Comparing Road Mortality of Lepidoptera between the Interoceanic Highway and a Secondary Growth Forest Road

Vanessa Portugal

Corresponding emails: vportugal201@gmail.com & info@sustainableamazon.org

Abstract

This study examines the impact of roads on Lepidoptera mortality and abundance in the Peruvian Amazon. Using transects along the Interoceanic Highway and a secondary dirt road, Lepidoptera remains and live specimens were collected. Results showed no significant difference in the abundance of live Lepidoptera between the two sites, but mortality was significantly higher near the highway. Pieridae was the most affected family along the highway, while Nymphalidae dominated in the forest road. The findings suggest that highways, with increased traffic, contribute to higher insect mortality, underscoring the need for further research on invertebrate road impacts in tropical ecosystems.

Introduction

Amazonia, a tropical forest region stretching across nine of the 12 South American countries, contains the most tropical biodiversity on Earth (Carlos et al., 2010). Peru alone is one of the ten most biodiverse countries in the world (*Comisión Nacional de Diversidad Biológica*). The Peruvian Amazon covers roughly 60% of the country, housing around 12,810 species (The Rainforest Trust).

To ensure the protection and conservation of these diverse species and their habitats, bioindicators are used to reveal the state of environment and act as first-line indicators of any disturbances to the environment due to anthropological or ecological perturbations. Insects are well suited for such monitoring due to their abundance and ecological importance (Chakravarthy et al., 1997). Lepidoptera specifically are widely used as bioindicators due to their distinguishable, often colorful appearance that makes them relatively easy to identify, thus simplifying the process of using them to detect changes in habitats, forest disturbance, heavy metal pollution, and environmental quality (Sajjad 2020).

Along with thousands of wildlife species, about 1.5 million people also live in the Amazon rainforest region in Peru (ARC Amazon). To connect those living in this region with other regions in Peru and with the neighboring country of Brazil, the Interoceanic Highway was completed in 2011. By spanning 2,600 km from thee three Peruvian ports of Juan de Marcona, Matarani, and Ilo to Brazil, the project aimed to expand ecotourism and trade (Bandura & Murphy, 2020). The highway is said to reduce transportation costs, offer more opportunities to consumer, and generative competitiveness for companies (CAF, 2013). In addition, Peruvians seeking economic gain migrated from the Andean highlands to fertile lowlands for better work opportunities. However, the highway made accessing regions of the Peruvian Amazon that were previously only reachable by river more achievable by vehicle. This saw an increase in illegal exploitation of wood, gold, and other natural resources (Bandura & Murphy, 2020). Besides modification of the natural landscape, the negative effects of roads on animal abundance outweighs the positive effects by a factor of 5 (Fahrig & Rytwinski, 2009). Animal mortality generally increases with traffic volume in particular (Trombulak & Frissell 2000; Rosen & Lowe, 1994; Fahrig et al., 1995). However, most studies investigating road mortality do so in temperate ecosystems and focus on taxa such as mammals, birds, and reptiles (Monge-Nájera, 2018). In fact, from 2011-2015, only five out of 215 studies investigating the effects of roads on wildlife focused on invertebrates and 76% of the total studies were conducted in North America or Europe (Bennett, 2017). In a literature review of tropical region studies, only one out of 73 studies included results of invertebrate road mortalities (Monge-Nájera, 2018).

In this study, I compare the mortality and abundance of Lepidoptera between transects near the Interoceanic Highway and a dirt road within a secondary growth forest. I chose to focus on Lepidoptera given their diversity in the Madre de Dios region, their ecological importance as bioindicators, and the current limited quantity of data on Lepidoptera road mortality. By walking along 4 total transects, collecting Lepidoptera remains, and capturing live Lepidoptera within the transects, I aim to evaluate whether there is a difference in mortality and abundance between the two transect habitats, along with analyzing which Lepidoptera families are most represented in the data.

Methods

Study Sites

Two study sites, or transects, were selected along the Interoceanic Highway near the town of Monterrey, about a 30-minute walk from the Finca Las Piedras research station. The two transects were located across from each other with the left transect starting at 12.21634 S, 69.13294 W and ending at 12.21509 S, 69.13225 W. Two other transects were selected along a dirt road within the secondary growth forest property of Finca Las Piedras. These transects were also located across from each other with the left transect starting at 12.22675 S, 69.11649 W and ending at 12.22547 S, 69.11714 W. All four transects were approximately 500 feet long and 1.5 m wide. The width was measured starting from the edge of the highway/road and horizontally away from the highway/road itself. The lengths were determined using a mobile app "GPS Tracks" and were accurate +/- 15 feet. The widths were measured with a measuring tape. Sticks were placed at the beginning and ending and at random intervals along the length of the transects to serve as boundary and width markers. These two sets of two transects were chosen for their difference in vehicular traffic and habitat in order to compare mortality and abundance of Lepidoptera. The highway is naturally used by more vehicles than the road as it is paved and wider, while the road is occasionally used by smaller personal vehicles. The two areas also have different types of vegetation as the highway is near human settlements while the road its within an area dedicated to regeneration of native plant species.

