ALLIANCE FOR A SUSTAINABLE AMAZON

Possible Human Impact on Mosquito Oviposition in Brazil Nut Shells

Jorge Rivera-Gonzalez and Clara Zheng

Corresponding emails: clara.z.zheng@gmail.com, jorgeirg@icloud.com & info@sustainableamazon.org

Abstract

Brazil nut tree concessions in the Madre de Dios region provides protection to the intact forest and substantial income to the local community. However, the stacked piles of harvested Brazil nut shells may store rainwater and act as a potential habitat for aquatic insects such as mosquito and mayfly larvae. As mosquitoes often act as a vector for disease transmission, these temporary bodies of water may be contributing to the dispersal of tropical diseases such as dengue and zika. In addition, overexploitation and piling Brazil nut shells close to the tree may also decrease the regeneration rate of the Brazil nut, which can only be observed in the long term. We investigated the abundance and variety of mosquito larvae in Brazil nut shells in two different locations, on piles and alone. The distance of the shells to the adult tree and the volume of water within the shell were measured and collected to take back to the lab. At the lab, the contents of the Brazil nut shells were divided and divided into morphospecies. This study shows that Brazil nut shells stacked in piles are closer and contain more larvae compared to those alone. Thus, harvesting Brazil nut may be contributing to the outbreak of diseases within a region; for this reason, humans should try to minimize the stand-still water sites aiding to the dispersal of different vector-borne tropical diseases.

Introduction

Brazil nut trees are protected by Peruvian law, making it an umbrella species protecting millions of hectors of intact forest (Ortiz, E.G., 2002). The Brazil nut (*Bertholleria excelsa*) is an economically profitable fruit-producing tree which serves as an important resource for local people (Shepard & Ramirez, 2011).

Brazil nut fruits are mainly dark brown and approximately 10-16cm in diameter. Fruit maturation takes 14 months, in which they fall during the rainy season (Wadt et al., 2008). The edible part of the fruit is its oil-rich seed, which can be roasted, pressed to produce nutritious milk, or consumed raw (Shepard, G. H. & Ramirez, H., 2011). The seeds are protected by an extremely hard outer shell which practically only humans and agoutis, which are large-bodied diurnal, terrestrial rodents with extremely sharp teeth, can breakthrough, making these two species a determining factor of the dispersal of the brazil nut seeds (Shepard, G. H. & Ramirez, H., 2011). Studies show that agoutis consume a quarter of the Brazil nuts on-site, whilst 65% are hoarded within 5m of origin providing a short-range method of dispersal (Peres, Carlos A. & Baider, 1997). Depleting the Brazil nuts from the agoutis may have a long-term impact on the dispersal of the Brazil nuts (Levin 2007).

Given its widespread success in the international market as a non-timber product, Brazil nuts have been considered as a role-model in promoting sustainable forest use (Wadt et al., 2008). Its high productivity has given farmers and residents an alternative to forest conversion which changes the native forest to other profitable practices, such as cattle farming or papaya lands. In the Madre de Dios region, rapid change in land use has occurred since the construction of the Interoceanic Highway, which has provided a link between residents and a larger potential market. In the year 2003, there were 970 legal Brazil nut concessions recorded by The Ministry of Agriculture of Madre de Dios, averaging a coverage of 1,000ha which each produces 9,500 kg per year (Escobal & Aldana, 2003). The number of concessions had remained somewhat constant, due to strict legislation on land management.

On the other hand, selective logging that is carried out in Brazil nut concession still leads to some extent of habitat transformation, which has been suggested to act as a catalyst to shift the vector abundance towards species more tolerant of human-dominated habitats and introduce new pathogens (Johnson, Gómez & Pinedo-Vasquez, 2008). In addition, this loss of forest cover may also affect the abundance and behavior of mosquitoes (Foley et al., 2007). For example, malaria transmission patterns have been demonstrated to change with different land use such as deforestation or agriculture (Johnson, Gómez and Pinedo-Vasquez, 2008).

