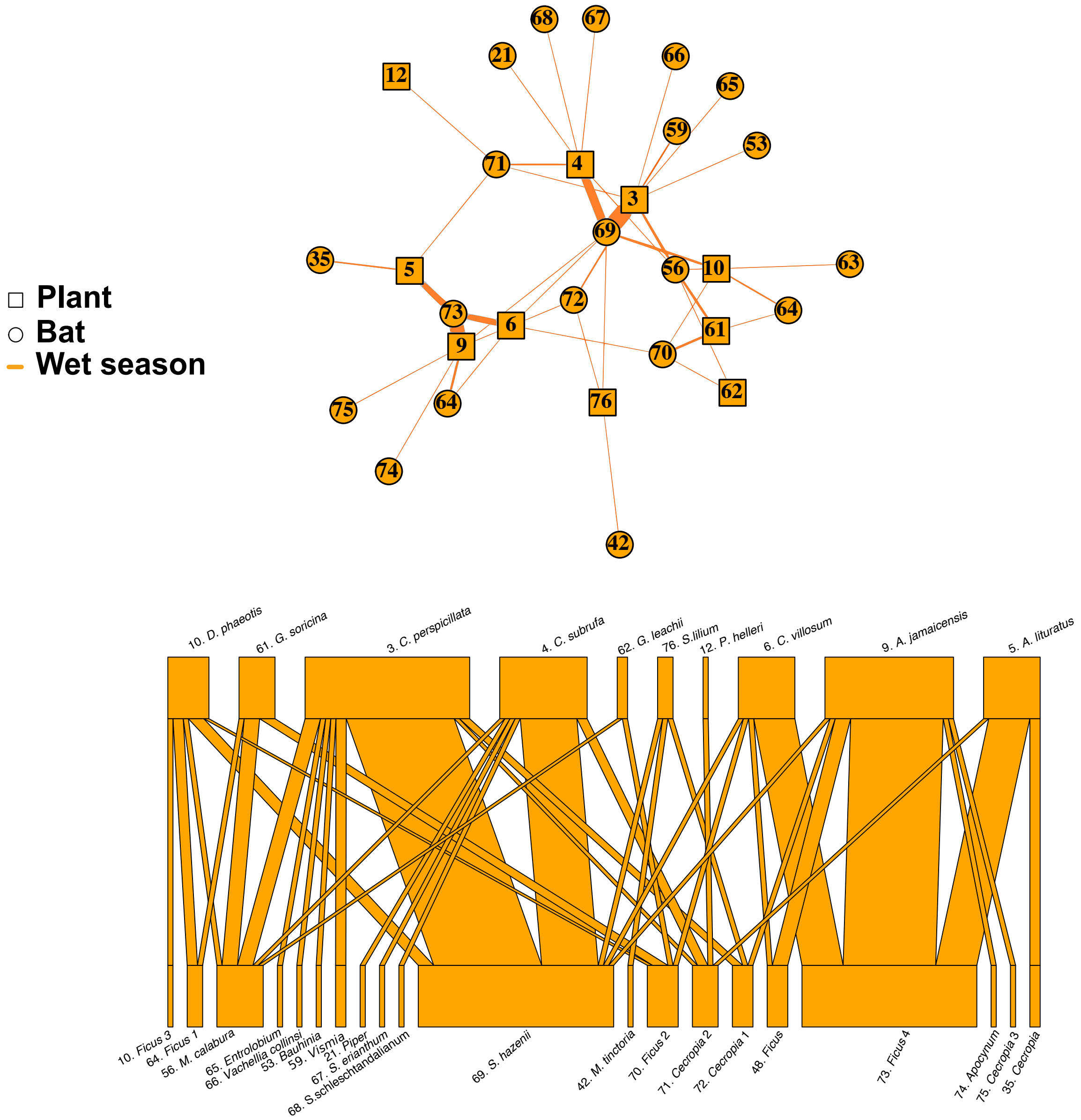


Figure 2.5. Mutualistic networks showing interactions of frugivorous bats and the plants they eat in the wet season of the dry forest of Sector Santa Rosa (of ACG) during a non-El Niño year (2009). Bats are represented by the boxes at the top of the bipartite plot while plants are represented by boxes at the lower level of the plot width is proportional to frequency of detection.

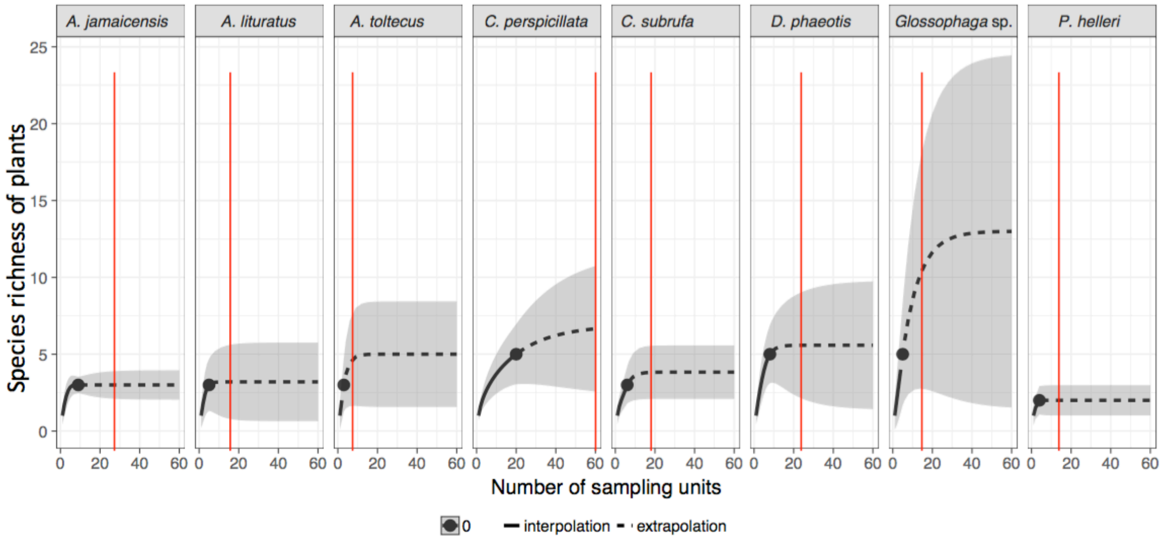


Figure 2.6. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the dry season of an extreme El Niño event in the dry forest of Sector Santa Rosa (of ACG) (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

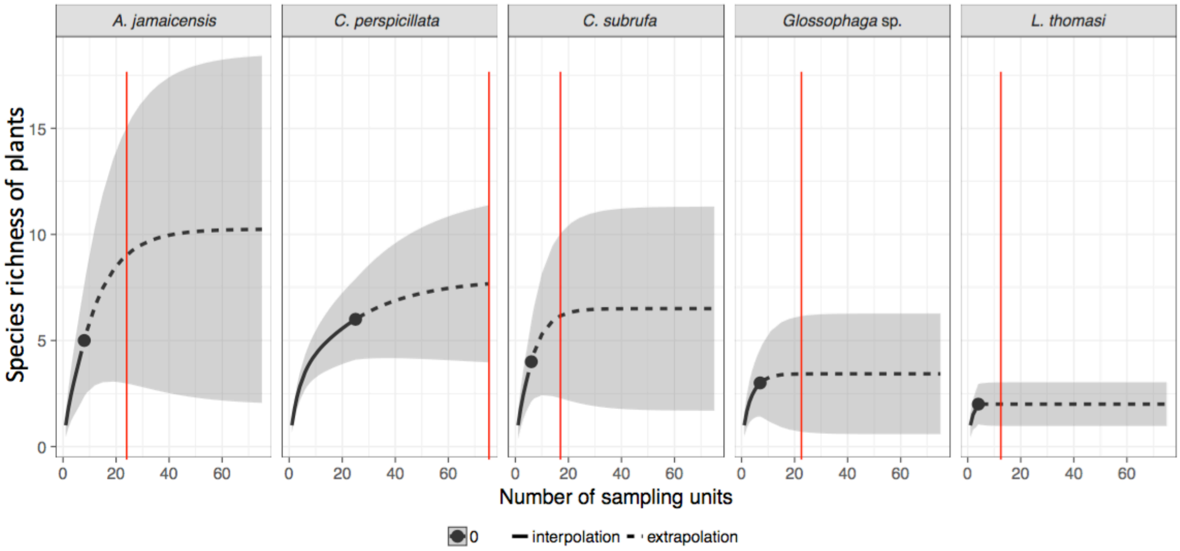


Figure 2.7. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the wet season of an extreme El Niño event in the dry forest of Sector Santa Rosa (of ACG) (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

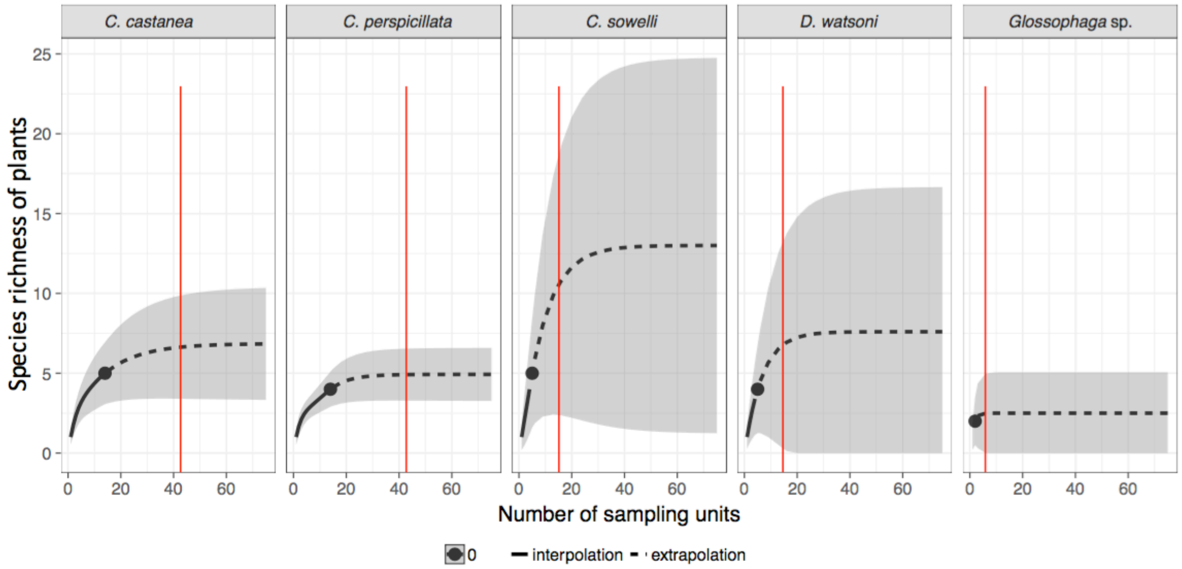


Figure 2.8. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the dry season of an extreme El Niño event in the rainforest of La Selva Biological Station (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

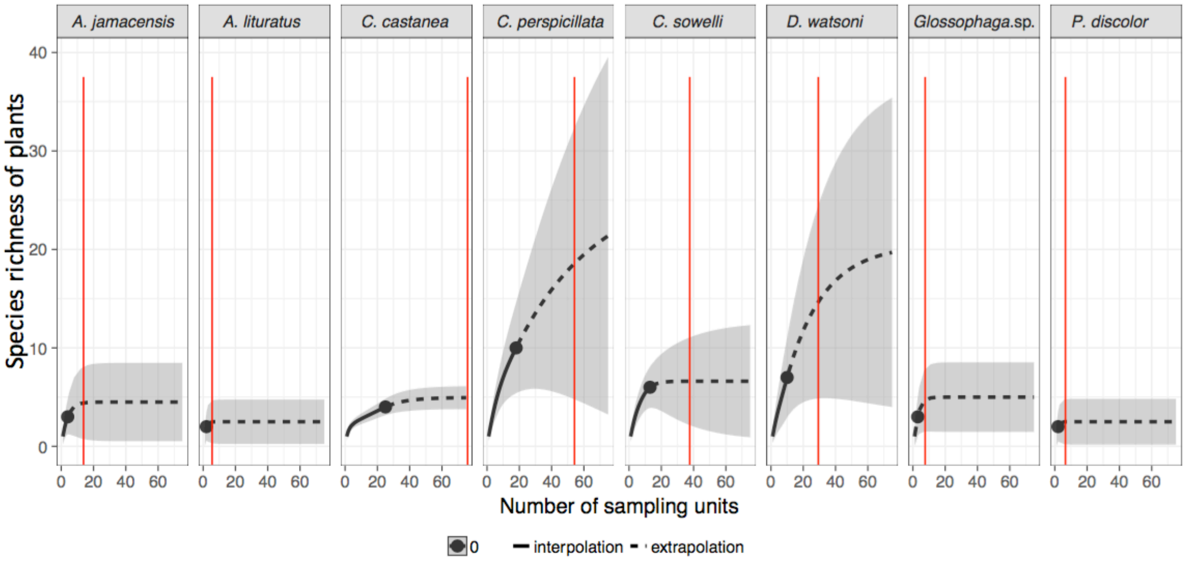


Figure 2.9. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the wet season of an extreme El Niño event in the rainforest of La Selva Biological Station (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

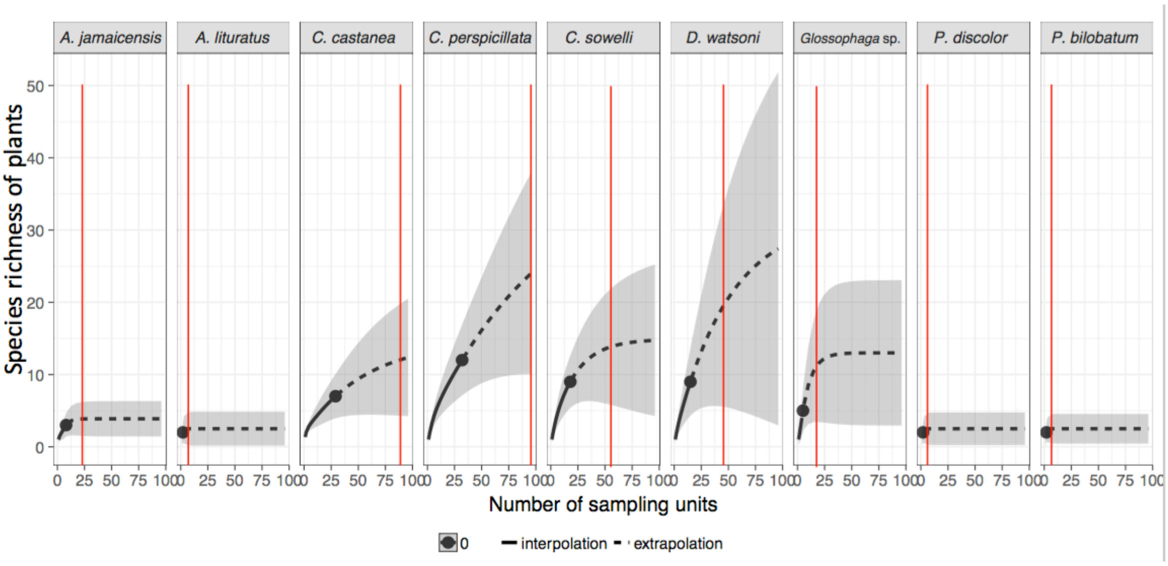


Figure 2.10. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the whole year of an extreme El Niño event in the rainforest of La Selva Biological Station (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

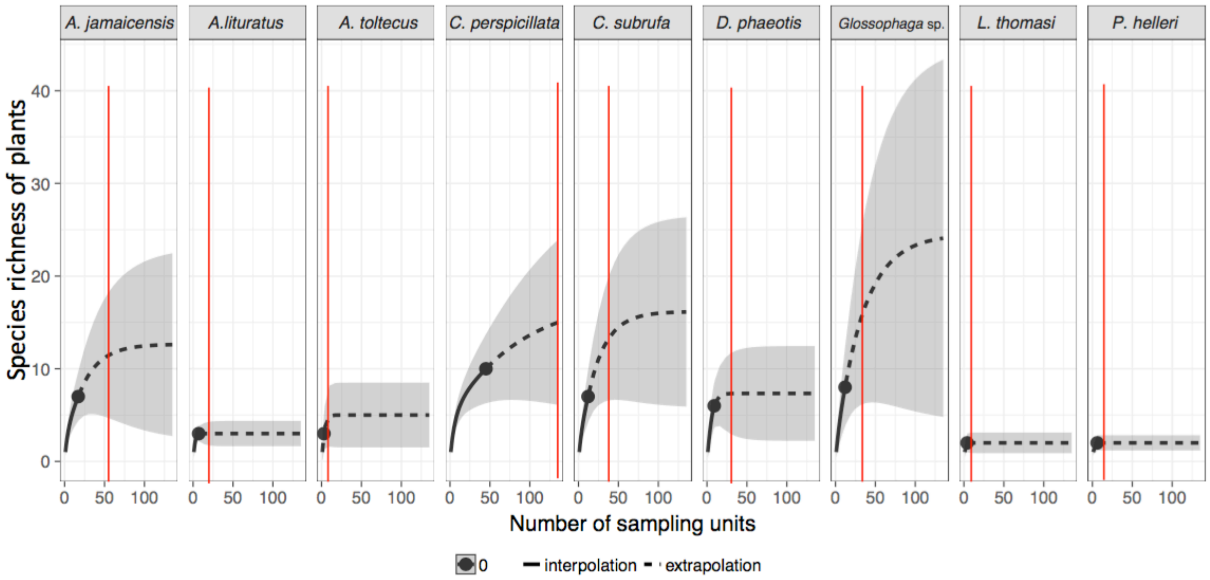


Figure 2.11. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the whole year of an extreme El Niño event in the dry forest of Sector Santa Rosa (of ACG) (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

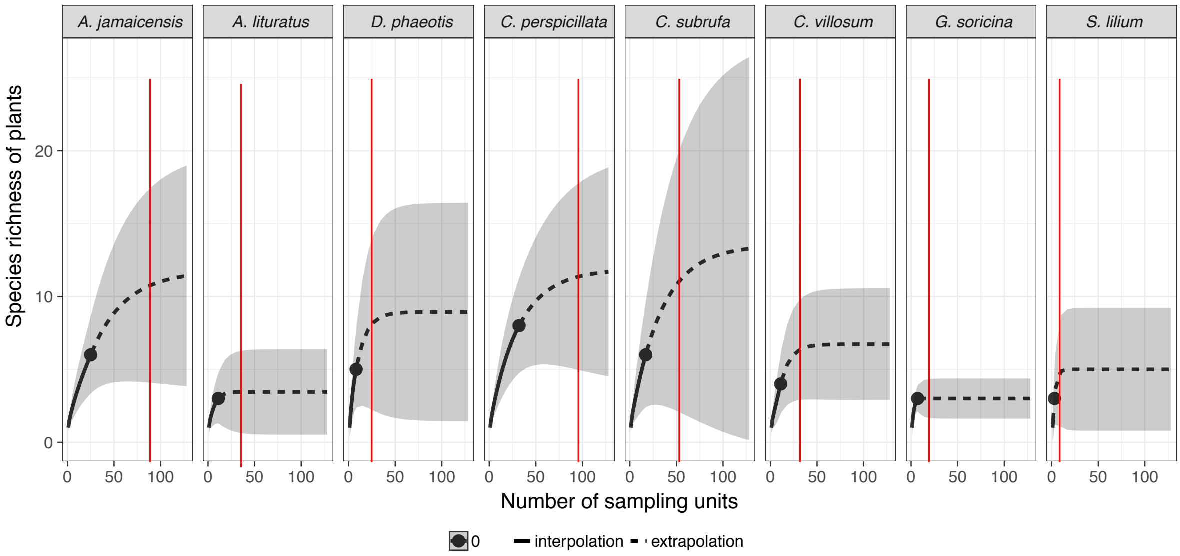


Figure 2.12. Individual-based rarefaction curves comparing the species richness of plants present on species diet of bats during the wet season of a non-El Niño year in the dry forest of Sector Santa Rosa (of ACG) (2009). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

Supplementary material

Due to the indication that for some plant genera, my taxonomic assignment method might not have been sufficient to differentiate between different taxa, I also ran my analysis to test whether my conclusions were robust when assigning all plant sequences at the genus level.

