THE ROLE OF CLIMATE IN THE SEASONALITY PATTERNS AND COMMUNITY ASSEMBLY OF NEOTROPICAL BUTTERFLIES ALONG AN ENVIRONMENTAL GRADIENT

By

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To my beloved angels, Inti and Daniel, and my dearly loved parents and sister, Papa, Susita and Karlita, who have been a beautiful, guiding light in my life.
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The goal of the present study was to measure the seasonality patterns of butterfly communities in relation to climate, and quantify the effects of different collection techniques on the observed patterns. Additionally, I determined the relative contribution of ecological filters and competition in structuring butterfly communities along a climatic environmental gradient through a phylogenetic approach using functional traits.

A 3-year survey was carried out at three sites (i.e., wet, transition and dry forests) across a climatic gradient in western Ecuador. Butterflies were sampled using traps baited with rotting banana and prawn, along with hand-nets, every two months from Nov 2010 to Sep 2013. Traps were set up at two heights, in the understory and canopy. DNA was extracted to sequence the barcode’ section of the mitochondrial gene cytochrome oxidase 1 (COI) for phylogenetic analyses. Measurements of morphological traits, forewing length and aspect ratio were done using digital photographs of specimens. A total of 1534 butterflies and approximately 230 species were collected.
using hand-nets, and, 7046 butterflies representing 214 species were collected with traps across all study sites.

Collection techniques, bait traps and hand-nets had an effect on analyses of butterfly communities in both temporal (e.g., seasons) and spatial (e.g., strata and microhabitat) dimensions. Combining both methodologies maximized species sampled. Butterfly communities exhibited conspicuous intra- and inter-annual variation in population dynamics with certain elements of seasonality patterns likely synchronized in seasonal forests across years but not in aseasonal forests. Rainfall was significantly positively associated with temporal abundance. Species displaying stronger seasonality were significantly negatively associated with higher rainfall periods in seasonal forests. Phylogenetic- and trait-based analyses revealed mostly non-random patterns of phylogenetic structure within butterfly communities along local (i.e., strata) and regional (i.e., wet to dry forests) environmental gradients. Competition was more prevalent within dry forest, whereas ecological filters were significant drivers of community composition within wet forest. Random patterns of phylogenetic structure were mostly observed for dry forest communities. The present study therefore provided insights into community assembly mechanisms in one of the richest butterfly faunas worldwide, revealing competition along with ecological filters as significant drivers of community composition.
Climate is clearly a major factor influencing biological assemblages. Understanding the role of climate in community ecology is not only of relevance at small spatial and temporal scales, but also has practical and theoretical interest in many other fields, including macroecology (e.g., the role of climate in influencing spatial patterns of biodiversity) and conservation biology (e.g., the susceptibility of species to climate and habitat change).

Studies of tropical insect faunas have a particularly high potential to provide insights into how climate and organisms interact. Insects are the richest group of living organisms comprising approximately 62% of the 1.6 million species described so far (Wiens et al. 2015), with nearly half of the world’s species inhabiting tropical forests (Olson & Dinerstein 2002).

Insects occupy the widest possible diversity of ecosystems, microhabitats and niches, and also play key ecological roles (Kremen 1994). Insects are ectothermic, depending on environmental temperature to raise or maintain their internal temperature. This condition makes insects susceptible to changes in their environment even at very small scales because of their relatively large ratios of surface area to volume and, due to their short generation time, they might provide timely information about the effects of environmental changes on the biota (Sparrow et al. 1994). Hence, insects, particularly butterflies, have been used as biological indicators (Kremen 1992, 1994, Bonebrake et al. 2010) as well as model groups in biology owing to their high abundance and species richness (Valtonen et al. 2013), relatively resolved taxonomy, large size and conspicuousness, ease of sampling, and sensitivity to environmental change (Kremen
1992, 1994; Brown & Freitas 2000, Bonebrake et al. 2010). One group of butterflies, those attracted to baits, are used as models in ecological studies since their diversity is apparently positively correlated with total butterfly diversity (Ribeiro & Freitas 2011).

However, despite this potential, studies of climate and insect communities are complex. Insect communities are known to show marked temporal fluctuations independent of climate. Wolda (1978) found that insect populations from a seasonal forest in Panama showed every conceivable pattern of temporal fluctuation and concluded that it was unlikely that a major environmental factor such as climate was causing the observed patterns. This study therefore showed that factors other than climate are likely to affect insect populations. Additionally, insect communities also show rapid spatial turnover (Novotny et al. 2007), from very large scales (biogeographic regions) to small scales such as microhabitat. Even in insects with relatively high potential dispersal ability, such as butterflies, microhabitat can be influential at a very small scales, such as between understory and canopy (see DeVries & Walla 2001), or between shaded and open areas (forest gaps, Hill et al. 2001), or across topography such as between ridgetops and valleys (Prieto & Dahners 2009, Checa et al. 2014). Adding complexity to this natural spatial and temporal variation in insect diversity, different collection techniques, such as baited traps or hand-nets, might further bias results (see Molleman et al. 2005, Holloway et al. 2013).

Studies of the effects of climate variability on population dynamics of insects, for example, require standardized sampling over long periods of time across a range of spatial scales to discriminate between the influence of climate and other factors. Here, I propose to measure the seasonality patterns in species richness and abundance of
butterfly communities in relation to climate across one of the world’s steepest terrestrial environmental gradients, the wet to dry forests of western Ecuador. I will additionally quantify the effects of different collection techniques on the observed population patterns in space and time.

Spatial variation in species richness is one of the most obvious attributes of biological communities and climate is hypothesized to play a key role in driving this pattern through energy availability, measured as primary productivity (Williams & Hero 2001, Williams & Middleton 2008, Hanya et al. 2011). In the Neotropics, climatic factors (temperature and precipitation) are important determinants of richness and community structure of butterflies at both the local scale (Atlantic forest butterflies, Brown & Freitas 2000) and at the regional scale (48 sites from Mexico to southern Brazil, Brown 2003). Nevertheless, the relative importance of biotic interactions and the abiotic environment as assembly mechanisms shaping insect tropical communities remains little explored (see Donoso 2013). Another objective of this dissertation is therefore to determine the relative contribution of ecological filters and interspecific interactions in structuring butterfly communities along a climatic environmental gradient (i.e., forest strata and ecosystems with varying climatic seasonality) through a phylogenetic approach using functional traits.

1.1 Study Area

The west Ecuadorian dry forest is one of the most important areas of endemism worldwide (Davis et al. 1997), but even within this region species may occur at extremely local scales or at single localities (Gentry 1992, Hamilton et al. 2007), which increases the vulnerability of such species to extinction. Around 20% of plant species are endemic (Dodson & Gentry 1991, Parker & Carr 1992), representing as high a level
of endemism as in the wet forests in the north of the country. In the case of birds, the
dry forests of western Ecuador are part of one of the most biologically important and
threatened Endemic Bird Areas of the world, the Tumbesian region (Stattersfield et al.
1998). Endemism in other groups, such as mammals and herpetofauna, shows similar
patterns (see Parker & Carr 1992), and the region likely contains more than 100
endemic butterfly species or geographically isolated populations (Willmott unpub. data).
Moreover, the wet forests of northwestern Ecuador are also part of one of the top 25
global hotspots of diversity and endemism (Myers et al. 2000).

Unfortunately, western Ecuador has been cited as one of the most globally
threatened areas in terms of biological extinctions due to habitat alteration and
fragmentation (Dodson & Gentry 1991). Indeed, by 1998 the Tumbesian region of
Ecuador had less than 5% of its original dry forests remaining, and was facing
exceptional threats of extinction (Stattersfield et al. 1998). In fact, the dry forests in
Ecuador are the most endangered worldwide (Portillo-Quintero & Sánchez-Azofeifa
2010), as their remnants are heavily fragmented and degraded (Espinosa et al. 2016).
In recent years deforestation has increased in western Ecuador (Checa 2008) and the
percentage of original vegetation cover has therefore decreased still further, which
emphasizes the urgency of increasing our knowledge about this area's biodiversity.
Indiscriminate wood exploitation and the expansion of monocultures of African oil palm
are the main deforestation factors in the wet forests of northwestern Ecuador (Checa
2008), whereas agriculture and population expansion are the main deforestation factors
in the dry forests (Linares-Palomino et al. 2010).
Western Ecuador presents a gradient of life zones due to a differential
distribution of precipitation over the year. The presence of two opposite ocean currents
is responsible for this pattern, the cold Humboldt Current coming from the south, which
swings westward away from the land near 2˚ S latitude, and the warm Equatorial
Current El Niño moving from the north (Lynch & Duellman 1997). As a result, pluvial
and wet forests dominate in the north, but increasingly change to moist forest and dry
forest (around 2˚ S latitude) in the southern part, where several months of drought occur
(see Cañadas & Estrada 1978). During January through March, El Niño shifts further
south before moving westward, producing an increase in precipitation in the north and
the arrival of the rainy season in dry and moist forests (Lynch & Duellman 1997).

This dissertation was carried out in three different forest reserves, which cover
the three main types of life zones in western Ecuador: wet forests in the north (Canandé
River Reserve), moist/dry transition forests (Lalo Loor Dry Forest Reserve) and dry
forests in the south (Jorupe Reserve) (Fig. 1-1).

The Canandé River Reserve (CRR, 00°28’ N, 79°12’ W) is located in the Chocó
Region, in a small mountain chain with an altitude not higher than 500 msnm
surrounded by valleys converted to monocultures of African oil palm. This wet forest is
characterized by evergreen forest (Sierra 1999), usually cloudy as the mountains retain
humidity coming from the Pacific Ocean. The CRR is part of the Chocó-Darién, one of
the 21 global hotspots of biodiversity and endemism (Myers et al. 2000), and some
species of mammals and birds that are critically endangered inhabit the reserve (Tirira
2001).
The Lalo Loor Dry Forest Reserve (LLDFR, 0°6' S, 80°10' W) is located in the Tumbesian Region, at the transition between moist and dry forest, a vegetation type that is nearly extinct in the region due to deforestation (Clark et al. 2006). The reserve has 200 ha of preserved forest and the altitude ranges from 10 to 300 m. The dry season may last 5 months, resulting in all trees of the lower hillsides and the coastal plain being deciduous (Clark et al. 2006), in addition to those of some ridgetops, due to their greater exposure to direct sunlight and the resultant higher variability in temperature and humidity. Moist evergreen forests are also present, over hillsides and in the valleys between ridges, which makes this reserve suitable for comparisons of butterfly faunas at a local scale. The Jorupe Reserve (4°18' S, -79°37' W) is in the southwest corner of Ecuador, close to the border with Peru. It is characterized by very dry forest, where up to 8 months of drought occur, and it is also part of the Tumbesian Region. Throughout the dissertation, the term ‘seasonal forests’ referred to both the transition and dry forests due to the presence of deciduous forests and drought within these ecosystems, whereas the wet forest was considered as ‘aseasonal’ (it is an evergreen forest).

1.2 Census Techniques

Butterflies were sampled using entomological hand-nets and Van Someren-Rydon bait traps. Two different types of baits were used: carrion (rotting shrimp fermented for 13-18 days), and fruit (banana fermented for 2 days). Fruit and carrion baits attract partially different taxonomic groups of butterflies (Hall & Willmott 2000, Hamer et al. 2006), and the use of different baits therefore increases the diversity of species sampled. I carried out fieldwork from November 2010 to September 2013, hence completing a 3-year period of butterfly monitoring. Sampling visits were done
every 2 months (i.e., six times a year) for 7 days each sampling month. The sampling effort invested at each study site was 13,824 hour/trap (or 180 sampling days).

I checked the traps daily during the first 7 days of each sampling month; traps were opened and baited on the first trapping day, and the baits were renewed daily. Over the next 6 days traps were checked and the majority of trapped butterflies were killed by a pinch to the thorax and placed in glassine envelopes, except for several very common species that were marked and released. The latter technique is known as ‘mark-release-recapture’ and involves marking the butterfly the first time it is collected with a unique number on its wings using permanent markers. After noting collection data (e.g., species name, place and date of collection), the butterfly is released and if collected again, it is easily identified by the number on its wings.

1.3 Experimental Design

I established two transects (each had a minimum of 280 m in length) in each reserve, and in the case of LLDFR each transect was established in two clearly different microhabitats, ridgetop and valley, differing by 150 m in elevation (Figure 1-1). Eight sampling positions were located within each transect and the distance between two neighboring positions was at least 40 m. From experience of previous field research, 40 m represents a minimum distance for different positions to be considered independent replicates, since traps located less than 40 m apart can be considered to occur in essentially the same space and therefore are not expected to sample distinct faunas.

At each position, two baited traps were set up in two different strata, understory (1.5 m) and canopy. The canopy height varied among the reserves, ranging from 20-25 m in the wet forest of CRR to 10-14 m in the dry forest of JR. The use of fruit and carrion baits alternated between positions, thus neighboring positions had different
types of bait, whereas canopy and understory traps in the same position were baited with the same type of bait.

Collection using hand-nets was also performed at the same transects established for trapping at CR (wet forest) and JR (dry forest). After checking traps, collectors remained in the sampling position for 15 minutes collecting all butterflies flying inside a 30-m diameter circle (with the understory trap located at the center) and at a maximum height of 5 m above the ground.

Data loggers were set up in each reserve from September 2010 to register the temperature, relative humidity and rain during the 3-year period of butterfly monitoring. All collected material was examined and identified to species; the higher classification follows Wahlberg et al. (2009). Legs from dried specimens were detached and stored in vials for subsequent DNA analyses. All collected specimens were deposited in the Section of Invertebrates, QCAZ Museum of the Pontifical Catholic University of Ecuador, and the McGuire Center for Lepidoptera, Florida Museum of Natural History, University of Florida.