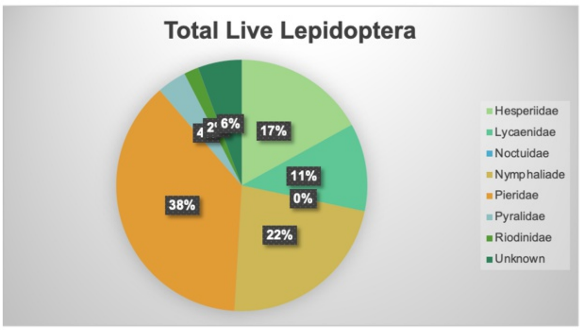
Data Collection

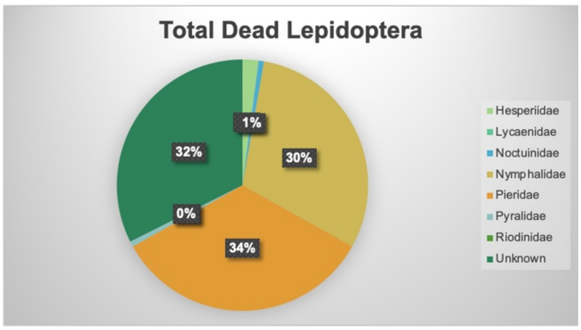
There were eight data collection dates on 7/11, 7/15, 7/18, 7/29, 7/31, 8/2, 8/5, and 8/7. The time range for collection was 9:00 am to 4:00 pm. On a typical collection day, I went to one of the sets of transects in the morning. I wrote down the start time on my phone and started a timer on my phone. I walked down the center of the left transect at a consistent pace. I focused on looking down for Lepidoptera wings or other remains while also keeping track of Lepidoptera that flew within my transect. Whenever I stopped to collect any Lepidoptera remains or capture a live Lepidoptera with my butterfly net I also stopped the timer on my phone. Using forceps, I placed remains and live Lepidoptera in envelopes labeled with the date of collection and which transect site the sample was collected from. Specimens that were located near each other and were of the same species were counted as a single individual. Once I reached the end of one transect, I stopped the timer and noted the time on my phone. I aimed to finish each transect in 30 minutes of walking time (not including the time I took collecting remains or catching live specimens.) I then walked across the highway or road to the beginning of the next transect. I repeated the same procedure at this transect. In addition, I monitored the number of vehicles that passed by for the first hour of collection by marking tallies on my arm with a pen. I visited the second set of transects in the afternoon and repeated the same process. Which set of transects, highway or road, I visited in the morning or afternoon alternated every collection day so that in the end I visited the highway transects four times in the morning and four times in the afternoon and the same frequency for the road transects.

However, data collection was interrupted on 8/5 in the highway transects. On this day, I had finished walking the left transect and was in the process of walking the right transect when a few dogs chased after me. I could not return to the transect for some time and the weather had turned unpleasant, thus rendering me unable to complete this transect that day.

After collecting specimens, I identified each one to the best of my ability

Figure 1: Families represented in the data set of total number of live and dead Lepidoptera, total live Lepidoptera, and total dead Lepidoptera.





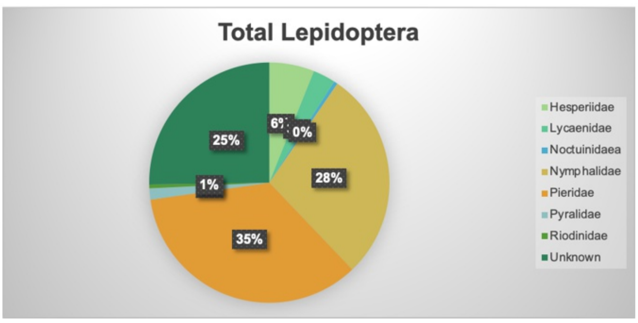
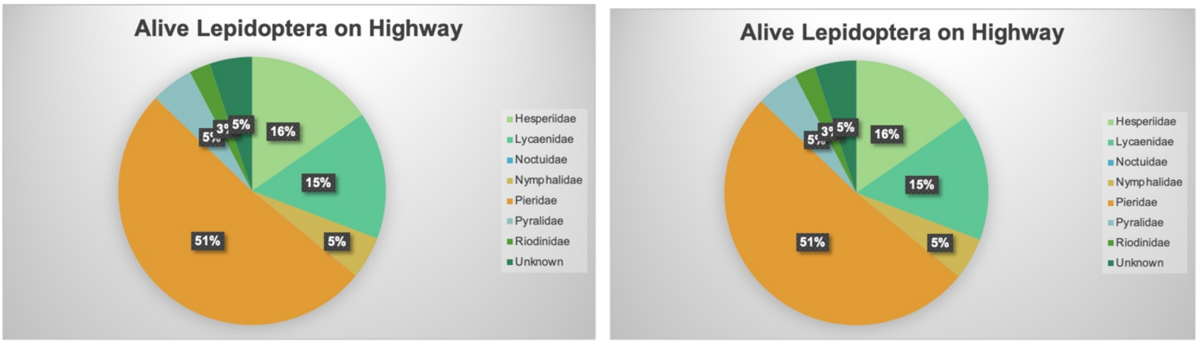


