**Zamorano Pan-American Agricultural School**

**Department of Environment and Development**

**Environment and Development Engineering**

Graduation Special Project

# Change in diversity and abundance of butterflies over five years of monitoring on the Zamorano Agroecological Farm

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Honduras, august 2023

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**Abstract**

Conventional agriculture has significant impacts on ecosystem health thus it is necessary to balance food production with preserving natural ecosystems. The present study analyzed the impact of agroecological practices on ecosystem health via long-term continuous monitoring of butterflies as bioindicators. Monitoring was done with replacement and mark recapture. A total of 2,843 individuals were captured and 90 species registered during 7 months from 2022 to 2023, yielding a 17% recapture rate. Even though only 13.95 ha of 40 ha are under land use change, there has been measurable impact on diversity, independent of seasonal patterns. Seasonality alters the abundance and diversity of butterflies due to food availability, habitat quality, and climate conditions. Time of the day (morning vs afternoon) did not influence capture frequencies. In addition, migratory species tend to alter population dynamics and increase apparent population size estimation during the early dry season.

*Key words:* Agricultural practices, bioindicator, ecosystem health, land use change, mark and recapture.

**Resumen**

La agricultura convencional tiene impactos significativos en la salud de los ecosistemas, por lo que es necesario equilibrar la producción de alimentos con la preservación de los ecosistemas naturales. El presente estudio analizó el impacto de las prácticas agroecológicas en la salud del ecosistema a través del monitoreo continuo a largo plazo de las mariposas como bioindicadores. El seguimiento se hizo con mediante marcado y recaptura de mariposas. Se capturaron un total de 2,843 individuos y se registraron 90 especies durante los 7 meses 2022-2023, lo que arroja una tasa de recaptura del 17%. Aunque solo 13.95 ha de las 40 ha están bajo cambio de uso de la tierra, ha habido un impacto medible en la diversidad independientemente de los patrones estacionales. La estacionalidad altera la abundancia y diversidad de las mariposas debido a la disponibilidad de alimentos, la calidad del hábitat y las condiciones climáticas. El horario del día en que se realiza la práctica de captura no mostró diferencia significativa entre el horario de la mañana y el de la tarde. Además, las especies migratorias tienden a alterar la dinámica de la población y aumentan la estimación del tamaño aparente de la población durante la estación seca temprana.

*Palabras clave*: Bioindicadores, cambio de uso de suelo, ecosistema saludable, marca y recaptura, prácticas agrícolas.

## Introduction

Deforestation and habitat fragmentation are causing a dramatic decline in the amount of biodiversity on the planet, which have significant effects on how the ecosystems operate (Bunker et al., 2005). Any change in land use can damage the ecosystem's health in several ways. One of the most significant ways is by reducing the biodiversity of the ecosystem. When natural areas are converted to monocultures or simplified landscapes, the number and variety of species that can thrive in the area are reduced. This reduction in biodiversity can lead to imbalances in the ecosystem, with some species thriving and others declining or disappearing entirely. An example of these extensive practices is a study done in 1995 that investigated the presence of pesticide residues on the environment in the Choluteca River, Honduras. Results found that river water samples with higher concentrations of pesticide residues were associated with intensive agricultural production, while the lowest concentrations of pesticides were found in the small sub-watershed characterized by traditional agricultural production (Kammerbauer & Moncada, 1998). Undeniably, agricultural practices are needed, yet they must be sustainable. Otherwise, the ecosystem will be at risk. According to Salas-Zapata and Ortiz-Muñoz (2019), sustainability consists of fulfilling the needs of current generations without compromising the needs of future generations, ensuring the balance between economic growth, environmental care, and social well-being.

Members of the European Parliament agreed on a motion that mentions concern regarding the widespread use of pesticides and a lack of public knowledge about the hazards and risks of pesticide use to the ecosystem, leading to inappropriate management guidance and undervaluation of the importance of biodiversity. Due to this concern, some agronomists decided to take a different path and produce under agroecological practices. Agroecology is a new paradigm that intends to redesign farming and agricultural systems. This principle engages farmers in a radical transformation of their practices, their way of reasoning, and their participation in production and innovation processes for the ecosystem´s health (Lacombe et al., 2018).

Healthy ecosystems are essential to purifying water and air, nourishing soil, regulating climate, provide nutrients, raw materials, and resources to produce energy. They form the basis of all civilizations and underpin our economy (Chivian & Bernstein, 2010). Ecosystem changes caused by humans have the potential to disturb population dynamics and change the population size of wild species. The consequences of alterations on an ecosystem's resilience can be estimated through the monitoring of biodiversity (Oliver et al., 2015). Numerous studies indicate that biodiversity may be especially crucial for the multifunctionality of ecosystems (Lohbeck et al., 2016). Biodiversity provides the stability of natural ecosystems and their ability to supply a wide range of services and benefits to humans and other organisms.

An indicator to gauge how well the ecosystem is doing and how it changed over time is bioindicators, which include species, communities, and biological processes. However, only a certain number of biological processes, species, or groups have the characteristics to qualify as bioindicators. Their modest resiliency to environmental change makes bioindicator species accurately reflect the state of the ecosystem. Rare species or species assemblages with limited tolerances may be either too sensitive to change or too uncommon to accurately reflect the overall biotic response (Shepard et al., 2021).

Butterflies are good bioindicators due to their host plant association since they can provide insight into the health of the ecosystem as a whole. Also, monitoring butterfly populations is an essential component of their conservation. Some survey techniques are based on the presence or absence of species, whereas others measure butterfly abundance (Taron & Ries, 2015). This is why Calderón Vásquez (2020) analyzed butterfly diversity at the Zamorano agroecological farm. This farm provides students with a space to put into practice what they learn about agroecology through learning by doing modules during their second year of college. The impact of the creation of grazing areas and farm plots in previously forested areas is affecting diversity and abundance of butterflies, emphasizing the importance of conserving habitats with high cover, and allowing reforestation

(Medina et al., 2004). Students participate in butterfly monitoring, Calderón Vásquez´s 2020 results showed a decrease in species diversity during four years of monitoring, attributed to land use change. In other words, while all species still appear to be present, some have become more dominant and/or others rarer. Yet as sampling is done with replacement, this practice may inflate the number captured through pseudo replication and similarly overestimate dominance in butterflies recorded and without marking, there was no way to distinguish if students caught the same butterfly several times during the same session or in subsequent sessions (as in morning vs. afternoon of the same day). The present study provides the missing information to critically assess the previous database (Calderón Vásquez, 2020) and to adjust it. This will help to determine if the agroecological activities impact ecosystem health. The following objectives were proposed: a) study the population dynamics of butterflies in the agroecological farm from July 2022, until February 2023; b) estimate the error margin inherent in sampling with replacement in this system; c) compare the abundance and diversity of butterflies during the dry and rainy seasons.