1.4 Phylogenetic Analyses

DNA extraction was done from 1-2 legs (depending on butterfly size) of dried specimens using the Qiagen DNEasy extraction kit. The ‘barcode’ section of the mitochondrial gene cytochrome oxidase 1 (COI) was sequenced for all Nymphalidae species collected across study sites; phylogenetic analyses therefore only included this taxonomic group. Sequences for a few species were obtained from GenBank. The phylogeny based on COI sequences was constrained by the generic level phylogeny of Wahlberg et al. (2009) to confidently resolve deeper nodes. In order to quantify morphological traits, one photograph per individual was taken and the software ImageJ
imaging was used to carry out measurements on the digital photographs. Ten individuals per species were measured and traits averaged, excepting in cases where abundance species was <10 and solely 1-2 specimens were measured. Only males were analyzed owing to low captures of females with baited-traps, as well as their higher morphological variability (DeVries et al. 2010). Variables measured included forewing length as a body size proxy, and aspect ratio (AR), calculated as $4 \times \text{forewing length}^2 \times \text{wing area}^{-1}$ (Betts & Wooton 1988, Dudley 1989).
Figure 1-1. Map showing the reserves where fieldwork was carried out. The sampling design (2 transects with 8 sampling positions each) is illustrated in the lower part, which corresponds to the Lalo Loor Dry Forest Reserve. In this case, each transect is located in 2 different microhabitats, ridgetop and valley.
CHAPTER 2  
QUANTITATIVE ANALYSIS OF METHODOLOGICAL BIAS ASSOCIATED WITH DIFFERENT BAIT TYPES IN NEOTROPICAL BUTTERFLY RESEARCH 

2.1 Background Information 

Nectar is often viewed as the most common and widespread source of carbohydrates for butterflies (Gilbert & Singer 1975, Scoble 2002). Indeed, the majority of butterfly taxonomic groups are considered nectar-feeders, including Papilionidae, Pieridae, Lycaenidae, Riodinidae, Hesperiidae and some subfamilies of Nymphalidae. But for other groups of Nymphalidae (Satyrinae, Charaxinae and some genera of Nymphalinae), fruits or other non-floral substrates are the major food source, and together these groups comprise the guild referred to as fruit-feeding butterflies (DeVries 1988). Two collection methods have thus predominantly been used for butterflies: fruit-baited traps (typically with fermented banana) for fruit-feeding species and hand-nets for all taxonomic groups (Lamas et al. 1991, Daily & Ehrlich 1995, Robbins et al. 1996). The utilization of bait traps has been more prevalent in the tropics compared to temperate zones, and has considerably increased in recent years for ecological research, using bait-attracted butterflies as a model system to improve our understanding about theoretical and practical aspects of butterfly ecology (see Freitas et al. 2014 for a review). 

Sodium is a very scarce nutrient in adult butterfly diet (Arms et al. 1974, Boggs & Jackson 1991). In addition, even though nectar and fruits are a rich source of sugar providing fuel for flight and carbon for egg production (Fischer et al. 2004, O’Brien et al. 2005), these sources provide a limited amount of nitrogen and an incomplete or unbalanced supply of amino acids (Bosque & Pacheco 2000, Fischer et al. 2004, but see Molleman et al. 2005). Some butterfly species have therefore evolved different
strategies to cope with this scarcity of sodium and amino acids, by feeding on sap, sweat, animal excretions, mud-puddles, carrion or dung (Gilbert & Singer 1975, Alm et al. 1990, Boggs & Jackson 1991, Beck et al. 1999). Such behavior is often called ‘puddling’ (Boggs & Jackson 1991). Researchers have taken advantage of this natural attraction of butterflies to such substrates, and utilized a wide variety of baits other than fruits for trapping, including feces, urine or ‘imitation’ bird droppings (Lamas et al. 1991, Robbins et al. 1996, Montero et al. 2009). For ecological studies however, carrion (i.e., decaying prawn or fish) has been mostly employed along with fermented banana or as an alternative to it (see Checa et al. 2009; Checa et al. 2014). Using carrion baits may provide advantages since fruit and decaying fish/prawn attract different guilds of butterflies (Hall & Willmott 2000, Hamer et al. 2006, Checa et al. 2014,), and the latter may attract a more diverse butterfly community, including the so-called fruit-feeding nymphalids but also nectar-feeders (Checa et al. 2009, Holloway et al. 2013).

In spite of this general understanding, information about bait attractiveness is very scarce (Freitas et al. 2014), and very few comparative studies are available that quantitatively tested differences in performance of baits, and those that have been published were carried out in Asia (a comparison of carrion vs. banana, Holloway et al. 2013) or Africa (a comparison among different fruits, Molleman et al. 2005). To the best of my knowledge, no such study has been done in the Neotropics, where butterfly diversity reaches its peak. The relative performance of carrion/banana baits remains poorly understood, and how the use of different baits may affect or bias results is not clear. Quantitative comparisons between net-collection techniques and traps are also rare but critical, as both sampling methods are biased towards different taxonomic

An improved understanding of how different collection techniques perform might contribute to help design and standardize long-term butterfly monitoring schemes in the Neotropics, and set up guidelines for regional efforts. As biodiversity loss continues to increase, repeatable and quantitative sampling is essential to assess how diversity and abundance changes as a consequence of anthropogenic disturbances. Such methodological standardization has been widely applied in temperate areas using the butterfly transect method or Pollard Walk (Pollard 1977, Pollard & Yates 1993), and since the establishment of the butterfly monitoring scheme in the UK in 1976, monitoring has now extended to at least 10 European countries (Van Swaay et al. 2008). Nevertheless, long-term monitoring remains scarce in the Neotropics (Bonebrake et al. 2010, Grøtan et al. 2012), although there is an increasing effort to establish guidelines for regional and global efforts (see Basset et al. 2012).

The goal of this study was, therefore, to contribute towards these initiatives by comparing the performance of different sampling techniques of butterflies, namely hand-nets and traps (with carrion and fruit baits), across an environmental gradient in western Ecuador. Furthermore, I examined how the patterns of butterfly abundance, diversity and species richness at temporal (seasons/years) and spatial scales (strata/ecosystems) differ among techniques.
2.2 Methods

2.2.1 Census Techniques

Butterflies were sampled with bait-traps and nets along established transects at three study sites: wet forest (CR), transition forest (LLDFR) and dry forest (JR). Sampling using traps was performed for three consecutive years from November 2010 to September 2013. Net sampling was carried out for two consecutive years at two study sites, from July 2011 to May 2013 in the dry forest, and from May 2011 to July 2013 in the wet forest. Butterflies were not sampled with nets in the transition forest. Sampling visits were made every 2 months (i.e., six times a year) for 7 days each month. The sampling effort invested at each study site was 13,824 hour/trap (or 180 sampling days), and 252-294 hour/net in the dry and wet forests, respectively.

Van Someren-Rydon bait traps were used with two types of baits: carrion (rotting prawn fermented for 13-18 days), and fruit (banana fermented for 2 days). Traps were checked daily during the first 7 days of each sampling month from 9 am to 3 pm; traps were opened and baited on the first trapping day, with the bait renewed daily, and over the next 6 days the traps were checked. Most trapped butterflies were collected and killed by a pinch to the thorax and placed in glassine envelopes, except for some very common species that were marked and released.

2.2.2 Experimental Design

Two transects were established in each reserve, with eight sampling positions located within each transect; the distance between two neighboring positions was at least 40 m. At each position, two baited traps were set up in two different strata, understory (1.5 m) and canopy. The use of banana and prawn baits alternated between positions, and thus neighboring positions had different types of bait. Canopy and
understory traps in the same position were baited with the same type of bait. The two transects represented different microhabitats: one was set up in a top ridge and another in a valley.

Net collection was also performed at the same transects established for trapping in the wet and dry forest. After checking traps, collectors remained in the sampling position for 15 minutes collecting all butterflies flying inside a 30-m diameter circle (with the understory trap located at the center) and at a maximum height of 5 m above the ground. All collected material was examined and identified to species, with the higher classification following Wahlberg et al. (2009).

2.2.3 Statistical Analyses

Individual-based rarefaction curves assisted in comparing observed species richness among study sites and in comparing collection techniques. Rarefaction curves are produced by repeatedly re-sampling the pool of total individuals at random, plotting the mean number of species represented by each individual (Gotelli & Colwell 2004).

Additionally, the Simpson diversity index and Chao 1 were calculated for each strata, season and microhabitat across study sites. The Simpson index is among the most robust for diversity estimation (Magurran 2003), being independent of sample size (Lande et al. 2000). Both measurements were estimated with 100 randomizations and without species replacement using the software EstimateS 9.1 (Colwell 2013).

T-tests were used to compare abundance, species richness and butterfly diversity between strata, seasons and microhabitats in each study site. I also used t-tests to examine for any bias in the sex ratio of butterflies attracted by prawn and banana baits. In cases where data did not fit a normal distribution, the non-parametric
U-Mann Whitney test was applied. Kolmogorov-Smirnov tests served to test normality distribution of data. Analyses were run in R software.

2.3 Results

A total of 1534 butterflies and approximately 230 species were collected using hand-nets over two consecutive years of sampling. Higher species richness was observed in the wet forest compared to the dry forest, with 180 and 89 species being recorded, respectively, but higher abundance existed in the latter community (Table 2-1). Meanwhile, 7046 butterflies representing 214 species were collected with traps across all study sites.

Hand-net and trapping techniques sampled different taxonomic groups in terms of abundance and observed species richness. Traps mostly recorded Nymphalidae and Hesperiidae, and, to some extent, Riodinidae; whereas the hand-net technique sampled families not recorded with traps such as Papilionidae and Pieridae, and resulted in conspicuously fewer Nymphalidae. These results were consistent across ecosystems (Fig. 2-1). Moreover, the hand-net technique sampled a larger number of butterfly species in the wet and dry forest (almost three times more in the latter) compared to species solely collected with traps (Fig. 2-2). In contrast, the number of species shared between both techniques was low regardless of the ecosystem examined.

In the case of traps, more species were recorded in the wet forest and diversity continued to decrease towards the more seasonal dry forests in the south; in fact, close to three times as many species were sampled in the former compared to the latter (129 and 57, respectively) (Table 2-1). Conversely, butterfly abundance was higher in the dry forest, with 3611 individuals trapped, and progressively decreased towards the north, with only 1396 butterflies trapped within the wet forest. Few species dominated the
samples across ecosystems but dominance was more conspicuous in the dry forest. *Fountainea rypha, Hamadryas amphichloe* and *Hamadryas fornax* comprised 71% of butterflies collected in the dry forest with 2564 individuals; meanwhile, the most abundant species (*F. rypha, Hamadryas amphinome* and *Achlyodes busirus*) accounted for 45% of individuals in the transition forest, and this percent further decreased to 34% in the wet forest (where the three most common species were: *H. amphinome, Memphis cleomestra* and *Nessaea aglaura*).

Prawn baits consistently attracted more species compared to banana across study sites, although banana-baited traps were more likely to attract a higher number of individuals (Fig. 2-3). For example, in the transition forest, 72 species were recorded in prawn-baited traps compared to 47 recorded with banana. On the other hand, almost three times more individuals were sampled in the dry forest compared to the wet forest. Furthermore, nearly twice as many species were attracted exclusively to prawn in comparison with banana, and this pattern was consistent across ecosystems (Fig. 2-2). In contrast, the number of species shared between both baits varied, with a higher proportion found in the dry forest. Hence, using just prawn as bait in all locations resulted in the collection of at least 75% of species recorded using trapping techniques.

Prawn baits were more efficient at attracting males than females in the transition forest, 1056 and 43 respectively, and this difference was highly significant (U Mann Whitney, U= 64, p=0.00001). Meanwhile, there were no significant differences between the number of females and males trapped with banana baits at the same study site (t= -2.09, p= 0.06) (Fig. 2-4). Although, quantitative comparisons are not available for the
wet and dry forest communities, a similar pattern of a higher number of males attracted to prawn but not banana were detected.

The taxonomic groups sampled varied depending on the type of bait. Riodinidae, and to a lesser extent Hesperiidae, were exclusively collected with prawn in the wet forest and transition forest, whereas in the dry forest these families showed no preference for one bait. In the case of Nymphalidae, both baits collected similar numbers of butterfly species in the dry and transition forest, but in the wet forest a higher species richness was recorded with banana, due to the higher number of satyrines collected with this bait compared to prawn (27 and 14, respectively).

When sample size was standardized through rarefaction curves, butterfly species richness was higher in the wet forest and decreased towards the dry forest in the south, regardless of the collection technique or bait used (Fig. 2-5). The curves also revealed more species were likely to be included in the sample from the wet forest with more sampling effort.

Collection techniques employing prawn or banana as baits provided similar vertical stratification patterns in terms of butterfly abundance over all study sites, but rather inconsistent results for species richness and diversity. Regardless of bait type used, butterfly abundance in the canopy and understory did not significantly vary in the transition forest and dry forest, whereas in wet forest the canopy had approximately twice as many butterflies as the understory (prawn: U=64, p=0.003; banana: U= 59, p= 0.0001) (Table 2-2). In terms of species richness and butterfly diversity, however, data gathered with different baits are concordant or contradictory depending on the ecosystem analyzed. For seasonal forests (i.e., transition and dry forest), both bait
types showed non-significant differences for estimated species richness between canopy and understory. However, in the wet forest, results from baits differed, with no significant difference between canopy and understory diversity being found for prawn feeders ($t = -1.57, p = 0.148$), whereas for banana feeders, almost twice as many species were estimated to occur in the canopy compared to the understory, with this difference in results being significant ($U = 55, P = 0.015$) (Table 2-2).

At the microhabitat scale (ridge vs. valley), in the transition forest, both baits found no significant difference in spatial patterns of abundance and butterfly diversity (Table 2-3). However, the estimated species richness of the valley was almost exactly twice that of the ridge ($U = 16, p = 0.029$) for prawn baits, whereas banana baits detected no significant difference among microhabitats.

Further inconsistent patterns were recovered for the two baits in terms of temporal patterns of abundance, estimated species richness and butterfly diversity across ecosystems. For most analyses, samples obtained by different baits produced dissimilar results. For example, butterfly species richness was significantly higher during the wet season compared to the dry season in both the transition and dry forest in samples collected with prawn, whereas no such differences were detected when using banana as bait (Table 2-2).

Butterfly populations showed inter- and intra-annual temporal variation across study sites, with the two seasonal forests ($i.e.,$ transition and dry forests) being more similar to one another regardless of bait type (Fig. 2-6, 2-7). Both baits resulted in a general pattern of highest numbers of species and individuals during the rainy season, but peaks were more conspicuous for prawn-attracted butterfly communities. In the
transition forest for instance, almost twice as many species and individuals were sampled with prawn in May 2012 compared to banana baits (species richness 39 and 21, respectively), in a similar way, one third more butterfly numbers were recorded during Mar 2013 using prawn baits.

In terms of intra-annual variation in butterfly communities, less concordant results were found in the wet forest for prawn and banana samples, with peaks and lows not occurring simultaneously. Two peaks in species richness occurred with prawn samples during Mar and Sep 2013 that were absent for banana-attracted communities. In addition, abundance showed a greater variation in 2012 with three peaks, one at the onset of the rainy season (Jan) and the others through the dry season (Jul and Nov) for banana samples, but not for prawn-attracted communities, which peaked mainly in Mar and to a lesser extent in Sep. Interestingly, these differences are likely reflecting changes in bait preferences by the same species group. When plotting abundances of individual species that were attracted to both baits, some species such as *Memphis cleomestra* appeared to change bait preference from one sampling period to the next, particularly through 2012 (Fig. 2-8).