I found differences and similarities in the changes in network metrics when I assigned sequences at the genus level. Changes in the network metrics measured were not consistent across habitats anymore with weighted connectance, weighted NODF, niche overlap and robustness using the abundance model of species extinctions only being significant in the rainforest. On the other hand, changes across seasons had a similar pattern to what I found when assigning sequences at multiple levels. In the dry forest, the wet season did not show any significant difference in the network metrics measured while the dry season had four metrics significantly different than expected from my null models (number of compartments, weighted NODF, weighted connectance and modularity (QuanBiMo)). In the rainforest, a similar pattern with the matches at multiple levels was also found with niche overlap and weighted NODF being consistently lower during both seasons and different metrics being significantly different at each season (wet season: number of compartments, weighted connectance, modularity (QuanBiMo), niche overlap; dry season: robustness (abundance).

S2.1. Interaction matrix of frugivorous bats and the plants present in their diet in the dry forest of Sector Santa Rosa (of ACG) (Costa Rica) during an extreme El Niño year (2015). Frequencies in the matrix represent the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***Carollia perspicillata***	***Carollia subrufa***	***Artibeus lituratus***	***Chiroderma villosum***	***Glossophaga* sp.**	***Lonchophylla thomasi***	***Artibeus jamaicensis***	***Dermanura phaeotis***	***Platyrrhinus helleri***	***Artibeus toltecus***	***Micronycteris microtis***	***Centurio senex***
Cecropia	2	1	0	0	1	0	1	0	5	0	0	0
Karwinskia	1	1	0	0	0	0	0	0	0	0	0	0
Maclura tinctoria	6	0	0	0	0	0	1	1	0	0	0	0
Solanum	12	3	0	0	0	0	0	0	0	0	0	0
Moraceae	5	0	0	0	0	0	1	0	0	0	0	0
Piper	1	1	0	0	0	0	0	0	0	0	0	0
Piper marginatum	12	4	0	0	1	0	0	0	1	0	0	0
Bauhinia ungulata	1	0	0	0	1	0	0	0	0	0	0	0
Bernardia nicaraguensis	1	0	0	0	0	0	0	0	0	0	0	0
Annona reticulata	4	0	0	0	0	0	0	0	0	0	0	0
Erythroxylum	0	1	0	0	0	0	0	0	0	0	0	0
Piper auritum	0	1	0	0	0	0	0	0	0	0	0	0
Ficus	0	0	3	8	2	0	7	1	0	1	0	0
Manilkara chicle	0	0	2	0	0	0	3	2	0	1	0	0
Sapotaceae	0	0	2	0	0	0	3	2	0	1	0	0
Helicteres	0	0	0	0	1	0	0	0	0	0	0	0
Piper amalago	0	0	0	0	4	3	1	1	0	0	0	0
Bauhinia	0	0	0	0	1	0	0	0	0	0	0	0
Pinus	0	0	0	0	1	0	0	0	0	0	0	0
Muntingia calabura	0	0	0	0	0	1	0	0	0	0	0	0
Ficus citrifolia	0	0	0	0	0	0	0	2	0	0	0	1
Casearia	0	0	0	0	0	0	0	0	0	0	1	0

Table S2.2. Interaction matrix of frugivorous bats and the plants present in their diet in the dry forest of Sector Santa Rosa (of ACG) (Costa Rica) during the dry season of an extreme El Niño year (2015). Frequencies in the matrix represent the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***Carollia perspicillata***	***Carollia subrufa***	***Artibeus lituratus***	***Chiroderma villosum***	***Glossophaga* sp.**	***Artibeus jamaicensis***	***Dermanura phaeotis***	***Platyrrhinus helleri***	***Artibeus toltecus***	***Micronycteris microtis***	***Centurio senex***
Piper marginatum	12	4	0	0	1	0	0	1	0	0	0
Bauhinia ungulata	1	0	0	0	1	0	0	0	0	0	0
Bernardia nicaraguensis	1	0	0	0	0	0	0	0	0	0	0
Annona reticulata	4	0	0	0	0	0	0	0	0	0	0
Solanum	2	0	0	0	0	0	0	0	0	0	0
Karwinskia	0	1	0	0	0	0	0	0	0	0	0
Piper	0	1	0	0	0	0	0	0	0	0	0
Ficus	0	0	1	3	0	3	1	0	1	0	0
Manilkara chicle	0	0	2	0	0	3	2	0	1	0	0
Sapotaceae	0	0	2	0	0	3	2	0	1	0	0
Bauhinia	0	0	0	0	1	0	0	0	0	0	0
Pinus	0	0	0	0	1	0	0	0	0	0	0
Cecropia	0	0	0	0	1	0	0	3	0	0	0
Ficus citrifolia	0	0	0	0	0	0	2	0	0	0	1
Piper amalago	0	0	0	0	0	0	1	0	0	0	0
Casearia	0	0	0	0	0	0	0	0	0	1	0

Table S2.3. Interaction matrix of frugivorous bats and the plants present in their diet in the dry forest of Sector Santa Rosa (of ACG) (Costa Rica) during the wet season of an extreme El Niño year (2015). Frequencies in the matrix represent the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***Carollia perspicillata***	***Carollia subrufa***	***Artibeus lituratus***	***Chiroderma villosum***	***Glossophaga* sp.**	***Lonchophylla thomasi***	***Artibeus jamaicensis***	***Dermanura phaeotis***	***Platyrrhinus helleri***
Cecropia	2	1	0	0	0	0	1	0	2
Karwinskia	1	0	0	0	0	0	0	0	0
Maclura tinctoria	6	0	0	0	0	0	1	1	0
Moraceae	5	0	0	0	0	0	1	0	0
Solanum	10	3	0	0	0	0	0	0	0
Piper	1	0	0	0	0	0	0	0	0
Erythroxylum	0	1	0	0	0	0	0	0	0
Piper auritum	0	1	0	0	0	0	0	0	0
Ficus	0	0	2	5	2	0	4	0	0
Helicteres	0	0	0	0	1	0	0	0	0
Piper amalago	0	0	0	0	4	3	1	0	0
Muntingia calabura	0	0	0	0	0	1	0	0	0

Table S2.4. Interaction matrix of frugivorous bats and the plants present in their diet in the rainforest of La Selva Biological Station (Costa Rica) during an extreme El Niño year (2015). Frequencies in the matrix represent the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***Carollia sowelli***	***Carollia castanea***	***Carollia perspicillata***	***Artibeus lituratus***	***Glossophaga* sp.**	***Artibeus jamaicensis***	***Dermanura watsoni***	***Platyrrhinus helleri***	***Uroderma bilobatum***	***Vampyressa nymphaea***	***Vampyressa thyone***	***Phyllostomus discolor***	***Ectophylla alba***
Piper	6	13	3	0	1	0	0	0	0	0	0	0	0
Piper multiplinervium	3	20	10	0	1	0	1	0	0	0	0	0	0
Solanum	2	0	8	0	0	0	1	0	0	0	0	0	0
Vismia macrophylla	2	0	3	1	0	0	0	0	0	0	0	0	0
Epipremmum	1	0	0	0	0	0	0	0	0	0	0	0	0
Piper glabrescens	1	0	0	0	0	0	0	0	0	0	0	0	0
Pourouma	1	0	0	0	0	0	0	0	0	0	0	0	0
Zingiberales	1	1	0	0	0	0	1	0	0	0	0	0	0
Columnea purpurata	0	1	0	0	0	0	0	0	0	0	0	0	0
Piper sanctifelicis	0	2	0	0	0	0	0	0	0	0	0	0	0
Piper reticulatum	0	1	0	0	0	0	0	0	0	0	0	0	0
Piper peltatum	0	1	0	0	0	0	0	0	0	0	0	0	0
Pinus	0	0	1	0	0	0	0	0	0	0	0	0	0
Juglandaceae	0	0	1	0	0	0	0	0	0	0	0	0	0
Cecropia	0	0	1	1	1	1	6	1	0	2	0	0	0
Piper auritum	0	0	1	0	0	0	0	0	0	0	0	0	0
Piper marginatum	0	0	1	0	0	0	1	0	0	0	0	0	0
Senna papillosa	0	0	1	0	0	0	0	0	0	0	0	0	0
Saxifragaceae	0	0	1	0	0	0	0	0	0	0	0	0	0
Philodendron	0	0	1	0	0	0	0	0	0	0	0	0	0
Moraceae	1	0	0	0	0	0	1	0	0	0	0	0	0
Ficus	0	0	0	0	0	6	2	0	1	0	0	0	5
Manilkara chicle	0	0	0	0	0	0	0	0	0	0	0	1	0
Sapotaceae	0	0	0	0	0	0	0	0	0	0	0	1	0
Bromeliaceae	0	0	0	0	1	0	0	0	0	0	0	0	0
Ochroma pyramidale	0	0	0	0	1	0	0	0	0	0	0	0	0
Ficus citrifolia	0	0	0	0	0	1	1	0	0	0	1	0	0
Ficus dewolfii	0	0	0	0	0	0	1	0	0	0	0	0	0
Vismia	0	0	0	0	0	0	0	0	1	0	0	0	0

Table S2.5. Interaction matrix of frugivorous bats and the plants present in their diet in the rainforest of La Selva Biological Station (Costa Rica) during the dry season of an extreme El Niño year (2015). Frequencies in the matrix represent by the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***Carollia sowelli***	***Carollia castanea***	***Carollia perspicillata***	***Glossophaga* sp.**	***Artibeus jamaicensis***	***Dermanura watsoni***	***Uroderma bilobatum***
Piper	1	5	0	0	0	0	0
Pourouma	1	0	0	0	0	0	0
Piper multiplinervium	1	5	6	0	0	1	0
Piper glabrescenes	1	0	0	0	0	0	0
Zingiberales	1	0	0	0	0	0	0
Piper sanctifelicis	0	2	0	0	0	0	0
Piper reticulatum	0	1	0	0	0	0	0
Piper peltatum	0	1	0	0	0	0	0
Saxifragaceae	0	0	1	0	0	0	0
Solanum	0	0	6	0	0	0	0
Philodendron	0	0	1	0	0	0	0
Ochroma pyramidale	0	0	0	1	0	0	0
Cecropia	0	0	0	1	0	2	0
Ficus	0	0	0	0	4	1	0
Piper marginatum	0	0	0	0	0	1	0
Vismia	0	0	0	0	0	0	1

Table S2.6. Interaction matrix of frugivorous bats and the plants present in their diet in the rainforest of La Selva Biological Station (Costa Rica) during the wet season of an extreme El Niño year (2015). Frequencies in the matrix represent the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***Carollia sowelli***	***Carollia castanea***	***Carollia perspicillata***	***Artibeus lituratus***	***Glossophaga* sp.**	***Artibeus jamaicensis***	***Dermanura watsoni***	***Platyrrhinus helleri***	***Uroderma bilobatum***	***Vampyressa nymphaea***	***Vampyressa thyone***	***Phyllostomus discolor***	***Ectophylla alba***
Piper	5	8	3	0	1	0	0	0	0	0	0	0	0
Piper mutliplinervium	2	15	4	0	1	0	0	0	0	0	0	0	0
Solanum	2	0	2	0	0	0	1	0	0	0	0	0	0
Vismia macrophylla	2	0	3	1	0	0	0	0	0	0	0	0	0
Moraceae	1	0	0	0	0	0	1	0	0	0	0	0	0
Epipremmum	1	0	0	0	0	0	0	0	0	0	0	0	0
Zingiberales	0	1	0	0	0	0	1	0	0	0	0	0	0
Columnea purpurata	0	1	0	0	0	0	0	0	0	0	0	0	0
Pinus	0	0	1	0	0	0	0	0	0	0	0	0	0
Juglandaceae	0	0	1	0	0	0	0	0	0	0	0	0	0
Cecropia	0	0	1	1	0	1	4	1	0	2	0	0	0
Piper auritum	0	0	1	0	0	0	0	0	0	0	0	0	0
Piper marginatum	0	0	1	0	0	0	0	0	0	0	0	0	0
Senna papillosa	0	0	1	0	0	0	0	0	0	0	0	0	0
Bromeliaceae	0	0	0	0	1	0	0	0	0	0	0	0	0
Ficus	0	0	0	0	0	2	1	0	1	0	0	0	5
Ficus citrifolia	0	0	0	0	0	1	1	0	0	0	1	0	0
Ficus dewolfii	0	0	0	0	0	0	1	0	0	0	0	0	0
Manilkara chicle	0	0	0	0	0	0	0	0	0	0	0	1	0
Sapotaceae	0	0	0	0	0	0	0	0	0	0	0	1	0

Table S2.7. Interaction matrix of frugivorous bats and the plants present in their diet in the rainforest of Sector Santa Rosa (of ACG) (Costa Rica) during the wet season of a non-El Niño year (2009). Frequencies in the matrix represent the number of times that a plant species was found in a bat's diet. Plant taxa were identified at multiple taxonomic levels using DNA barcoding.

Plant taxa	***A. jamaicensis***	***A. lituratus***	***A. phaeotis***	***C. perspicillata***	***C. subrufa***	***C. villosum***	***G. soricina***	***G. leachii***	***P. helleri***	***S. lilium***
Apocynum	1	0	0	0	0	0	0	0	0	0
Bauhinia	0	0	0	1	0	0	0	0	0	0
Cecropia	0	2	0	0	0	0	0	0	0	0
Cecropia 1	1	0	0	2	0	0	0	0	0	1
Cecropia 2	0	1	0	1	2	0	0	0	1	0
Cecropia 3	1	0	0	0	0	0	0	0	0	0
Enterolobium	0	0	0	1	0	0	0	0	0	0
Ficus	3	0	0	0	0	1	0	0	0	0
Ficus 1	0	0	2	0	0	0	1	0	0	0
Ficus 2	0	0	1	0	0	1	3	1	0	0
Ficus 3	0	0	1	0	0	0	0	0	0	0
Ficus 4	18	8	0	0	0	8	0	0	0	0
M. tinctoria	0	0	0	0	0	0	0	0	0	1
M. calabura	0	0	1	3	1	0	0	3	1	0
Piper	0	0	0	0	1	0	0	0	0	0
S. erianthum	0	0	0	0	1	0	0	0	0	0
S. hazenii	1	0	3	21	11	1	0	0	0	1
S.schlechtendalianu	0	0	0	0	1	0	0	0	0	0
V. collinsii	0	0	0	1	0	0	0	0	0	0
Vismia	0	0	0	2	0	0	0	0	0	0

CHAPTER THREE

El Niño, seasonality and modularity of tropical antagonistic food web

Abstract

Dry periods such as dry seasons and droughts are expected to lead to reduced food resources in tropical areas, and are predicted to increase in frequency and severity with climate change. Despite this, the impacts of droughts on interaction networks (food webs) are poorly understood. According to optimal foraging theory, at times of low food availability animals tend to adopt more generalist dietary strategies, leading to food webs becoming less modular but with an increased diversity within modules. Alternatively, niche theory suggests that reduced resource availability may increase niche differentiation promoting the opposite trend. To test these two alternative hypotheses, I constructed antagonistic bat-arthropod networks in the dry forest of Costa Rica, during a non-El Niño and during a drought plagued wet season caused by the severe El Niño cycle of 2015-2016 and contrast this to an El Niño dry season. To resolve trophic links between insectivorous bats and their prey, and characterise the consequences of drought on the structure of food networks, I applied DNA barcoding to the arthropod fragments contained within bat faeces. I found that, in line with predictions of niche theory, higher modularity was found during the dry season of the ENSO year. There was also a higher number of Molecular Operational Taxonomic Units (MOTUs) consumed and higher species dietary overlap during the non-El Niño wet season. Closeness centrality was lower during the ENSO event and there was a switch in the network position of Coleoptera, which had its lowest centrality values during the dry season. Betweenness centrality was higher during the non-El Niño wet season, with no difference found between orders. These changes suggest that bats do not adopt a more generalist strategy under extreme stress but niches become increasingly small and segregated. This may increase network stability but reflect a severe reduction in resource exploitation and increased vulnerability for any species.

Introduction

Major droughts are predicted to increase in frequency and severity with climate change, and are strongly associated with a growing incidence of extreme El Niño events (Cai *et al.* 2014). Such droughts can have profound ecological consequences (Holmgren *et al.* 2001; Magaña *et al.* 2003), altering the distribution and abundance of resources (Glynn 1988). Insects, for example, which are key prey sources for many vertebrates, face population crashes during extreme dry periods (Holmgren *et al.* 2001), with potential cascading negative effects for insectivores. Despite this, the impacts of drought on ecological interactions are poorly understood. In particular, although a small number of studies have examined how changes in rainfall during El Niño events can influence population dynamics and mutualistic interactions (Lima *et al.* 1999; Harrison 2000; Harrison 2001; Marshal *et al.* 2002), there has been little or no consideration of how droughts might impact on antagonistic interactions, including predator-prey relationships.

Attempts to understand how predators respond to prey availability come from optimal foraging theory (Stephens & Krebs 1986). When resources are scarce, species are predicted to adopt a more generalist foraging strategy (MacArthur & Pianka 1966; Schoener 1971; Pulliam 1974), with increased niche overlap at the community-level. Alternatively, niche theory suggests that increased competition due to food scarcity drives niche segregation, which decreases diet overlap between species (Hardin 1960).

Interaction networks (food webs) provide a powerful means of describing trophic interactions (e.g. pollinators and plants; predators and prey) at the community-level (e.g. Ings *et al.* 2009), and have been used to assess how communities respond to environmental change (Carey *et al.* 2013). Yet constructing ecological networks can be challenging, with even ecologically-important links often difficult to detect (McCann 2000). In this regard, the use of DNA for species identification in dietary studies has proven particularly important in detecting rare interactions. In the case of insectivores (e.g. Clare *et al.* 2009), a large proportion of the consumed resources might be accounted for by rare or difficult-to-detect arthropod species (Novotný & Basset 2000; Coddington *et al.* 2009).

Although molecular tools have been applied widely to detect prey of single predators (reviewed in Pompanon *et al.* 2012), they have only recently been used in food web ecology (reviewed by Roslin & Majaneva 2016). Despite this, early results suggest molecular methods are able to detect missing links, and have the potential to change our understanding of network structure by revealing different levels of specialization and increasing interaction types (Kaartinen *et al.* 2010; Wirta *et al.* 2014). For example, in a high Arctic host-parasitoid food web, the addition of molecular methods led to the identification of new taxa, as well as nearly three times the number of observed links, and a threefold increase in the values of generality, vulnerability and linkage density in comparison to traditional methods (Wirta *et al.* 2014).

A key property of ecological networks, which might provide useful insights into how predator-prey interactions are structured and change in times of drought, is modularity. This metric reflects the extent to which species share and exploit resources in the community. Specifically, modularity describes the extent to which interactions among species are confined to subgroups ('modules') (Olesen *et al.* 2007). High modularity is associated with increased network stability because species inside modules are buffered from impacts in other parts of the network; thus perturbations that reduce modularity might also lower networks resilience (Guimerà *et al.* 2010). Other metrics can also provide important information on network structure dynamics. For example, 'centrality' is generally used as a measure of importance of a species (e.g. whether or not it is a keystone species) in the network, and can be measured in several ways. Closeness centrality measures how connected one particular species is to all others in the network and how rapidly an impact on one species spreads across the network (Rocchi *et al.* 2017). Betweenness centrality, on the other hand, measures species importance in linking different modules in the network, acting as a bridge between these otherwise distinct communities (Clauset *et al.* 2004; Rocchi *et al.* 2017).

Applying optimal foraging theory to network ecology suggests that in times of low food availability, species that adopt a more generalist feeding behaviour are expected to become more connected within the network, leading to lower modularity. This arises because species form new connections in the network and explore the niche of other species. On the other hand, according to niche theory, food scarcity will lead to increased niche segregation due to ecological specialization, resulting in higher modularity.