## Methodology

### Study Area

Zamorano´s agroecological farm is in the Valle de Yeguare, San Antonio de Oriente 13°59′16.18″N y 86°58′43.86″E, department of Francisco Morazán, Honduras, with 40.03 ha of dry forest that has recovered after 30 years of lying fallow. The capture sessions, however, concentrated on an area of 10 ha, centered on the farmhouse. The annual average temperature is 24 °C and the average precipitation is 1,100 mm. There are 210 species distributed in 53 families of angiosperms. Most species are native (86%), and only 14% are introduced. Most introduced species are edible, *Mangifera indica* and *Citrus reticulata* being the most abundant (Ferrufino–Acosta et al., 2018).

### Scope of Investigation

This investigation is part of an ongoing monitoring study initiated in 2016; it will update and correct Calderon´s 2020 analysis with the new results. Examining the significant impact of pseudo-replication arising from the practice of sampling with replacement.

### Marking Process

Data collection was done with groups of second-year students during their learning by doing module on agroecology. Groups consist of 9 to 12 students; each group participates only once without any previous experience. The capturing exercise was executed with entomological nets, with a diameter of 30 cm and 80 cm in depth. Capture sessions took place from 7:00 to 9:20 a.m. and from 1:00 to 3:20 p.m., one morning and one-afternoon session every 3 weeks on average. Prior to field work, students receive an introductory talk covering capture/handling of butterflies. To earn full credit for the day, students must achieve a certain amount of captures. The amount is adjusted depending on the weather, it normally goes from 40 to 50 captures per student on a sunny or partly cloudy day. To have a basis for evaluating student effort and keep them motivated.

Identifications and data recording are handled by the instructor and the person in charge of marking. The marking process was performed using the thumb and index fingers to restrain the thorax of butterflies to draw a small mark on the underside of the fore or hind wings using a Sharpie felt pen.

**Data Recording**

The data sheet used from 2016 until 2021 for capturing data was used again, but a column was added for the number of recaptures. Consequently, the new database includes columns for the name of the species, the number of captures, the number of recaptures, the date, and time of day. In addition, there was one species and three families not marked due to their fragility or color characteristics, considered too dark for the mark to be visible. The species were *Cissia similis,* and all species in Hesperidae, Riodinidae, and Lycaenidae.

**Contrast Between Dry and Rainy Season**

The difference between the rates of recaptures during the dry and rainy seasons served to probe if the climate is relevant to the abundance and richness of butterflies. This study had as dependent variables the species and individuals, while the independent variable was the season. By using the precipitation levels shown in Table 1, it was determined which months were considered rainy season and dry season.

**Table 1**

*Precipitation during the seven months of study*

| Month | Precipitation (mm) |
|---|---|
| July | 102.37 |
| August | 165.50 |
| September | 215.83 |
| October | 229.28 |
| November | 37.20 |
| January | 8.55 |
| February | 10.02 |

**Data Analysis**

This is an observational study since there was no manipulation of the variables. Variables were analyzed with an analysis of variance (ANOVA). Since field work took place during morning and afternoon sessions, it was also analyzed whether the time of the day alters abundance and the number of recaptures. ANOVA was used to evaluate the effects of morning versus evening on abundance. This analysis was done by the generalized linear model (GLM) procedure on the program SAS® version 2020.

**Correction for Recaptures**

Microsoft Excel® version 2020 was used to calculate the variance stemming from recaptures, with a level of confidence of 95%. The actual number of captures per species was used for Formula 1. The number was obtained by doing a subtract of the number of recaptures from the total captures. Species that were not marked were excluded from the analyses, making sure residuals were normally distributed.

$$\ell = \left(\frac{Va - Ve}{Ve}\right) \times 100\% \qquad [1]$$

Where:

$\ell$ = correction adjustment

Va = approximate value

Ve = actual value

**Lincoln Index**

The Lincoln Index was used to estimate the population size based on the data recaptures during the 7 months of this study. This index is a method of estimating animal populations by the process of capturing, marking, and releasing them back into the population from the point where the marking was done, and using the proportion recaptured to estimate the population as a whole (Hayne, 1949). Formula 2 was applied to the data obtained from each month of field work.

$$N = \frac{n1 \times n2}{\mathrm{m}} \qquad [2]$$

Where:

N = total population size of animal of interest in the study site

n1 = number of animals captured on the first trapping

n2 = number of animals captured on the second trapping

m = number of marked animals in the sample recaptures on the second trapping

## Results and Discussion

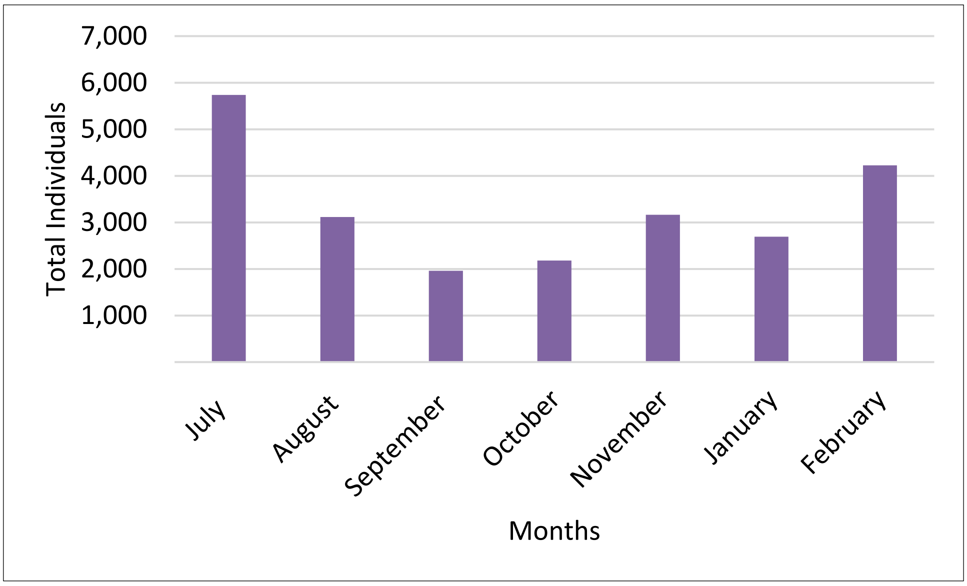
### Population Dynamics

Butterflies rely on specific host plants for food and breeding, and these plants may only be available during certain seasons. Butterfly population size increases during rainy season since the weather affects the host-plant quality and population size (Kingsolver, 1989). During dry season months some species may enter a state of dormancy, while others may migrate in search of food and breeding opportunities.