Pooled data from both banana and prawn baits showed clear peaks in abundance through the rainy season across years and study sites (Fig. 2-9, 2-10). Hand-net and trapping showed similar patterns of butterfly abundance within the wet forest, but not in the dry forest. In the latter, butterfly communities sampled with nets peaked at the beginning of the dry season in Jul and Sep over the 3-year period due to most abundant taxonomic groups that were exclusively collected with nets peaking during these months (*i.e.*, Pieridae and Riodinidae).
Net-collected butterfly communities also showed intra- and inter-annual variation across study sites in a similar manner to trapped butterflies. The highest species richness and abundance were recorded in 2012 in the dry forest but during 2013 within the wet forest, a result obtained regardless of the collection technique employed at either site (Fig. 2-9, 2-10).

2.4 Discussion

In this study, different methods resulted in the same conclusions for some questions but not for others. These results suggest that great care must be taken in planning butterfly monitoring studies if data are to be comparable, and that making general inferences from data collected using only a single method needs to be justified. As far as I am aware, this study represents the first attempt to quantitatively compare the performance of different sampling techniques of Neotropical butterflies. Butterfly collection employing hand-nets exclusively sampled a larger number of butterfly species in the wet and dry forest (almost three times more in the latter) compared to species solely collected with traps. In terms of bait traps, prawn attracted a significantly higher number of species from a wider range of taxonomic groups compared to fruit. At regional scales, hand-nets and baited-traps showed concordant results for species richness patterns, namely a decrease from wet forests towards dry forests. However, different methods could result in either similar or different conclusions for temporal (e.g., seasons) and spatial (e.g., strata and microhabitat) analyses at local scales. Furthermore, there was a tendency for data on abundance, diversity and seasonality patterns to be more consistent within seasonal forests regardless of collection technique or bait employed.
Nectar and fruits are major carbohydrate sources for butterflies, but these substrates may provide an inadequate supply of amino acids and salts (Bosque & Pacheco 2000, Fischer et al. 2004). Butterflies therefore seek these limiting nutrients in alternative food sources such as dung and carrion (Boggs & Jackson 1991, Beck et al. 1999) but in addition, carrion is a major food source rather than merely an alternative for many butterfly taxa including the Riodinidae, Nymphalidae and Lycaenidae (Hall & Willmott 2000). This might partially explain the large number of individuals attracted to prawn compared to banana found here. It is important to emphasize that the results of the present study were based on a 3-year dataset, whereas analyses based solely on a 1-year dataset in the LLDFR transition forest resulted in different conclusions, with banana attracting far more species and individuals compared to prawn (Rodriguez 2013), a result further emphasizing the importance of multi-year butterfly monitoring.

Females and males presumably use different strategies to meet their nutrient budget given the differential attraction of both sexes to prawn; the prawn was significantly more attractive to males than females, a result previously found in other studies focused on riodinids (Hall & Willmott 2000). Butterflies can actively seek and prefer flowers with high concentration of amino acids (see Baker & Baker 1986, Lewis 1989, Mevi-Schütz & Erhardt 2002); indeed, flowers visited by butterflies have higher concentrations of amino acids compared to flowers pollinated by other insects (Baker & Baker 1983, Baker & Baker 1986, Alm et al. 1990). This is particularly true for females, which are able to detect and prefer nectar sources with higher amino acid content compared to males (Erhardt & Rusterholz 1998, Rusterholz & Erhardt 2000, Mevi-Schütz & Erhardt 2002, Bauerfeind & Fischer 2009). In experiments with *Pieris rapae,*
males were not able to discriminate between sugar-only nectars and sugar-amino acid nectars (Alm et al. 1990). As a response to this trait, males may more frequently seek limited nutrients in additional sources such as carrion or mud; indeed, the puddling behavior is predominantly found in males, which consists mainly in absorbing dissolved salts and minerals from mud (Arms et al. 1974, Hall & Willmott 2000). But not all taxa displaying puddling behavior, such as Papilionidae and Pieridae (Arms et al. 1974), visit prawn-baited traps, suggesting other factors come into play.

Males may also visit prawn more frequently than females to gather nutrients to provide to females in the spermatophore. Males can significantly contribute nutrients to females through nuptial gifts transferred during mating (Boggs & Gilbert 1979, Oberhauser 1997), which enhance male mating success and reproductive success in females (Boggs, 1990). This behavior demands a high energy and resource input from males, necessitating foraging at alternative nutrient sources such as carrion, dung, mud-puddles and others (Boggs & Jackson 1991, Mevi-Schütz & Erhardt 2002). This additional requirement and their limited ability to find amino acids compared to females might explain why prawn attracted both more males and species from a wider range of taxonomic groups compared to banana baits. Since older females of some species do not mate again, and their reserves of sodium or other nutrients can become depleted through time, they might also display puddling behavior (Boggs & Jackson 1991) or visit carrion sources, hence explaining why prawn also attracted some females, although in lower frequency compared to banana, across study sites.

Furthermore, the variation in relative attractiveness of baits among taxa might reflect the differential needs of species as adults to meet nutrient budgets. Butterflies
are generally considered income breeders (Wheeler 1996) with egg production also relying on consumed resources during adult stage, in contrast to capital breeders, species depending only on larval resources to sustain fecundity (Tammaru & Haukioja 1996). The tropical pollen-feeding Heliconius is the best example of an income breeder as most of the amino acids required for eggs come from pollen acquired by adults (Gilbert 1972). In other genera, most proteins come from larval food sources and carbohydrates derived from adult feeding (Wheeler 1996). Therefore, larval diet can greatly influence the use and need of adult resources for key aspects such as reproduction and survival (Bauerfeind & Fischer 2009). Indeed for riodinids, carrion feeders tend to have large thoraces and low abdominal volumes compared to species not recorded on this food source, limiting their nutrient storage during larval feeding to sustain reproduction and rapid flight as adults, and thus necessitating adult feeding on carrion to boost nutrient reserves (Hall & Willmott 2000).

The results of the present study suggest that choosing the most suitable collection technique for butterfly research depends on the study goal (e.g., maximization of sampled diversity, taxonomic group to be studied, ecological studies, monitoring), and available resources (e.g, number/experience of collectors, time and funding available).

Collection with hand-nets within transects allows the sampling of a more varied and ecologically diverse butterfly community (Caldas & Robbins 2003). Indeed, species sampled utilizing this technique comprised 63-80% of total species collected within the wet and dry forest, respectively. On the other hand, in terms of bait traps, prawn resulted in the sampling of 75% of all species caught by traps in the wet forest, and this
percent progressively increased towards the south where diversity decreased, comprising 79% in the transition forest, and 88% in the dry forest.

Furthermore, each collection technique and bait were better at sampling certain taxonomic groups, hence the utilization of traps with different baits and hand-nets greatly complemented each other in terms of maximizing sampled species diversity. Hand-nets were useful to collect butterflies feeding mainly on nectar such as Danainae, Pieridae, Papilionidae and Lycaenidae, and certain groups of Riodinidae and Hesperiidae. Trapping was more suited for most nymphalids (Biblidinae, Charaxinae, Heliconiinae, Limenitidinae, Nymphalinae and Satyrinae), and, more specifically, banana baits were particularly attractive for the tribe Satyrini (subfamily Satyrinae), whereas prawn was more effective for attracting Hesperiidae and Riodinidae.

In addition to the performance of each technique in terms of diversity and taxonomic groups sampled, other specific advantages or disadvantages should be considered when choosing an appropriate technique. Trapping has a number of associated advantages. It allows standardization in sampling efforts permitting accurate comparison among samples in space and time (DeVries et al. 1999). Standardization may also be achieved by collecting with hand-nets whenever collectors have similar levels of experience, but this makes hand-netting less accessible to novel researchers in the field of standardized long-term monitoring projects. Another potential disadvantage of hand-netting is that it might be challenging to maintain the same collectors over a long time period, and the changing of personnel over time could cause bias in results.
Perhaps most importantly, trapping also allows the collecting of butterfly species inhabiting the forest canopy, since this stratum is easily accessible through the establishment of traps at different heights in trees. Trapping might be less resource-intensive in terms of collectors needed and associated logistical costs, as one person can check a large number of traps per day (e.g., 50) distributed over different habitats and strata (see Checa et al. 2009). Traps can also continuously attract butterflies throughout the sampling period despite weather conditions. Additionally, minimal training is required to manage trapping including checking traps, preparing baits and identification of the most common species to mark and release, which in turn increases the possibility of training local people to conduct butterfly research, a task carried out in the present study in order to implement a feasibility analysis for butterfly farming in LLDFR (see Checa 2015). This capacity-building opportunity in monitoring schemes is still rarely implemented despite the obvious potential advantages of both cost savings and local communities involvement in scientific research. Involving local people living close to sample sites and training them as field workers should make logistical costs lower and conservation efforts more effective (Sekercioglu 2011).

Nevertheless, the bait-trap technique has some clear disadvantages related to lower butterfly diversity sampled in particular with respect to fruit baits, and still unclear aspects of bait performance at the species level. The trapping technique is biased due to variance among species in attraction to baits (Muirhead-Thomson 1991), which can also vary over seasons and microhabitats (Rodríguez 2015) as found here, with some species such as *Memphis cleomestra* distinctly preferring either banana or prawn on alternate months. This variation is likely due to the availability of naturally occurring
fruits and carrion influencing the relative attractiveness of baits for butterflies (Caldas & Robbins 2003). These aspects therefore affect the detection probability of species, which is also a challenge and potential bias while using hand-nets (Nowicki 2008). The relative attraction of species to baits can only be further understood with intensive mark-recapture studies or observation of diet preference in species (DeVries et al. 1999), and further research into this topic would prove very useful.

Collection technique had an effect on analyses in both temporal (e.g., seasons) and spatial (e.g., strata and microhabitat) dimensions at local scales, thus suggesting caution is needed in interpreting results whenever just one technique is used. This finding was particularly true for aseasonal forests, likely owing to their higher diversity compared to seasonal dry forests, as a tendency was found for higher consistency in butterfly seasonality patterns within seasonal forests regardless of collection technique or bait employed. Combining net and bait-trap methodologies not only maximizes species sampled but can also be very useful because the bias associated with each of these methods is different (Caldas & Robbins 2003) and different methods can therefore provide complementary abundance data (Sparrow et al. 1994). While budget and other factors might pose constraints in the utilization of multiple collection techniques for standardized butterfly monitoring, the use of both prawn and banana baits is recommended in order to correct for observed bias in attractiveness of different baits on species over space and time.

The results presented here will hopefully contribute towards developing efficient but effective standard methods for long-term butterfly monitoring schemes in the tropics. These schemes have provided influential data about the effects of environmental
change and highlighted priorities for conservation in Europe (Isaac et al. 2011) and could do so in the Neotropics, where biodiversity is overwhelmingly concentrated and faces serious threats from high levels of deforestation and associated extinction risks (Sodhi & Ehrlich 2010).
Table 2-1. Butterfly abundance and observed species richness determined using baited traps and hand-nets as collection techniques in wet, transition and dry forests from Western Ecuador. Only trapping technique was utilized in the transition forest.

<table>
<thead>
<tr>
<th></th>
<th>Traps</th>
<th></th>
<th>Nets</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abundance</td>
<td>Species richness</td>
<td>Abundance</td>
<td>Species richness</td>
</tr>
<tr>
<td>Wet forest</td>
<td>1396</td>
<td>129</td>
<td>652</td>
<td>139</td>
</tr>
<tr>
<td>Transition</td>
<td>2039</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry forest</td>
<td>3611</td>
<td>57</td>
<td>882</td>
<td>89</td>
</tr>
<tr>
<td>Total</td>
<td>7046</td>
<td>214</td>
<td>1534</td>
<td>210</td>
</tr>
</tbody>
</table>

Table 2-2. Abundance, estimated species richness (Chao 1 estimator) and diversity (Simpson index) of butterfly communities occurring at different spatial (strata) and temporal scales (seasons) in Western Ecuador: Canande Reserve (wet forest), Lalo Loor Dry Forest Reserve (Transition Forest) and Jorupe Reserve (dry forest). Results are separated according to type of bait, prawn or banana, used to sample butterflies. Asterisks shown represent significant (*) or highly significant differences (**) were found between strata or seasons according to U-Mann Whitney or $t$ tests.

<table>
<thead>
<tr>
<th></th>
<th>Wet forest</th>
<th>Transition</th>
<th>Dry forest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prawn</td>
<td>Banana</td>
<td>Prawn</td>
</tr>
<tr>
<td>Abundance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td>420**</td>
<td>555**</td>
<td>577ns</td>
</tr>
<tr>
<td>Understory</td>
<td>179**</td>
<td>242**</td>
<td>462 ns</td>
</tr>
<tr>
<td></td>
<td>103 ns</td>
<td>40*</td>
<td>58.31 ns</td>
</tr>
<tr>
<td>Canopy</td>
<td>127 ns</td>
<td>89*</td>
<td>76.05 ns</td>
</tr>
<tr>
<td>Understory</td>
<td>103 ns</td>
<td>40*</td>
<td>58.31 ns</td>
</tr>
<tr>
<td>Simpson Canopy</td>
<td>13.85 ns</td>
<td>10.58 ns</td>
<td>8.79 ns</td>
</tr>
<tr>
<td>Simpson Understory</td>
<td>13.45 ns</td>
<td>10.54 ns</td>
<td>5.49 ns</td>
</tr>
<tr>
<td>Chao 1</td>
<td>220**</td>
<td>340 ns</td>
<td>117**</td>
</tr>
<tr>
<td>Abundance Dry season</td>
<td>379**</td>
<td>457 ns</td>
<td>922**</td>
</tr>
<tr>
<td>Chao 1</td>
<td>106 ns</td>
<td>80.03 ns</td>
<td>51.96**</td>
</tr>
<tr>
<td>Abundance Wet season</td>
<td>128 ns</td>
<td>80.33 ns</td>
<td>74.74**</td>
</tr>
<tr>
<td>Simpson Canopy Dry season</td>
<td>17.08*</td>
<td>12.36 ns</td>
<td>9.31 ns</td>
</tr>
<tr>
<td>Simpson Canopy Wet season</td>
<td>17.07*</td>
<td>16.32 ns</td>
<td>9.05 ns</td>
</tr>
</tbody>
</table>
Table 2-3. Abundance, estimated species richness (Chao 1 estimator) and diversity (Simpson index) of butterfly communities occurring in the topridge and the valley at the Lalo Loor Dry Forest Reserve (Transition Forest) in Western Ecuador. Results are separated according to type of bait, prawn or banana, used to sample butterflies. Asterisks shown represent significant differences (*) were found between microhabitats according to the U-Mann Whitney or t tests.