Figure 2: Families represented in the data set of alive Lepidoptera between the highway and road transects.



with the assistance of the Lepidoptera team, online guides, and iNaturalist. Photos of each specimen were also taken using my phone.

Results

After eight data collection days, I gathered 198 specimens. The families represented in thee data are *Hesperiidae, Lycaenidae, Noctuidae, Nymphalidae, Pieridae, Pyralidae*, and *Riodinidae*. There is also an additional group I labeled "Unknown" to represent specimens that were not identified. As seen in Figure 1., out of the 198 specimens, both dead and alive, *Pieridae* made up 35% of the individuals, followed by *Nymphalidae* at 28%, Unknown at 25%, *Hesperiidae* at 6%, *Lycaenidae* at 3%, *Pyralidae* at 1.5%, and both *Noctuidae* and *Riodinidae* at 0.5%. Of the live Lepidoptera, *Pieridae, Nemphalidae* and *Hesperiidae* were the largest groups. Of the dead Lepidoptera, *Pieridae*, "Unknown", and *Nymphalidae* were thee largest.

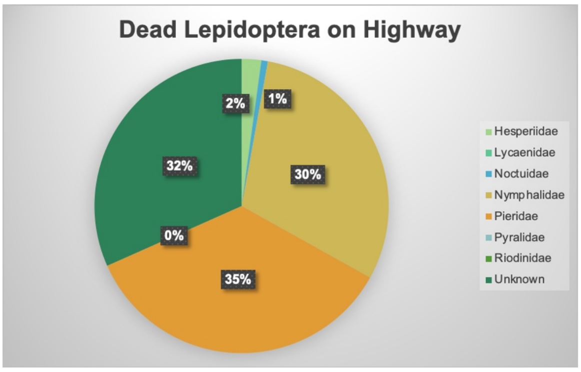
By separating the data of live Lepidoptera by transect type, we see in Figure 2 that *Pieridae* is the most represented family on the highway whereas it is not represented in the road. On the other hand, *Nymphalidae* is the largest family represented in the road while only making up 5% of the live Lepidoptera in the highway. To test if there is a significant difference between abundance of live Lepidoptera between the highway and road transects I conducted a Shapiro test using R and found my data was normally distributed. I then ran a t-test and received a p-value of 0.5311. As $p > 0.05$, this suggests there is not a significant difference between abundance between the highway and road. Exact values of these tests can be found in Figure 3.

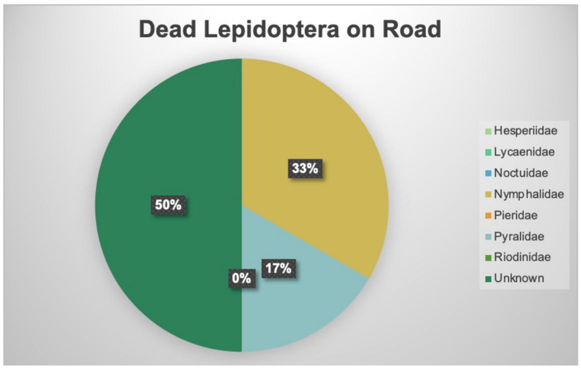
Figure 3: R code with the results of the Shapiro test and t-test for live Lepidoptera on the highway and road.

```
+   shapiro_test(number_in)
# A tibble: 2 x 4
  transect variable  statistic     p
  <chr>    <chr>         <dbl> <dbl>
1 highway  number_in     0.942 0.630
2 road     number_in     0.856 0.110

> t.test(number_in~transect,data=Data1)

        Welch Two Sample t-test

data:  number_in by transect
t = 0.64232, df = 13.931, p-value = 0.5311
```