After applying the Lincoln Index to the data obtained, results showed that population size during the rainy season was elevated, as expected. Yet during the dry season population size estimation was higher than expected. As shown in Figure 1, there was an apparent increase during the dry season, which is not expected for butterfly population dynamics.

**Figure 1**

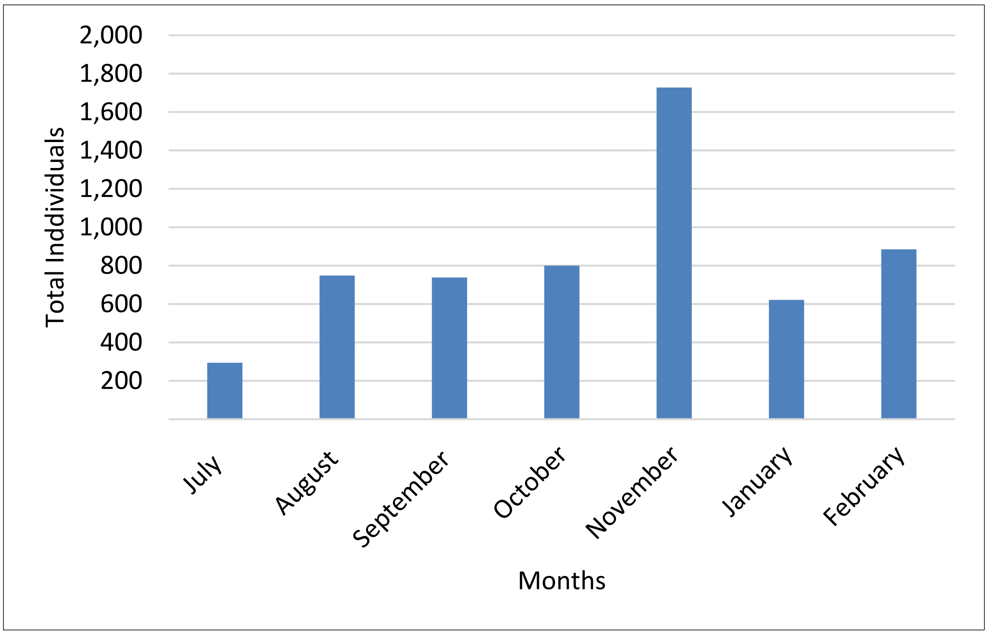
*Population dynamic of butterflies at the Zamorano Agroecological Farm estimated by the Lincoln index (July 2022- February 2023)*



An explanation for this “phenomenon” is migration. According to Cambraia Alves et al. (1920), there is a tendency for some insects including certain species of butterflies and dragonflies to journey into localities that will provide them with food. But not all species migrate, so for them to survive until the rainy season they will estivate. Thus, the estimation of the population size during the early dry season increases due to the movement of marked individuals out of the sampling area, and a continuous flow of unmarked individuals into the area. Meaning we are sampling not the local population, but a much larger one outside the intended area. To test it and see the contrast, the Lincoln index was used on two species present during the whole investigation, Figure 2 *Anartia fatima* (a non-migratory species) and Figure 3 *Eurema daria* (a migratory species).

**Figure 2**

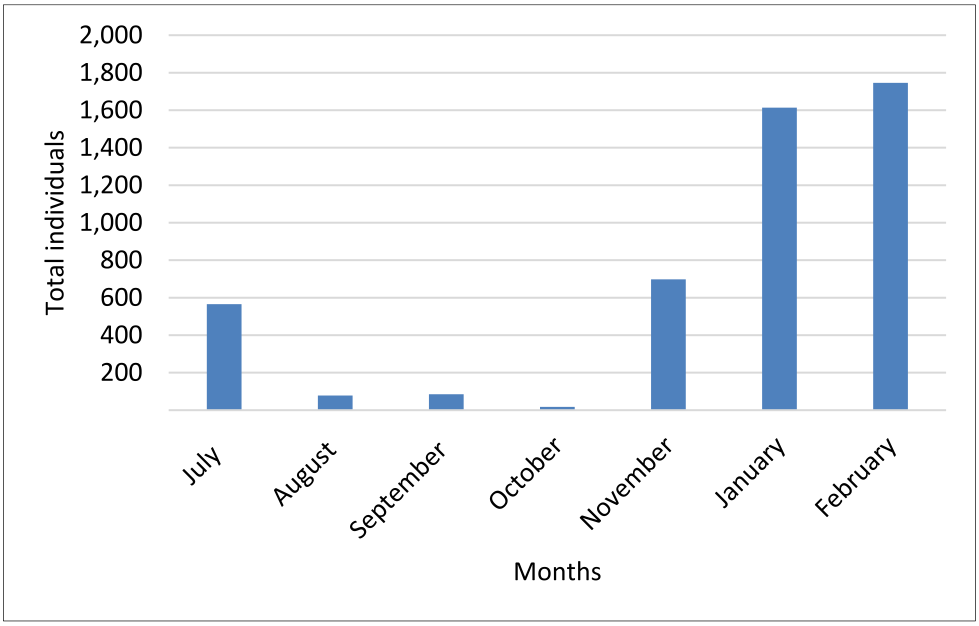
*Population dynamic of the non-migratory species Anartia Fatima estimated by Lincoln Index (July 2022- February 2023)*




According to Cambraia Alves et al. (2020), *Anartia fatima* is considered as non-migratory or as a sedentary species. This species shows the expected population dynamic considering the climate conditions they were exposed to.