<table>
<thead>
<tr>
<th></th>
<th>Prawn</th>
<th>Banana</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abundance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valley</td>
<td>659&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>260&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Top ridge</td>
<td>380&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>740&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Chao 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valley</td>
<td>87.96*</td>
<td>136.6&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Top ridge</td>
<td>43.98*</td>
<td>54.31&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Simpson</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valley</td>
<td>9.28&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Top ridge</td>
<td>6.17&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>6.27&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>WET FOREST</td>
<td>Trap: Banana</td>
<td>Trap: Prawn</td>
</tr>
<tr>
<td>------------</td>
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<td>-------------</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>Nymphalidae</td>
<td>796</td>
<td>513</td>
</tr>
<tr>
<td>Biblidinae</td>
<td>252</td>
<td>162</td>
</tr>
<tr>
<td>Charaxinae</td>
<td>145</td>
<td>225</td>
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<tr>
<td>Danainae</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Heliconiinae</td>
<td>0</td>
<td>13</td>
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<td>Limenitidinae</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>Nymphalinae</td>
<td>142</td>
<td>11</td>
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<tr>
<td>Satyrinae</td>
<td>248</td>
<td>71</td>
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<tr>
<td>Hesperiidae</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>Lycaenidae</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pieridae</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Riodinidae</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>797</td>
<td>599</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>DRY FOREST</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Nymphalidae</td>
</tr>
<tr>
<td>Apaturinae</td>
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Figure 2-1: Number of species (S) and individuals (N) collected with hand-nets and traps (using banana and prawn as baits) across study sites: wet, transition and dry forests. Only trapping was carried out in the transition forest. Subfamilies of Nymphalidae are also shown (i.e., Biblidinae, Charaxinae, Danainae, Heliconiinae, Limenitidinae, Nymphalinae, Satyrinae), and values included in the Nymphalidae rows correspond to total values of N and S.
Figure 2-2: Number of species recorded using different collection techniques. A) Species exclusively collected with hand-nets or traps, and those sampled with both techniques (shared). B) Number of species exclusively collected with banana/prawn, or collected with both baits (shared) across ecosystems in Western Ecuador.
Figure 2-3: Specimens collected using prawn or banana in Canande Reserve (wet forest), Lalo Loor Dry Forest Reserve (transition forest) and Jorupe Reserve (dry forest). Bars represent standard deviations. A) Butterfly abundance recorded across ecosystems. B) Species richness registered.
Figure 2-4: Abundance of females and males collected with prawn or banana at the Lalo Loor Dry Forest Reserve (LLDFR). Standard deviations represented with bars.

Figure 2-5: Individual-based rarefaction curves of butterfly communities sampled with different collection techniques in wet, dry and transition forests from Western Ecuador. A) Butterflies sampled with hand-nets B) Specimens collected with prawn baited-traps. C) Results from banana-baited traps.
Figure 2-5. Continued.
Figure 2-6: Fluctuation patterns of abundance of butterfly communities across study sites. A) Wet forests. B) Transition forests. C) Dry forest.
Figure 2-5. Continued.

Figure 2-7: Fluctuation patterns of observed species richness of butterfly communities across study sites. A) Wet forests. B) Transition forests. C) Dry forest.
Figure 2-7. Continued.
Figure 2-8. Abundances of species sampled by both prawn and banana baits throughout the study period in the CRR wet forest. This plot excluded species exclusively sampled by either prawn or banana.

Figure 2-9. Fluctuation patterns of abundance of butterfly communities across study sites. A) Wet forest. B) Dry forest.
Figure 2-9. Continued.

Figure 2-10: Fluctuation patterns of observed species richness of butterfly communities across study sites. Each line represents different collection techniques, hand-nets or bait traps, used to sample butterflies. A) Wet forest. B) Dry forest.
Figure 2-10: Continued.
CHAPTER 3
RAINFALL AS A SIGNIFICANT CONTRIBUTING FACTOR TO BUTTERFLY SEASONALITY ALONG A CLIMATIC GRADIENT IN THE NEOTROPICS

3.1 Background Information

The mechanisms driving higher biodiversity towards the equator have puzzled ecologists for decades. Recently, studies into insects have contributed to our understanding of these mechanisms by focusing on host-specificity in insect herbivores and spatial turnover (Dyer et al. 2007, Novotny et al. 2007). A further, little explored contributing factor might be the temporal partitioning of resources, thereby decreasing competition and promoting species coexistence (Grimbacher et al. 2009). For most insects a ‘temporal window’ exists when environmental conditions are most favorable for certain stages of their life cycle; synchronization of critical stages of the life cycle with this temporal window is thus expected to prevent fitness consequences (van Asch & Visser 2007). For example, numerous herbivores use specific plant resources during short periods of time, when the quality of these resources is optimal (Hellmann 2002), and as a consequence a peak in insect herbivore abundance is expected to coincide with leaf flush (Murakami et al. 2008, Srygley et al. 2010) or other resource availability (e.g., rotten fruit, flowers) (Hamer et al. 2006, Checa et al. 2009). Competition for such resources could therefore drive temporal niche partitioning.

The population dynamics of insects in the form of seasonality patterns is one potential mechanism by which species might partition resources. For decades, it has been known that some tropical insects have marked seasonal changes in their relative abundance with climate as a key factor controlling their population dynamics (Wolda 1988, see review Kishimoto-Yamada & Itioka 2015). A pattern repeatedly found in the majority of studies of the temporal dynamics of tropical insects is an abundance peak
during the wet season (Wolda 1978, Novotny & Basset 1998, Grimbacher et al. 2009 and citations therein), whereas other studies have found abundance to be higher during the dry season (see Valtonen et al. 2013). In addition, opposing patterns might occur even at local scales. For example, in one study, the spatiotemporal dynamics of insects differed in moist/shaded sites versus drier sites (Janzen 1973, Richards & Windsor 2007), with insect abundance reaching a peak in the wet season in the former, but peaking in the dry season in the latter. Butterfly data apparently support these results at regional scales. In Neotropical dry forests, butterfly abundance peaks during the months with highest precipitation and relative humidity in Mexico (Pozo et al. 2008, Torres et al. 2009), Venezuela (Shahabuddin et al. 1999), and western Ecuador with relative humidity, but not temperature as a significant associated factor with this temporal abundance variation (Checa 2010, Checa et al. 2014). In contrast, butterfly communities of relatively aseasonal or rainforests in the Neotropics may show decreased species richness and abundance during the wet season but peaks through the transitional months (see DeVries & Walla 2001, Checa et al. 2009, Grøtan et al. 2012, Valtonen et al. 2013, Grøtan et al. 2014) or warmest part of the year (Ribeiro et al. 2010). Conversely, annual fluctuation of butterfly numbers are significantly and solely correlated with temperature within these ecosystems (Checa et al. 2009, Ribeiro et al. 2010, Grøtan et al. 2015). It is thus possible that temporal patterns of insect abundance and their associated climatic factors diverge across forests with varying seasonality. Further research is therefore needed to better understand the role of climate on insect communities from different biome types. The role of environmental factors influencing insect seasonality in the tropics is still poorly understood due to the challenges involved

Improved understanding of insect seasonality and underlying factors is valuable for identifying how insects will respond or adapt to climate change (Valtonen et al. 2013). Climate change will increase rainfall seasonality, which might increase the length or severity of the dry season, and drought incidence, as a consequence of El Niño–Southern Oscillation (ENSO) (Walsh & Ryan 2000). Hence, studies that analyze seasonality and species richness patterns in Western Ecuador, an area directly affected by ENSO could provide more insights about possible effects of seasonality changes of rainfall in the biota. This information might be in turn useful to determine the susceptibility to climate variability of butterfly species displaying different traits. Body size, population density and mobility contribute to extinction vulnerability of taxa (Brown 1971, Kattan et al. 1994, Shahabuddin & Ponte 2005, Graves & Gotelli 1983). Butterfly seasonality, however, might be an additional important and hitherto unstudied contributing factor as more seasonal species can have a more restricted ecological niche, which in turn might make them more vulnerable to climate and habitat change.

Western Ecuador offers an ideal opportunity to examine how the dynamics of multi-species butterfly communities from seasonal and aseasonal forests are related to climate, because it presents a marked gradient of life zones, with wet forests dominating in the north, which gradually change to moist forest and dry forest in the south (Dodson & Gentry 1991). Here, I analyze the dynamics of multi-species butterfly communities along a climatic gradient with varying precipitation regimes for three consecutive years,
and determine how climatic variables associate with observed butterfly seasonality. To provide a baseline for future studies of how climate change might affect these butterfly populations, I additionally explored the role of butterfly seasonality as a potential contributing factor for their susceptibility to climate variation.

3.2 Methods

3.2.1 Census Techniques

Butterflies were sampled using baited-traps at three study sites: Canande River Reserve (CR, wet forest), Lalo Loor Dry Forest (LLDFR, transition forest) and Jorupe Reserve (JR, dry forest). Sampling was performed every two months (i.e., 6 times a year) over 7 days each sampling period for three consecutive years from November 2010 to September 2013.

Van Someren-Rydon bait traps were used with two different types of baits: rotting shrimp fermented for 13-18 days, and banana fermented for 2 days. Traps were checked daily during the first 7 days of the sampling month from 9 am to 3 pm; traps were opened and baited on the first trapping day, and checked over the next 6 days with trapped butterflies being collected, except for the most abundant species, which were marked and released.

Two transects were established at each reserve with each transect containing eight sampling positions, with a minimum distance between sampling positions of 40 m. Two baited traps were set up at each sampling position in two different strata, understory (1.5 meters above the ground) and canopy (10-25 m depending on the ecosystem sampled). The use of banana and shrimp baits alternated between positions, thus neighboring positions had different types of bait. Canopy and understory traps in
the same position were baited with the same type of bait. The order at which sampling positions were visited was modified every sampling day in order to correct any bias produced by both daily temporal (Wolda 1988, Sutcliffe et al. 1996) and spatial distribution of butterflies at each sampling point.

Data loggers were deployed at each reserve to register daily rainfall, temperature and relative humidity, and climatic data for the entire sampling period (i.e., from Nov 2010 to Sept 2013) was thus obtained for subsequent analyses.

All collected material was examined and identified to species, and classified following the higher taxonomic classification of Wahlberg et al. (2009). Moreover, butterfly species were further classified as wet or dry species. Due to limited published data about geographic distribution of most species included in the present study, a qualitative approach was used for this classification. Butterfly species recorded at CRR (wet forest) but not at JR (dry forest) were considered as wet species, whereas species registered at JR but not at CRR were classified as dry species. All collected specimens were deposited in the Museum of Invertebrates of the Pontificia Universidad Católica del Ecuador (QCAZ), and the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History at the University of Florida (MGCL).

3.2.2 Statistical Analyses

Generalized linear models were used to test whether changes in both butterfly species richness and abundance were associated with local climatic variables (i.e., rainfall, temperature and relative humidity) across study sites. Generalized linear models have applications in circumstances where the assumptions of the standard linear model do not hold, as in the case where the distribution of the data is not normal (Littell et al. 1996) or when data are autocorrelated in terms of space or time. Hence,
generalized linear models permit analyses of response variables such as counts, binary, proportions and positive valued continuous distributions (Hilbe 1994, Hoffman 2004). Typically, count data do not follow a normal distribution, but a Poisson or a negative binomial. Key assumptions of GLN are homogeneity, normality and independence of residuals (Dobson 2002, Hoffman 2004).

Two different models were performed to analyze each dependent variable, butterfly species richness and abundance per study site. In order to perform these analyses, data from different strata and transects collected during a single month were pooled together. Each point in the analyses thus consisted of the number of butterflies collected in a given month, regardless of collection point (i.e., strata, sampling position and transect), and the associated climatic variables registered for that specific month. Best fit models were selected using their values of the Akaike’s Information Criterion (AIC) and residual variance. Scatterplots of the residuals and fitted values were checked to examine whether the model met the assumption of homogeneity, in addition to normal probability plots of residuals to verify normal distribution of residuals (see Lindsay 1997, Dobson 2002). The negative binomial distribution fitted the data better than a Poisson or normal distribution, and the best models included total rainfall and mean temperature. Therefore, further interpretation is based on the outputs of these models.

Secondly, I investigated whether butterflies that were more seasonal, showing more restricted emergence peaks, were associated with any particular climatic variables, in the JR dry forest. As a measure of seasonality I used the coefficient of variation (CV) of abundance, calculated by dividing the standard deviation of log-
transformed species abundance in each of the 18 consecutive months over the average log-transformed abundance of the 18 months. Abundance data were log-transformed to correct bias induced by species abundance. The CV constitutes a useful measure of seasonality or temporal heterogeneity of species distributions (see Hanya et al. 2011, Tello & Stevens 2010), and rather than reflecting seasonality patterns (i.e., timing or length of species occurrence), it measures the evenness of the temporal distribution of species over sampling time. Higher values indicate broader variability of species occurrence whereas low values reveal a more even distribution. To measure the climatic niche when species were active as adults, the climatic variables of the months when species were recorded were selected and averaged. Climatic variables included total monthly rainfall, maximum and minimum values of relative humidity and temperature, and standard deviation of both temperature and relative humidity. The climatic niche measured corresponds to the realized climatic niche and not the fundamental niche (see Schweiger et al. 2014). Finally, I used General Linear Models to test whether climate (explanatory variables) explained seasonality (response variable) across butterfly species. The GLM excluded singletons and doubletons to improve fit. Best fit models were based on the Gaussian distribution, and included total monthly rainfall, and the standard deviation of both relative humidity and temperature.

3.3 Results

In total, 7046 individuals of 212 species were sampled over 180 sampling days, representing the families Nymphalidae (Apaturinae, Biblidinae, Charaxinae, Cyrestinae, Danainae, Heliconiinae, Limenitidinae, Nymphalinae and Satyrinae), Hesperiidae, Riodinidae and Lycaenidae. Observed species richness was two-fold higher in the wet forest (129) compared to the dry forest (57), even though nearly three times greater
butterfly abundance was recorded in the latter (3611). The transition forest showed intermediate numbers of species richness (91) and abundance (2039). Diversity and evenness of species abundance therefore decreased from wet forests towards dry forests in the south.

The climate of the study sites was distinctly seasonal (Fig. 3-1). Most of the rainfall occurred during the wet season from January to May, but was nearly absent in the transition and dry forest after August. The wet forest received considerably more precipitation throughout the study duration with annual cumulative values constantly exceeding 3,000 mm\(^3\), a two- and three-fold increase compared with the annual precipitation in the transition and dry forests, respectively. Furthermore, cumulative rain achieved highest values in 2012 ranging from 3886 mm\(^3\) in the wet forest to 1203 mm\(^3\) in the dry forest. Mean monthly temperature fluctuated by as much as 2-3\(^\circ\)C from the warmest to the coldest months across years and reserves, although temperature varied more and reached higher maximum values in LLDFR (transition) and JR (dry forest) (Fig. 3-1).