Other negative impacts Brazil nut harvesting may bring includes overexploitation. This may ultimately lead to a decreased regeneration rate as the number of Brazil nut seeds left for regeneration may be insufficient to support sustainable recruitment (Wadt et al., 2008). By this means, altering the food chain might lead to a shift in the population of disease organisms and their vectors, as rainforests have been demonstrated to regulate their population (Foley et al., 2007).

An example of this includes the shift in oviposition preference of mosquitoes. After Brazil nuts are harvested, the empty shells are often left in a pile near the tree. These stacked empty shells will not only store rainwater but also retain humidity within the pile, consequently providing more temporary habitats for aquatic species such as mosquitoes, midges and damselfly larvae compared to alone brazil nuts dispersed randomly by agoutis.

Therefore, there is a possibility that the act of harvesting Brazil nuts has altered the abundance of waterborne insects, including mosquitoes that act as a vector for spreading diseases such as malaria and dengue. Mosquitoes viral strands can significantly deter an individual's health and can even be fatal. The truth is no animal on earth has touched so directly and profoundly the lives of so many human beings (Spielman, A. & D'Antonio, M. 2001). Some of the world's most serious infectious diseases like dengue, malaria, leishmaniosis and several arboviruses are found in this region. (Foley et al., 2007). During harvest season, every employee working in the concession must be in ideal health condition, as not only their families rely on their income, but also because it is a seasonal job. The Madre de Dios region provides the ideal conditions for the transmission of numerous tropical diseases, which pose a risk for those exposed to precarious housing and working conditions (Tauil, P.L., 2009).

The potential threat to both the ecosystem and human health is obvious and requires further investigation. This study is directed to assess the human impact on the tropical rainforest by harvesting Brazil nuts. We do this by gathering Brazil nut shells that contain water, measuring the distance of the nutshell to the nearest adult tree, and analyzing the specimens found within. Assuming that harvesting Brazil nut will both decrease the dispersal range and provide more sites of oviposition for mosquitoes, we predict that the shells in piles will be found closer to the adult tree, have a larger volume of water, and have a higher abundance of mosquito larvae.

Methods

Field Site

The field site of Finca Las Piedras (S 12°13.570'; W 069°06.850'), located in the Madre de Dios region of Peru, is located three kilometers away from the Interoceanic Highway. The site is comprised of 54 hectares of mainly terra firme rainforest, aguajal, and a new developing agroforestry plot. The aguajal are riparian forests mostly composed of aguaje palms *(Mauritia flexuosa).* The developing agroforestry plot is adjacent to the terra firme forest and includes a wide variety of plants like *Inga edulis, Theobroma cacao, Theobroma grandiflorum,* ironwood, and other native plants. Brazil nut trees are the dominant species along the terra firme. There are 22 Brazil nut concessions adjacent to the site within Monterey, Madre de Dios.

Brazil Nut Collection

The collection process took place in the dry season, during the first and second week of August 2019, spanning over 10 days in total. All shells were collected along the main trail of Finca Las Piedras which laps most of the terra firme ecosystem. Fifteen Brazil nutshells were collected from man-made piles that were opened and arranged into a pyramid-like structure during Brazil nuts harvest. These piles usually consist of over 50 shells that can stack up to 40cm. Fifteen other Brazil nutshells were collected from the ground away from the piles, which were most likely opened and dispersed by agoutis. 23 shells were collected on-site while 7 shells were collected from an adjacent Brazil nut concession. The Brazil nutshells were carefully selected due to the fact that stand-still water was present in the interior. The stand-still water provides an ideal environment for the oviposition of mosquitoes (Sunahara, T., Ishizaka, K. and Mogi, M., 2002)

The GPS location of each shell was recorded along with an estimate of the relative distance in relation to the nearest Brazil nut tree, assuming that each nut was produced by the adult tree closest to it. Using a syringe, the water was measured, noted down and extracted into a container. The inside of the shell was then fully rinsed to ensure that all the organisms were collected.