Most studies in network ecology have focused on mutualistic interactions (Bascompte & Jordano 2007; Bastolla *et al.* 2009; Donatti *et al.* 2011; Suweis *et al.* 2013), with very few investigations of antagonistic interactions such as predators and their prey (Pires & Guimarães 2013; Rhor & Bascompte 2014). As such, little is known about how such networks respond to changes in resource availability. In the tropics, bats consume an extraordinary diversity and volume of insects, with energetic demands suggesting that some species must regularly consume up to 70% of their body weight in insects each night (Kunz *et al.* 2011). Yet bats are elusive animals whose actual trophic interactions have only recently started to be understood with the use of molecular techniques (e.g. Clare *et al.* 2009; Bohmann *et al.* 2011; Clare *et al.* 2011). Insectivorous bats are thought to change diet in relation to resource fluctuations in their environment (Clare *et al.* 2011) with oscillations in insect availability playing an important role in defining dietary breadth (Agosta *et al.* 2003). Using molecular techniques to resolve such interactions are likely to be especially powerful in resolving interactions in tropical systems, where arthropods are hyper diverse and poorly sampled.

Here I use molecular methods and network analysis to analyse the effect of a severe drought during an extreme El Niño event on modularity and food web structure of an entire community of insectivorous bats in a seasonal dry forest in Central America. By comparing food web structure from a non-El Niño year to that of the El Niño year, I tested for significant changes in modularity in drought conditions. Decreased modularity is predicted if bats become more generalist and thus share more prey taxa, as might be expected from optimal foraging theory, whereas increased modularity is expected if individual bats become more specialist and share fewer taxa, as predicted from niche theory (Table 3.1).

Methods

Study area

The study was conducted in Sector Santa Rosa (10°48'53" N, 85˚36'54" W) in the Área de Conservación Guanacaste (ACG) Costa Rica, which undergoes an extreme seasonal variation in precipitation and plant phenology (Murphy & Lugo 1986; Janzen 1988). In typical years, the dry season (December to May) is followed by the wet season (June to November) and the annual precipitation ranging from 915 to 2,558 mm/year (Hilje *et al.* 2015). However, during the El Niño year of 2015, Sector Santa Rosa (of ACG) registered the lowest annual rainfall recorded in the last 30 years.

Bat sampling

A total of three collecting trips were performed: one during the wet season of a non-El Niño year (May - Jul, 2009), and one during the dry season (Jan - Feb) and wet season (Jul - Aug) of an extreme El Niño event (2015). I captured bats using four to six mist nets (6m - 12m) opened along trails and near watercourses in the study area from 18h - 22h. In addition, a canopy net and harp trap were used in 2009 but these had low capture rates and so were not used in 2015. Sampling effort was equal to approximately 2,250 m^2.hours during each of the seasons during the El Niño year, and approximately the same during the non-El Niño year. Bat captures during the non-El Niño year were conducted by Dr. Elizabeth L. Clare. Each bat captured had its forearm measured with callipers (0.1 mm precision), and was identified to species using field keys (Reid 1997; Timm & Laval 1998; Laval & Rodríguez-Herrera 2002). Bats were held in cloth bags for a maximum of 2 hours so that faecal samples could be collected. These were then stored in 70% ethanol at -20°C. Bats captured in 2009 had their taxonomic identities confirmed using DNA barcoding *sensu* Clare *et al.* (2007) (data not shown).

Sample collection, DNA extraction, PCR amplification and sequencing

I extracted DNA from faecal samples of the El Niño year, and data from the normal year was acquired from Clare *et al.* (2018). DNA extractions were made using the QIAamp Stool Mini Kit (Qiagen UK) following manufacturer's instructions with the modifications suggested by Zeale *et al.* (2011) and Clare *et al.* (2014b). Amplification, gel electrophoresis and amplicon size selection, clean up and sequencing were all performed at the Biodiversity Institute of Ontario, University of Guelph (Canada). Primers based on COI primers ZBJ-ArtF1c and ZBJ-ArtR2c were used to amplify prey DNA (Zeale *et al.* 2011); these primers were modified using the dual adaptor system for the Ion Torrent (Clare *et al.* 2014b). Each 20µL PCR reaction contained 10µL of Qiagen multiplex PCR (Qiagen, CA) master mix, 6µL of water, 1µL of each 10µM primer and 2µL of DNA. PCR amplification was as follows: 95°C, 15 min; 50 cycles of 95°C, 30 s; 52°C, 30 s; 72°C, 30 s and 72°C, 10 min. Amplicons were visualized on a 2% agarose 96-well precast E-gel (Invitrogen, Life Technologies). Size selection was performed using a PCRClean DX kit (Aline Biosciences). The product was eluted in water, and the concentration measured using a Qubit 2.0 spectrophotometer and the Qubit dsDNA HS Assay Kit (Invitrogen, Life Technologies). The products were normalized to 1ng /µL prior to final library dilution. Sequencing was performed on the Ion Torrent (Life Technologies) sequencing platform as per Clare *et al.* (2014b) with 192 samples (2 x 96 well plates) in a run using a 316 chip and following the manufacturers' guidelines but with a 2x dilution.

Data analysis

I analysed the data from the El Niño year following the same procedure used for the non-. El Niño year (Clare *et al.* 2018). In brief, sequences were analysed using the Galaxy platform (http//main.g2.bx.psu.edu/root, Giardine *et al.* 2005; Blankenberg *et al.* 2010; Goecks *et al.* 2010). I de-multiplexed the samples by forward and reverse MIDs (a maximum of two mismatches and two indels were allowed) and removed primer, MID and adapter sequences (http://han nonlab.cshl.edu/fastx_toolkit). I filtered out all sequences shorter than 147 bp or longer than 167 bp (target amplicon length was 157bp) and collapsed them into unique haplotypes and then excluded singleton sequences from further analyses (http://han nonlab.cshl.edu/fastx_toolkit). I clustered sequences into molecular operational taxonomic units (MOTUs) and picked representative sequence of each MOTU for analysis with the QIIME pick_otu and uclust methods (http://qiime.sourceforge.net/, Caporaso *et al.* (2010)). MOTUs were clustered with the similarity threshold of 92% (see a discussion in Clare *et al.* 2016 for the appropriateness of MOTU cluster levels). I used MOTU reference sequences to identify MOTUs to the order level using BLAST analyses and a database of >600,000 reference DNA barcodes extracted from GenBank. I used MEGAN version 5.6.3. (Huson *et al.* 2011) to screen out unidentified sequences and those not resolved to the level of a taxonomic order with the LCA parameters: Min score=150.1, Max expected=0.001, Top percent=10.0, Min support=1, LCA percent=100.0, Min complexity=0.2. I screened for chimeric sequences from each reference sequence using UCHIME as implemented in MOTHUR (Schloss *et al.* 2009), and for contaminants by looking for similar BLAST matches to nontarget taxa (e.g. bacteria) in MEGAN (with the same parameters as above). The identified MOTUs were used for network analysis.

Network matrices

I compiled the observed interactions into frequency matrices where each cell value represents the number of observed interactions between each species pair (bats and arthropod MOTU). I considered one realized interaction when the DNA of an arthropod MOTU from an identified Order was detected in the faeces of one individual bat species. For example, individual Bat X may have consumed Lepidoptera MOTU 2, 3 and 56 and Diptera MOTU 7. For each bat species-prey item combination, I calculated the interaction frequency as the number of bat individuals captured whose faeces were found to contain the given arthropod MOTU; e.g. if 10 individuals of the bat *Pteronotus mesoamericanus* were found to consume Lepidoptera MOTU 6, the frequency is 10. I constructed the following matrices: (1) the network of interactions for the dry forest during the dry season of the El Niño year; (2) the network of interactions for the dry forest during the wet season of the El Niño year, and (3) the network of interactions for the dry forest during the wet season of the non-El Niño year.

Network metric and statistical analyses

I calculated modularity using the fast greedy algorithm (Newman 2004). The fast greedy algorithm is a modularity-based maximization function that tries to optimize modularity in a greedy manner by merging the modules iteratively until an optimum is found (Pons & Latapy 2005). It starts with each node being a member of a unique community, then repeatedly merges two communities in order to search for the highest value of modularity (Clauset *et al.* 2004). Such fast greedy methods are considered among the best choices for weighted networks (Leger *et al.* 2015). I also recorded the number of modules in the network. For each module, I recorded the number of taxa present based on the number of nodes (hereafter termed “module size”) as well as calculated the diversity of taxa (hereafter termed “module diversity”) using the Shannon index (H) (Shannon 1948) with the *diversity* command in the vegan package for R (Oksanen *et al.*

2012). In order to determine the sampling completeness of my networks and the proportion of the total arthropod species richness present in bat diets that have been sampled, I used the Chao 1 index according to the method proposed by Macgregor *et al.* (2017) for the networks, and individual based rarefaction curves for the estimation of each bat species' diet.

Other metrics can also provide important information on network structure dynamics. For example, 'centrality' is generally used as a measure of the importance of a species (e.g. whether or not it is a keystone species) in the network, and can be measured in several ways. Closeness centrality measures how connected one particular species is to all others in the network and how rapidly an impact on one species spreads across the network (Rocchi *et al.* 2017). Closeness centrality values range from 0 to 1 where 0 represents low closeness centrality and 1 is considered high (Rocchi *et al.* 2017); prey species with high values are under greater predatory pressure as they are more closely connected to all other species in the network and are thus more likely to be preyed upon. If nodes with high closeness centrality are removed from the network, this can lead to an increase in secondary extinctions (Jordán 2005; Quince *et al.* 2005). Betweenness centrality, on the other hand, measures species importance in linking different modules in the network, acting as a bridge between these otherwise distinct communities (Clauset *et al.* 2004; Rocchi *et al.* 2017). Species with high betweenness centrality are important in propagating or buffering cascading effects between modules during events that cause perturbations in the network. Networks with many species with low betweenness centrality suggest either one big module of interactions or that most species interact only with their own module members. This can indicate increased use and perhaps competition for the same resources (Sokhn *et al.* 2013). I calculated closeness and betweenness centrality using the package igraph (Csárdi & Nepusz 2006) in R.

Since the drought during the El Niño year was extreme in comparison to non-El Niño years, which would very likely reduce the arthropod availability for bats, I expected bats in the network to increase their niche breadth, leading to a more connected and low modular network, or for them to have their interactions constrained by species traits, such as: echolocation frequency or wing morphology. In order to test the direction and magnitude of the change in observed networks, I used a Monte Carlo method, and a Erdõs-Rényi approach for the randomization of the observed networks, where the chance of a predator interacting with a prey species was 50% (Erdõs & Rényi 1960). This algorithm produces networks with low modularity (~0.100), but simulate realized interactions in a way that is ideal to test if species in the network are forming more generalist or more specialized interactions than would be expected from a binomial distribution with probability 0.5. To assess the significance of each network and for pairwise comparisons, I used a Monte Carlo procedure with 1,000 randomizations (α = 5%).

To compare differences between the number of MOTUs consumed by individual bats between El Niño wet and El Niño dry and between El Niño wet and non-El Niño wet, I used a student t test for normally distributed data with equal variances. To compare the module diversity and size between the dry and wet season of the El Niño, and between the wet seasons in the El Niño and non-El Niño years, I used factorial ANOVAs by resampling with 10,000 iterations and a Tukey post-hoc HSD test. To test for differences in the closeness centrality values of the different orders of arthropods between seasons during the non-El Niño and El Niño year, I used a MANOVA, in which I excluded all orders with fewer than 10 MOTUs. To test for which orders were responsible for the changes in the centrality values, I used univariate ANOVAs for each order between seasons and subsequent post-hoc Tukey HSD tests. I also used a two-way factorial ANOVA to test for differences in betweenness centrality values among seasons and orders and subsequent post-hoc Tukey HSD tests to assess differences.

To determine whether there was a significant species niche overlap was different from null distributions within each season, and whether there was a change between El Niño wet and El Niño dry and between El Niño wet and non-El Niño wet, I also used a Monte Carlo approach. However, I transformed the matrices of interaction to binary values representing presence-absence of arthropod prey, and calculated the bat species diet overlap for each season in each year using the Jaccard index (Jost 2007). I then randomized the matrix of interactions using the MGEN algorithm (Vázquez *et al.* 2009). This algorithm does not constrain for marginal totals or connectance and randomizes the matrix of interactions by making each interaction between two species in the matrix equiprobable (equal chances of the interaction of an arthropod prey to happen with each of the bat species). I choose this null model to simulate a complete random choice of prey for each bat species as I expected initially that they would adopt a generalist approach due to the low availability of food resources. Niche overlap values were calculated using the function network level in the Bipartite R package (Dormann *et al.* 2008), which gives one single value for bat species niche overlap in each network. To assess the significance of each network with resepct to null models and for pairwise comparisons, I used a Monte Carlo procedure with 1,000 randomizations (α = 5%).

To understand the impact of changes in precipitation across seasons on the identity of the MOTU in the networks, as well as on the overall structure, I used the R package betalink (Poisot *et al.* 2012) and calculated the dissimilarity of interaction matrices between habitats and between seasons within each habitat. The values for network dissimilarities were calculated in several ways, based on, in turn, the dissimilarity in the species composition of communities in the networks (β_{S}), the differences in the interactions observed between species common to both networks (β_{OS}), the differences in the interactions between both networks (β_{WN}), and the dissimilarity of the interaction structure that was induced by the dissimilarity in species composition (β_{ST}) (Poisot *et al.* 2012).

Results

A total of 253 bats from 13 species and five families were captured during the three sampling periods. Barcoding of these bats' diets revealed a total of 875 arthropod MOTUs from 15 orders. Separate ecological networks constructed for the dry season and wet season of an El Niño year, and the wet season of a non-El Niño year, contained 7, 7 and 11 bat species, respectively, representing 61, 64 and 128 bat individuals. The numbers of arthropod MOTUs recorded for these three sampling periods were 333, 382 and 586, respectively. In all three networks, the dominant order of arthropods present was Lepidoptera, which accounted for 67.27% (n= 224) of MOTUs during the El Niño dry season, and 65.97% (n= 252) during the El Niño wet season, and 70.65% (n= 414) during the non-El Niño year wet season (see Figure 3.1 for networks and Table 3.2 for values of each taxon in the network).

Dissimilarities between the networks were highest between the El Niño dry season and the non-El Niño wet season (Table 3.3) with values ranging from 0.327 for dissimilarity of interactions due to species turnover (β_{ST}) to 0.836 in relation to the total dissimilarity of interactions (β_{WN}). Values of sampling completeness ranged from 78.39% (non-El Niño wet season) to 88.80% and 89.59% during El Niño wet and dry season, respectively. None of the rarefaction curves built for any bat species present in my networks during each season have reached the plateau (Figures 3.5 - 3.7).

Comparisons of network structure revealed that modularity recorded for the network of the dry season of the El Niño year was higher than that recorded for the wet season of the same year (0.419 and 0.241, respectively) (mean = 0.066; p-value < 0.01), but not for the wet season of the non-El Niño year (0.345). Although these results are consistent with the expectations from niche theory, I found no associated decrease in either the number of modules (mean= 0-1; p-value= 0.15-0.39), or their associated Shannon diversity values. The number of modules per network ranged from five to six with module size varying from nine to 233 species, and module diversity from H'= 1.99 to H'= 4.76. Modularity was higher than expected by chance in all networks compared to null models with no differences detected between networks (mean= 0.12; SD= 0.006; p-value=0). The number of modules was only higher than expected by chance compared to null models during the wet season of the El Niño year (mean = 8.69; SD= 1.98, p-value= 0.04) (Figure 3.2 and Table 3.4). The number of MOTUs consumed by individual bats during the dry season of the El Niño year was significantly lower than the wet season of the El

Niño year (student t test, t= -2.378, df= 118.6, $p < 0.05$) (Figure 3.3). The degree of dietary niche overlap between bat species was significantly lower in the dry season of the El Niño year in comparison with the wet season of the same year (mean=0.010; SD=0.007; p-value=0.00) and the dietary niche overlap during the wet season of El Niño year was significantly lower than the niche overlap during the wet season of the non-El Niño year (mean= 0.007; SD= 0.006; p-value=0.047) (Figure 3.4).

There was no overall difference in either the size of the modules (permutational ANOVA; p=0.765) or the species diversity within them (permutational ANOVA; p= 1.000) across the three networks constructed. Estimates of closeness centrality values revealed significant differences among the three datasets (Wilks lambda= 0.967, $F_{(8, 2542)}$= 5.445, p= 0.000), which were driven by MOTUs of the order Coleoptera, which were significantly lower during the El Niño dry season (Tukey's; $P < 0.01$). Comparable analyses of betweenness centrality values for all species in the networks revealed significant differences ($F_{(6, 2)}$= 6.365, p= 0.001), driven by the higher values of the non-El Niño wet season (Tukey's; $P < 0.01$). However, when individual orders were examined I observed no differences in betweenness centrality ($F_{(6, 2)}$= 1.964; p= 0.06) and no interaction between order and season ($F_{(12, 8)}$= 0.894; p= 0.552).

Discussion

To examine the consequences of seasonality and drought on antagonistic interactions, I constructed and compared ecological networks, and assessed dietary overlap, for a community of insectivorous bats and their arthropod prey between a non-El Niño and El Niño wet season and then contrast this with the extreme El Niño dry season. Few studies have used network approaches to assess insectivorous species' responses to reduced resources at a community-scale; however, predictions from optimal foraging theory suggest that individual taxa might become more generalist, thus leading to greater niche overlap at the community level.

Comparisons of network structure revealed that modularity recorded for the dry season of the El Niño year was higher than that recorded for a wet season of the El Niño year, but not for the wet season of the non-El Niño year. Although these results are consistent with the expectations from niche theory, I found no further decrease in either the number of modules, or their Shannon diversity values. Furthermore, while I make a direct comparison between a non-El Niño and El Niño wet season I also contrast this with an El Niño dry season (but without a direct non-El Niño year comparison). Thus, this remains an extrapolation and should be considered in that light throughout. Similarly, I do not have enough replicates to test for the effect of internal variation and thus my conclusions remain speculative.

When comparing networks across the two seasons in the El Niño year, the clearest difference was the reduced total number of prey items recorded in the dry season, with ~14% fewer MOTUs consumed. This change cannot be explained by the numbers of bat taxa or individuals, which were approximately the same across sampling periods, although I cannot rule out an effect of bat species identity. Combining these data, I found that the total number of prey items (n= 577) consumed in the El Niño year was similar to that recorded in the wet season of the non-El Niño year. While this is interesting as a comparison of two seasons to one, it is based on comparable numbers of individual overall bats but around one third fewer bat species which makes it hard to infer a pattern.

In contrast to results based on network-wide total numbers of MOTUs, examination of the diets of individual bats revealed significant variation among sampling periods. Bats sampled in the dry season of the El Niño year were found to eat fewer MOTUs compared to those captured in the wet season of the same year. This trend was also associated with a concomitant reduction in dietary overlap among bat species in the dry season, providing further support for niche theory.

Interestingly, my findings run counter to previous results of bats recorded in the dry forest of Mexico, where two of the insectivorous species recorded (*P. mesoamericanus* and *P. personatus*) were seen to increase their dietary diversity during the dry season (Salinas-Ramos *et al.* 2015). On the other hand, the same study reported no clear pattern in the dietary overlap between bat species. My finding that the decrease in the number of MOTUs consumed by individual bats during El Niño-induced drought was not coupled with decreases in the size and diversity of modules in the network, suggests that this decrease is followed by a restructuring of the interactions in the network, such that the overall structure has remained stable over time. However, this result needs to be carefully analysed as changes in insect availability among years (interannual variation) might also be a potential source of bias that could lead to differences in module diversity and other network metrics, especially when comparing different seasons across different years.

I found that two of the networks (El Niño during dry and wet season) had a slightly lower value of sampling completeness in relation to the 90% proposed as a rule of thumb by Macgregor, Evans, and Pocock (2017) (El Niño dry 89.59%; El Niño wet= 88.80%), while the network during the non-El Niño wet season had a lower value (~78%). This indicates that there are still many species and interactions to be added to the network, particularly of the non-El Niño year, which might potentially change the number of modules (either higher or lower), depending on how the new connections are added to the network, and any functional redundancy of these species. In addition, contrary to the high sampling completeness found for the two networks, none of the rarefaction curves estimating the sampling completeness of bat species' diets have reached a plateau, indicating that more individuals needed to be sampled for a better estimation of the full diet breadth of the bat species in the network.