Butterfly communities showed conspicuous intra- and inter-annual variation in temporal dynamics with important similarities between the seasonality patterns of seasonal forests (i.e., both transition and dry forests) compared to aseasonal forests (i.e., wet forest) (Fig. 3-2). Furthermore, the greatest species richness and abundance were recorded during the wettest part of the year across all study sites. In both wet and transition forests, butterfly numbers reached a peak in March for both 2011 and 2013, and in May of 2012 (Fig. 3-2). In contrast, the highest numbers of species and individuals were recorded in January (2011 and 2013) and March (2012) in the dry
forest. A second, weaker peak occurred in July and/or September across all study sites over all years. These temporal patterns remained similar for remaining species when extracting most abundant species (>100 individuals) from plots (Fig. 3-3).

In general, both intra- and inter-annual variation in butterfly abundance was correlated with rainfall in seasonal forests but not in the aseasonal forest. For example, butterfly communities from the southern seasonal forests in Manabi and Loja showed highest numbers of species and individuals in 2012, coinciding with the wettest year covered by the sampling period (cumulative rain per year equaled 1763 mm³ and 1203 mm³, respectively). Similarly, rain was considerably higher during 2012 in the wet forest (3886.43 mm³), nevertheless the most conspicuous butterfly peak occurred one year later (i.e., nearly three times more species and individuals sampled compared to previous years). In addition, this pattern remained even when splitting the butterfly community into wet forest and dry forest species (Fig. 3-4). Peak abundance of dry forest species was synchronized in both seasonal forests of Manabi and Loja through 2012, matching the year with highest water availability in these ecosystems (Fig. 3-1). On the contrary, the abundance of wet-forest species reached a peak during the wettest year in the Manabi transition forest, whereas this peak showed a 1-year time lag after the wettest year in the Canande wet forest (Fig. 3-4).

For some of the most common species, occurring in all three sites, the highest number of individuals occurred at the same time of year across all sites and years (Fig. 3-5). For example, the highest peak in abundance of *H. amphinome* coincided across ecosystems through the 2012 wet season but extended towards the driest part of the year in the wet forest. Conversely, *F. ryphea* exhibited the same highest peak across
years only in the seasonal forests, peaking through the 2012 wet season, but showed a 1-year time lag in the wet forest. For other species, there was not an obvious pattern; the population abundance of *S. blomfildia* peaked distinctively across study sites (Fig. 3-5).

Generalized linear models revealed that rainfall was significantly related to monthly butterfly abundance over all ecosystems, and to monthly species richness only in the wet forest (Table 3-1). Furthermore, rainfall coefficients were positive, indicating increased cumulative monthly rain is associated with higher butterfly numbers. Temperate was not a significant predictor in all models, except when species richness was analyzed for the wet and transition forest (estimate = -0.08, p <0.05, estimate = -0.34, p <0.05, respectively), and in contrast to rainfall, the temperature coefficient was negative indicating that warmest months had lower species richness.

Similarly, total monthly rainfall, but not temperature, was significantly negatively associated with butterfly species seasonality in the dry forest (Estimate = -0.13, p=0.00) (Table 3-2). These results suggest butterfly species with stronger seasonality as measured by the CV of their relative monthly abundance were significantly more constrained by rainfall variation. Among more seasonal species is *Ithomia cleora* (CV = 3.65), a range-restricted species confined to western Ecuador and northwest Peru, mostly sampled in May 2012 (6 out of 7 individuals).

### 3.4 Discussion

Butterfly communities exhibited conspicuous intra and inter-annual variation in temporal dynamics with certain elements (*e.g.*, maximum abundance recorded) of seasonality patterns likely synchronized in seasonal forests across years but not in aseasonal forests. In addition, the highest numbers of species and individuals occurred
during the wet season across all study sites and years; indeed, rainfall was significantly positively associated with temporal abundance. Likewise, butterfly species displaying stronger seasonality were significantly associated with higher rainfall periods in seasonal forests. As far as I know, this represents the first study that simultaneously sampled and described seasonality patterns of tropical butterfly communities across ecosystems with varying climatic seasonality, including data emphasizing variation in precipitation regimes might significantly affect more seasonal species.

The pattern of butterfly abundance peaking through the wet season found here is concordant with the majority of studies into temporal dynamics of tropical insects (Wolda 1978, Novotny & Basset 1998, Grimbacher et al. 2009, for a review Kishimoto-Yamada & Itioka 2015), and particularly with studies focused on butterfly communities distributed in Neotropical dry forests (Shahabuddin et al. 1999, Pozo et al. 2008, Torres et al. 2009, Checa et al. 2010, Checa 2014). Precipitation, but not temperature, was significantly associated with butterfly abundance across ecosystems. By contrast, temperature and species richness were significantly related in the wet forest, a result concordant with previous research in rainforest butterflies (Checa et al. 2009, Ribeiro et al. 2010, Grøtan et al. 2015). This result could be explained by the presence of different limiting climatic factors in seasonal dry forests compared to aseasonal wet forests, and the associated phenology of the flora and fauna. Maintaining a sufficiently high thoracic temperature for adequate flight performance is a greater problem for butterflies inhabiting wet forests compared to butterfly species from drier habitats, where water availability instead is more critical.
The close relationship between butterfly numbers and climatic variables was expected because butterflies are ectothermic and their thermal physiology is thus highly dependent on climate (Roy et al. 2001). Weather affects population growth by limiting the available time for adult flight needed for oviposition (Warren 1992) and by its effects on survival of the immature stages (Warren 1992, Hellmann 2002, Dooley et al. 2013) and fecundity (Boggs & Freeman 2005). Other factors are also important such as predator avoidance, courtship and adult feeding (see below).

Higher availability of food sources for adult butterflies (i.e., fruits, carrion) and larvae (leaves) could explain the peaks in butterfly numbers during the wet season, in addition to the significant association of rainfall with butterfly numbers since water availability is more critical in drier habitats and seasons where dehydration of both hostplants and butterflies is at risk. Fruiting peaks and increased leaf production occur during the rainy season in tropical forests (Foster 1996 in Grøtan et al. 2012). Indeed, weather-related density-independent processes (e.g., senescence timing of hostplants) are considered the main driver of population dynamics for some temperate butterfly species (Hellman et al. 2004, Yamamoto et al. 2007), and insect phenology thus follows and synchronizes with host-plant phenology, which also varies from year to year in response to environmental conditions (van Asch & Visser 2007, Valtonen et al. 2013). Additionally, the wet season is likely the main period of oviposition (Shahabuddin et al. 1999) since leaf-feeding butterflies prefer new shoots and leaves for this task (Rodrigues & Pires 1999, van Asch & Visser 2007, Srygley et al. 2010). In the seasonal forests, the abundance peak of adults in March, a few months after the start of the rainy season in December, could result from the time-lag due to the development of the
immature stages. As climate change has affected the phenology of temperate butterflies (Roy & Sparks 2000, Stefanescu et al. 2003, Altermatt 2010), disrupting the relationship between butterflies and their hostplants and adult resources, similar consequences can be expected in dry forests owing to the close relationship between rainfall and the temporal dynamics of their butterfly communities.

The abundance of adult butterflies does not necessarily reflect total population abundance, since individuals may be present at a site but undetected as diapausing adults or immature stages (Kishimoto-Yamada & Itioka 2015). Diapause, a state of low metabolic activity prevents development during unfavorable conditions increasing resistance to adverse environmental conditions (van Asch & Visser 2007). It is possible butterflies survive the dry season as adults in reproductive diapause (see Shahabuddin et al. 1999), although Lepidoptera can also diapause as pupae, eggs or more rarely larvae (Torres et al. 2009).

Species detection probability or detectability may additionally bias the observed seasonal patterns. For example, sunny days enhance butterfly activity, hence increasing probability of trap captures, and the level of bait attractiveness may differ over seasons (Torres et al. 2009, Ribeiro et al. 2015). Although these factors can certainly explain some variation, the bias produced is likely to be negligible in this study since butterfly abundance peaks were detected during the wettest part of the year (the dry season encompasses a larger number of sunny days which increases the probability of both trap captures and scheduling sampling during warmer days), and differences in abundance among years and seasons were relatively large (see Checa et al. 2014). Additionally, in Neotropical forests, detectability of butterfly species attracted
to baits has been reported to positively correlate with observed abundance, which means probability detection raises as butterfly abundance increases (Ribeiro et al. 2015).

Peaks in abundance and species richness has been reported for rain forests during the transitional months rather than throughout the wet season in Costa Rica (Grøtan et al. 2014) and Amazonia (see DeVries & Walla 2001, Checa et al. 2009, Grøtan et al. 2012), results that contrast with those found here for wet forests, where butterfly numbers reached a peak during the most humid part of the year. These differences are likely related to species detectability and underlying factors regulating temporal dynamics and assembly mechanisms shaping communities, and how they vary across space (i.e., biogeography regions, biomes) (see below).

Similarities were detected in butterfly community dynamics across the two seasonal sites, in particular with both showing the greatest butterfly abundance during the 2012 wet season, which coincided with highest rainfall recorded across years and ecosystems. Meanwhile in the aseasonal wet forest, the highest annual abundance occurred one year later. This pattern may indicate that regional processes are more prevalent in regulating butterfly populations in dry forests (e.g., climatic variables) compared to local mechanisms (e.g., predation, natural enemies), which may be more prevalent drivers of temporal patterns in the wet forest. Further evidence to support this idea of variable drivers of community dynamics comes from varying seasonal patterns of wet/dry species depending on the sampled ecosystem. The highest abundance for dry forest species was synchronized in both seasonal forests in 2012, whereas wet forest species also reached a peak during the wettest year in the Manabi transition
forest, but one year later in the wet forest. In addition, different regulating mechanisms may operate at the species level. Regional climatic factors might be more relevant for *H. amphinome* populations since its abundance peak coincided across ecosystems through the 2012 wet season. On the other hand, although *F. ryphea* also reached its peak annual abundance in 2012 during the wet season within the seasonal forests, it reached peak abundance one year later in the wet forest, which may perhaps further indicate the importance of local processes for population regulation.

In temperate areas, the synchrony of population dynamics among species can be explained by a strong effect of environmental forces (Nowicki *et al.* 2009). In England, butterfly populations are also often regionally synchronized (see Pollard 1991) due to the regional correlation in climatic patterns (Sutcliffe *et al.* 1996). Synchrony occurs because species show similar responses to environment (*i.e.*, positive covariation) with intra- or inter-specific interactions playing a weak role in shaping temporal abundance patterns of species within a community (Mutshinda *et al.* 2010).

Furthermore, time-lags of peaks in abundance in response to weather conditions occur particularly if different stages of the life cycle are affected in overlapping populations (Boggs & Inouye 2012). There is a frequent correlation between increased humidity/precipitation in one year and increased butterfly abundance in the following year (Pollard 1991, Forister *et al.* 2011, Dooley *et al.* 2013). In one study, the mechanisms involved included an increase in nectar availability through higher precipitation, which in turn enhanced fecundity (O'Brien *et al.* 2004); but precipitation can also depress natural enemies and permit increased growth rates in the population (Forister *et al.* 2011). Therefore, weather conditions can lead to density-dependent
effects the following year. Positive effects of temperature during one previous year on growth rates of the following year have also been reported (Roy et al. 2001, Forister et al. 2011, WallisDeVries et al. 2011). These effects may occur because higher temperatures can extend flight periods and hence permit more oviposition opportunities, or warmer environments may enhance plant growth supporting higher larval survival.

Predators and parasitism are considered major regulatory factors of tropical butterflies (Gilbert 1972), with, for example, selection from bird predators (e.g., jacamars, flycatchers, kingbirds) believed to be responsible for the remarkable mimicry observed in butterflies (Langham 2004). Nevertheless, our understanding of the importance of competition, predation and parasitoids in limiting butterfly abundance is very limited (Grøtan et al. 2012). In studies focused on population dynamics, the detection of density-dependence is greatly limited by short-term abundance studies (Woiwood & Hanksi 1992). Therefore, a lack of detection of density dependence can result from short times series analyzed (<20 years) rather than weak contributions to population regulation (Mittelbach 2012). Moreover, some authors argue that density-dependence has been underestimated in butterflies due to a greater attention on the adult stage (Nowicky et al. 2009). As a consequence of the long-term abundance data required and the need for monitoring factors shaping fluctuations, very few studies have quantitatively analyzed the relative impact of density-dependent factors and density-independent factors in the population regulation of butterflies, and the few studies that have were focused on very well-known species from temperate areas (Azerefegne et al. 2001, Mclaughlin et al. 2002, Nowicky et al. 2009, Dooley et al. 2013). Further research is therefore needed on density-dependent factors (e.g., competition, predation,
parasitism) to have a more complete understanding of the population dynamics of tropical butterflies and to verify what has been suggested here, that local density-dependent mechanisms (e.g., predation, competition) are more significant in controlling abundance in wet forests compared to dry forests.

Our understanding of mechanisms influencing seasonal patterns and the evolution of insect seasonality might also benefit from research at the species level, taking into account generation cycles, different life stages and specific behaviors such as diapause (see Kishimoto-Yamada & Itioka 2015). In addition studies are needed of the variation of populations across microhabitats and geographic space, since the relative importance of density-dependence or density independence for butterfly populations can vary across space and time (Flockhart et al. 2012, Dooley et al. 2013). In terms of distribution, temperate butterfly populations show greater fluctuations at the edges of their distributions (Warren 1992; Thomas et al. 1994), and are more strongly affected by environmental stochasticity compared to populations occurring in the center (Nowicky et al. 2009). At very local spatial scales, in adjacent habitats, different factors become increasingly important; butterflies from homogenous habitats may be more influenced by weather (i.e., rainfall in the preceding year) rather than by endogenous factors (McLaughlin et al. 2002).

Finally, a significant result found in the present study is that butterflies with stronger seasonality were significantly negatively associated with total monthly rainfall but not variability of both temperature and relative humidity in seasonal forests. As a consequence, butterfly species with uneven temporal distribution (i.e., restricted ecological niche) might be more influenced by changes in the precipitation regime within
forests with marked climatic seasons. It is likely some strongly seasonal species may also be restricted in geographic range, as is the case with *Ithomia cleora*, which could amplify its susceptibility to climate change if effects of rainfall variation prove to be negative (*i.e.*, narrowing even more peaks of occurrence). Hence, seasonality might be a further trait contributing to extinction vulnerability of taxa, along with other previously studied traits, including body size (Brown 1971, Kattan et al. 1994, Shahabuddin & Ponte 2005), population density (Pimm et al. 1988, Bolger et al. 1991, Shahabuddin & Ponte 2005) and mobility (Graves & Gotelli 1983). In addition, more seasonal nymphalid species tend to also have larger body sizes (Ribeiro & Freitas 2011), a trait related to increased vulnerability to extinction in some Neotropical nymphalids (Shahabuddin & Ponte 2005). Further research about this topic will shed more light into whether seasonality is another contributing factor for extinction vulnerability of insect species.