**Figure 3**

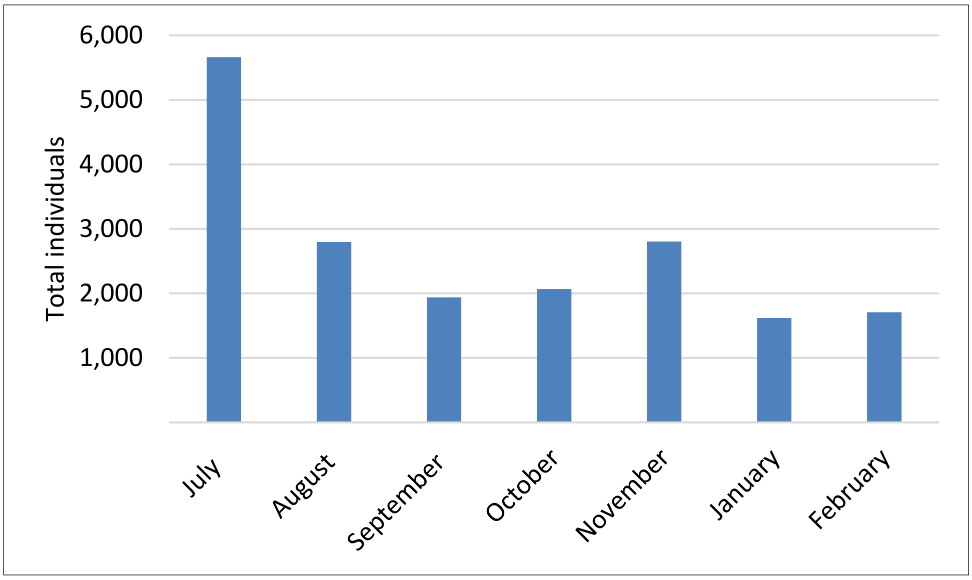
*Population dynamic of the migratory species Eurema daria estimated by Lincoln Index (July 2022-February 2023)*




*Eurema daria* switches movement northward in the early fall and southward during the late fall (Walker, 2001). This is clearly shown in Figure 3 where there is an increase in captures and a decrease in recapture rate during the months of January and February. After analyzing this factor, butterflies' population size was estimated once more without including *Eurema daria* data in the analysis (Figure 4), to show how by not including this migratory species during the whole study, the graph reflects a decrease that is aligned. Thus, migratory species data raised the population size estimation during the dry season, and omitting the migratory species brought the abundance estimate in line with the diversity trend.

**Figure 4**

*Population dynamic of butterflies without including Eurema daira, using the Lincoln Index (July 2022-February 2023)*

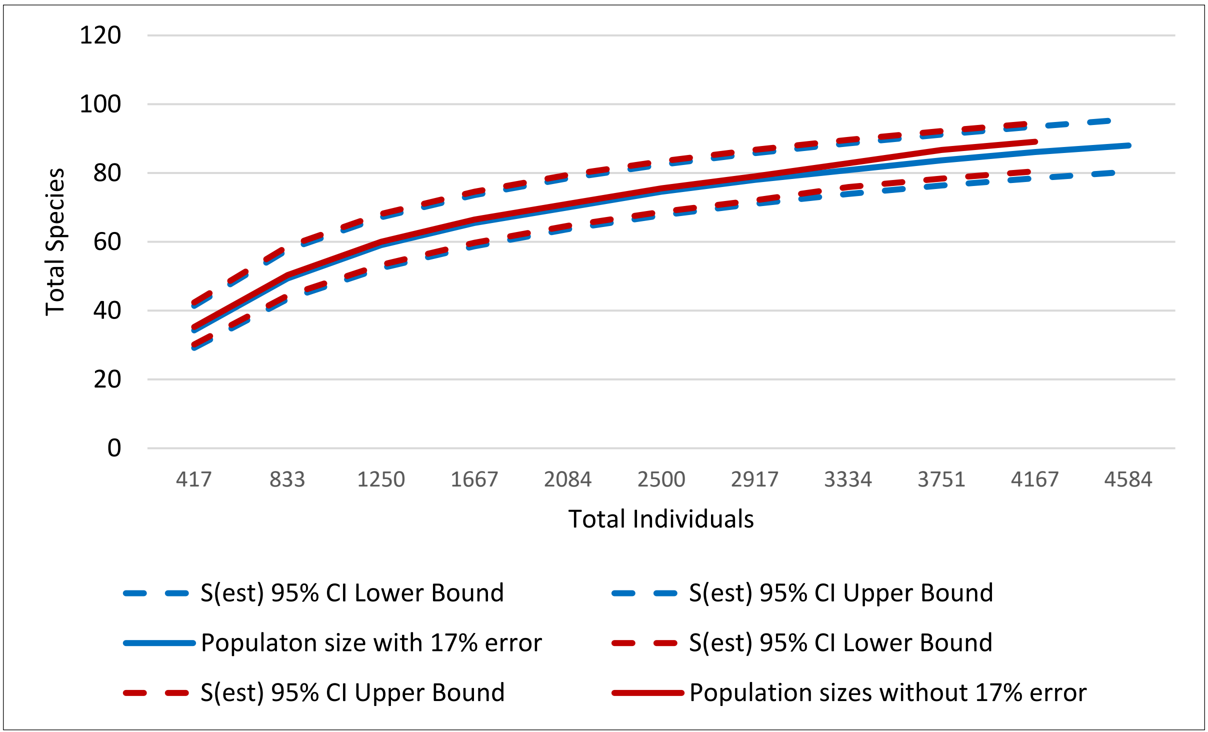



**Error from Recaptures and Confidence Interval**

Recaptures of marked butterflies revealed that the total number of butterflies captured was overestimated by 17%. Consequently, the total numbers of captures reported by (Calderón Vásquez, 2020) include about 17% recaptures, meaning that they need to adjust downward by 17% for a net effect of steepening the species accumulation curves slightly but not raising them, as the curve height (the total species number) was unaffected. The experimental value was the number obtained from the total of individuals captured without considering the recaptures. The exact value was the number of individuals captured minus the number of recaptures shown in Figure 5. However, the number of species is not affected by recapture, corroborating that the effect on the graph is negligible. The conclusion of decreased diversity by Calderón Vásquez, 2020) was thus robust and substantiated. The addition of another year of reduced diversity also strengthens the previous conclusion that diversity in the sampling site had been reduced.

**Figure 5**

*Species accumulation curve for butterflies obtain from the experimental value with a 17% adjustment compared to the unadjusted curve, at the agroecological Farm of Zamorano (2022- 2023). The net effect on the graph is, however, negligible*




In this analysis, the margin of error formula was additionally employed, encompassing a 95% confidence interval centered around the mean. This formula defines the upper limit for the anticipated variance between the actual number of species and the observed value. A wider margin of error corresponds to diminished confidence in the sample estimates aligning closely with the actual values. This effect is particularly noticeable in graphical representations, such as the accumulation curve. However, the inclusion of recaptures, as demonstrated in Table 2, had only a minimal impact on this phenomenon when contrasted with outcomes derived from the precise dataset. Yet the curves showed significant difference ($P > 0.05$) on the error margin, meaning that the adjustment for sampling with replacement reduces the number of butterflies sampled represented on the graph but doesn't affect the total number of species encountered. This is because each species has a different probability of being sampled, regardless of whether other individuals of the same species have already been sampled.