For all three of my networks, comparisons of modularity values with null distributions generated by Monte Carlo randomisation revealed higher values than expected by chance, indicative of non-random structuring. Interestingly, the greatest magnitude in deviation from the expected value was seen in the dry season of the El Niño year, associated with the most severe drought conditions. Networks with high modularity are thought to be more stable because they are resilient to the spread of perturbations, and thus buffer against secondary extinctions across modules (Thébault & Fontaine 2010; reviewed by Tylianakis *et al.* 2010). In my study, the observed increased modularity in the dry season, together with the lower number of MOTUs recorded, could conceivably have arisen via the extinction of vulnerable taxa. In this scenario, the remaining MOTUs, and the resulting increased modular structure of the network, would be composed of more resilient taxa.

To the best of my knowledge, mine is the first study to use network approaches to study seasonal changes in the diets of insect-eating bats. Although it has long been known that bats alter their diets seasonally, likely tracking fluctuations in insect abundance (Clare *et al.* 2011; Clare *et al.* 2014a; Sedlock *et al.* 2014), the community-level consequences of such shifts have not been documented. Salinas-Ramos *et al.* (2015) recently reported contrasting responses to resource availability in three related species of mormoopid bat species in Mexico, with dietary breadth in the dry season appearing to narrow in one taxon but widen in the other two species. In my study, I show that, by examining the entire community, dietary niche narrows with lower rainfall, and that this results in greater modularity; thus niches become more clearly defined across the network. More work is needed to determine whether increased modularity arises through bats excluding rarer insect species in their diets.

Changes in network structure, including those observed, can also be driven by the position and presence of key species associated with high values of closeness and betweenness centrality. In particular, taxa that link multiple modules (i.e. high betweenness centrality) play an important role in the ecosystem as they allow perturbations to be transferred from one module to another, but at the same time can also confer resilience against fragmentation of the network (Gauzens *et al.* 2015). It has been shown that seasonal variation in resources might also result in the formation of temporal modules or compartments, with different species composition and functional groups in food webs (McMeans *et al.* 2015). I found higher values of betweenness centrality during the non-El Niño wet season compared to values from both seasons in the El Niño year, whereas values of closeness centrality were lower during the dry season of the El Niño compared to values from both other networks (though, as stated above, this comparison is tenuous due to no control year data). Thus there appears to be a shift in the importance of arthropod groups between seasons.

Comparisons of centrality values for individual arthropod orders suggests that the observed lower closeness centrality in the El Niño dry season is likely to be driven by changes in the position of members of the Coleoptera. Such changes might reflect responses to fluctuations in rainfall, since previously it has been shown that beetle communities in tropical dry forest can be highly sensitive to rainfall, with lower abundances and species richness found during dry seasons (Andersen 2005). Species with high centrality values can serve as important key resources and their removal can lead to significant reconfigurations of food networks (Rocchi *et al.* 2017). Although closeness centrality estimates for MOTUs assigned to Coleoptera were not higher than for other orders, the observed significant decrease in the El Niño could indicate that their importance as a prey resource for bats is diminished in periods of drought, perhaps with negative bottom-up effects (from prey to predators). Perturbations in nodes with high closeness centrality are known to spread to the rest of the network more rapidly (Rocchi *et al.* 2017).

My finding that drought leads to a rewiring of networks, involving changes in node position and increased modularity, points to greater species niche segregation. This in turn, suggests important ecological consequences, such as reduced competition for resources. Such findings are more consistent with predictions set out by niche theory than optimal foraging theory. Interestingly, this result appears to corroborate wider trends; Sih & Christensen (2001) reviewed 134 studies and found that optimal foraging theory was only able to explain interactions in 37% of cases where the prey was mobile, compared to 74% of cases where the prey was less mobile or immobile. At the same time my results support the idea that, during times of reduced resources, food web networks are likely to be more susceptible to fragmentation through the loss of nodes connecting modules, particularly if these connectors are key prey species. More work is now needed to determine whether the observed changes in dietary niche in bats are a function of intrinsic factors such as variation in morphology (e.g. Weinstein & Graham 2017) or shifts in prey availability and the intensity of competition during extreme climatic events.

Tables

Table 3.1. Expected changes in observed metrics of antagonistic networks of insectivorous bats and the arthropods they eat in the dry forest of Sector Santa Rosa (of ACG) during paired wet seasons of a non-El Niño year (2009) and an extreme El Niño event (2015) in contrast to an extreme El Niño dry season. This is considered in relation to null models (network metrics) and historical patterns (food resources).

Dry forest	**Network metrics**	**Rainfall (obs.)**	**Food resources**
Dry season (El Niño)	Low modularity and number of modules, but high module diversity	Drought	Very reduced arthropod availability
Wet season (El Niño)	Intermediate modularity, number of modules and module module diversity	Reduced precipitation	Reduced arthropod availability
Wet season (non-El Niño year)	High modularity and number of modules, but low module diversity	Precipitation within historical trends	Arthropod availability within historical trends

Table 3.2. Composition of bat-arthropod antagonistic networks of interaction during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) in the dry forest of Sector Santa Rosa (of ACG) in Costa Rica.

Taxa	El Niño dry	El Niño wet	Non-El Niño wet
Bat species			
N. leporinus	0	0	2
M. microtis	0	0	1
M. hirsuta	0	0	2
G. soricina	0	0	19
B. plicata	7	0	31
L. brachyotis	4	4	4
P. mesoamericanus	24	38	63
P. personatus	5	4	0
P. davyii	0	0	2
R. tumida	18	13	2
S. bilineata	2	2	0
S. leptura	1	2	1
N. mexicanus	0	1	2
Arthropod order			
Araneae	2	4	5
Blattodea	1	9	9
Coleoptera	40	22	19
Collembola	0	0	2
Decapoda	0	0	1
Diptera	43	73	87
Ephemeroptera	0	0	2
Hemiptera	11	10	33
Hymenoptera	4	8	8
Lepidoptera	224	252	414
Mantodea	0	1	1
Neuroptera	4	1	1
Polydesmida	3	0	1
Psocoptera	0	2	2
Trichoptera	1	0	1
Arthropod richness	333	382	586
Bat species richness	7	7	11
Total bat	61	64	128
Total number of interactions	833	1165	2554

Table 3.3. Dissimilarity values between bat-arthropod antagonistic networks during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica. Interactions were revealed through the identification of the arthropods in bat diets via DNA barcoding.

Dissimilarity measures		**El Niño dry *versus* El Niño wet**	**El Niño dry *versus* non-El Niño wet**	**El Niño wet *versus* non-El Niño wet**
Dissimilarity in species composition	(β_S)	0.556	0.637	0.540
Dissimilarity of interactions between common species	(β_{OS})	0.393	0.510	0.417
Dissimilarity of interactions	(β_{WN})	0.694	0.836	0.793
Dissimilarity of interactions due to species turnover	(β_{ST})	0.301	0.327	0.376

Table 3.4. Comparisons between modules of interactions in bat-arthropod antagonistic networks during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica.

Season	**El Niño – Dry season**	**El Niño – wet season**	**non-El Niño year – wet season**
Modularity	0.42 (p = 0.00)	0.24 (p = 0.00)	0.34 (p = 0.00)
Number of modules	5 (p = 0.07)	5 (p = 0.04)	6 (p = 0.78)
Module A	109 (H’= 4.19)	227 (H’= 4.69)	233 (H’= 4.76)
Module B	047 (H’= 3.44)	072 (H’= 3.96)	135 (H’= 4.43)
Module C	143 (H’= 4.40)	059 (H’= 3.75)	179 (H’= 4.28)
Module D	025 (H’= 2.69)	022 (H’= 2.78)	022 (H’= 2.87)
Module E	016 (H’= 2.55)	009 (H’= 1.99)	019 (H’= 2.81)
Module F	---	---	009 (H’= 2.15)
Total	340 (H’=3.43)	389 (H’= 3.62)	597 (H’= 5.34)

Table 3.5. Observed, mean, standard deviation and significance values of niche overlap for one observed and 1,000 random generated networks of bat-arthropod antagonistic networks during the dry and wet season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica.

Seasons	**Obs.**	**Mean**	**SD**	**p-value**
El Niño dry	0.062	0.029	0.003	0.000
El Niño wet	0.123	0.035	0.004	0.000
Non-El Niño wet	0.107	0.038	0.002	0.000
Differences El Niño dry - El Niño wet	0.044	0.010	0.007	0.000
Differences El Niño wet – Non-El Niño wet	0.016	0.007	0.006	0.047

Figures

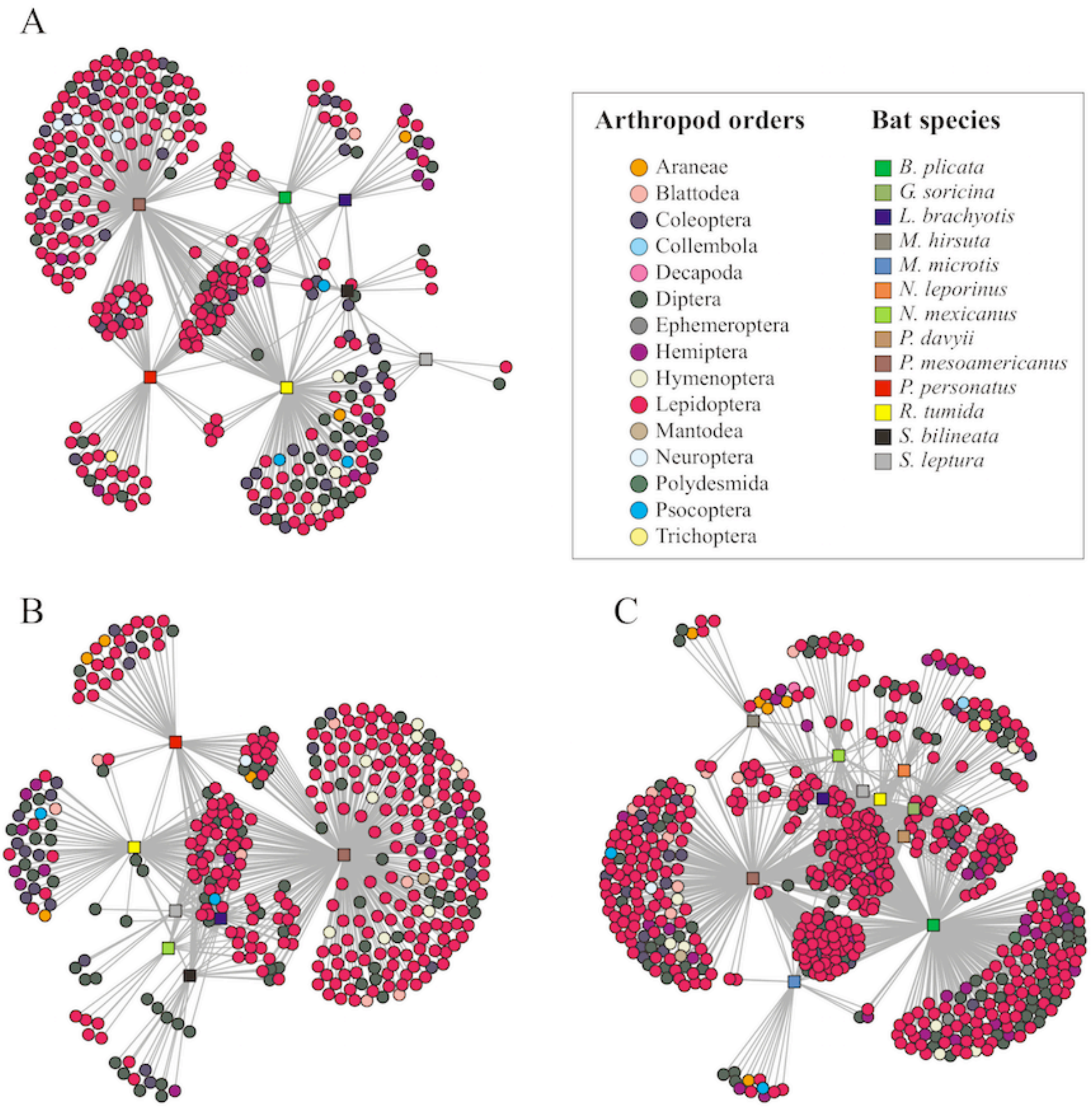
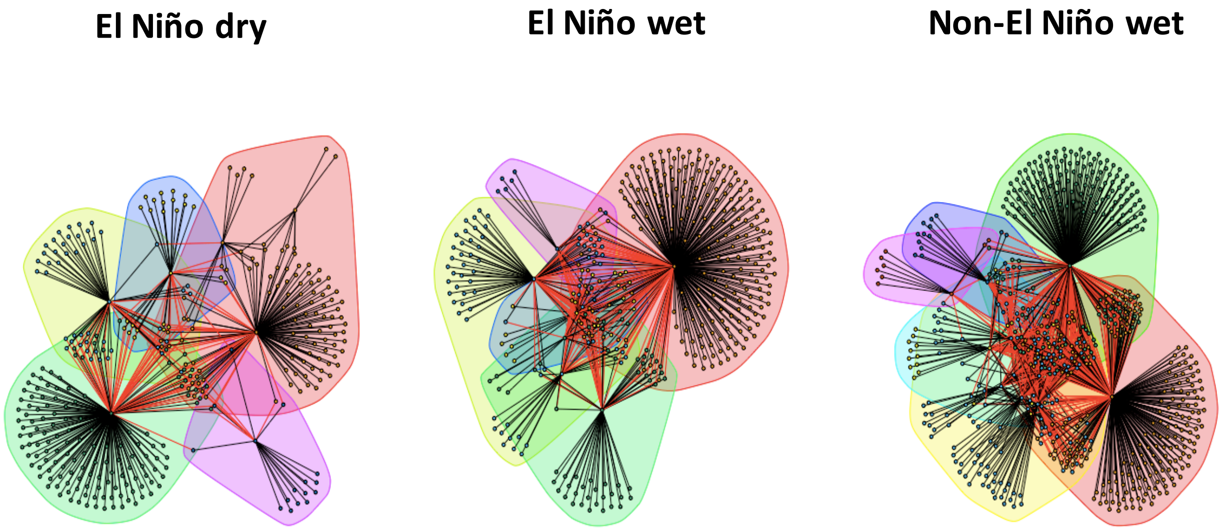


Figure 3.1. Antagonistic networks of insectivorous bats and their prey items in the dry forest of Sector Santa Rosa (of ACG) during the dry (A) and wet season (B) of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) (C). Interactions were revealed through the identification of the insects in bat diets via DNA metabarcoding.



El Niño dry

El Niño wet

Non-El Niño wet

Figure 3.2. Modules of interactions in antagonistic networks of insectivorous bats and their prey items in the dry forest of Sector Santa Rosa (of ACG) during the dry and wet season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009). Interactions were revealed through the identification of the insects in bat diets via DNA metabarcoding. Red lines represent interactions between species that connect two modules of interaction. Modules are represented by the colours of the circles, but colours do no correspond between seasons.

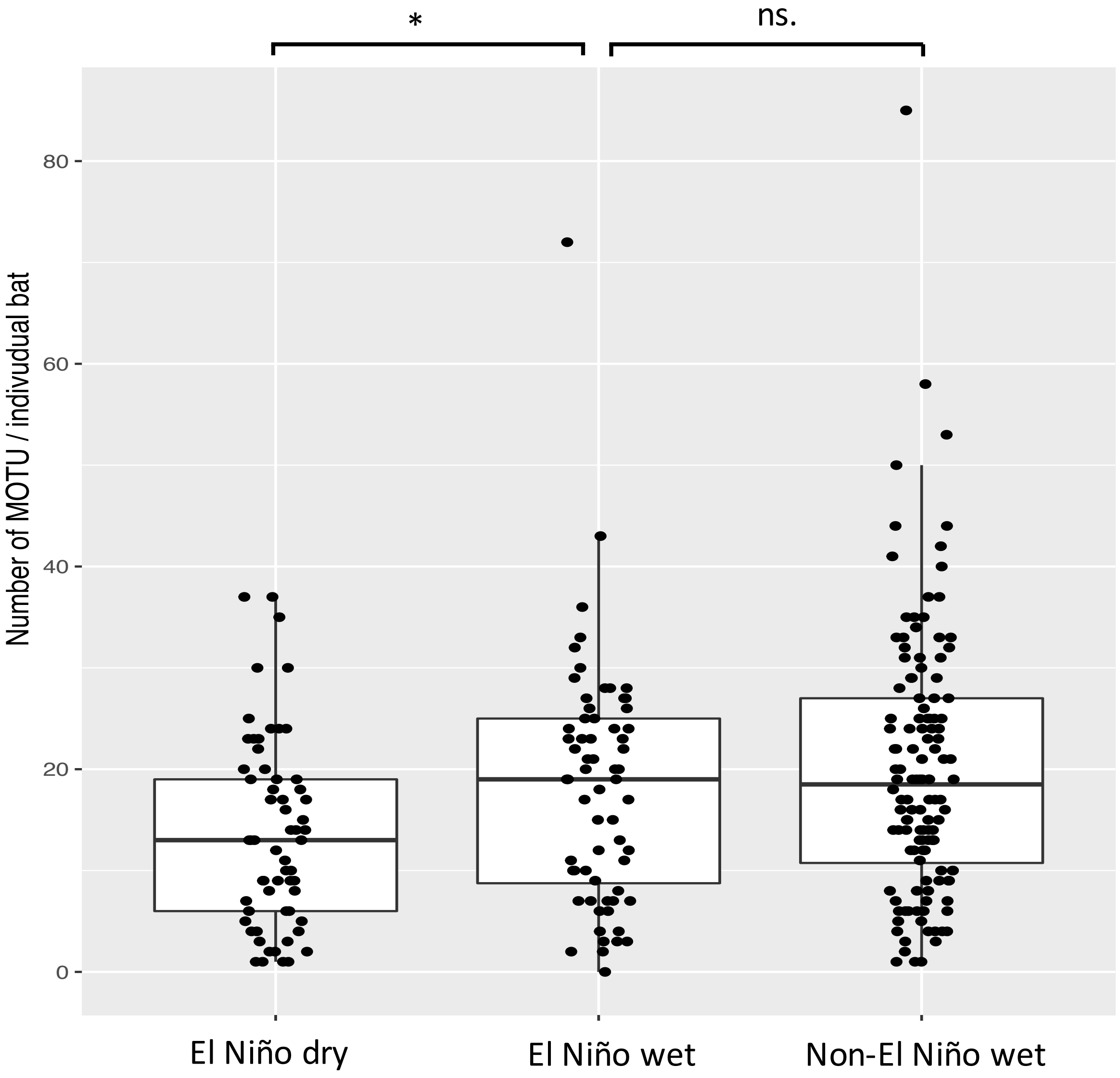


Figure 3.3. Number of MOTUs consumed per individual bat during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica. * represent p-values <0.05 and ns. represent not significant after a student t test comparison between samples.