The results of this study are also relevant for determining the possible impacts of climate change on tropical ectotherms, which several authors regard as potentially more sensitive than temperate organisms (Wright et al. 2009, Laurance et al. 2011) due to their narrower thermal tolerance compared to temperate species (Deutsch et al. 2008). Further research into insect seasonality taking into account species traits such as host specialization or geographic distribution, are needed to achieve that goal. For example, such studies might shed light on how insects may adjust to unfavorable seasons when extending their distribution to higher altitudes (Kishimoto-Yamada & Itioka 2015), or provide information about the climatic niche of species at local scales, which in turn can further contribute to understanding species distribution and latitudinal patterns of species richness. This type of research is a high priority for the fragmented dry forests
in Ecuador as climate change is a major threat because of the positive feedback between forest fragmentation and drought (Laurance & Williamson 2001). Moreover, these fragmented forests are more vulnerable to the impacts of El Nino-Southern Oscillation (ENSO) droughts, whose frequency and therefore impact on butterfly ecology (Srygley et al. 2010) is expected to be altered by climate change, in addition to an increased variability of weather patterns (Easterling et al. 2000).
Table 3-1. Results of GLM per each study site (i.e., wet, transition and dry forests) using monthly abundance or observed species richness as the response variable and monthly measures of climatic variables as predictors. Models included the negative binomial distribution for the response variables. Asterisks and double asterisks represent significant and highly significant associations between butterfly numbers and climate, respectively.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Abundance Coefficients</th>
<th>t value</th>
<th>P</th>
<th>Species Coefficients</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total rain</td>
<td>0.001</td>
<td>2.48</td>
<td>0.01*</td>
<td>0.00</td>
<td>2.97</td>
<td>0.00**</td>
</tr>
<tr>
<td>Mean temperature</td>
<td>-0.10</td>
<td>-1.72</td>
<td>0.09</td>
<td>-0.08</td>
<td>-2.15</td>
<td>0.03*</td>
</tr>
<tr>
<td>Transition forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total rain</td>
<td>0.01</td>
<td>3.08</td>
<td>0.00**</td>
<td>0.00</td>
<td>1.29</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean temperature</td>
<td>-0.34</td>
<td>-1.39</td>
<td>0.17</td>
<td>-0.34</td>
<td>-2.16</td>
<td>0.03*</td>
</tr>
<tr>
<td>Dry forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total rain</td>
<td>0.00</td>
<td>3.01</td>
<td>0.00**</td>
<td>0.00</td>
<td>0.74</td>
<td>0.46</td>
</tr>
<tr>
<td>Mean temperature</td>
<td>-0.09</td>
<td>-0.62</td>
<td>0.54</td>
<td>-0.16</td>
<td>-1.50</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 3-2. Results of GLM for the dry forest communities using the coefficient of variation of butterfly abundance (measure of seasonality) as dependent variable and climatic variables as predictors. The response variable better fit the normal distribution. Double asterisks represent rainfall highly significantly affects butterfly seasonality.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Coefficients</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rain (mm3)</td>
<td>-0.13</td>
<td>-3.63</td>
<td>0.00**</td>
</tr>
<tr>
<td>Std.Temperature (%C)</td>
<td>0.39</td>
<td>0.68</td>
<td>0.50</td>
</tr>
<tr>
<td>Std.Relative Humidity (%)</td>
<td>-0.21</td>
<td>-1.04</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Figure 3-1. Monthly average temperature (line with dots) and monthly total rainfall (bars) recorded along a climatic gradient in Western Ecuador from November 2010 to September 2013. A) Wet forest located in the northern province of Esmeraldas (Canande River Reserve, CRR), B) Transition forest within Manabi province (Lalo Loro Dry Forest Reserve LLDFR) and C) Dry forest in the southern corner (Jorupe Reserve JR).
Figure 3-2. Monthly observed butterfly species richness (line with dots) and abundance (line with triangles) from Nov 2010 to Sep 2013 across study sites. A) wet forest, B) transition forest and C) dry forest.
Figure 3-3. Monthly butterfly abundance recorded across study sites and years in Western Ecuador from Nov 2010 to Sept 2013. A) Wet forest, B) Transition forest and C) Dry forest.
Figure 3-4. Monthly abundance of wet and dry species recorded across study sites from Nov 2010 to Sep 2013. A) Wet species recorded in the wet forest (red line with squares) and transition forest (blue line with triangles), B) Dry species registered in the transition forest (line with squares) and dry forest (line with triangles).
Figure 3-5. Monthly abundance of species distributed in all three study sites: wet forest (red line), transition forest (blue line) and dry forest (green line). A) *Fountainea ryephea*, B) *Hamadryas amphinome* and C) *Smyrna blomfildia*. 
4.1 Background Information

Spatial variation in species richness is one of the most obvious attributes of biological communities, but the causes of such variation remain one of the most actively studied and debated areas in ecology (Mittelbach 2012). Species richness peaks in equatorial regions and gradually decreases towards the poles for virtually all taxonomic groups; several hypotheses have been proposed to explain this gradient including ecological, biogeographic and evolutionary processes as determinant factors (Schemske et al. 2009, Wiens & Donoghue 2004, Valentine et al. 2008, Schemske 2009).

A majority of studies have shown a correlation between environmental factors and diversity patterns, with the energy hypothesis being one of the most broadly accepted hypothesis to explain these patterns (see Tello & Stevens 2010). Climate is hypothesized to play a key role driving the latitudinal pattern of species richness through energy availability, measured as primary productivity (Williams & Middleton 2008, Williams & Hero 2001, Hanya et al. 2011). Evidence supporting the energy hypothesis has been provided by studies with birds (Evans et al. 2006, Williams & Middleton 2008), bats (Tello & Stevens 2010) and primates (Stevenson 2001, Hanya et al. 2011). Climatic variables are frequently used as reasonable surrogates of primary productivity, but seasonality of climate can likely better predict species richness than total or mean measurements (Pyrcz et al. 2014), particularly in seasonal forests (Williams & Middleton 2008). Areas with high seasonal rain variation have lower species richness of frogs (Williams & Hero 2001) and birds (Williams & Middleton 2008). Hanya et al. (2011)
found that species richness increased as seasonality of resource availability decreased and the effects of biogeographic factors, if any, were small, revealing the importance of seasonality in determining energy availability.

Despite strong correlations between climate and species diversity, however, the mechanisms by which climate controls diversity are more poorly understood. Resource-niche partitioning is hypothesized to promote greater species diversity (Finke & Snyder 2008) since it decreases interspecific competition, a condition denoted in early ecological models (Hutchinson 1959, MacArthur 1970, Chase & Leibold 2003) to foster species coexistence and promote biodiversity. One possibility is therefore that more stable climates, which host higher species diversity permit greater niche specialization as a result of continual competition, allowing more species to coexist, whereas less stable climates favor broader niches, that perhaps require specific adaptations, and thus result in less diverse communities. Phylogenetic and phenotypic analyses are an increasingly common approach to examine the relative importance of these two kinds of community assembly mechanisms, namely competition and habitat filtering (Mittelbach 2012). These analytical tools allow inferring about the action of niche-based processes in community assembly by examining patterns of phylogenetic and trait dispersion within communities (Cavender-Bares et al. 2009, Donoso 2013). The distribution of traits and phylogenetic distance observed within a community is then compared to ‘expected’ values generated within null communities formed by randomly selecting species from a regional pool of potential colonists (Webb 2000, Kraft et al. 2007).

One of the most familiar stabilizing mechanisms (i.e., processes that give advantage to species when rare) that occur in tropical forests is niche partitioning with
respect to heterogenous light environments across different habitats (Harms 2001) and strata (Terborgh 1985), which is expected to generate communities with co-occurring species sharing adaptations to the environment, consisting in traits to use shared resources (Kraft et al. 2010). This mechanism along with other examples encompassing different habitat characteristics are referred to as habitat filtering, a process that influences the range of 'viable' trait values existing at a given site (see Cornwell & Ackerly 2009).

On other hand, competition influences the spacing of trait values within a community, imposing limits to the similarity of coexisting species, a prediction of classical models of competition (MacArthur & Levins 1967, Cornwell & Ackerly 2009). For traits exhibiting phylogenetical signal (i.e., phylogenetic conservatism), ecological filters result in communities formed of closely related species (i.e., phylogenetic clustering) (Kraft et al. 2007, Lessard et al. 2009, Kraft et al. 2010), whereas competition produces an even dispersion of traits and co-occurring species are less related than expected (Webb 2002). With respect to convergent traits, assembly processes can generate completely opposite patterns of community phylogenetic dispersion (Kraft et al. 2007). Furthermore, if traits are irrelevant as drivers of community assembly, a precept of neutral theory (Hubbell 2001), random patterns of trait and phylogenetic dispersion are expected outcomes.

The relative importance of habitat filtering and competition might differ along environmental gradients. A study of hummingbird communities in Ecuador revealed an interesting pattern: competition constituted the dominant mechanism driving community assembly within moist lowland forests, whereas communities inhabiting seasonally dry conditions...
forests were predominantly shaped by habitat filtering (Graham et al. 2009). Likewise, similar phylogenetic clustering within ‘harsh’ environments compared to more ‘benign’ conditions has been observed for plant traits across latitude and/or elevation (Sweson & Enquist 2007, Cornwell & Ackerly 2009). Nevertheless, it is unknown whether these patterns apply to other biological communities such as tropical insects, as studies focused on assembly mechanisms of tropical communities remain scarce. This is particularly true for insects (Chazot et al. 2014, Donoso 2013) along environmental gradients, such as wet moist lowland to dry forests. Nevertheless, studies of insects have great potential, since insects are the richest group of living organisms (Wiens et al. 2015), concentrated in the tropics, with nearly half of the world’s species inhabiting these forests (Olson & Dinerstein 2002).

It nevertheless seems likely, however, that tropical insect communities may show patterns similar to other organisms, with competition predominantly influencing community composition in wet forest while in dry seasonal forests, habitat filtering due to stronger environmental seasonality, is a major community assembly driver. This might be expected because of the hypothesis of biotic interactions being stronger in more diverse communities and weaker in less diverse communities (Schemske et al. 2009). Moreover, the intensity or frequency of biotic interactions is likely to vary within tropical forests with varying degrees of seasonality. For example, Coley & Barone (1996) found higher rates of leaf herbivory occurring in wet forests compared to dry forests.

Vertical strata within rainforests offer much ‘steeper’ climatic gradients compared to those found across latitude and elevation dimensions (Scheffers et al. 2013). Indeed,
abiotic conditions shift progressively from the understory to the canopy, particularly in terms of temperature, humidity and light (Johansson 1974, Fetcher et al. 1985), with canopy conditions within lowland forests exhibiting warmer and drier conditions compared to the understory. The varying abiotic environment is accompanied by a conspicuous change in the biological communities inhabiting the forest strata. This is the case for Neotropical butterfly assemblages, which show clear stratification patterns in terms of abundance and composition (DeVries & Walla 2001, Fermon et al. 2003, Fordyce & DeVries 2016 and citation therein).

As a consequence, species exhibit morphological traits potentially associated with flight height. Understory species are hypothesized to have high wing-area/thoracic-volume ratios, associated with slower flight (Hall & Willmott 2000), and higher wing aspect ratio (e.g., long and narrow wings) and wing loading (Mena & Checa in prep.). Wings with high aspect ratio are aerodynamically more efficient for gliding (Betts & Wooton 1988), a flight mode adapted to lower temperatures in the understory as it is energetically less expensive (Ellington 1985). It is less clear how these traits vary across sites with varying seasonality, but it is likely that lower aspect ratio values occur in species inhabiting the wet forest compared to those within dry forests, since wet forest butterfly species tend to be rarer, more territorial and display perching behavior to find mates (see Rutowski 1991, Hall & Willmott 2000), traits more frequent in more diverse, competitive environments.

The goal of the present study is, therefore, to test the hypothesis that assembly processes that limit species similarity predominantly occur in more ‘stable’ abiotic environments, whereas habitat filtering is a major driver of community composition.
within more variable environments at regional (e.g., aseasonal vs seasonal forests) and local scales (e.g., understory vs. canopy). In other words, I expect the importance of competition as an assembly mechanism to decrease from the wet forest to dry forest, and at local scales from the understory to the canopy, whereas the contrary pattern is expected to occur for habitat filtering, being prevalent in the dry forest and understory strata. A combined approach of phylogenetic- and trait-based analyses using forewing length and aspect ratio as traits, were used to test these hypotheses.

4.2  Methods

4.2.1  Census Techniques

Butterflies were sampled using bait traps at three study sites: Canande River Reserve (CR, wet forest), Lalo Loor Dry Forest (LLDFR, transition forest) and Jorupe Reserve (JR, dry forest). Sampling was performed every 2 months (e.g., six times a year) over 7 days each sampling period for three consecutive years from November 2010 to September 2013. Van Someren-Rydon bait traps were used with two different types of baits: rotting prawn fermented for 13-18 days, and banana fermented for 2 days. Traps were checked daily during the first 7 days of the sampling month from 9 am to 3 pm; traps were opened and baited on the first trapping day, and checked over the next 6 days with trapped butterflies being collected, except for the most abundant species, which were marked and released.

Two transects were established at each reserve with each transect containing eight sampling positions. Two baited traps were set up at each sampling position in two different strata, understory (1.5 meters above the ground) and canopy (10-25 m depending on the ecosystem sampled). The use of banana and shrimp baits alternated between positions, thus neighboring positions had different types of bait. Canopy and
understory traps in the same position were baited with the same type of bait. All collected material was examined and identified to species, and classified following the higher taxonomic classification of Wahlberg et al. (2009). Legs from dried specimens were detached and stored in vials for subsequent DNA analyses.

**4.2.2 DNA extraction and phylogenetic analyses**

DNA extraction was done from 1-2 legs (depending on butterfly size) of dried specimens using the Qiagen DNEasy extraction kit. The ‘barcode’ section of the mitochondrial gene *cytochrome oxidase 1* (COI) was sequenced for all Nymphalidae species collected across study sites, only nymphalids were included as the DNA extraction and amplification procedures were better understood for these species, and owing to resource constraints to sequence more species. Phylogenetic analyses therefore only included this taxonomic group. Sequences of two species, *Hamadryas arinome* and *Nessaea aglaura*, were obtained from GenBank. Additionally, sequences of the following species were provided by colleagues currently researching these taxa: *Memphis artacaena, M. aureola, M. cleomestra, M. mora, M. nenia, Archaeoprepona amphimachus, Prepona gnorima, P. werneri, Junonia genoveva* and *Marpesia chir*.