Separating the data of dead Lepidoptera by transect type, we see in Figure 4 that both share "Unknown" and *Nymphalidae* as two of the largest groups represented. However, 35% of dead individuals on the highway are *Pieridae*, whereas *Pieridae* is not represented on the road transects. Instead, *Pyraildae* makes up the third group on the road. Comparing this to the data of alive individuals collected at

Figure 4: Families represented in the data set of dead Lepidoptera between the highway and road transects.





each transect, it follows that since the largest group of live individuals on the highway belong to the family *Pieridae* then the most represented family in the group of dead individuals on the highway also belong to *Pieridae*. Meanwhile, *Nymphalidae* are the most represented of the live Lepidoptera while the most represented in the dead data set was the "Unknown" group.

This data was found to not be normally distributed by the Shapiro test on R as seen in Figure 5, so I used the Mann-Whitney U test to test the relationship between dead Lepidoptera in the highway transects and the road transects. I received a p-value of 0.000771 ($p < 0.05$.) This suggests there is a significant difference

Figure 5: R code with the results of the Shapiro test and Mann-Whitney U test for dead Lepidoptera on the highway and road.

```
+   shapiro_test(number.in)
# A tibble: 2 x 4
  transect   variable  statistic        p
  <chr>      <chr>         <dbl>    <dbl>
1 "highway " number.in     0.926 0.482
2 "road"     number.in     0.628 0.000337

> wilcox.test(number.in~transect,data=Data, exact=FALSE)

        Wilcoxon rank sum test with continuity correction

data:  number.in by transect
W = 64, p-value = 0.000771
```

between the number of dead Lepidoptera at each transect type. Since there was no significant difference between live Lepidoptera at each transect type but there is for dead Lepidoptera, this suggests the highway is playing a role in Lepidoptera mortality.

Figure 6: R code with the results of the Spearman test testing for correlation between dead and alive Lepidoptera in the road transects (top) and highway transects (bottom).

```
> cor.test(x,y,method="spearman",exact=FALSE)

        Spearman's rank correlation rho

data:  x and y
S = 72.453, p-value = 0.7455
alternative hypothesis: true rho is not equal to 0
sample estimates:
      rho
0.1374643

> cor.test(x,y,method="spearman",exact=FALSE)

        Spearman's rank correlation rho

data:  x and y
S = 96.072, p-value = 0.7342
alternative hypothesis: true rho is not equal to 0
sample estimates:
       rho
-0.1437152
```

In addition, I tested for a correlation between dead and alive Lepidoptera within the same transect type. As shown in Figure 6, I used the Spearman test in R and received a p-value of 0.7455 for the road and 0.7342 for the highway. Since for both values $p > 0.05$, this suggests there is no correlation between dead or alive Lepidoptera per transect type. In other words, more alive individuals in one type of transect does not mean there will be more dead in individuals in the same transect.

Lastly, although I did not test for significance in the difference in number of vehicles between transects, there was an average of 74 vehicles per day on the highway and 1.8 vehicles per day on the road. I counted the number of vehicles for six of the eight collection days.

Discussion

The finding that there is a significant difference in Lepidoptera mortality between the highway transects and road transects followed my hypothesis given data from previous studies. Since this is a very preliminary study and data on road mortality in tropical regions, especially on invertebrates, is limited, I see potential and importance in further research. More research can reduce room for error. For example, someone with more experience catching butterflies can get more accurate abundance data or someone with more experience identifying Lepidoptera could reduce the number of unknown data points.

There are also some things that would nevertheless be difficult to control for. For example, a butterfly specimen found on the side of the road does not necessarily mean that it died due to vehicle collision. There is also the issue that predators can quickly reach Lepidoptera bodies quickly, eat them, and degrade the wings, making identification harder. This is especially the case in the tropics compared to temperate regions as biological matter is used as quickly as it becomes available. There may also be bias in regards to which Lepidoptera families are more heavily represented if the host plants of species within those families grow near the road or highway at a greater degree than host plants of species in other families. To control for this consideration, more research into plant identification and butterfly-host plant relationships would have to be done for wherever transects are selected.

Nevertheless, further research into road mortality of Lepidoptera should be conducted as roads continue to be built that will increase the number of vehicles passing through previously inaccessible areas. Peru has several proposed road projects in its future. These often consist of local governments proposing to resurface or repave an already existing road. In addition, 28 regional roads and 5 national roads in the Amazon are scheduled for expansions (Bandura & Murphy, 2020). Although making it easier for communities to connect within and between countries can be beneficial for economies and generate work opportunities, greater care should go into selecting where roads will be built given the potential harm vehicles traveling on them have on wildlife.

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