Thus, using a sampling method with replacement reduces the number of distinct individuals sampled but does not affect the total number of species encountered. This is because the number of species encountered is determined by the diversity of the habitat being sampled and the effectiveness of the sampling method, which are not affected by sampling with replacement.

**Table 2**

*Statistical results from experimental and exact data (July 2022- February 2023)*

| Data analyzed | Experimental data (%) | Exact data (%) |
|---|---|---|
| F(z) | 0.97 | 0.97 |
| X bar (mean) | 6.71 | 5.73 |
| Sample size | 491 | 491 |
| Standard deviation | 12.63 | 10.43 |
| Margin of error | 0.01 | 0.01 |
| Lower bound | 6.69 | 5.71 |
| Upper bound | 6.73 | 5.74 |

**Effects of Season and Time of the Day on Abundance**

For this analysis, migration status was considered once again, excluding data from *Eurema daria*, to estimate the abundance of non-migrant individuals. The analysis of the difference in abundance during the dry and rainy season show a significant difference (P < 0.05). Additionally, the time of day shows no significant difference in abundance (P > 0.05), Table 3.

**Table 3**

*Abundance analysis on season and time of day done by GLM procedure*

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Time of day | 1 | 915.18 | 915.18 | 0.56 | 0.4739 |
| Seasons | 1 | 13588.36 | 13588.36 | 8.29 | 0.0182 |

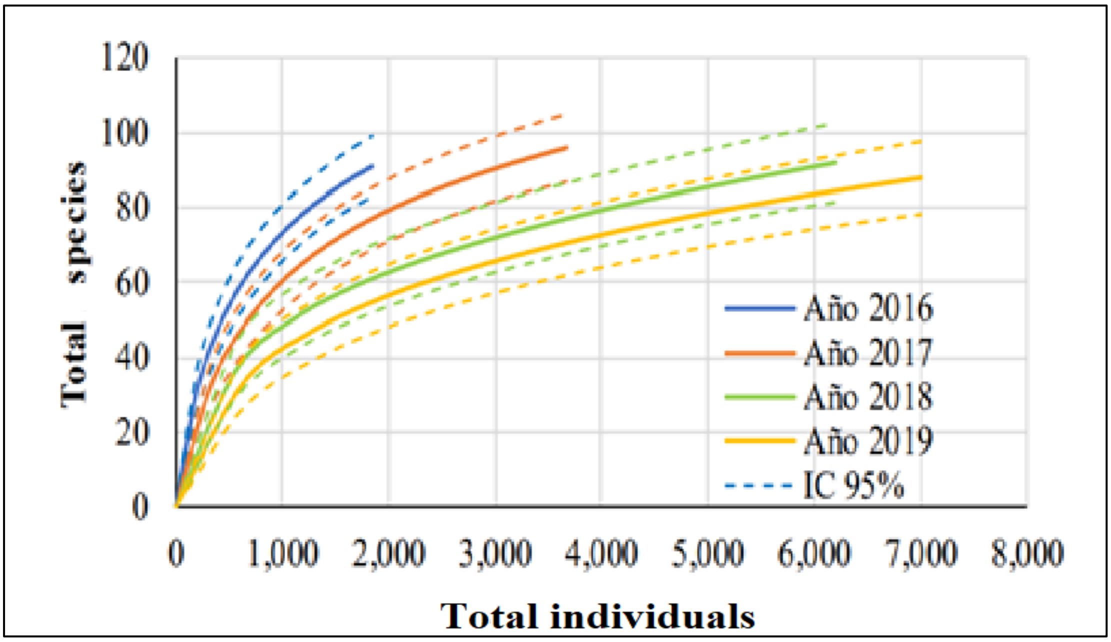
Seasonality is reduced greatly in the tropics, many species remain active throughout the year and reproduce continuously, even though wet and dry seasons can differentially affect both species' abundance and diversity (Grotan et al., 2012). Results showed 558 individuals captured during the dry season with a total of 48 species and in the rainy season 2,285 individuals were captured with 74 species registered. To analyze diversity a Chi-Square test was applied on the number of species identified during dry and rainy seasons, showing significant differences ($P < 0.05$) in the number of species for each season. Data shows an increase in the richness and abundance of butterflies during the rainy season, proving that seasons do have effects on the abundance and diversity of butterflies. Tropical butterflies adapt to their environment to improve their survival chance. Vicencio et al. (2014), studied the development of butterfly larvae at different temperatures and found that the internal hormonal signal can be modified by the environment. Changes are triggered by hormone signals that transmit information about temperature to the butterflies' tissues.

**Adjustment of Previous Results**

Calderón Vásquez (2020) showed a clear decrease in biodiversity attributed to land use change from 2016 until 2019 (Figure 6). Throughout the four years she analyzed, there were 150 species registered, however there were less than 100 species registered in any given year. Accumulation curves from the years 2016, 2017, and 2018 showed significant differences ($P < 0.05$), however the ones from 2018 and 2019 were not significantly different from each other but both were significantly below the first years, meaning that either the common species got more abundant and/or other species became rarer, a shift to “pest” species. Figure 7 shows land use in 2019 for the farm, with 38% production and grazing area, 59% secondary forest and scrub, including Teak plantation, and 2%  road (Calderón Vásquez, 2020).

**Figure 6**

*Species accumulations curves, by year, for butterflies from 2016 until 2019 from Calderon (2020)*