The phylogeny based on COI sequences was constrained by the generic level phylogeny of Wahlberg et al. (2009) to confidently resolve deeper nodes. In order to quantify morphological traits, one photograph per individual was taken and the software ImageJ imaging was used to carry out measurements on the digital photographs. Ten individuals per species were measured and traits averaged, except in cases where species abundances was <10, where only 1-2 specimens were measured. Only males were measured owing to low captures of females with baited-traps, as well as to females’ higher morphological variability (DeVries et al. 2010). Variables measured
included forewing length, measured from the base to the apex, and aspect ratio (AR), calculated as $4^{*}\text{forewing length}^{2}{*}\text{wing area}^{\frac{1}{2}}$ (Betts & Wooton 1988, Dudley 1989). Forewing length was used as a proxy of body size, as previous research found it is a robust proxy (Shahabuddin & Ponte, 2005, Ribeiro & Freitas 2011).

4.2.3 Statistical Analyses

Blomberg’s K statistic was used to measure the phylogenetic signal in morphological tratis, AR and FWL (Blomberg et al. 2003), based on the species-level phylogenetic tree. This statistic estimates the trait variability within a phylogeny, with K>1 meaning more trait conservatism than expected under a Brownian model of evolution, in contrast to more trait convergence than expected under the same model of evolution when K<1. Significance of Blomberg’s K statistic was assessed generating 999 random combinations of trait values by shuffling the traits across the tips of the phylogeny 999 times; the observed k statistic was further compared to the distribution of k values generated by the null model. Trait conservatism exists if observed K values fall in the upper 2.5% of the null distribution (Kraft et al. 2010).

4.2.4 Phylogeny-based analyses

Phylogenetic analyses were performed using the constrained species-level phylogenetic tree. Phylogenetic structure of butterfly communities was estimated using two indices: the Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI). These metrics allow determining whether communities include a random set of species from the regional pool or, by contrast, a deterministic process drives community composition (e.g., phylogenetic clustering or even dispersion is observed). NRI refers to the standardized effect size of the mean phylogenetic distance (MPD) across species in the local communities, and NTI corresponds to the standardized effect size of the mean
nearest taxon distance (MNTD) (Donoso 2013). Positive values of NTI and NRI denote phylogenetic clustering, whereas negative values indicate communities that are phylogenetically evenly dispersed. Standardized effect sizes were obtained by comparing observed values to those generated by null models, standardized by the standard deviation of the null distribution. Two null models were used, ‘Taxa Labels’ which generate random communities by shuffling the tips of the phylogeny, and ‘Sample Pools’ that creates null communities by drawing species from the sample pool. Communities were considered significantly clustered or evenly dispersed if the observed distance of MPD and MNTD was above or below 2.5% of those within the null distributions. In addition, higher NTI values with respect to NRI mean more clustering at the tips of the phylogeny, rather than tree-wise.

4.2.5 Trait-based Analyses

Following the approach developed by Kraft et al. (2008) and Kraft & Ackerly (2010), I assessed assembly mechanisms of butterfly communities by analyzing trait dispersion within these communities. I estimated several indices sensitive to niche differentiation and habitat filtering. First, I calculated the indices ‘range’ and ‘variance’ of traits, which are expected to decrease within a community in the presence of an environmental filter. In addition, three indices sensitive to trait dispersion were estimated: standard deviation of nearest neighbor distance along trait axes divided by trait range (SDNNNr), standard deviation of successive neighbor distances along trait axes divided by trait range (SDNDr), and kurtosis. The standard deviation of NN and ND is an index representing how regularly spaced are species along the trait axis, whereas division by range is used to partially correct for effects of habitat filtering, thus detecting niche differentiation in an environment of ecological filters. Moreover, both SDNNNr and
SDNDr are estimated by detecting the most similar species to each successive species within the assemblage. In communities where biotic interactions act as assembly mechanisms, the spacing of trait values remains constant, and as a consequence kurtosis, SDNDr and SDNNr are expected to decrease. One-tailed Wilcoxon tests were used to determine whether these metrics significantly differed from zero. All phylogenetic analyses were performed using the R package ‘Picante’ (Kembel et al. 2010).

4.3 Results

A total of 6466 specimens representing 142 species of Nymphalidae were recorded from November 2010 to September 2013 within wet, transition and dry forests from western Ecuador. Observed species richness decreased gradually from wet forests (98 species), to transition (60) and dry forests (32) in the southern part. Few species dominated the samples; in particular, within dry forest butterfly communities, two species, *Fountainea ryphaea* and *Hamadryas amphichloe*, comprised 65% of specimens collected (1518 and 767 individuals, respectively). Species abundances were more even within the wet forest since the most abundant species, *Memphis cleomestra* and *Hamadryas amphinome*, were represented by 158 and 143 individuals, respectively, which corresponded to 23% of the total sample.

4.3.1 Phylogenetical Signal In Traits

Butterfly species significantly differed in terms of body size and wing aspect ratio across study sites (Fig. 4-1). The former gradually decreased from wet forest to dry forests with butterfly species' forewing length varying in mean values from 35.73 mm to 32.48 mm, respectively; whereas this trait averaged 35.50 mm in the transition forests. In terms of AR, butterfly species from the transition forests presented the highest mean
value of 5.78, followed by dry forest species (5.71) and wet forest species (5.65). Hence, butterflies inhabiting dry forests on average tended to have smaller body size (lower values of FWL) and longer, narrower wings (high AR) compared to those from the other study sites. Wilcoxon tests revealed that the standardized mean values of both traits highly significantly differed among butterfly communities across ecosystems. Moreover, Blomberg’s K values for AR and FWL revealed that traits were more convergent than expected under the Brownian model of evolution ($k = 0.20 \ p = 0.00$; $k = 0.18 \ p = 0.00$, respectively), but more conserved than predicted by a random association of traits and phylogeny as shown by their associated p values (all p values < 0.05). Hence, owing to p values less than 0.05, traits were considered as conserved (showing phylogenetic signal) for the interpretation of results, with clustered patterns of phylogenetic relatedness indicating the mechanisms limiting similarity of species are shaping community composition. By contrast, even phylogenetic dispersion indicated ecological filters as assembly mechanisms. K values also showed that AR is more evolutionarily labile, while forewing length was more conserved.

**4.3.2 Phyllogenetic-based Tests Of Community Composition**

Phylogenetic-based tests revealed significant levels of phylogenetic clustering (Fig. 4-2). According to NRI values, the wet forest community was composed of more phylogenetically closely related species than expected, regardless of the null model used, namely by shuffling the tips of the phylogeny (Taxa Label, TL) or drawing species from the sample pool (Sample Pool, SP). (Fig. 4-2). Likewise, NTI values revealed phylogenetic clustering in dry forest communities, but solely for SP null models when considering NRI (NRI= 0.3 $p = 0.00$). The distribution of observed NRI and NTI values for wet and dry forest communities was significantly shifted above null expectation.
Species within butterfly communities from transition forest were random with respect to phylogeny (non-significance detected in both NRI and NTI indices).

In terms of phylogenetic structure of communities across strata within sites, similar patterns of phylogenetic clustering were detected. In cases using Taxa Label null models, co-occurring taxa from the canopy and understory communities within wet forest tended to be more closely related than expected based on values of NRI and NTI (Table 4-1). Moreover, phylogenetic clustering was detected for canopy (NTI= 0.98 p<0.01) and understory communities (NTI= 1.38 p<0.01) distributed in the dry forest. With respect to transition forests, species within canopy communities were random or phylogenetically more related than expected (NTI= 0.43 p>0.05, NTI= 0.26 p< 0.05), whereas species in understory communities were random (NRI= -0.47 p> 0.05, NTI= -0.36 p>0.05) (Table 4-1). Similar trends were revealed when using the Sample Pool algorithm, namely by drawing species from the sample pool to generate null communities.

4.3.3 Trait-based Tests of Community Composition

Non-random patterns of even spacing with respect to AR were solely detected for butterfly community composition within transition forests, with two metrics sensitive to niche differentiation showing a significant reduction for AR: SDNNr (p= 0.01) and SDNDr (p= 0.02) (Fig. 4-3). All other metrics including variance, range and kurtosis were not significantly reduced relative to the null expectation for any study site, revealing random patterns of AR distribution.

Trait-based tests using body size detected nonrandom patterns solely within dry forests as SDNDr and kurtosis were highly significantly lower relative to the null expectation, revealing even dispersion within this community (Fig. 4-4). There was no
significant decrease in range and variance in traits within wet forest communities (p = 0.054 and p = 0.074, respectively), although p values close to 0.05 suggested a trend of phylogenetic clustering. Random patterns of body size distribution were detected for transition forest communities, wet forest communities in relation to metrics sensitive to niche differentiation, and dry forest communities with respect to variance and range.

Along the vertical forest dimension, similar patterns of trait even spacing were detected for dry forest communities within the canopy and understory. With respect to AR, even dispersion was detected since SDNDr significantly decreased within understory and canopy communities, along with kurtosis for understory assemblages (Table 4-2). Likewise, all metrics sensitive to niche partitioning significantly decreased within understory and canopy communities for body size analyses (Table 4-3). Analyses focused on body size additionally revealed even dispersion within the understory from wet forest communities (e.g., kurtosis and SDNNr significantly decreased), along with a clustering pattern within the canopy community (i.e., range and variance significantly decreased). On the other hand, metrics based on AR also revealed a clustering pattern in the understory community within wet forest communities (variance decreased compared to null expectation, Table 4-3). Summaries of the outcomes from phylogeny- and trait-based analyses are shown in Table 4-4, 4-5 and 4-6.

4.4 Discussion

Based on the results of the present study, I rejected the hypothesis that assembly processes that limit species similarity (i.e., competition) are likely to predominantly occur in more ‘stable’ abiotic environments, whereas habitat filtering can be a major driver of community composition within more variable environments at regional (i.e., aseasonal vs seasonal forests) and local scales (i.e., understory vs.
canopy). My study of assembly mechanisms revealed the opposite pattern, with stronger evidence for the action of ecological filters in the assembly of butterfly communities from the wet aseasonal forests, and competition likely to be a major assembly process within dry seasonal forests.

Phylogenetic- and trait-based analyses revealed mostly non-random patterns of phylogenetic structure within butterfly communities along local and regional environmental gradients in western Ecuador. I found both traits AR and body size were evenly dispersed within communities from the dry forest even at local scales across strata communities, which is consistent with the competition hypothesis. Additionally, phylogenetic tests revealed co-occurring species were more closely related than expected by chance within canopy and understory communities from the dry forest, pattern consistent with the habitat filtering hypothesis. Similarly, habitat filtering was found as a significant assembly mechanism within both the canopy and understory communities from wet forests, with less evidence of trait even dispersion (i.e., competition) along this gradient (i.e., significant outcomes solely from body size analyses). With respect to transition forest communities, random patterns of phylogenetic structure were mostly observed, except for local scale analyses that revealed phylogenetic clustering within canopy butterfly assemblages and trait-based analyses revealing even dispersion of AR, results consistent with habitat filtering and competition, respectively.

These general conclusions do not mean that competition or habitat filtering are mutually exclusive depending on the ecosystem analyzed, or that other factors (e.g., evolutionary) have affected community composition, only that at the scale of the
analyses presented here and the approach used (Kraft et al. 2007), the role of one mechanism might be prevalent above the effect of the other (see Graham et al. 2009). Indeed, other biotic interactions such as mutualistic mimicry have been found to shape Andean butterfly communities (Chazot et al. 2014). Furthermore, predation (see Schulze 2001) and distribution of hostplants (see Beccalon 1997, Willmott & Mallet 2004) can also structure butterfly community composition across strata within tropical forests.

Our understanding of the importance of competition in tropical butterfly communities is very limited (Grøtan et al. 2012). The results found here confirm that it is challenging to predict how competition can vary along environmental gradients where other processes to prevent it commonly occur, such as niche partitioning in time (i.e., seasonality). It is likely that, indeed, that the latter prevents competition being a prevalent assembly mechanism within wet forests, in contrast to what was expected of this mechanism shaping butterfly communities in more ‘benign’ aseasonal environments.

Temporal species turnover has been reported to occur in tropical butterfly communities from aseasonal forests (Checa et al. 2009) with community similarity displaying annual cycles that peak in the driest months (Valtonen et al. 2013, Grotan et al. 2014). It is likely that the incidence of drought throughout 4-6 months strongly constrains the ‘time’ resource within dry forests, which can further impose a challenge for species survival thereby increasing competition within communities, explaining the prevalence of competition as an assembly mechanism. However, other factors might come into play in order to explain my results (see below).
Most stabilizing assembly mechanisms, including competition and habitat filtering, are not mutually exclusive; in fact, these processes can generate interacting effects on biological communities (Kraft et al. 2007, Kraft et al. 2010). As a consequence, trait or phylogenetic distribution is altered and random patterns can occur, as suggested by Swenson & Enquist (2009) for a research study focused on tropical plant communities within dry forests, where the simultaneous occurrence of clustering and even dispersion of traits led to random phylogenetic community structure. As a consequence, it is not possible to disentangle whether the random patterns of trait and phylogenetic distribution found here, particularly for wet and transition forests (see summary Table 4-5, 4-6), are indeed showing equalizing mechanisms (the most remarkable example being Hubell’s neutral theory) as assembly processes within these communities, or by contrast, are the result of interacting effects of competition and habitat filtering.

Furthermore, outcomes derived from trait-based analyses might depend on studied traits (Kraft et al. 2010). For example, Sedio et al. (2010) found that hydraulic but not photosynthetic traits largely explained phylogenetic clustering within species communities of the hyperdiverse plant genus Psychotria in Panama. Hence, further research incorporating other functional traits of butterflies into analyses of community structure could provide more evidence to patterns described in the present study. In addition, including more taxonomic groups (not only Nymphalidae) into the analyses could further contribute towards our understanding as it will increase the phylogenetic diversity present in the local and regional communities, as well as an increased range of trait variation.
An interesting result is that butterfly species are using the traits of AR and body size as adaptations to different processes depending on the ecosystem and strata analyzed. For example, niche partitioning involved both traits within understory and canopy communities from dry forests, whereas solely body size remained relevant for competition within the wet forest (understory) and AR within the transition forest. On the other hand, body size and AR are relevant adaptations for the abiotic environment acting as ecological filters in wet forests within the canopy and understory, respectively.

Furthermore, a trend existed of smaller body size and higher AR (i.e., long and narrow wings) in butterfly species inhabiting dry forest compared to wet forest. The genus Heliconius showed the highest AR values in the present study, and population studies about this well-known genus in the tropics revealed competition occurs for larval resources for some species but not others (Rodrigues & Moreira 2004) as populations are below carrying capacity of hostplants (Ehrlich & Gilbert 1973) and niche partitioning occurs among instars (e.g., younger instars feed on apical and new shoots) (Rodrigues & Pires 1999). Intra-specific competition for mates can also affect population dynamics of Heliconius (see Delnert et al. 1994).