Specimen preparation and identification

At the lab, a pipette was used to extract the organisms present in order toseparate them from the standstill water and dirt. 70% ethanol was added to euthanize the organisms. For each sample, all the organisms were laid out on a white acrylic background to be photographed. The photographs were

Figure 1: 10 morphospecies were identified according to characteristics of the head, thorax, abdomen, and siphon

used as a key to identify different morphospecies by comparing characteristics of the head, thorax, abdomen and siphon. Once divided, the total number and total morphospecies were accounted for.

R was used to analyze the data, including using one-way ANOVA test to determine if the abundances of mosquito larva were significantly different for the two situations, calculating Shannon-Weaver diversity indices, and linear regression to test the linear relationship between the factors.

Results

The total number of mosquito larvae and pupae recorded in the stacked and alone Brazil nuts were 143 and 134 respectively. A total of 10 morphospecies were recorded (Fig.1 & Fig.2), with all 10 presents in the piled shells and 9 present in the alone shells (Fig. 3). The number of the morphospecies found differed between the piled and alone nuts (Fig.4). For example, the most abundant morphs were orange and light blue respectively. The distance of the shell to the nearest adult tree averaged at 7.29m for the piled shells and 12.47m for the alone shells. The average volume in the shells for each were 28.27mL and 32.37mL. Mayfly larvae, a predator of the mosquito larvae, were found in 6 of the samples, 4 in alone shells and 2 in piled shells. In addition, one sample had a water beetle present.

The Shannon-Weaver diversity indices of the piled and alone shells were 1.93 and 1.67 respectively. An ANOVA test was performed for the volume, abundance, distance and density (calculated by dividing the number of mosquitoes by the volume of water) for the piled and alone Brazil nut shells. Only the distance was revealed to be significantly different between the two categories (df = 1, F = 4.489, p = 0.0431) (Fig. 4). A linear relationship between the number of mosquitoes and the number of morphospecies was also demonstrated (Fig. 5).