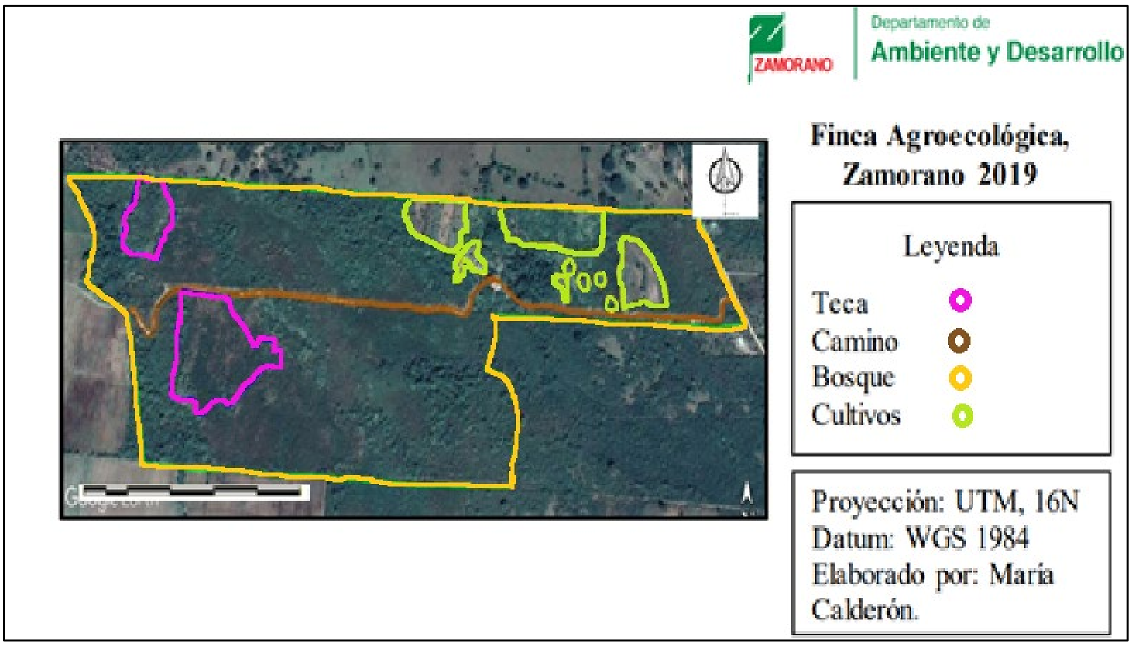



Note.

Source Calderón Vásquez (2020)

**Figure 7**

*Land use at the Zamorano Agroecological Farm in 2019*




Note.

Source Calderón Vásquez (2020)

For the present study, a new map and accumulation curve was made to compare results over time (Figure 8). The agroecological farm has as objective promoting knowledge to students and other small producers of the adaptation to climate change, mitigation of risks and disasters. Some of the practices done on the farm are composting, vermiculture, production of microorganisms, direct sowing, minimum tillage, permanent ground cover for crops, polyculture, use of live barriers, apiculture (non-native bees) permaculture, and sustainable livestock.

The productive area for each agroecological practice was measured with a Geographic Information System, QGIS® (Table 4). Giving a total of 1.29 ha under production practices from the 40 ha the farm has in total. However, some areas are not under production but have been altered in some way, adding up to 13.95 ha subject to land use change; 5.68 ha of these are in the area where butterfly capture is focused.

**Table 4**

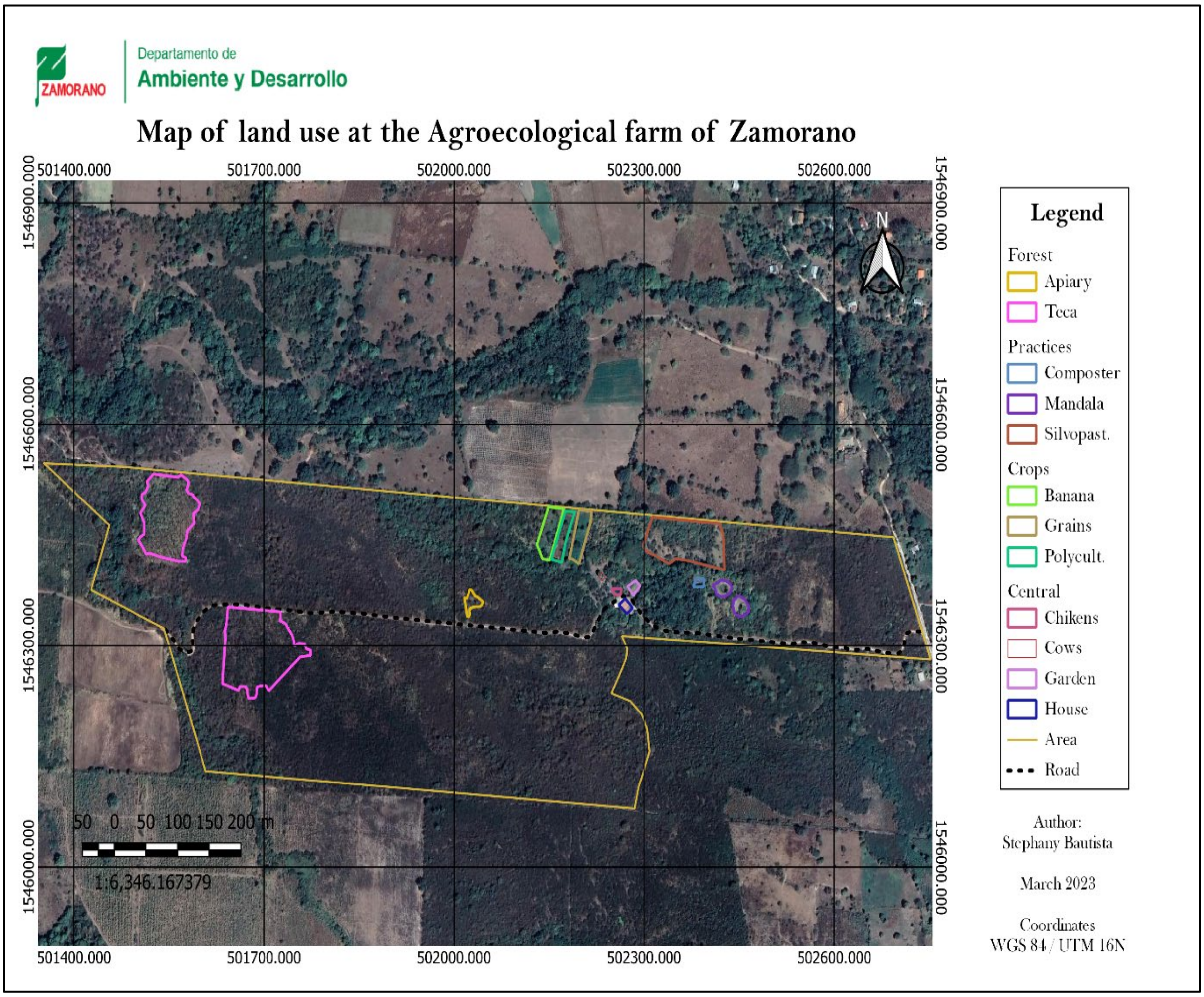
*Productive practices and area at the agroecological farm of Zamorano*

| Practices | Area (ha) |
|---|---|
| Banana production | 0.18 |
| Basic grains | 0.14 |
| Polyculture production | 0.1 |
| Mandalas | 0.09 |
| Pastures | 0.67 |
| Composter | 0.02 |
| Family garden | 0.02 |
| Stables | 0.01 |
| Chicken coop | 0.01 |
| Apiary | 0.05 |
| Total | 1.29 |

Land use change can further isolate butterfly populations and reduce gene flow between them. On a larger scale, this can lead to reduced genetic diversity and an increased risk of inbreeding depression. Also, when natural grasslands are converted to agricultural land or pastures, the vegetation may become less diverse, which can lead to a decline in butterfly populations that depend on specific plant species for food, shelter, or the contamination of their food.