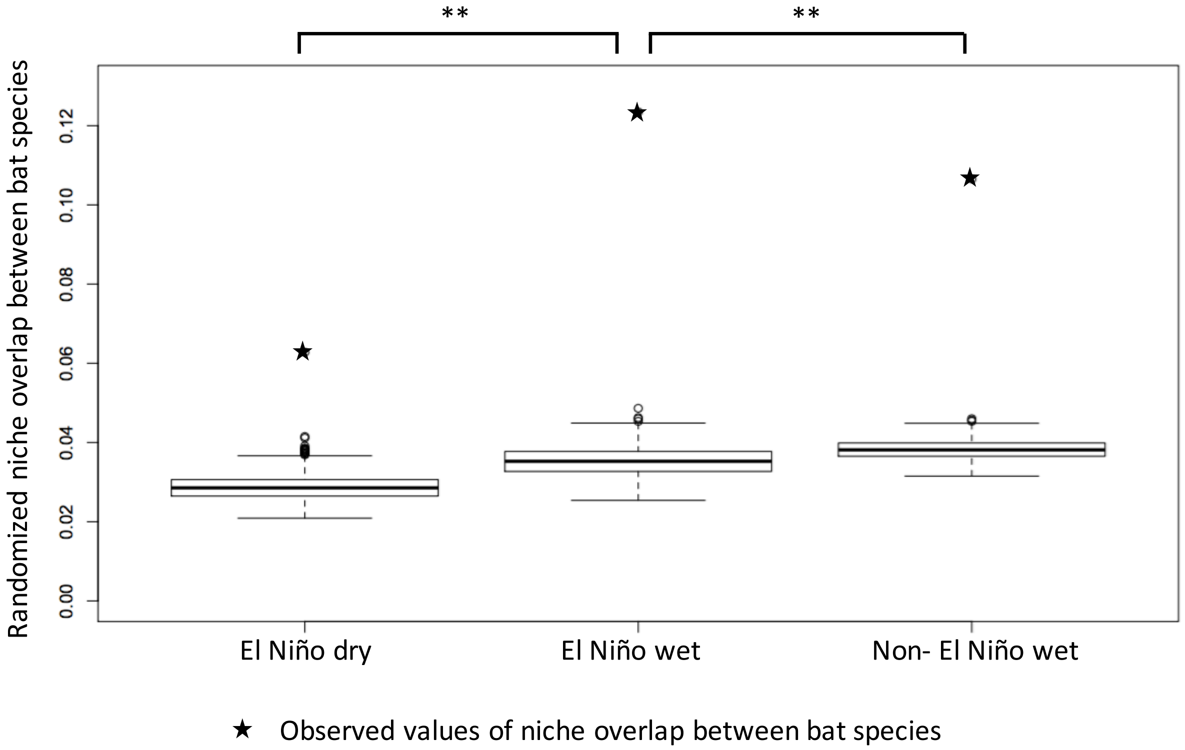


Figure 3.4. Randomized and observed bat species niche overlap during the dry and wet season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009) in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica. ** indicates p-value < 0.01.

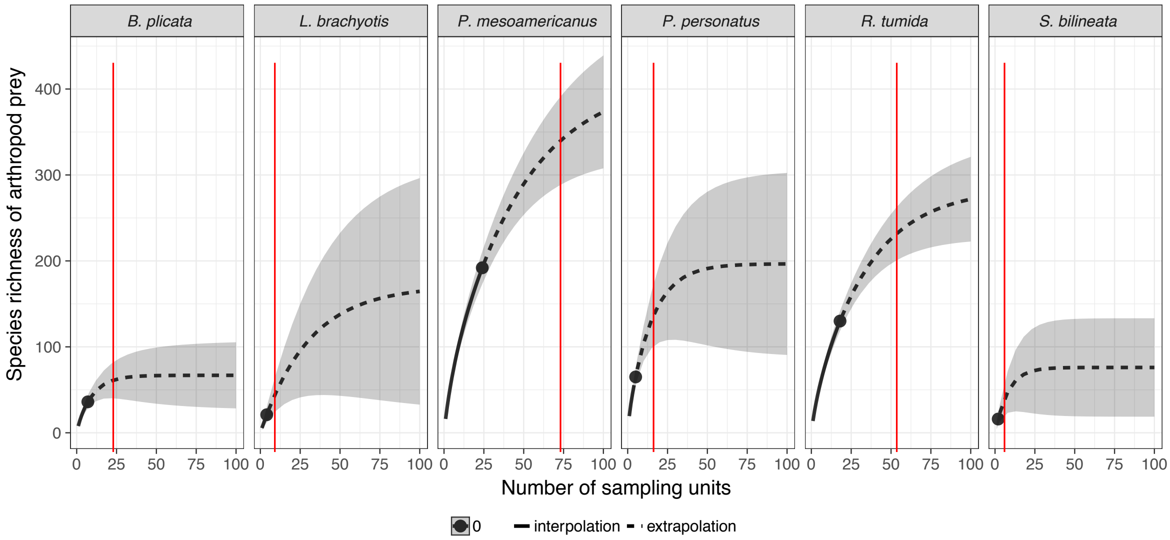


Figure 3.5. Individual-based rarefaction curves comparing the species richness of arthropod prey present on species diet of bats during the dry season of an extreme El Niño event in the dry forest of Sector Santa Rosa (of ACG) (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

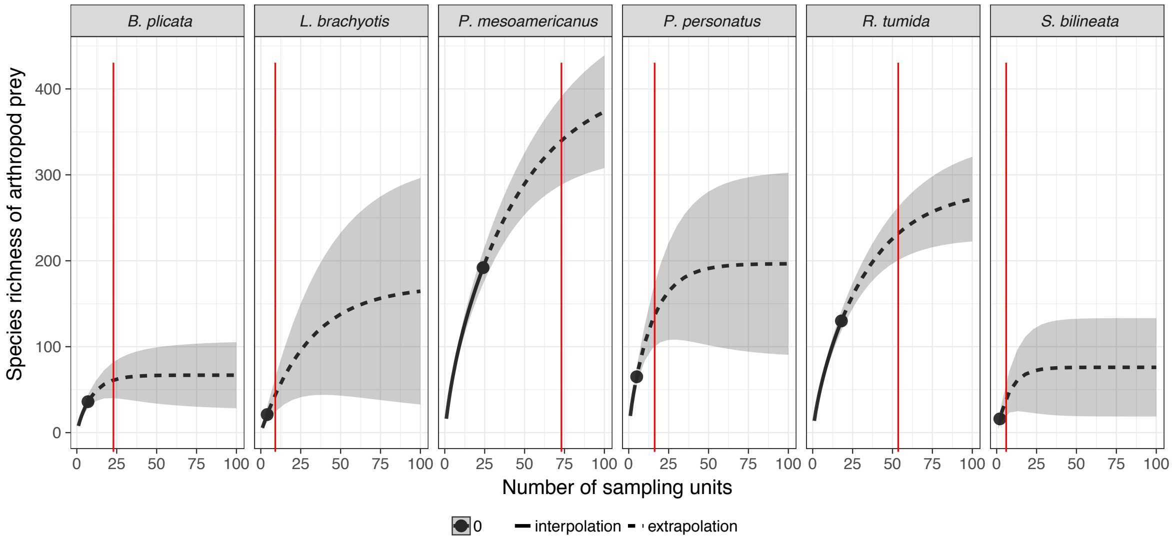


Figure 3.6. Individual-based rarefaction curves comparing the species richness of arthropod prey present on species diet of bats during the wet season of an extreme El Niño event in the dry forest of Sector Santa Rosa (of ACG) (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

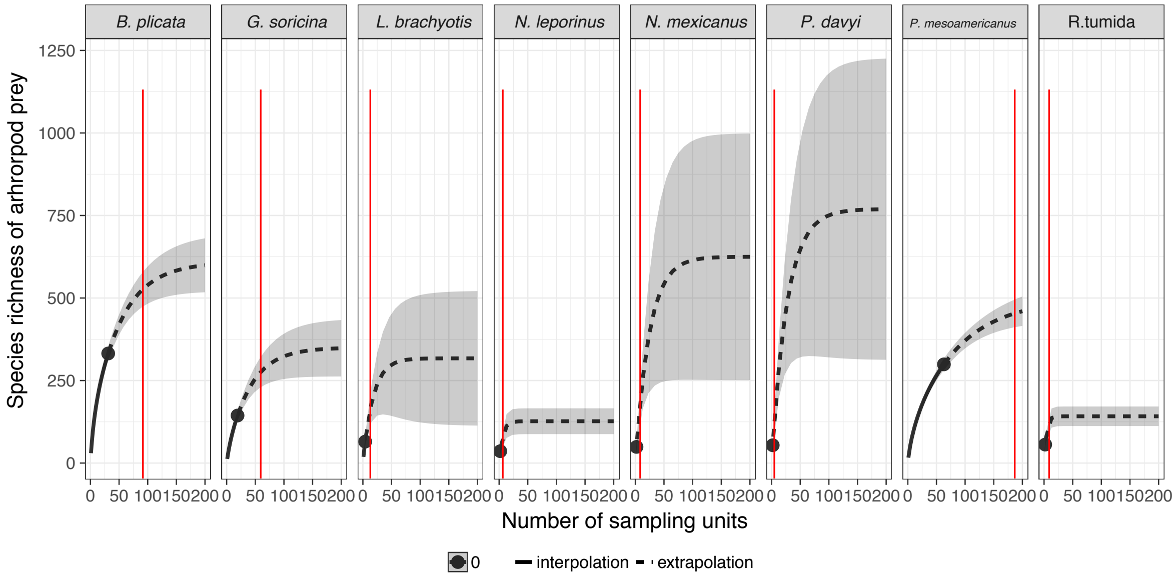


Figure 3.7. Individual-based rarefaction curves comparing the species richness of arthropod prey present on species diet of bats during the wet season of non-El Niño year in the dry forest of Sector Santa Rosa (of ACG) (2009). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for each bat species.

Supplementary material

Tables

Table S3.1. Mean, standard deviation and significance values for modularity, number of modules and differences of values calculated using the fast greedy algorithm for 1,000 random networks simulated using the Erdõs-Rényi approach and the observed antagonistic networks of insectivorous bats and the insect MOTUs that they eat in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of non-El Niño year (2009). Significance values were obtained using a Monte Carlo procedure.

Network metric (season)	**Observed**	**Mean**	**Standard deviation**	**P-value**
Modularity (El Niño dry)	0.419	0.151	0.010	0.000
Modularity (El Niño wet)	0.241	0.150	0.009	0.000
Modularity (non- El Niño wet)	0.345	0.119	0.006	0.000
Modularity (Diff. non- El Niño wet versus El Niño wet)	0.066	-	-	0.798
Modularity (Diff. El Niño dry versus El Niño wet)	0.041	-	-	0.001
Modularity (Diff. El Niño dry versus non-El Niño wet)	0.072	-	-	0.521
Number of modules (El Niño dry)	5	8.149	1.825	0.074
Number of modules (El Niño wet)	5	8.693	1.981	0.040
Number of modules (non-El Niño wet)	6	6.023	0.936	0.785
Number of modules (Diff. non- El Niño wet versus El Niño wet)	1	-	-	0.324
Number of modules (Diff. El Niño dry versus El Niño wet)	0	-	-	0.149
Number of modules (Diff. El Niño dry versus non-El Niño wet)	1	-	-	0.388

Table S3.2. Results of the Tukey tests in the post hoc of a MANOVA comparing closeness centrality values of different arthropod order in the antagonistic network of insectivorous bats in the dry forest of Sector Santa Rosa (of ACG) of Costa Rica during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of a non-El Niño year (2009).

Comparisons	**Coleoptera**	**Diptera**	**Hemiptera**	**Lepidoptera**
El Niño dry *versus* El Niño Wet	0.001	0.743	0.887	0.811
El Niño dry *versus* non-El Niño wet	0.000	0.744	0.213	0.699
El Niño wet *versus* non-El Niño wet	0.266	0.194	0.052	0.257

CHAPTER FOUR

Wing morphology predicts individual niche specialization in *Pteronotus mesoamericanus* (Mammalia: Chiroptera)

Abstract

The niche variation hypothesis states that within a population, variation in morphological characters among individuals is determined by the diversity of habitats and foods available. Therefore, morphological variation increases niche segregation, which has been shown to decrease intraspecific competition in many animal species. A prediction from this theory is that populations are composed of heterogeneous individuals that explore their environment in different ways. Among bat species, wing shape correlates with flight manoeuvrability and habitat use, with species possessing broader wings forage in more cluttered habitats. However, few studies have investigated the role of morphological variation in bats for niche partitioning at the population level. To determine the relationship between wing shape and diet, I studied a population of the insectivorous bat species *Pteronotus mesoamericanus* in the dry forest of Costa Rica. Individual diet was resolved using DNA metabarcoding, and bat wing shape was assessed using geometric morphometric analysis. Inter-individual variation in wing shape showed a significant relationship with both dietary dissimilarity based on Bray-Curtis estimates, and nestedness derived from an ecological network. Overall, bats with broader and more rounded wings were found to feed on a greater diversity of arthropods. I conclude that bats with broader wings are more adapted to exploit dense vegetation and/or feed on different prey items, leading to the observed overall patterns of diet specialization and differentiation within the population.

Introduction

The niche variation hypothesis states that within a population, variation in morphological characters among individuals is determined by the diversity of habitats and food resources available (Van Valen 1965). Inter-individual variation in a morphological trait may allow the exploration of different micro-niches, leading to enhanced survival of a greater number of individuals with lower intraspecific competition (Van Valen 1965; Svanbäck & Bolnick 2007; Camargo *et al.* 2014). Individuals that differ in morphology are expected to show greater dissimilarity in diet (Price 1987; Bolnick & Paul 2009). It follows that overall inter-individual variation in resource use leads to an increase in apparent dietary breadth at the population level. Therefore, a key prediction from the niche variation hypothesis is that populations are composed of heterogeneous individuals that explore their environment in different ways.

Network approaches can provide useful insights into inter-individual variation in resource use. Any variability in the diversity of diets promoted by trait variation is expected to have an impact on food web structure and dynamics (Bolnick *et al.* 2011). Most studies of ecological networks focus on interactions among multiple species in a community (Ings *et al.* 2009; Thébault & Fontaine 2010; Mougi & Kondoh 2012; Rafferty & Ives 2013), whereas such interactions have been relatively unexplored at the level of populations or individuals (Pires *et al.* 2011). A population might be composed of many generalist individuals, many specialists or a mix of generalists and specialists, with a profound impact on the structure and resilience of ecological communities (Araújo *et al.* 2010). The few existing studies that have examined networks composed of individuals within a population have reported different levels of specialization (Dupont *et al.* 2011), where the diets of specialist individuals are nested within the diets of the generalists (Araújo *et al.* 2010). More nested structures suggest higher ecological redundancy (Nordström *et al.* 2015) and resilience to change (Tylianakis *et al.* 2010). Although it has been shown for many species that individual variation in diet is linked with differences in morphological traits (Price 1987; Bolnick & Paul 2009), there are few, if any, studies evaluating how trait variation between individuals may explain their position in ecological networks.

Although inter-individual variation in diet has been a major ecological focus (Lehmann *et al.* 2015; Forsman & Wennersten 2016; Camprasse *et al.* 2017; Costa-Pereira *et* *al.* 2017), properly identifying dietary items still remains one of the biggest challenges in foraging ecology (Deagle *et al.* 2005; Casper *et al.* 2007; Clare 2014). Multiple approaches to diet tracing have been applied with varying levels of success (reviewed by Nielsen *et al.* 2017). The digestion level of prey items in scats can make it almost impossible to generate a detailed description of diet using morphological approaches, particularly in some of the most generalist trophic groups such as insectivores (Clare 2014). Molecular techniques, such as DNA barcoding, have added more power to the detection of interactions (Nielsen *et al.* 2017), and when this increased resolution is applied to food webs (interaction networks), network metrics (e.g. vulnerability, linkage density and nestedness) are suggested to be orders of magnitude different from previous descriptions based on poorly or unevenly resolved taxa (Wirta *et al.* 2014). Interaction networks (food webs) are a powerful way to assess ecological structure but they are at their best when the resolution of nodes (taxa) is complete. As such, DNA barcodes have been increasingly advocated as an important method to assess species interactions and build ecological networks (Evans *et al.* 2016; Roslin & Manajeva 2016). While resolving species identity using Sanger sequencing (Wirta *et al.* 2014) is one approach, another approach is to apply metabarcoding to mixed samples (Clare *et al.* 2018). This approach based on molecular operational taxonomic units (MOTU) (Floyd *et al.* 2002) is not equivalent to species level resolution (reviewed in Clare *et al.* 2016) but applies an identical level of resolution to all nodes in a network making comparisons more standardised (Clare *et al.* 2018).

Bats are a highly diverse group and the only mammals to have evolved powered flight. Wing shape in bats varies considerably among species, and correlates with differences in flight mode and speed (Norberg 1990; Norberg 1994). As such, variation in wing shape is associated with differences in habitat use (Norberg & Rayner 1987; Norberg 1994). Species with broad wings and rounded wing tips are more adapted for slower manoeuvrable flight, and thus tend to forage in cluttered environments such as dense vegetation. Conversely, species characterised by narrower pointed wings are more adapted for faster flight in open areas, and are less manoeuvrable (Norberg 1994). Differences in habitat use could have potential consequences for bat-arthropod interactions as vegetation density is a good estimator of prey availability for bats, with more cluttered habitats showing higher prey abundance (Kalcounis & Brigham 1995; Müller *et al.* 2012).

In addition to influencing habitat use, flight manoeuvrability might also confer advantages in hunting, increasing the probability of successful prey capture (Asís *et al.* 2011;

Coelho 2011; Chai & Srygley 1990; Hedenström & Rosén 2001). Most investigations of the variation in bat flight ability, diet and morphology have consisted of comparisons between bat species (Norberg & Rayner 1987; Norberg 1994) with little consideration given to the impact of variation within populations (Kalcounis & Brigham 1995; Camargo & Oliveira 2012). *Pteronotus mesoamericanus* is an interesting model for this problem as it appears to exhibit variation in individual diet and habitat use, with marked differences between individuals exploring cluttered versus non-cluttered environments (Oliveira *et al.* 2015).

To determine the relationship between wing shape and diet, I studied a population of the insectivorous bat species *P. mesoamericanus* in the dry forest of Costa Rica. My objective was to explore individual micro-niche variation within a population of *P. mesoamericanus* and to use DNA metabarcoding and geometric morphometric analysis to test the hypothesis that variation in ecological network position of individual predators is related to differences in individual wing shape. More specifically, I made the following predictions. First, individuals with broader wings and thus more manoeuvrable flight will be able to exploit a wider range of prey items. Therefore, they would have a more generalist diet and be associated with less nested positions in the network. Second, wing shape is a good predictor of individual differentiation in niche use. Individuals with different wing shapes will be specialised to exploit different types of prey (more mobile versus less mobile prey). Thus, variation in wing shape among individuals will be associated with increasing diet dissimilarity, due to the exploration of different resources that reflect differences in hunting efficiencies and the possible exploration of different habitats.

Methods

Study area

The study was conducted in the dry forests of Sector Santa Rosa (10°46.7′N, 85°39.8′W) in the Area de Conservación Guanacaste (ACG), which faces large variation in precipitation due to its extreme seasonal climate (Murphy & Lugo 1986; Janzen 1988). Dry season ranges from December to late May when there is virtually no rain, with the wet season occurring in the remaining months when most of the precipitation occurs, and an annual precipitation varying from 900 to 2400 mm (Janzen 1986; Chapman 1989). During my study there was a strong El Niño (2015) when the park witnessed an unusually low annual rainfall of only ~600 mm.

Bat sampling, diet and network analysis

One sampling trip was made in the wet season (Jul - Aug) of 2015. I captured bats using four to six mist nets opened along trails and near watercourses from 18h - 22h with a total estimated effort of 2,250 m^2.h. Bats were sampled for wing membrane biopsies as part of a different study and thus I was able to assess whether individuals were recaptures by the presence of a hole or scar. For each individual, I measured forearms with callipers (0.1 mm precision) and I identified them according to the taxonomic information present in the field keys of Reid (1997). Each bat was held in a cloth bag for a maximum of two hours to collect faecal samples and each sample was stored in 70% ethanol and frozen (-20° C). These samples were analysed to determine diet using metabarcoding approaches. For molecular and bioinformatics steps see Chapter 3.

Geometric morphometric analysis

Geometric morphometrics is a powerful tool for the detection of variation in shape that has been developed and incorporated into the analysis of morphological structures (Adams *et al.* 2004; Adams *et al.* 2013). It has been shown to improve species' discrimination (Schmieder *et al.* 2015) as it allows the detection and discrimination of variation in morphological traits with higher accuracy than traditional morphometric measures (Parsons *et al.* 2003; Maderbacher *et al.* 2008). However, most of the variation in wing shape has been used to explain variations within bat assemblages using traditional morphometrics (Aldridge & Rautenbach 1987; Birch 1997; Moreno *et al.* 2006), with few studies analysing differences using geometric morphometric approaches (Camargo & Oliveira 2012; O'Mara *et al.* 2016).