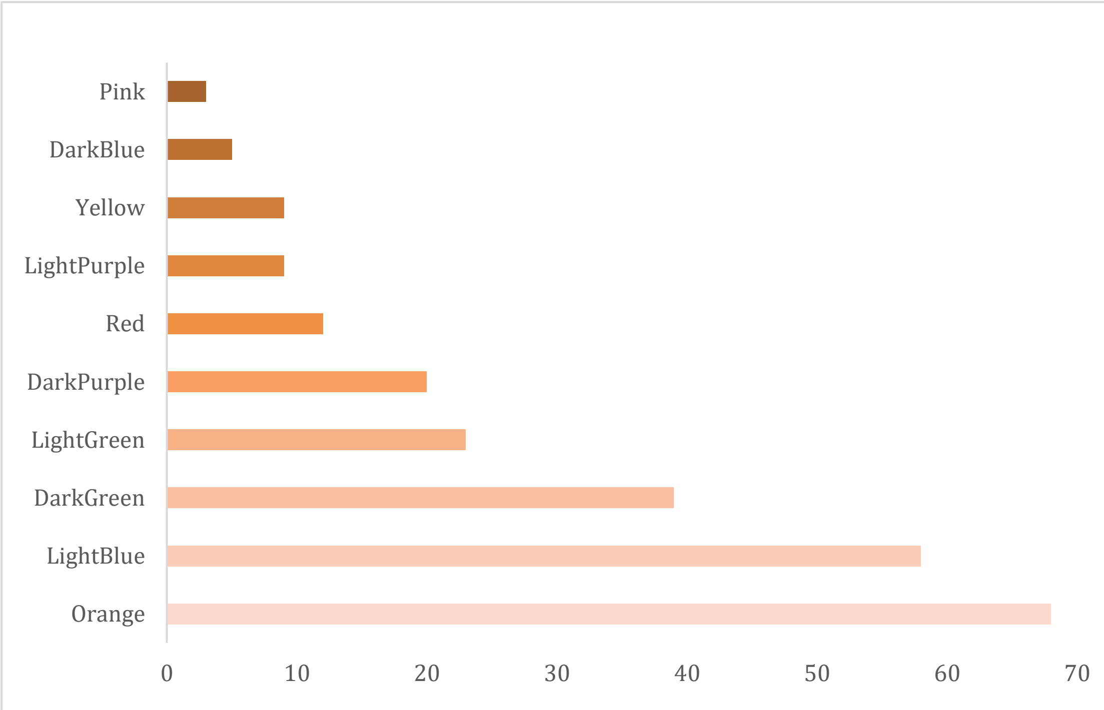

Figure 2: The total count of mosquito larvae found

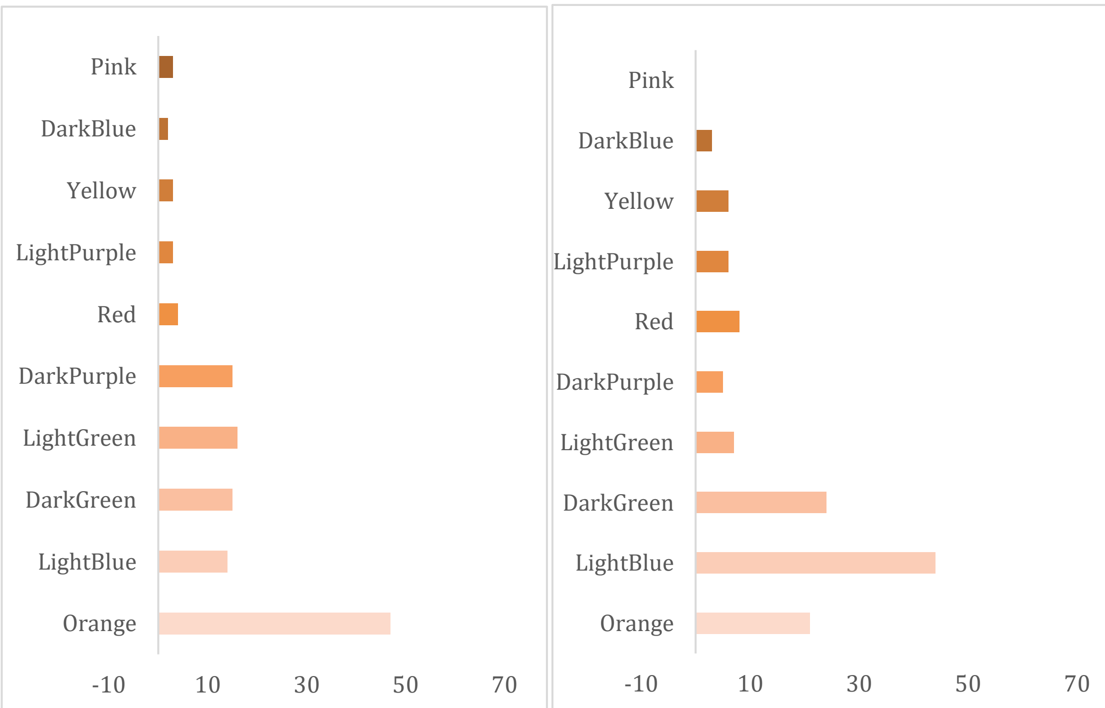

Figure 3: The total number of morphs of mosquito larva found in the piled Brazil nut shells and alone.