**Figure 8**

*Land use at the Zamorano agroecological farm in 2023*




The new accumulation curve was made with the program EstimateS® (version 9.1), with data recovered from the seven months of the study; missing data to get an annual curve of the population dynamic of butterflies was obtained from the previous year. An exact value of 2,843 individuals were captured, and 90 species were registered during the investigation, similar to the accumulation curve from 2017 and 2018 from the previous investigation (Figure 9). The 17% recapture rate was not a factor in the significant measurable decrease in diversity since the establishment of the farm. Since, as mentioned before, the practice concentrates on an area of 10 ha around the farmhouse, and 5.68 ha of those are under agroecological practices.

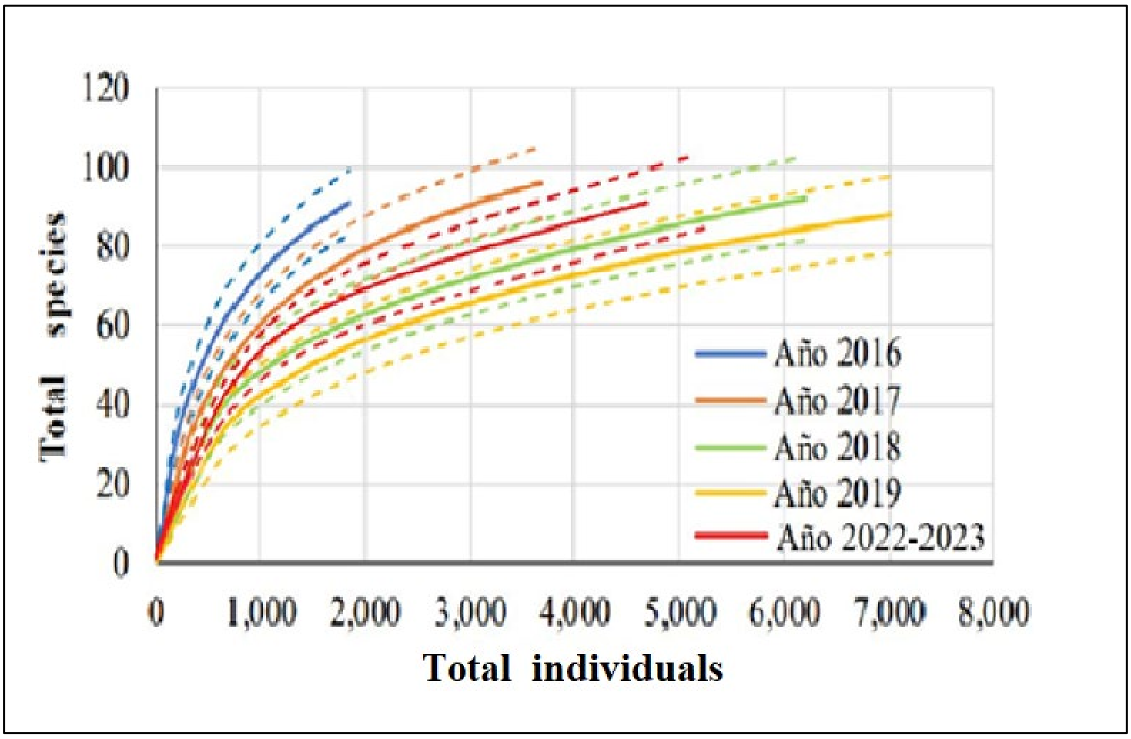
Agroecological practices focus on creating a sustainable and diverse agricultural system that works in harmony with nature, rather than against it. Even so, these practices seem to have measurably eroded local biodiversity, reflected in the lower accumulation curves. In contrast, normal agriculture often relies on a high level of chemical input such as pesticides and fertilizers, leading to even more serious environmental impacts, health risks, and loss of biodiversity in agrosystems (Dudley & Alexander, 2017). A study of analysis of environmental damage on tropical forest conducted in North Sumatra Province showed that due to conventional agricultural damage there was a loss of flora and fauna, a reduction of oxygen productivity and $CO_2$ absorption, hydrology cycle disruption, causing catastrophes such as floods, droughts, landslides, erosion, and sedimentation, changes in environmental geology including aesthetic conditions, a reduction of soil productivity, microclimate change, as well as various socioeconomic problems (Wasis et al., 2019).

Overall, agroecological practices have been shown to promote more sustainable and resilient ecosystems, while conventional practices tend to degrade ecosystems over time. So, if this investigation was conducted on a conventional farm, the most likely result will show a more prominent decrease in biodiversity and consequently reduced ecosystem health that can have far-reaching consequences beyond the environment, affecting human societies and economies as well. A study done by Hernández et al. (2005), characterized and compared the community of diurnal butterflies in a fragmented landscape in Matiguas, Matagalpa, in the north of Nicaragua; sampling six types of different habitats disturbed landscapes. Their results showed a total of 843 individuals of diurnal butterflies and 57 species registered, thus most species recorded were characterized by being from disturbed areas (41% of individuals) and few from non-disturbed areas (24% of individuals).

Some of the most representative genders, and also common during this study, were *Cissia* and *Eurema* which are usually found in areas that have been altered by man for pasture areas (Wezel et al., 2014), as is the case for the Agroecological Farm of Zamorano. Although fragmented landscapes have generally been assumed to be of little value for conservation, both studies showed the preference butterflies have toward non disturbed areas and how land use change decreases diversity at an ecosystem.

**Figure 9**

*Species accumulation curves by year for butterfly populations at the Zamorano Agroecological Farm, 2016-2023*



**Conclusions**

Despite ecologically friendly agricultural practices, there has been a significant reduction in butterfly diversity on a local scale at the Agroecological Farm.

The sampling with replacement in combination with mark-recapture showed that the total number of individuals recaptured is 17%, hence the number of different individuals captured needs to be adjusted downward by 17%. However, this adjustment doesn't affect the number of species registered, or the shape of the accumulation curves. It just lowers the total individuals capture number.