I performed geometric morphometric analyses from information collected from photos of bat wings. To photograph specimens, the left wing of each individual was extended against a grid with 1 cm marking guides and photographed with a digital camera (Canon EOS DIGITAL REBEL T1i - Canon EF-S 18–55 lens), which was mounted on a tripod at a fixed height. For the standardization of wing position, I have considered the fifth finger parallel to the body of the animal and the largest possible stretching of major (digits IV and V) and medius (digits III and IV) dactylopatagium membranes (Figure 4.1). Additionally, I considered the maximum angulation between the humerus and the radius/ulna (Figure 4.1). The arm extension of bats is related to the stretch capacity of the propatagium membrane. Therefore, if there was any resistance in the extension of the bat's arm, to avoid injury, I have considered this as the maximum angulation between humerus and radius/ulna. For each individual I extended and photographed its wings three different times for further evaluation of the standardization of the method. Wing images were taken of non-pregnant females and males to avoid stress to pregnant females and as their diet may vary due to their physical condition. For the evaluation of the wing shape of *P. mesoamericanus*, I selected fourteen anatomical landmarks with the support of the software TpsDig v.1.4 (Rohlf 2004). Anatomical landmarks were used as a way to sample homologous portions of the wing, and were represented only by tissues joints (phalanges, cartilage and wing membrane; Figure 4.1).

To test the amount of error variance related to the standardization method used to extend the bat wings before obtaining the images, I checked the repeatability of anatomical landmarks measurements in 20 individuals (eleven males and nine females) using the three photos taken from each of them. For this, I used the intraclass correlation coefficient from an analysis of variance on the x and y coordinates of each anatomical landmark. From this analysis I was able to verify the error in locating the anatomical landmark position and the differences between individuals. I confirmed that all landmark locations were highly repeatable (Fleiss 1996) across samples, with intraclass correlation coefficients ranging from 0.95 to 0.99 (experimental error between 0.02 and 0.020 pixels; variance between 0.12 and 0.83 pixels). Thus, I assumed that the method adopted to extend the wings was standardized throughout the study. Mean variation in measurements for any one individual was 81.25 pixels while mean variation between individuals was 791.0 pixels.

After recording the 14 landmarks for each analysed individual, I obtained the wing shape variables (partial warps and uniform components) from the superimposition of anatomical landmarks (Procrustes algorithm) using the software TpsRelW v.1.62 (Rohlf, 2016). This method involves the centralization and minimization of distances between anatomical landmarks and the standardization of anatomical landmarks configuration from the Centroid Size (CS) (Rohlf 1999; Bookstein 1997). The CS is a multivariate measurement of size of the structure analysed; this value is obtained by the square root of the sum of the square distance of each anatomical landmark to the mass centre of each configuration (centroid) (Bookstein 1997).

Network analysis

I compiled the observed interactions into a presence and absence matrix with that each cell value representing the interactions between each individual pair (individual bat and insect MOTU). I considered one realized interaction to be when the DNA of an insect order from a determined MOTU was detected in the faeces of one individual bat. I constructed the antagonistic network using the 20 individuals of *P. mesoamericanus* and their arthropod MOTUs.

Differences in individual niche use (interaction specialization) were assessed in the network using values of nestedness. In this case, nestedness is a measure of the level to which the interactions are specialised or generalised (Dormann *et al.* 2009). To quantify nestedness values for each individual, I used the function nestedrank with the binmatnest algorithm from the Bipartite R package (Dormann *et al.* 2008). Nestedrank rearranges the network of interactions according to its maximal possible nestedness and then quantifies the level of specialization of a given node (individual bat) through its rank in the matrix (Alarcón *et al.* 2008) with higher values indicating more specialized individuals.

Statistical analysis

Wing morphology

In order to identify significant changes in wing morphology between individuals, I performed a Principal Component Analysis (PCA) using the wing shape variables (i.e., partial warps and uniform components). I obtained two new variables (PC1 and PC2) that summarize 34.2% and 26.7%, respectively, of the information contained in the original set of variables. I used the software TpsRelW v.1.62 to visually evaluate the variation of wings shape across individuals within these two axes. For evaluating any sex-related bias in the wing shape, I performed a Hotelling T^2 test using the PC1 and PC2. For this analysis, I considered each sex as an independent variable and the scores of each individual obtained in the PCs as dependent variables. Additionally, to investigate any size-related bias in the wing shape of *P. mesoamericanus* (i.e., allometry), I performed simple regressions using individual scores of each PC as dependent variables and the centroid size of each individual as independent variable. Centroid size is defined as the square root of the sum of the squares of the distance of each landmark from the centroid of the configuration (i.e., the mean of all coordinates) (Bookstein 1997). From this measurement, I was able to detect any allometric contribution of the wing shape in further analysis.

Relationship between wing morphology and diet

To quantify the similarity of the diet among individuals, I performed a Principal Coordinate Analyses (PCoA) using the Bray-Curtis dissimilarity index in the vegan package (Oksanen 2007) in R (R Development Core Team 2017). For this, I generated a new matrix containing the different food items pooled by arthropod order, based on the presence absence matrix described above. For example, if individual bat 1 had consumed 8 MOTU assigned to Coleoptera, this individual was assigned a Coleoptera frequency of 8. In order to determine the sampling completeness of my networks and the proportion of total arthropod species richness present in the diet of *P. mesoamericanus*, I used the Chao 1 index according to the method proposed by Macgregor *et al.* (2017) for the networks, and individual based rarefaction curves for the estimation of the bat species diet.

To test for the relationship between variation in wing shape and individual specialization in diet (number of food items), I performed a multiple regression between the individual scores obtained in the first two principal components (summarized wing shape as independent variables) and the values of individual nestedness (dependent variable). Similarly, to assess how much of the difference between individual diet (diet dissimilarity) could be related to differences in wing shape, I performed a multiple regression between the first two axis of the PCA (PC1 and PC2) (independent variables) and the first axis of the PCoA (dependent variable). For this analysis, I selected only the first axis of the PCoA because it represented a relatively high percentage (about 61%) of the total variance of the individuals' diet (see supplementary material for more details).

I ran all statistical analysis using the R statistical language and environment (R Development Core Team 2017).

Results

Diet description and network analysis

I analysed the diet of 20 individuals of *P. mesoamericanus* spanning a time range of 20 days. The diet was composed of nine arthropod orders, with Lepidoptera recorded as the most diverse order with 152 MOTUs, followed by Diptera with 16 MOTUs, and Hymenoptera and Blattodea with four MOTUs each (Figure 4.2). Individuals consumed from three to 36 MOTUs ($\bar{x}$= 19.6, SD= 8.99) of one to five orders ($\bar{x}$= 2.5, SD= 1.19), with Lepidoptera recorded as the most prevalent order present in the diet of all individuals (See Supplementary material for a description of the diet). Values of nestedness from the ecological network showed an extreme variation ranging from 0 (indicating an extreme generalist diet composed of a large number of arthropod MOTUs) to 0.947 (indicating an extreme specialist diet composed only of few arthropod MOTUs). However, on average individuals had an intermediate level of diet specialization (nestedness, $\bar{x}$= 0.474, SD= 0.30) (See Supplementary Material for additional information). The rarefaction curve (Figure 4.5) did not reach plateau, and sampling completeness of the network was estimated as 36.23%.

Wing morphology

The analysis of the landmarks using the program TpsRelw generated 24 shape variables. The first axis of the PCA (PC1) using these variables explained 34.2% of the total variance of the wing shape, whereas the second axis (PC2) explained 26.7%. For both components (PC1 and PC2), low PC scores suggested more narrow and pointed wings (i.e. more triangular in shape) while higher scores were associated with more broad and rounded wings. Over all landmarks, the second and the third (Figure 4.1) accounted together for most of the variation in bat wing shape (73.72%), with each representing 38.61% and 35.11% of relative contribution for wing shape variation, respectively (Table 4.1). However, I found no differences in the wing shape between males and females (Hotelling $T^2_{2,17} = 0.621$; $P = 0.250$). Moreover, I found no association between the wing shape and the centroid size indicating no allometric effects considering both PC1 ($r^2 = 0.18$; $F_{1,18} = 4.017$; $P = 0.06$) and PC2 ($r^2 < 0.01$; $F_{1,18} = 0.097$; $P = 0.758$).

Relationship between wing morphology and diet

The multiple regression analysis demonstrated a significant relationship between wing morphology (PC1 and PC2) and individual values of nestedness from the ecological network (global adjusted $r^2 = 0.60$; $F_{2,17} = 15.512$; $P < 0.01$) (Figs 4.3-4.5; Table 4.2) showing that individuals with pointed wings had a more specialized diet. The analysis between the PCs and diet similarity (first axis of PCoA) also showed a significant association (global adjusted $r^2 = 0.40$; $F_{1,18} = 7.388$; $P = 0.005$) (Figures 4.3 - 4.4) (see Supplementary Material for additional information), revealing that individuals with similar wing shape also present similar diet. However, these associations were significant only for PC1 (Table 4.2).

Discussion

The niche variation hypothesis rests on the assumption that individuals within a population are heterogeneous in the way that they use resources (Nakano 1995; Bolnick *et al.* 2002) and that the diversification of niche use in a population reduces intraspecific competition through the exploitation of different parts of the environment by individuals (Svanbäck & Bolnick 2007). Niche diversification is more likely to happen when the population is formed by individuals with different ecological requirements, where each individual uses a subset of the niche of the whole population (Bolnick *et al.* 2002). One of the main factors that account for differences in individual niche use is phenotypic variation, which can influence foraging behaviour, resource preferences, physiological requirements and even social status and dominance (Araújo *et al.* 2011). In this project, I set out to test these conditions by assessing the relationship between individual morphological variation and resource use in a population of *Pteronotus mesoamericanus*. By relating diet inferred from metabarcoding to variation in wing shape, my hypothesis was supported and I found that round winged individuals employ a more generalist approach and have a diet characterised by a greater number of arthropod MOTUs, than those with more pointed wings. I observed no differences between males and females.

The relationship I observed between wing shape and diet specialization and differentiation suggests that inter-individual variation might be related to differences in the ability of individuals to capture different prey types. Indeed, most of the prey items detected in the bats' diets consisted of members of the Lepidoptera (86.5% of all feeding items), and these insects are known to show species variation in flight performance, ranging from fast and erratic to regular and slow flying species (Dudley 2000; Berwaerts *et al.* 2002). It is thus conceivable that bat morphology might relate to specific lineages of lepidopterans, however, the current metabarcoding approach did not allow me to narrow down the identification of prey to family or genus level, and so I was not able to test this. Although I found differences at the individual level, I observed no differences between males and females further supporting the conclusion that this variation is related habitat exploitation and hunting efficiencies rather than sexual selection. However, dietary rarefaction curves did not reach plateau and sampling completeness for the network was low, indicating that there are many more arthropods present in the diet of the species. So while the relationships for the individuals that I captured appears robust, sampling more individuals would increase confidence that the diet has been well sampled and the results should thus be treated with caution.

Dietary breadth might relate not only to different flight styles, but also to differences in habitat use, and it is difficult to separate these two variables. Bats with pointed wings are more adapted to hunt high-flying insects that are more common in open spaces, while bats with broad and rounded wings are more adapted to hunt insects in the vegetation (Patterson *et al.* 2003). In open spaces, densities of insects, and thus prey availability, tend to be lower, leading to a narrower niche and more specialised diet (Kalcounis & Brigham 1995; Müller *et al.* 2012). Intraspecific differences in habitat use related to differences in wing morphology, or wing loading capacity (defined as the total bat body mass divided by the area of its wing), have been reported for at least two other bat species (*Myotis lucifugus* and *Miniopterus schreibersii*) (Kalcounis & Brigham 1995; Jacobs 1999). For *Myotis lucifugus*, wing loading explained 20% of the variation in habitat use (Kalcounis & Brigham 1995). In the case of *Miniopterus schreibersii*, bats captured in clutter had shorter wingspans, and lower aspect ratios (defined as wingspan^2/wing area), than bats captured in open areas (Jacobs 1999). Morphological variation related to habitat use within populations has also been recorded in other groups, such as the bluegill sunfish, where individuals inhabiting open waters are more fusiform with a shorter pectoral fin in comparison to individuals found on the littoral zone of the same lake (Ehlinger & Wilson 1988). The Three-spined Stickleback (*Gasterosteus aculeatus*) also shows a high intraspecific morphological variation that may lead to important implications for resource interactions (Durston & El-Sabaawi 2017; Leal *et al.* 2017).

P. mesoamericanus was originally included in *P. parnellii* and was only recently described as a separate species in Central America, distinct from other *Pteronotus* lineages elsewhere in Mexico, the Antilles and South America (Clare *et al.* 2013). Thus, analyses of ecological variation in this taxon are still lacking, however, previous work on *P. parnellii* lineages from the Amazon forest (a sister-taxa, previously considered the same species) has shown a preference for more cluttered habitats where insect availability is higher, but with the behavioural flexibility to forage in more open habitats (Oliveira *et al.* 2015). *P. parnellii* has the wing shape of a generalist species, which makes it possible to exploit different habitats (Marinello & Bernard 2014), including highly cluttered sites. Generalising for the cryptic species complex, this suggests environmental flexibility in terms of space use. Oliveira *et al.* (2015) has also showed variation in the diets of individual *P. parnellii* depending on their use of cluttered environments (Oliveira *et al.* 2015), indicating that dietary variation is habitat-linked. However, until now no studies have linked this flexibility to morphological variation among individuals. My analysis suggests that inter-individual morphological variation may lead to different habitat exploitation with diet as a consequence. What is still unknown is whether this micro-habitat and micro-niche partitioning is fixed in an individual, or whether individuals can exploit alternative micro-niches when food availability is low.

In my study year, the El Niño was extreme with a cascade of consequences including extremely low abundances in arthropod resources (D. Janzen pers. comm.). This has had a number of consequences for the bat-insect food web (Chapter 3) and may have led to more extreme pressure on individual dietary niches measured here. Since periods of low resources are more strongly associated with variation in individual niche use (Svanbäck & Bolnick 2007; Huss *et al.* 2008) and I have a sample size of only 20 individuals, it is unknown whether the relationship between wing shape and diet that I report in this study would still exist under more normal weather conditions or in other populations in different areas of the park. Even in the absence of El Niño, dry forests are highly seasonal and show a pronounced difference in habitat structure and insect abundance across seasons (Denlinger 1980; Murphy & Lugo 1986; Pinheiro *et al.* 2002; Neves *et al.* 2010). Dry forest trees show a seasonal loss of leaves and tree growth (Reich & Borchert 1984), which can dramatically change the landscape and forest structure. Variation in wing morphology within the population might enable different individuals to cope with foraging in different habitats (open *versus* cluttered) across the whole year, including variation in forest structure and prey availability across seasons. An interesting prediction from this hypothesis would be the expectation of lower individual variation in morphology in less seasonal habitats.

While morphological variation is fixed within an individual bat, echolocation can be highly plastic and in some bat species, individuals appear to alter aspects of their echolocation signals with habitat use (Schnitzler *et al.* 2003). For example, individuals of the species *Tadarida brasiliensis* can modify their call rates depending on whether they are flying alone or in proximity to other individuals, which is thought to reduce signal interference and increase obstacle avoidance (Adams *et al.* 2017). Higher frequencies give better resolution over short distances and are associated with more cluttered environments while lower frequency are associated with more open area foraging (Jones 1997). In comparison, high duty-cycle bats, such as members of the Old World families Rhinolophidae and Hipposideridae, and some species of the New World genus *Pteronotus*, might not be as flexible in their calls, and do not make such alterations in frequency or rate (Fawcett *et al.* 2015). While less is known about echolocation in the *Pteronotus* complex than in rhinolophid bats, one of the main criteria for differentiating species in this complex is frequency (Clare *et al.* 2013) and very small differences in echolocation frequency are associated with slight niche partitioning in sibling species of European rhinolophids (Arrizabalaga-Escudero *et al.* 2018). The role in inter-individual differences in frequency would be an obvious next avenue for investigation in inter-individual differences in niche use. If frequency use is less variable in *Pteronotus*, as may be the case in high-duty cycle bats, inter-individual morphological variation (as measured here) may be much more important in the determining micro-niches.

This is the first study to use geometric morphometrics coupled with DNA metabarcoding to evaluate the role of morphological variation in determining dietary specialization of individuals. My data suggest that in some circumstances individual morphological variation in wing shape might be an important mechanism for the exploitation of different environmental micro-niches potentially leading to a reduction in intraspecific competition. This may be due to access to different micro-habitats or through better individual prey capture performance. More studies are needed to determine whether this is a general pattern across species, under what conditions these small variations in individuals impact the structure of food webs and what extrinsic factors drive the maintenance of individual variation and individual niche specialization.

Tables

Table 4.1. Results for the Principal Component Analysis (PCA) evaluating the diet of 20 individuals of *Pteronotus mesoamericanus.* The analysis was performed with the Bray-Curtis dissimilarity index using a matrix containing the different food items pooled by arthropod order for each individual.

Analysis	**Eigenvalue**	**Variance explained (%)**
PCA1	0.000435	34.19
PCA2	0.000340	26.74
PCA3	0.000147	11.54
PCA4	0.000134	10.50
PCA5	0.000080	6.27
PCA6	0.000062	4.90
PCA7	0.000019	1.49
PCA8	0.000018	1.38
PCA9	0.000012	0.97
PCA10	0.000009	0.73
PCA11	0.000007	0.55
PCA12	0.000003	0.27
PCA13	0.000002	0.18
PCA14	0.000002	0.12
PCA15	0.000001	0.07
PCA16	0.000001	0.05

Table 4.2. Partial results for each dependent variable from the two multiple regressions investigating the relationship between wing morphology (PC1 and PC2) and individual specialization (nestedness), and wing morphology and diet similarity (PCoA).

Analysis	**PCA Axe**	**Beta**	**Partial correlation**	**T(17)**	**P-value**
Nestedness	PC1	-0.683	-0.754	-4.730	<0.001
Nestednes	PC2	-0.424	-0.581	-2.941	0.009
PCoA	PC1	-0.578	-0.620	-3.259	0.005
PCoA	PC2	-0.362	-0.443	-2.038	0.057

Figures

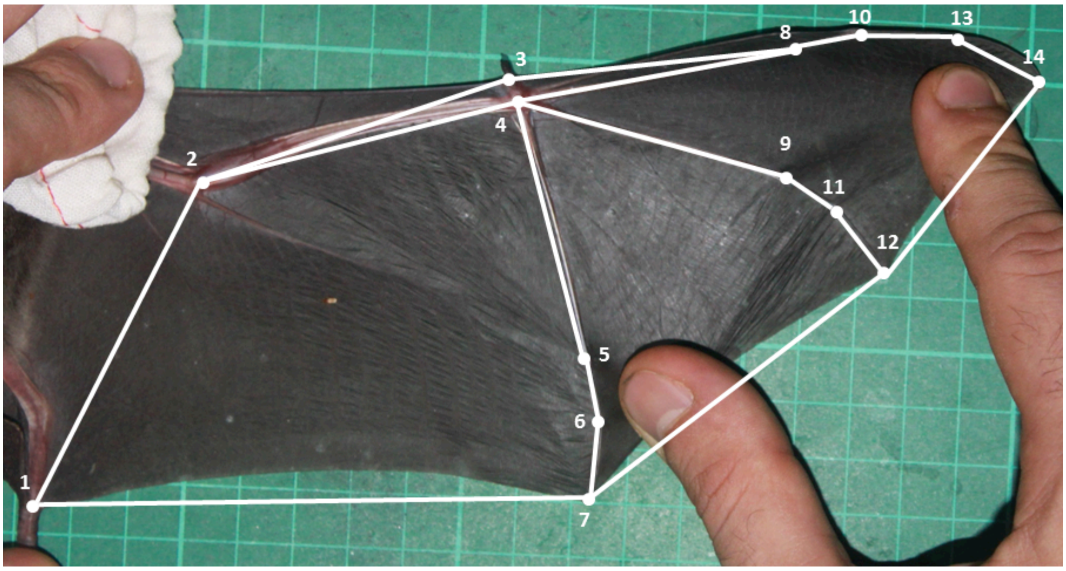


Figure 4.1. Photograph showing the method by which the bat wing was stretched in order to record the following anatomical landmarks: 1) Tissue junction between the wing and the hind foot; 2) Articulation between the humerus and radius/ulna; 3) Tissue junction between the propatagium membrane and digit I; 4) Center of the carpus; 5) Articulation between metacarpus and proximal phalange of digit V; 6) Articulation between proximal and distal phalanges of digit V; 7) Tissue junction between distal phalange of digit V and propatagium membrane; 8) Articulation between metacarpus and proximal phalange of digit III; 9) Articulation junction between metacarpus and proximal phalange of digit IV; 10) Articulation between proximal and intermediate phalanges of digit III; 11) Articulation between proximal and distal phalanges of digit IV; 12) Tissue junction between distal phalange of digit IV and dactylopatagium major membrane; 13) Articulation between intermediate and distal phalanges of digit III; 14) Tissue junction between distal phalange of digit III and dactylopatagium medius membrane.