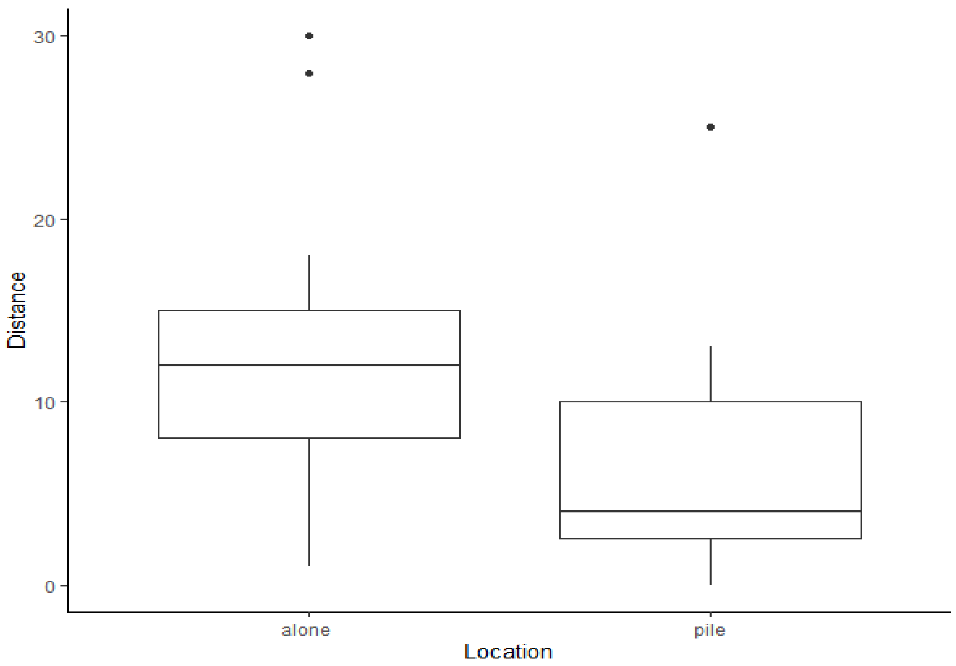

Figure 4: The difference between distances for the two locations.

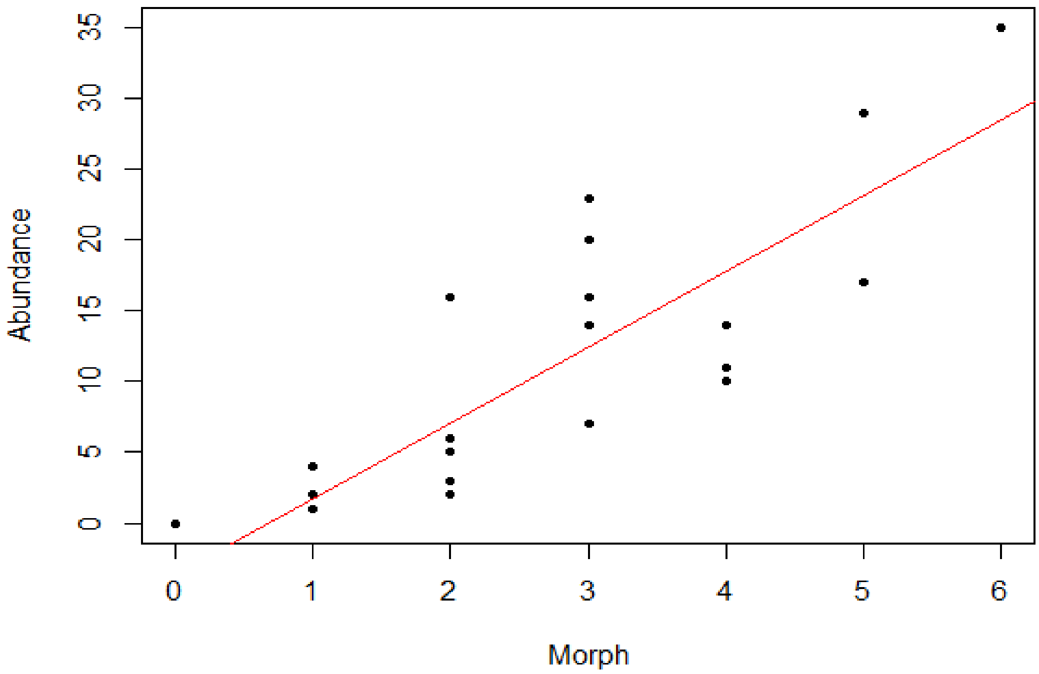

Figure 5: The linear relationship between abundance and morphospecies.