Seasons do influence significantly on abundance and diversity of butterflies. During the dry season butterfly populations decline because of food availability, habitat quality, and climate conditions.

## Recommendations

Continue with the monitoring of butterflies as bioindicators at the agroecological farm to keep track of the effects of any future change in land use.

Apply the 17% adjustment to the data collected in the future to get a more accurate population estimation of butterflies at the Agroecological Farm of Zamorano.

Use different colored markers for each capture period, to determine mortality, longevity, and life cycles of butterfly species at the farm.

Identify migratory species of butterflies at the Agroecological Farm, to analyze their behavior, during which season and for how long do they stay at the farm. This will also improve understanding of certain species' population dynamics throughout seasons.

In order to consistently apply agroecological practices on the farm, it is essential to formulate a comprehensive plan addressing the potential long-term implications of land use change. This entails a thorough assessment of the foreseeable outcomes associated with any contemplated alterations in land use.

Promote more practices that increase pollination such as permaculture and diversification of vegetation to improve the health of this ecosystem.

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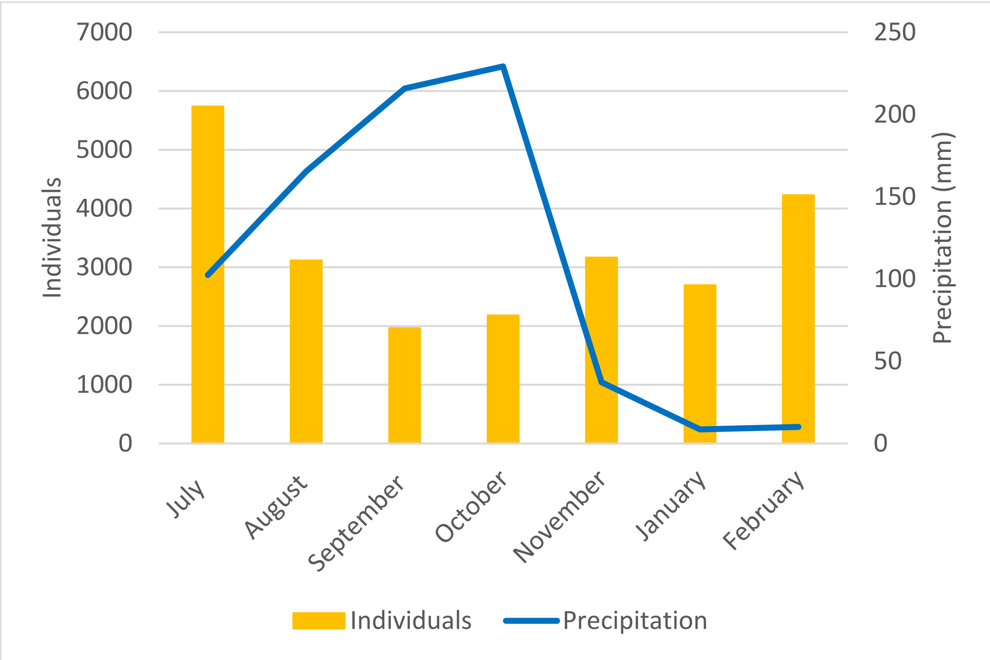
**Annexes**

**Annex A**

*Graph of population size estimation done by Lincoln Index with precipitation (mm) data for the Agroecological Farm from July 2022 to February 2023*



**Annex B**

*List of taxa found during the study*

| **Taxa** | | |
|---|---|---|
| *Adelpha bassiloides* | *Euptoieta claudia* | *Parides eurythalion* |
| *Adelpha naxia* | *Eurema daira* | *Parides eurymides* |
| *Agraulis vanillae* | *Eurema dina* | *Parides montezuma* |
| *Anartia fatima* | *Eurema lacina* | *Phoebis argante* |
| *Anartia jatrophae* | *Eurema lisa* | *Phoebis sennae* |
| *Anteras rurina* | *Eurema mexicana* | *Riodinidae* |
| *Anthanassa ptolyca* | *Eurema nise* | *Siproeta epaphus* |
| *Anthanassa tegosa* | *Eurema proterpia* | *Siproeta stelenes* |
| *Anthanassa texana* | *Eurema salome* | *Taygetis andromeda* |
| *Anthanassa tulcis* | *Eurema sennae* | *Tegosa anieta* |
| *Appias drusilla* | *Ganyra josephae* | *Temenis laothoe* |
| *Ascia* | *Greta morgane* | *Tessalamia* |
| *Battus polydamas* | *Greta otto* | *Zerene cessonia* |
| *Biblis hyperia* | *Hamadryas guatemalense* | |
| *Chlosyne erodyle* | *Hamadryas februa* | |
| *Chlosyne janais* | *Hamadryas glauconome* | |
| *Chlosyne lacina* | *Heliconius charithonius* | |
| *Cissia similis* | *Heliconius erato* | |
| *Colias cessonia* | *Heliconius hecalesia* | |
| *Colobura dirice* | *Hesperidae* | |
| *Danaus gillippus* | *Historis acheronta* | |
| *Danaus plexippus* | *Junonia evarete* | |
| *Diaethra astala* | *Leptophobia arepa* | |
| *Dione juno* | *Leptophobia arite* | |
| *Dircenna chiriquensis* | *Lybetheana carinenta* | |
| *Dismorphia amphinome* | *Lycaenidae* | |
| *Doxocopa callianaria* | *Lycorea cleobaea* | |
| *Dryadula phaetusa* | *Marpesia petreus* | |
| *Dryas iulia* | *Mechanitis polymnia* | |
| *Dynamine glauce* | *Megneuptychia libye* | |
| *Dynamine postverilum* | *Melete lysemnia* | |
| *Dynamine dyonis* | *Mestra dorcas* | |
| *Dynamine postverite* | *Microtia elva* | |
| *Dynamine theseus* | *Nathalia iole* | |
| *Eunica monima* | *Nica monimus* | |
| *Epiphele adrastra* | *Eunica monimus* | |
| *Eueides isabella* | *Papilio thoas* | |
| *Eunica modesta* | *Paraeuptychea hezione* | |
| *Euptoieta claudia* | *Pareuptychia metaleuca* | |

**Annex C**

*Paraeuptyhia hezione representing the mark done on each individual from the study.*

**Annex D**

*Group of students from sophomore year and Dr.Eric Van den Berghe during the marking and recapturing exercise at the farm*

**Annex E**

*Population variation of the species Eurema daria, with application of the Lincoln Index (July 2022-April 2023), showing apparent migration periods*