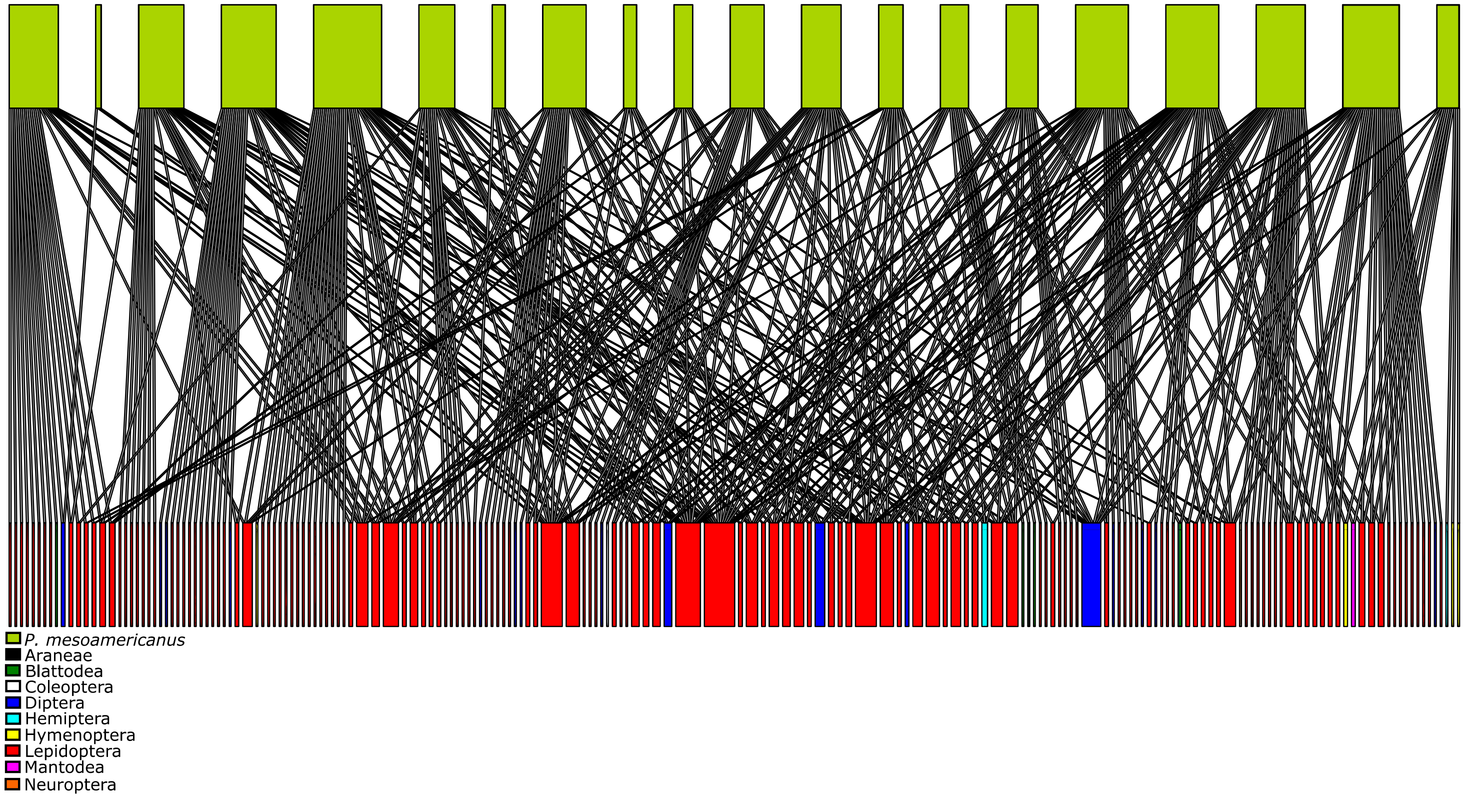


Figure 4.2. Antagonistic network of individuals of the bat species *Pteronotus mesoamericanus* and the prey items present on their diets. Links in the network representing species diets were revealed using DNA metabarcoding (gene COI). The width of the top bars represent the number of feeding items present in the diet of an individual while the width of the bottom bars represents the number of individuals that consumed that prey item.

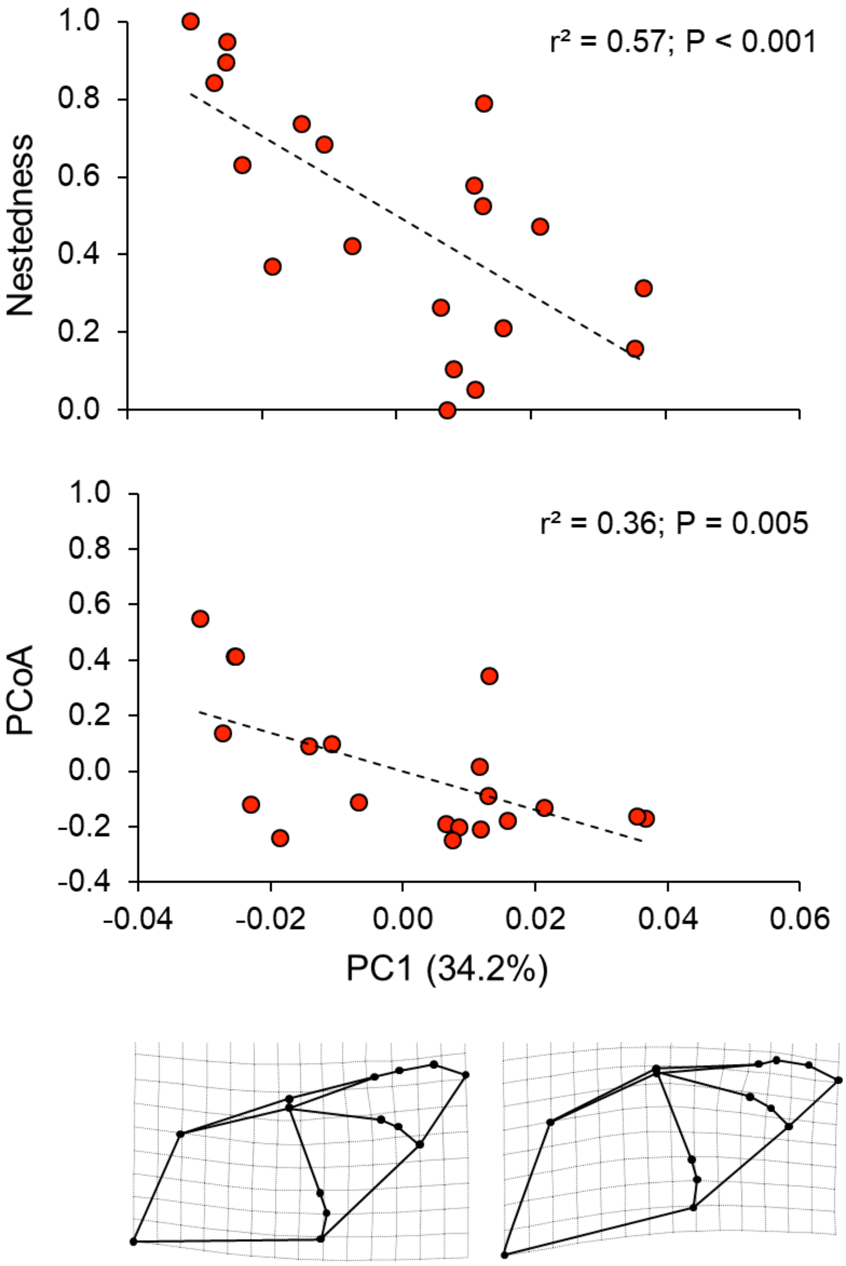


Figure 4.3. Relationship between wing shape and diet (nestedness and similarity [PCoA]) of *Pteronotus mesoamericanus*. The wing shape representations below the x-axis represent the extrapolated twofold values of the lowest (left inset) and highest (right inset) PC1 scores. Low scores represent wings with a more triangular shape while high scores represent a more rounded wing. The value in parenthesis indicates the proportion of the total wing shape variance; r^2 and P-values is indicated according to the partial correlation obtained in the multiple regression analysis (global adjusted $r^2 = 0.60$ for nestedness and global adjusted $r^2 = 0.40$ for PCoA; see results for more details).

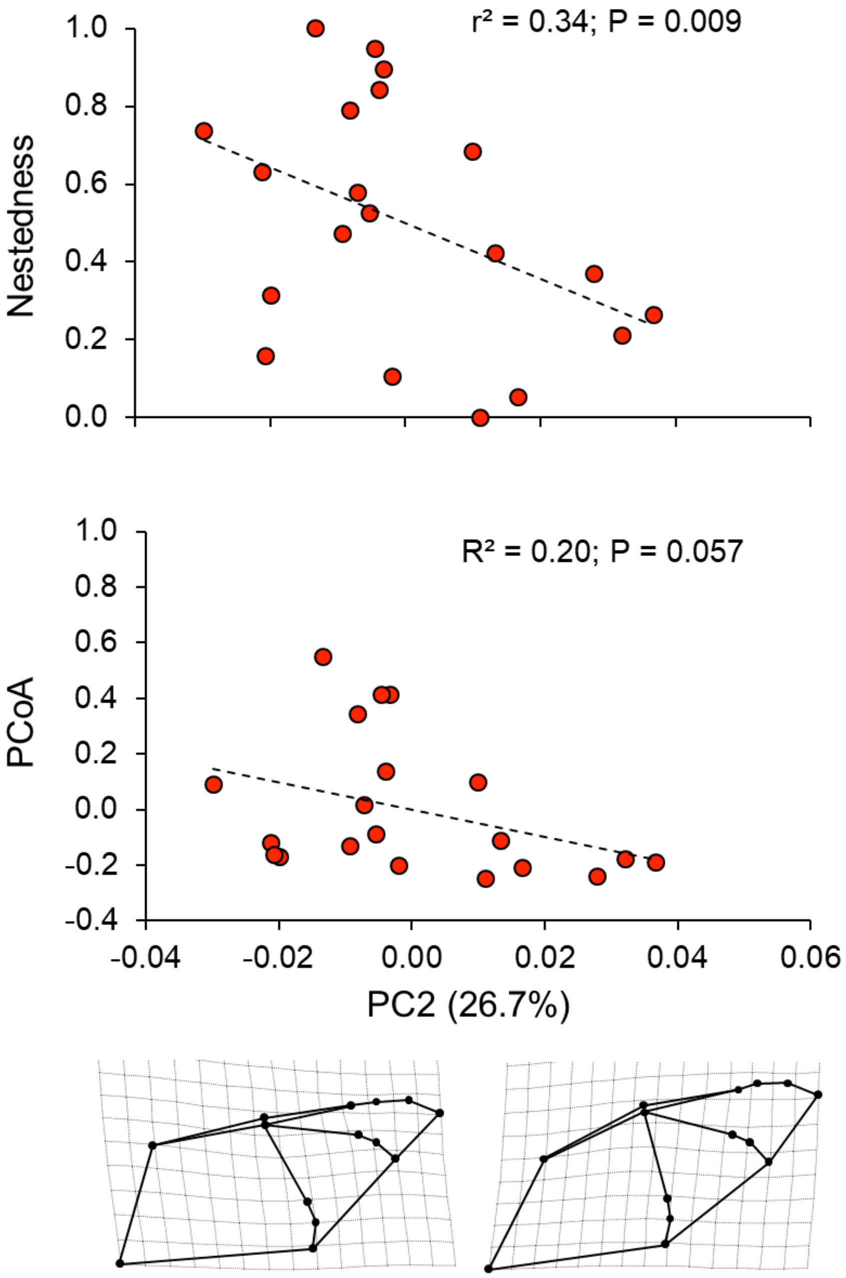


Figure 4.4. Relationship between wing shape and diet (nestedness and similarity [PCoA]) of *Pteronotus mesoamericanus*. The wing shape representations below the x-axis represent the extrapolated values twofold of the lowest (left inset) and highest (right inset) PC2 scores. Low scores represent wings with a more triangular shape while high scores represent a more rounded wing. The value in parenthesis indicates the proportion of the total wing shape variance; r^2 and P-values are indicated according to the partial correlation obtained in the multiple regression analysis (global adjusted $r^2 = 0.60$ for nestedness and global adjusted $r^2 = 0.40$ for PCoA; see results for more details).

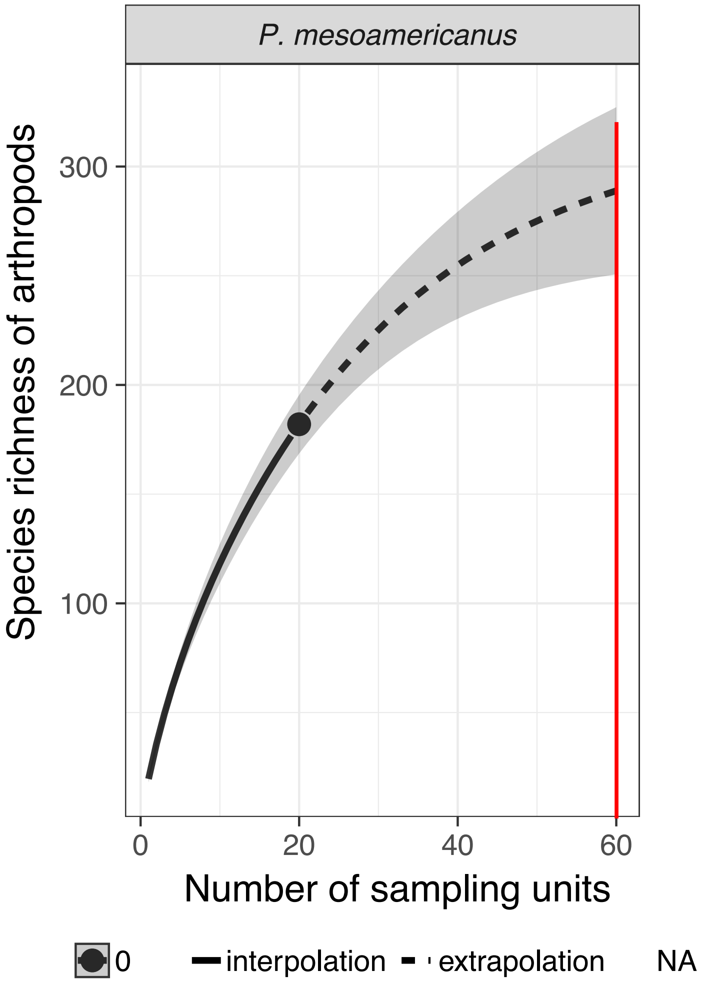


Figure 4.5. Individual-based rarefaction curves estimating the species richness of arthropods present on the diet of the bat species *Pteronotus mesoamericanus* during the wet season in the dry forest of Sector Santa Rosa (of ACG) (2015). Red line indicates the richness extrapolating 3 times the number of faecal samples analysed for the present bat species.

Supplementary material

Table S4.1. Results for the Principal Coordinates Analysis (PCoA) evaluating the diet of 20 individuals of *Pteronotus mesoamericanus.* The analysis was performed with the Bray-Curtis dissimilarity index using a matrix containing the different food items pooled by arthropod order for each individual.

Analysis	Eigenvalue	Variance explained (%)
PCoA1	1.178	61.3
PCoA2	0.344	17.9
PCoA3	0.147	07.6
PCoA4	0.118	06.2
PCoA5	0.063	03.3
PCoA6	0.044	02.3
PCoA7	0.022	01.1
PCoA8	0.06	0.3

Table S4.2. Consumption of MOTUs from different arthropod orders by individuals of *Pteronotus mesoamericanus* in the dry forests of Costa Rica. Cell values represent the number of Molecular Operational Taxonomic Units (MOTUs) consumed per individual. Nestedness, number of orders and total number of MOTU consumed are indicated.

	Individual 1	**Individual 2**	**Individual 3**	**Individual 4**	**Individual 5**	**Individual 6**	**Individual 7**	**Individual 8**	**Individual 9**	**Individual 10**	**Individual 11**	**Individual 12**	**Individual 13**	**Individual 14**	**Individual 15**	**Individual 16**	**Individual 17**	**Individual 18**	**Individual 19**	**Individual 20**
Araneae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Blattodea	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Coleoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Diptera	2	1	1	1	2	0	5	1	5	1	1	0	2	2	3	0	2	0	4	1
Hemiptera	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0
Hymenoptera	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Lepidoptera	5	23	38	6	23	17	18	20	21	12	2	24	28	14	26	10	5	10	23	24
Mantodea	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Neuroptera	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nestedness	0.89	0.3	0.53	0.79	0.26	0.6	0.42	0.4	0.2	0.7	1.0	0.37	0.05	0.58	0.10	0.84	0.95	0.68	0.16	0.00
Numbers of orders consumed	2	4	2	5	3	1	2	2	4	2	2	1	2	4	2	1	2	4	3	3
Total number of MOTUs consumed	7	26	19	12	26	17	23	21	28	13	3	24	30	18	29	10	7	15	28	36

CHAPTER FIVE

General Discussion

The central aim of this thesis was to analyse the effects of seasonality in rainforest and dry forest of Costa Rica on the antagonistic and mutualistic interactions of insectivorous and frugivorous bats and their food items. I study this against a background of an extreme El Niño event, which exacerbates these conditions and thus should maximise the probability of measuring an effect. Where possible, in the dry forest, I compare the measurements to a regular non-El Niño year. In addition, I analysed the effects of variation in wing shape on the diet of the insectivorous bat *Pteronotus mesoamericanus*. My studies have addressed several broad problems. First, I examined network metrics of mutualistic networks (bat-fruit interactions) in periods of extreme flooding and drought, and used null models to test for deviations from expectations. Second, I addressed how the severe decrease in the rainfall of the dry forest of Sector Santa Rosa (of the ACG) is likely to have impacted the modular structure of the antagonistic networks (bat-arthropod interactions) by comparing data from a non-El Niño year against an El Niño year. Finally, I applied a network approach to evaluate inter-individudal variation in diet in one common species, and tested whether differences in network position among indivudals, could be attributed to morphological variation in wing shape.

- *The effects of floods and droughts on the structure of mutualistic networks*

In the first data chapter, an examination of the patterns found for mutualistic networks in the dry forest and rainforest of Costa Rica have revealed that droughts and floods seem to be associated with similar network structures of interactions between bats and fruits (decreased values of nestedness and increased values of modularity in comparison with the null models). Although the total annual rainfall variation between sites was one order of magnitude different (~600 *versus* ~6,000 mm), the direction of the change for most of the network metrics in comparison with the null model was the same. When taking into account only the absolute values (independently of the null model comparisons), both metrics (nestedness and modularity) showed higher values during the dry season. While the behaviour of nestedness seemed to follow the expected pattern reported in the literature, modularity followed an opposite trend (Rico-Gray *et al.* 2012; Dalsgaard *et al.* 2013; Trojelsgaard & Olesen 2013; Schleuning *et al.* 2014).

Network metrics such as nestedness can be determined by different underlying factors; for example, by decreasing the niche of the generalists while keeping the niche of the specialists constant. Thus, the exact routes by which changes in rainfall might have been associated with differences in nestedness may be due to different causes for each of the networks (wet *versus* dry season / dry forest *versus* rainforest). Droughts are thought to impact forests by causing tree mortality rather than by reducing their growth (Philips *et al.* 2009) and thus it is possible that the low nestedness recorded during the drought in the dry forest is not only a result of a decrease in fruit productivity, but also an increase in plant mortality, which might have led to an increase in the generalization of species diet and a consequent increase in nestedness. Lower values of modularity recorded in the rainforest compared to the dry forest were not expected based on assumed higher fruit availability. One possible explanation for this is that the precipitation was too high, and that water stress caused by the excess of rain led to a decrease in fruit production.