Discussion

The goal of this study was to assess the human impact of harvesting Brazil nut. Assuming that harvesting Brazil nut will both decrease the dispersal range and provide more sites of oviposition for mosquitoes, we predict that the shells in piles will be found closer to the adult tree, have a larger volume of water, and have a higher abundance of mosquito larvae.

We found that mosquito larvae are more abundant in piled shells, whilst those shells also contained a larger volume of water, with piled shells averaging at 32.27mL and the alone shells averaging at 28.27mL. We found a positive linear relationship between the number of larvae found in the *Bertholleria excels* shells and morphospecies found (Fig 6). Considering that both mayfly and water beetles were found in the shells, this might demonstrate a possible high diversity component within the microhabitat of the Brazil nut shells in nature.

Experts believe that abiotic factors like precipitation limit mosquito abundance thus signifying that mosquito populations should positively covary with precipitation. (Chase, J.M. and Knight, T.M., 2003). Any standstill water provides a potential environment as a mosquito breeding site. If humans can minimize the amount of standstill water, we could control disease vectors which contributes to the spread of the many diseases around the world.

In this experiment, the morphospecies labelled in orange was the most abundant, with the morphospecies labelled pink as least abundant. Interestingly, the orange was the morphospecies smallest in size, while the pink morphospecies was one of the largest in size. The variance in the abundance of morphospecies could be accounted for by the preference of different sized habitats by the species (Sunahara, T., Ishizaka, K. and Mogi, M., 2002). This might indicate a relationship between habitat preferences in related to habitat size which could be further investigated.

We also found that the distance of the shell to the nearest adult tree was significantly different between the two situations, with shells in piles being closer to the adult tree (Fig 5). In nature, the agoutis will carry the Brazil nuts away from the parent tree allowing the sapling to escape competition. However, if brazil nuts are piled close to the parent tree, young saplings germinating close to it will be forced to compete with its parent for resources such as water and sunlight, while it will also be impacted by the host-specific parasitoids and pests attracted to the large adult tree. Thus, harvesting Brazil nuts might negatively impact the recruitment rate of Brazil nut trees which can only be seen in the long term.

The larger abundance of larva in piled shells could possibly be due to the longer period the shells contained water. This would indeed increase the chances of mosquitoes laying eggs in those bodies of water. Studies have shown that mosquitos tend to lay more eggs in containers with less larvae compared to those that contain a larger number of larvae to avoid intraspecific competition and maximize their fitness (Munga et al., 2014). Intraspecific competition is known to prolong larval stage of *Anopheles gambiae* and stunt the adult size which may reduce productivity (Gimnig et al. 2002). Thus, shells in piles, being able to hold water for a longer period of time, could possibly be more likely to have more oviposition for a trade-off of having minimal intraspecific competition. The moist environment of the shells in piles might also support the growth of algae and microbes, providing a more suitable environment for larvae survival and growth (Gimnig et al. 2002).

Although our predictions were met, differences in both volume and abundance were revealed to be insignificant. This could be due to the limited sample number. On the other hand, as we were collecting the samples, we noticed that the number of shells in piles that contained water was significantly larger than the ones that were randomly distributed. Future studies should sample all shells with water for multiple adult trees, both in the dry and wet season, to give an estimate of the total number of larvae in both situations. Being able to identify each morphospecies into species would be the key to understanding the disease spreading mosquitoes in the Amazon Rainforest. Many of the well-known diseases epidemiologic characteristics are changing due to the accelerating populations, environmental and climate changes Tauil, P.L., 2009). The more knowledgeable and self-aware we are, better choices can be made for a more eco-conscious lifestyle focusing on protecting the biodiversity and ourselves from possible health risk we might encounter.

References

Chase, J.M. and Knight, T.M., 2003. Drought-induced mosquito outbreaks in wetlands. *Ecology Letters*, *6*(11), pp.1017-1024

Escobal, J. & Aldana, U. (2003) Are nontimber forest products the antidote to rainforest degradation? Brazil nut extraction in Madre De Dios, Peru. *World Development*. 31 (11), 1873-1887.