These results indicated that morphological traits, namely body size and AR, are likely involved in competition among Neotropical butterfly species. Moreover, the results presented here provide insights into assembly mechanisms in one of the richest butterfly faunas worldwide, revealing competition along with ecological filters as significant drivers of community composition.
Table 4-1. Indices of phylogenetic community structure, Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) estimated using different null models (i.e., Taxa Label TL or Sample Pool SP) for butterfly communities within wet, transition and dry forests; asterisk or doubled asterisks mean observed indices were significantly or highly significantly different from expected values estimated with null models.

<table>
<thead>
<tr>
<th></th>
<th>NRI_TL</th>
<th>NRI_SP</th>
<th>NTI_TL</th>
<th>NTI_SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet forest Canopy</td>
<td>1.64**</td>
<td>1.70**</td>
<td>0.46*</td>
<td>0.44*</td>
</tr>
<tr>
<td>Understory</td>
<td>2.32**</td>
<td>2.15**</td>
<td>0.87*</td>
<td>0.77*</td>
</tr>
<tr>
<td>Transition Canopy</td>
<td>0.26*</td>
<td>0.29*</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Understory</td>
<td>-0.47</td>
<td>-0.50</td>
<td>-0.36</td>
<td>-0.37</td>
</tr>
<tr>
<td>Dry forest Canopy</td>
<td>0.29</td>
<td>0.30</td>
<td>0.98**</td>
<td>0.97**</td>
</tr>
<tr>
<td>Understory</td>
<td>-0.13</td>
<td>-0.10</td>
<td>1.38**</td>
<td>1.39**</td>
</tr>
</tbody>
</table>

Table 4-2. Standardized values of metrics estimated for wing aspect ratio of butterfly communities across strata within wet, transition and dry forests; metrics are sensitive to habitat filtering (variance and range) and niche differentiation (kurtosis, SDNDr and SDNNr). SDNDr stands for standard deviation of successive neighbor distances along trait axes divided by range, whereas SDNNr refers to standard deviation of nearest-neighbor distance along trait axes divided by range. Asterisk or asterisks refer to indices that significantly decreased with respect to their null distribution according to Wilcoxon tests (p<0.05 and p<0.01, respectively).

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>SDNNr</th>
<th>SDNDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet forest Canopy</td>
<td>0.9</td>
<td>0.25</td>
<td>0.6</td>
<td>0.5</td>
<td>-0.4</td>
</tr>
<tr>
<td>Understory</td>
<td>-0.3</td>
<td>-0.9*</td>
<td>0.2</td>
<td>0.35</td>
<td>-0.25</td>
</tr>
<tr>
<td>Transition Canopy</td>
<td>0.1</td>
<td>1.1</td>
<td>-0.3</td>
<td>-0.15</td>
<td>-0.55</td>
</tr>
<tr>
<td>Understory</td>
<td>0.9</td>
<td>0.75</td>
<td>-0.75</td>
<td>-0.9</td>
<td>-0.3</td>
</tr>
<tr>
<td>Dry forest Canopy</td>
<td>-0.5</td>
<td>0.75</td>
<td>-0.25</td>
<td>-0.6</td>
<td>-0.5*</td>
</tr>
<tr>
<td>Understory</td>
<td>0.8</td>
<td>1</td>
<td>-0.9**</td>
<td>0.85</td>
<td>-0.56*</td>
</tr>
</tbody>
</table>
Table 4-3. Standardized values of metrics estimated for forewing length of butterfly communities across strata within wet, transition and dry forests; metrics are sensitive to habitat filtering (variance and range) and niche differentiation (kurtosis, SDNDr and SDNNr). Asterisk or asterisks refer to indices that significantly decreased with respect to their null distribution according to Wilcoxon tests (p<0.05 and p<0.01, respectively).

<table>
<thead>
<tr>
<th>Habitat filtering</th>
<th>Competition</th>
<th>Range</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>SDNNr</th>
<th>SDNDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td>-2.4**</td>
<td>-1.4**</td>
<td>-1.2</td>
<td>-0.7</td>
<td>-0.7</td>
<td></td>
</tr>
<tr>
<td>Understory</td>
<td>-0.65</td>
<td>0.2</td>
<td>-0.9**</td>
<td>-0.9**</td>
<td>-0.9</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td>-0.35</td>
<td>-0.25</td>
<td>0.35</td>
<td>-0.2</td>
<td>-0.2</td>
<td></td>
</tr>
<tr>
<td>Understory</td>
<td>0.35</td>
<td>-0.1</td>
<td>-0.6</td>
<td>-0.6</td>
<td>-0.6</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td>0.4</td>
<td>0.8</td>
<td>-0.15**</td>
<td>-1.2*</td>
<td>-1.3**</td>
<td></td>
</tr>
<tr>
<td>Understory</td>
<td>0.5</td>
<td>0.9</td>
<td>-0.95**</td>
<td>-0.8**</td>
<td>-0.8**</td>
<td></td>
</tr>
</tbody>
</table>

Table 4-4. Summary of outcomes from phylogenetic-based analyses based on indices of phylogenetic community structure, Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) estimated using different null models (i.e., Taxa Label TL or Sample Pool SP) for butterfly communities within wet, transition and dry forests. The letter X denotes habitat filtering was detected as a prevalent assembly mechanism within these butterfly communities. Blank cells indicate random patterns of species distribution were found.

<table>
<thead>
<tr>
<th>NRI_TL</th>
<th>NRI_SP</th>
<th>NTI_TL</th>
<th>NTI_SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet forest</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry forest</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wet forest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Understory</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Dry forest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Understory</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
Table 4-5. Summary of outcomes from trait-based analyses based on the standardized values of metrics estimated for body size and aspect ratio (AR) of butterfly communities within wet, transition and dry forests; metrics are sensitive to habitat filtering (variance and range) and niche differentiation (kurtosis, SDNDr and SDNNr). The letter X denotes which assembly mechanism was detected as significant for each butterfly community. Blank cells indicate random patterns of trait distribution within communities were found.

<table>
<thead>
<tr>
<th>Body size</th>
<th>Wet forest</th>
<th>Transition</th>
<th>Dry forest</th>
<th>Wet forest</th>
<th>Transition</th>
<th>Dry forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Habitat filtering</th>
<th>Range</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>SDNNr</th>
<th>SDNDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry forest</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4-6. Summary of outcomes from trait-based analyses based on the standardized values of metrics estimated for body size and aspect ratio (AR) of butterfly communities across strata within wet, transition and dry forests; metrics are sensitive to habitat filtering (variance and range) and niche differentiation (kurtosis, SDNDr and SDNNr). The letter X denotes which assembly mechanism was detected as significant for each butterfly community. Blank cells indicate random patterns of trait distribution within communities were found.

<table>
<thead>
<tr>
<th>Body size</th>
<th>Habitat</th>
<th>filtering</th>
<th>Competition</th>
<th>SDNNr</th>
<th>SDNDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet forest</td>
<td>Canopy</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Understory</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>Canopy</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Understory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry forest</td>
<td>Canopy</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Understory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| AR        | Wet forest | Canopy  | X         |       |       |
|           | Understory |         |           |       |       |
| Transition | Canopy  |         |           |       |       |
|           | Understory |         |           |       |       |
| Dry forest | Canopy  |           | X         |       |       |
|           | Understory |           |           |       |       |

Figure 4-1. Box plots of standardized mean values of species traits within communities from wet, transition and dry forests; green color means highly significant differences were found among sites (p< 0.01) according to Wilcoxon Tests. A) Box plot corresponding to wing Aspect Ratio. B) Box plot corresponding to forewing length.
Figure 4-2. Indices of phylogenetic community structure, Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) estimated using different null models for butterfly communities within wet, transition and dry forests; blue color means taxa are more related than expected or phylogenetically clustered. A) NRI using Taxa Label null model. B) NRI using Sample pool null model. C) NTI using Taxa Label null model. B) NTI using Sample pool null model.
Figure 4-3: Standardized values of metrics estimated for wing aspect ratio of butterfly communities from wet, transition and dry forests; metrics are sensitive to habitat filtering (A-B) and niche differentiation (C-E). Red color means indices significantly decreased with respect to their null distribution according to Wilcoxon tests (p<0.05) showing evidence for competition. A) Variance. B) Range. C) Kurtosis. D) Standard deviation of successive neighbor distances along trait axes divided by range (SDNDr). C) Standard deviation of nearest-neighbor distance along trait axes divided by range (SDNNr).
Figure 4-4: Standardized values of metrics estimated for forewing length of butterfly communities from wet, transition and dry forests; metrics are sensitive to habitat filtering (A-B) and niche differentiation (C-E). Blue and red color mean indices significantly decreased with respect to their null distribution according to Wilcoxon tests (p<0.05) showing evidence for habitat filtering and competition, respectively. A) Variance. B) Range. C) Kurtosis. D) Standard deviation of successive neighbor distances along trait axes divided by range (SDNDr). C) Standard deviation of nearest-neighbor distance along trait axes divided by range (SDNNr).
CHAPTER 5
CONCLUSIONS

Collection techniques, namely bait traps and hand-nets, had an effect on analyses of butterfly communities in both temporal (e.g., seasons) and spatial (e.g., strata and microhabitat) dimensions. This finding was particularly true for aseasonal forests, likely owing to their higher diversity compared to seasonal dry forests, as a tendency was found for higher consistency in butterfly seasonality patterns within seasonal forests regardless of collection technique or bait employed (i.e., banana or prawn). Climatic seasonality drives greater similarity in abundance patterns across all taxa in seasonal forests, whereas in wet forests taxa differed more in terms of temporal patterns of abundance and distribution. Therefore, a small sample of the total butterfly community (i.e., obtained using one method only) is more likely to be representative of the whole community in dry forests rather than wet forests.

Different collection methods resulted in the same conclusions for some questions but not for others. These results suggest that great care must be taken in planning butterfly monitoring studies if data are to be comparable, and that making general inferences from data collected using only a single method needs to be justified. Moreover, combining net and bait-trap methodologies maximizes species sampled, in addition to partially correcting for the bias associated with each of these methods, as each technique differs in taxonomic focus, and a combined approach can provide complementary abundance data.

While budget and other factors might pose constraints in the utilization of multiple collection techniques for standardized butterfly monitoring, the use of both carrion and fruit baits is recommended in order to correct for observed bias in attractiveness of
different baits on species over space and time. The results presented here will hopefully contribute towards developing efficient but effective standard methods for long-term butterfly monitoring schemes in the tropics.

Butterfly communities exhibited conspicuous intra and inter-annual variation in temporal dynamics with certain elements (e.g., maximum abundance recorded) of seasonality patterns likely synchronized in seasonal forests across years but not in aseasonal forests. Synchrony of population dynamics among species can be explained by a strong effect of environmental forces, as is the case for butterfly populations from temperate areas, which are often regionally synchronized due to the regional correlation in climatic patterns. Synchrony occurs because species show similar responses to environment, with intra- or inter-specific interactions playing a weak role in shaping temporal abundance patterns of species within a community, which can in turn being important mechanisms underlying community dynamics in aseasonal forests. In addition, the highest numbers of species and individuals occurred during the wet season across all study sites and years; indeed, rainfall was significantly positively associated with temporal abundance.

Furthermore, species displaying stronger seasonality were significantly negatively associated with higher rainfall periods in seasonal forests. As a consequence, butterfly species with uneven temporal distribution (i.e., restricted ecological niche) might be more influenced by changes in the precipitation regime within forests with marked climatic seasons. It is likely some strongly seasonal species may also be restricted in geographic range, which could amplify their susceptibility to climate
change if effects of rainfall variation prove to be negative (i.e., narrowing peaks of occurrence).

Additional research is needed at the species level to shed light into temporal dynamics of multi-species assemblages and underlying mechanisms, taking into account generation cycles, different life stages and specific behaviors such as diapause. Further research is also needed on density-dependent factors (e.g., competition, predation, parasitism) to have a more complete understanding of the population dynamics of tropical butterflies and to verify what has been suggested here, that local density-dependent mechanisms (e.g., predation, competition) are more significant in controlling abundance in wet forests compared to dry forests.

Phylogenetic- and trait-based analyses revealed mostly non-random patterns of phylogenetic structure within butterfly communities along local and regional environmental gradients in western Ecuador. There exists strong evidence of competition as an assembly mechanism of community composition within dry forest even at local scales, within understory and canopy strata. Evidence comes from trait-based (including both traits, AR and body size) tests of community composition. Additionally, phylogenetic tests revealed habitat filtering (i.e., co-occurring species were more closely related than expected by chance) as another significant ecological process in the assembly of butterfly communities from both strata. Likewise, habitat filtering was found as a significant assembly mechanism within both the canopy and understory communities from wet forests, with less evidence of even trait dispersion, indicating competition, along this gradient. With respect to transition forest communities, random patterns of phylogenetic structure were mostly observed, except for local scale analyses
that revealed phylogenetic clustering within canopy butterfly assemblages and AR-based analyses revealing competition as an assembly mechanism of dry forest communities.

The present study therefore provided insights into assembly mechanisms in one of the richest butterfly faunas worldwide, revealing competition along with ecological filters as significant drivers of community composition.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

María Fernanda Checa was born in Quito, Ecuador. She got her bachelor's degree in biology from the Pontificia Universidad Católica del Ecuador (PUCE), and pursued a Master's program in the Department of Entomology at the University of Florida (UF) in 2010. She is passionate about butterflies, and her career goals focus on promoting multidisciplinary research aimed towards biodiversity conservation, and implementing capacity-building programs in her home country. In order to achieve her goals, María F. has strived to get a multidisciplinary academic formation at UF. She received a joint degree in entomology (PhD program, Department of Entomology) and sustainable development (Master for Sustainable Development Practice Program MDP, Center for Latin American Studies) in 2016. Moreover, she also graduated with a certificate from the Tropical Conservation and Development Program. María has published two books and several papers in scientific and popular journals. She has also received several awards, scholarships and assistantships. In 2015, based on her professional potential and PhD dissertation, she was elected as an AAW Emerging Scholar Award Finalist by the Association for Academic Women. Furthermore, her joint research (PhD and MDP) received the Schmink Award for Innovation in Tropical Conservation and Development (UF) in 2012. She has been a teaching assistant in the Department of Entomology and research assistant in the Florida Museum of Natural History (McGuire Center for Lepidoptera and Biodiversity) for several years. In addition, María has taught courses about butterfly biology and conservation in Ecuador, and mentored several undergraduate students. This has been possible thanks to her position as associate researcher and professor at PUCE since 2006.