Because my study is limited to a single year, which contained an extreme El Niño event, it is not possible to directly assess the impact of El Niño or control for interannual variation as I do not have replicate measures. To compensate for that, I have used null models to test for deviations from random measures and, where possible, I have compared my data against data from a non-El Niño year. While the extreme weather patterns should magnify the differences between forests, the limitation of my study is the lack of direct comparisons and replication. More studies are required, both to verify whether the trends I have measured hold across years and in forests in different parts of the world, and also to understand more fully the causes and effects of the environmental changes on the network metrics.

- *The effects of a severe drought on the structure of antagonistic networks*

Severe droughts can lead to big decreases in insect populations (Holmgren *et al.* 2001). However, few studies have examined the impact of severe droughts on antagonistic interactions, particularly any bottom-up cascades on insectivores that result from declines in insect populations. This is especially true of investigations using molecular techniques. In the second data chapter, I focus exclusively on the dry forest where I have data from a non-El Niño year and an El Niño year for the wet season and my own measure of the dry season. My analyses show that changes in rainfall can have important modifications in bat-arthropod interactions and the overall structure of antagonistic networks. The decrease in rainfall was followed by a decrease in modularity and changes in the position of some nodes represented by some insect orders in the network. Although I have detected these changes, it is hard to address the underlying causes that have led to them. Although I did not attempt to measure actual changes in the population sizes of bats and insects in the dry forest of Sector Santa Rosa (of ACG), bats are believed to migrate towards higher elevations during periods of extended droughts, and while there are no trends reported for insects, entomologists on site noted a marked decrease in some important groups like the Lepidoptera. A time series of recorded antagonistic interactions between bats and arthropods during multiple non-El Niño years would allow me to better understand the true magnitude of the changes witnessed during the El Niño year.

- *The role of wing morphology in individual niche specialization and diversification*

Few studies have analysed the importance of morphological differences for niche specialization and differentiation in individual bats (Kalcounis & Birgham 1995; Jacobs 1999). In the third data chapter I perform the first examination of the role of morphology on individual diet diversification and specialization. I found a significant relationship between wing shape of bats and their degree of diet specialization and differentiation, but it would be interesting to verify whether this relationship exists during non-El Niño years or is limited to the unusual El Niño induced effects of my field season when prey resource fluctuations may have been extreme. Another interesting point that needs to be investigated in further detail is whether the individual differences identified during this event were due to the exploration of different habitats, or whether they reflect differences in the efficiency of the individuals in capturing different types of prey, which seems to be a more likely explanation for the relationship I found.

- *Limitations*

For the analysis of insectivorous diet, I used a high-throughput sequencing approach based on the work of Clare *et al.* (2014). Although this form of molecular analysis can provide a more detailed and accurate analysis of animal species diets, there are limitations (Nielsen *et al.* 2017). There is the risk of sequence misclassifications during the description of the diet of insectivorous species, which could have led to erroneous inferences regarding arthropod prey taxa. The measurement of species richness in a sample using high throughput sequencing techniques can depend on the parameters chosen for the analysis of the data such as, the filtering choices made for the raw data; clustering thresholds for grouping DNA sequences into Molecular Operational Taxonomic Units (MOTUs); the choice of the clustering algorithms (mothur, UCLUST and UPARSE); the treatment of gaps in the sequences; and the removal of sequences with low copy numbers (singletons, doubletons, etc.) (Schloss *et al.* 2009; Edgar 2010; Flynn *et al.* 2015). The choice of the clustering thresholds for MOTU delimitation alone can shift the number of MOTUs generated across orders of magnitude (Flynn *et al.* 2015), which could potentially influence the network metrics evaluated and change the results. Another important factor in defining the number of MOTUs is the removal of unique DNA sequences (Flynn *et al.* 2015). Amplification and sequencing errors can lead to the formation of artefactual sequences (Kunin *et al.* 2010; Behnke *et al.* 2011; Bachy *et al.* 2013) and can be responsible for greatly inflating the number of MOTUs defined in a sample (Flynn *et al.* 2015). The most common strategy to deal with the removal of these artificially created sequences is to set a minimum sequence number below which haplotypes are excluded from the analysis (Alberdi *et al.* 2018). However, multiple criteria have been used to define the ideal number of sequences to be removed with numbers varying from singletons to sequences that are represented by many more copies (Giguet-Covex *et al.* 2014**;** Arrizabalaga-Escudero *et al.* 2015). My analysis targets a small region of the mitochondrial cytochrome C oxidase subunit 1 (COI) and as a coding gene I do not expect gaps in the sequences, thus gaps should not represent a significant factor in my MOTU detection (Clare *et al.* 2016). However, indels are possible and must be considered as a source of MOTU error. To attempt to correct for the generation of MOTU based on sequencing error, I chose to be conservative and use an OTU clustering threshold of 92% similarity and remove singletons as a way to remove artificially created sequences that could increase the number of MOTUs found in my samples (Flynn *et al.* 2015). This has the potential impact of collapsing real species together, but should reduce the overinflation of MOTU observed elsewhere. Another potential impact is that collapsing more genetic variation into fewer prey nodes using a lower MOTU threshold (92%) might reduce the values of some network metrics such as modularity, while increasing for others such as connectance. However, it is not clear whether this might have had an influence in my findings in relation to network comparisons against the null models.

For the analysis of seed dispersal, I used traditional sanger sequencing based on standard DNA barcoding approaches for plants (Fazekas *et al.* 2012). DNA barcoding of plant material has different limitations from that of insects. The main problem is the potential for taxon and sequence specific differences in the accuracy of the identification. Different plant genomic regions have been assessed as markers for plant identification (e.g. *rbcL*, *trnh-psbA*, ITS and *matK*) (Li *et al.* 2015). Each of the genes has their pros and cons and no gene alone has been shown to identify plants at the species level across all taxa (Li *et al.* 2015). I used a combination of *rbcL* and ITS to identify plant species and assigned matches based on existing reference collections. *rbcL* has the advantage of having a large dataset of sequences already deposited in Genbank (over 50,000 sequences), it is easily recoverable and allows for a good identification of plants at the family and genus level (Li *et al.* 2015). Although ITS is a better marker for the identification of plants at the species level than plastid regions, it has been suggested as a supplementary locus for plant identification as it has yielded previous difficulties in sequencing and amplification and the potential for fungal contamination (Hollingsworth *et al.* 2011).

Because of the variability in differentiating plant taxa with these two regions, different sequences were matched at different taxonomic levels: family, order, genus or species. This variation in sequence matching could theoretically influence the structure of the network if there are taxon specific differences in resolution, making it difficult to standardize comparisons across different networks generated using this method for datasets of different plant species. Several taxa in particular (*Ficus* and *Piper*) are thought to have rapidly radiated and species level identification may not be possible. To compensate for the variable identification rates from this methodology, I tested both a fully resolved network and one where I limited my identification of all plant DNA sequences to genus, which, for the recovered ITS2 and *rbcL* regions, can improve the probability of correct matches (Chen *et al.* 2010; Yao *et al.* 2010; Bruni *et al.* 2015) and as a result generates networks with even node resolution, a problem that has been noted by others (e.g. Ings *et al.* 2009). To test for the effect of node resolution, I estimated the significance of the metrics of interest, comparing the outcomes from networks with matches at the genus level to those where the networks were built with matches at multiple taxonomic levels. In the former case, I am ignoring cases where additional resolution is possible (e.g. to species) and in the latter case I risk type II errors in my identifications. In this case, I drew similar conclusions using both methods. Thus I do not expect the issues of potential type 2 errors or mixed resolution is a serious limitation and, as a result, I have focused on the most well resolved networks.

Null models are a commonly used method to assess the significance of changes in network metrics (Pellisier *et al.* 2018) when the difficulty of measuring interactions precludes the use of replication (Evans *et al.* 2016). Selecting the null model that best fits to randomize a network has been a challenge in network ecology. Many different null models have been created with different constraints and suggested as the best choices or alternatives for network analysis (Patefield, vaznull, shuffle, swap, Erdõs-Rényi). However, some of them generate unrealistic distribution of values in the matrix of interactions. The randomization of a matrix of interactions using the Patefield algorithm (which increases connectance), for example, leads to a very generalist network, which will very likely always be considered to be more generalist than the observed network according to the null model. However, these influences are often overlooked, leading to wrong interpretations of the patterns of the observed networks. The lack of a clear understanding and characterization of the patterns underlying the network structure of mutualistic and antagonistic food webs across the globe for many different biological systems helps to reinforce this problem, making it more difficult to correctly interpret the results of any analysis coming out of a null model. To try and compensate for this I followed the advice of Gotelli & Ulrich (2011) and chose a more constrained null model in order to avoid type I error. The swap algorithm constrains not only by total marginal sums, but also by connectance, which limits the ways that the observed matrix can be randomized, and tends to generate network metric values that are closer to the ones from the observed network.

Sampling completeness of the network also represents a potential limitation for network analysis and conclusions. Sampling species interactions is extremely difficult and even biological communities with low species richness with an extremely high sampling effort still have missing links (Wirta *et al.* 2014). On the other hand, the addition of missing links can lead to changes in the structure of ecological networks and increasing values of connectance (Olesen *et al.* 2011), which is ultimately linked with the behaviour of other network metrics, such as modularity and nestedness. Increasing connectance values tend to have an opposite effect on the values of modularity. Under higher connectance values, there is also a positive correlation between values of nestedness and modularity (Fortuna *et al.* 2010), which would lead to a decrease in nestedness as well. To overcome the problem of differences in network size, missing links and potential low sampling completeness of some networks, I chose to focus my analysis on network metrics that have little influence from sampling completeness and network size, such as NODF and modularity (QuanBIMO) (Fründ *et al.* 2015).

- *Future work*

Insectivorous bat species occur on all continents apart from Antarctic, whereas frugivorous species occur in both the Palaeotropics (family Pteropodidae) and Neotropic (family Phyllostomidae). In this analysis I have examined the response of interaction networks from two communities to fluctuations in season in the context of an extreme case of flooding and drought caused by El Niño. Where possible I have compared this to normal conditions. Moving forward, future investigations should include repeated sampling of non-El Niño years to allow us to draw direct conclusions about the impact of El Niño, the role of interannual variation and to increase the sampling completeness. In addition, including other locations, specially some where the extreme rainfall variations registered in the present study happen on a yearly basis, will be useful. For example, some parts of Asia where the precipitation is extremely high due to the monsoon or some desert areas of South America where there are big precipitation variations between wet and dry season, might increase the understanding about how consistent the pattern that I registered is and how resilient the interactions in the bat food webs are to extreme events. Thus, more work is needed to assess whether my results from two sites in Costa Rica are consistent over time and also true of other locations worldwide. Describing interactions for multiple species from different bat families using different sites around the world remains an important task if we want to understand the general patterns of how changes in rainfall are impacting the interactions of bats and their food items worldwide as well as in Costa Rica.

Molecular data has only recently started to be used for the reconstruction of trophic interactions. There is still a need for a framework or guidance on the impact on the integration of molecular data with networks which addresses potential limitations and impacts of changing sequencing platforms, number of PCR replicates per sample, minimum copy number of retained sequences, the impact of different OTU clustering thresholds and algorithms on the overall structure of the observed network. Without this framework, it is hard to know whether the findings of any study are robust to modifications in the laboratory or bioinformatics pipeline. At the same time, network analysis needs to advances in my understanding of the impact of missing or mixed node resolution and minimum sample size or minimum number of observed links needed in order to have an accurate estimation of stable network values for many metrics from different species interactions, while there are a number of analyses that attempt to assess this there are often alternative conclusions. For many networks and species interactions, it is not known what the general pattern and expected behaviour is for many network metrics, which makes it harder to detect and interpret deviations from a normal trend.

- *Conclusion*

Extreme changes in rainfall seem to produce similar effects in mutualistic networks of frugivorous bats across both dry forest and rainforest while droughts promotes an increase in modularity with changes in the position of arthropods in antagonistic interactions in the dry forest. These changes are important to understand as the world is rapidly facing instability provoked by the fast a changing climate and we know little about the impacts of it will have on the robustness of the interactions and ecosystem functions that different species realize and provide in these environments.

Appendix

Tables

Table 6.1. Yearly rainfall record (mm) in the dry forest of Sector Santa Rosa (of ACG) – Costa Rica (1985-2015).

Year	Total rainfall (mm)	Year	Total rainfall (mm)
1980	1708.3	1998	1986.3
1981	2239.6	1999	1837.1
1982	1821.2	2000	1138.0
1983	915.3	2001	1655.2
1984	1721.9	2002	1563.7
1985	1431.0	2003	1404.3
1986	1305.9	2004	1328.1
1987	1088.6	2005	3031.2
1988	2558.3	2006	1247.0
1989	1346.2	2007	3038.1
1990	1116.2	2008	2984.8
1991	880.1	2009	1242.1
1992	1020.2	2010	2819.3
1993	1325.1	2011	2568.2
1994	1120.1	2012	1258.0
1995	2103.7	2013	1591.8
1996	2260.6	2014	1114.9
1997	959.5	2015	627.0

Table 6.2. Yearly rainfall record (mm) in the rainforest of La Selva Biological Station – Costa Rica (1958-2015).

Year	Total rainfall (mm)	Year	Total rainfall (mm)
1971	3721.8	1994	4221.5
1972	4590.4	1995	2809.3
1973	3670.8	1996	4241.9
1974	3926.8	1997	4810.9
1975	4092.9	1998	3863.8
1976	4898.5	1999	4322.0
1977	4256.9	2000	4701.5
1978	4524.9	2001	5189.4
1979	5011.0	2002	5144.2
1980	4860.8	2003	4315.9
1981	5121.4	2004	5193.0
1982	4852.6	2005	4224.9
1983	3548.7	2006	4532.1
1984	3572.6	2007	3747.5
1985	3128.8	2008	4319.5
1986	3917.0	2009	4521.4
1987	3391.5	2010	4732.4
1988	4133.2	2011	4304.9
1989	3673.5	2012	3555.1
1990	4462.7	2013	3159.4
1991	4537.4	2014	4549.4
1992	3843.1	2015	5813.7
1993	3637.7		

Figures

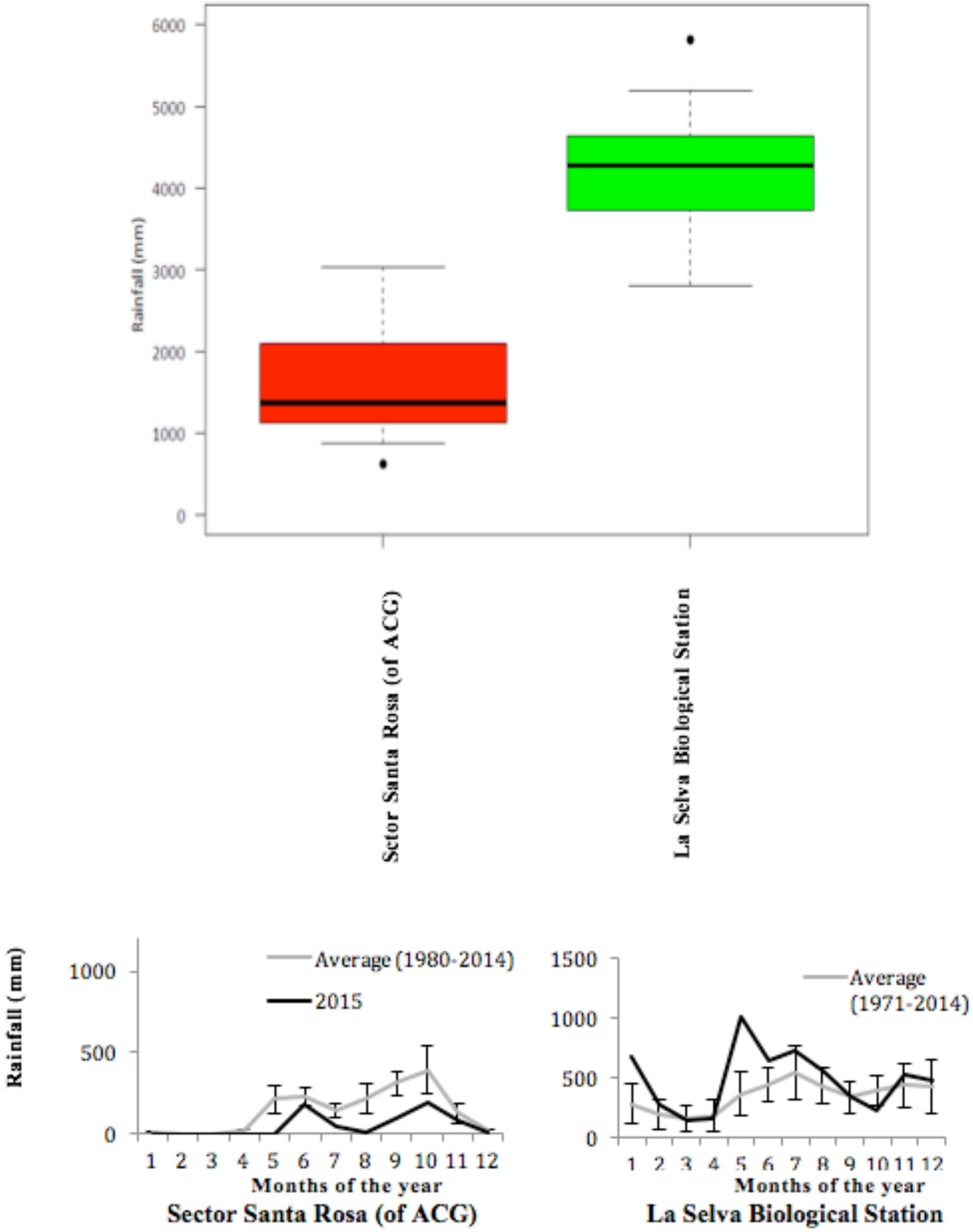


Figure 6.1. Annual and monthly rainfall variation in the dry forest of Sector Santa Rosa (of ACG) and rainforest of La Selva Biological Station (Costa Rica).

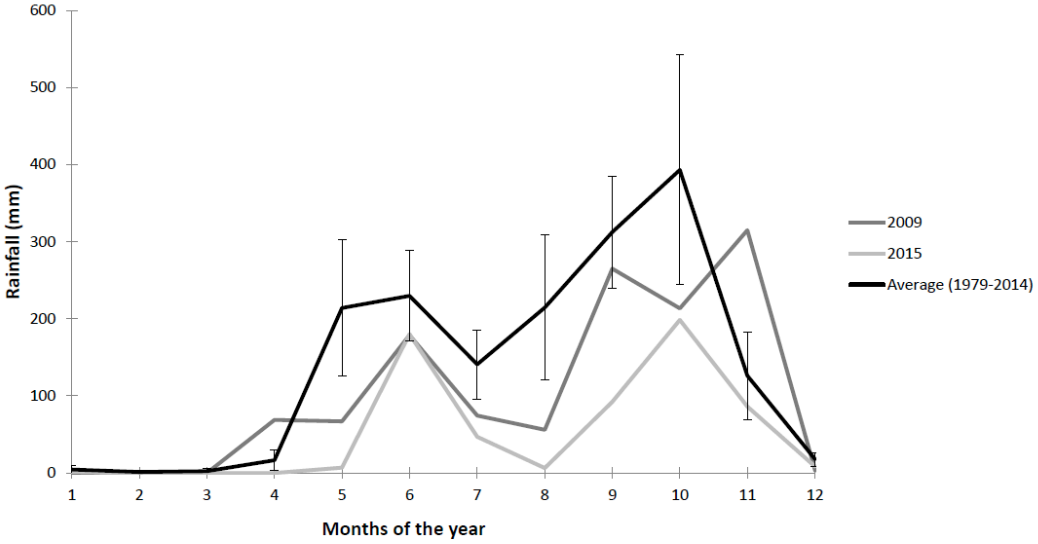


Figure 6.2. Comparison of the monthly seasonality in the dry forest of Sector Santa Rosa (of ACG) Costa Rica during the dry (A) and wet (B) season of an extreme El Niño event (2015) as well as a wet season of a normal year (2009).

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