Foley, J.A., Asner, G.P., Costa, M.H., Coe, M.T., DeFries, R., Gibbs, H.K., Howard, E.A., Olson, S., Patz, J., Ramankutty, N. and Snyder, P., 2007. Amazonia revealed: forest degradation and loss of ecosystem goods and services in the Amazon Basin. *Frontiers in Ecology and the Environment*, *5*(1), pp.25-32.

Gimnig, J. E., M. Ombok, S. Otieno, M. G. Kaufuma, J. M. Vulule, and E. D. Walker. 2002. Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *Journal of Medical Entomol*ogy. 39: 162-172.

Johnson, M. F., Gómez, A. & Pinedo-Vasquez, M. (2008) Land use and mosquito diversity in the Peruvian Amazon. *Journal of Medical Entomology*. 45 (6), 1023-1030.

Levin, S., 2007. Agoutis and Seed Dispersal in Tropical Rainforests. *Eukaryon*, *3*(1), p.32.

Munga, S., Minakawa, N., Zhou, G., Barrack, O. J., Githeko, A. K. & Yan, G. (2014) Effects of larval competitors and predators on oviposition site selection of Anopheles gambiae sensu stricto. *Journal of Medical Entomology*. 43 (2), 221-224.

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

Ortiz, E.G., 2002. Brazil nut (*Bertholletia excelsa*). Tapping the Green Market: Certification & Management of Non-timber Forest Products. *Earthscan Publications Ltd*., London, UK, pp. 61–74.

Peres, C. A. & Baider, C. (1997) Seed dispersal, spatial distribution and population structure of Brazil nut trees (Bertholletia excelsa) in southeastern Amazonia. *Journal of Tropical Ecology*. 13 (4), 595-616.

Shepard, G. H. & Ramirez, H. (2011) "Made in Brazil": human dispersal of the Brazil nut (Bertholletia excelsa, Lecythidaceae) in ancient Amazonia. *Economic Botany*. 65 (1), 44-65.

Spielman, A. & D'Antonio, M. (2001). *Mosquito: A Natural History of Our Most Persistent and Deadly Foe*. Hyperion, New York, NY.

Sunahara, T., Ishizaka, K. and Mogi, M., 2002. Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. *Journal of Vector Ecology*, *27*, pp.8-20.

Tauil, P.L., 2009. The status of infectious disease in the Amazon region. *Emerging infectious diseases*, *15*(4), p.625.

Wadt, L. d. O., Kainer, K. A., Staudhammer, C. L. & Serrano, R. (2008) Sustainable forest use in Brazilian extractive reserves: natural regeneration of Brazil nut in exploited populations. *Biological Conservation*. 141 (1), 332-346.

Appendix

```
> #Import data
> mosdata <- read.csv('../data/mosdata.csv')
> #Analysing distance with respect to location
> distancelm <- aov(Distance ~ Location, data=mosdata)
> par(mfrow = c(2, 2), mar = c(5, 5, 1.5, 1.5))
> plot(distancelm)
> summary(distancelm)
            Df Sum Sq Mean Sq F value Pr(>F)
Location     1  240.8  240.83   4.489 0.0431 *
Residuals   28 1502.1   53.65
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> #Shannon-Weaver analyses
> mossom <- read.csv("../data/mos_sum.csv")
> library(vegan)
> diversity(mossom[-1], index="shannon")
[1] 1.850532 1.837913
> #Linear relationship between number of mosquito larvae and the num
ber of morphs
> numbermorph<-lm(Number~Morph, data=mosdata)
> plot(numbermorph)
> summary(numbermorph)

Call:
lm(formula = Number ~ Morph, data = mosdata)

Residuals:
   Min     1Q Median     3Q    Max
-7.806 -2.090 -0.732  2.089 10.552

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -3.6261     1.6922  -2.143    0.041 *
Morph         5.3581     0.6085   8.805 1.48e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4.682 on 28 degrees of freedom
Multiple R-squared:  0.7347,	Adjusted R-squared:  0.7252
F-statistic: 77.53 on 1 and 28 DF,  p-value: 1.476e-09
```

