Assessing Neotropical Wasp Diversity Between Primary and Secondary Forest Using Colored Pan Traps

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Abstract

While the western Amazon has some of the highest levels of biodiversity on Earth, it's increasingly at risk of deforestation and forest loss, which is why assessing biodiversity in primary and secondary forests is becoming more important than ever. Wasps are some of the most diverse animals in the world and can serve as good indicators for ecosystem health. Colored pan traps are a widely used, easy to employ method for sampling Hymenoptera populations, and this study assessed wasp superfamily abundance, composition, richness, and diversity between primary and secondary forest in the Madre de Dios region of Peru using colored pan traps. Because relatively few studies have been done with pan traps in neotropical forest, this study also examined their effectiveness in a neotropical rainforest, and if the wasps sampled had a color preference. While no significant difference in superfamily abundance was observed between the primary and secondary forest, there was a difference in richness and overall diversity. Additionally, a higher collection rate was observed in yellow pan traps compared to blue pan traps which is consistent with other studies conducted in forested ecosystems. Though other methods of sampling are needed to obtain a more complete assemblage of wasp species, these results show that pan trapping can be an effective method for sampling Hymenoptera diversity in the neotropics and that secondary forests may be more biodiverse than is immediately clear.

Introduction

Tropical forests have the highest biodiversity levels on Earth and play critical roles in ecological, climatic, and biogeochemical cycles (Vera V. et al. 2019, Sanchez-Cuervo et al. 2020). The western Amazon contains some of the most intact and biodiverse tropical rainforest in the world, however it is increasingly at risk of deforestation and forest loss (Sanchez-Cuervo et al. 2020). While Peru contains the second largest portion of the Amazon forest after Brazil, approximately 24,334 km2 of its forest was lost between 2001 and 2019 (Rojas et al. 2021). Peru's Madre de Dios department is a particularly species rich biodiversity hot spot that has been undergoing increasing rates of forest loss and land use change over the past several decades which has led to it becoming a primary region for global conservation (Nicolau et al. 2019, Sanchez-Cuervo et al. 2020).

Forest loss in the Madre de Dios Region is complex and centered around a mix of market incentivized public policies, infrastructural and agricultural expansions, and mining developments (Ravikumar et al. 2016, Gallice 2017, Caballero Espejo et al. 2018, Marquardt 2018, Nicolau et al. 2019, Sanchez-Cuervo et al. 2020). Much of this loss is attributed to the Interoceanic Highway, which connects the Peruvian Pacific to the Brazilian Atlantic. The road was completed in 2010 and has led to increased immigration into the Madre de Dios region and greater access to previously isolated parts of the forest and gold mining areas via the creation of secondary roads (Nicolau et al. 2019, Sanchez-Cuervo et al. 2020). Another leading driver of forest loss is agricultural expansion (Socolar et al.

2018, Nicolau et al. 2019). Unlike the large-scale mechanized agriculture taking place in Brazil and other areas of the eastern Amazon, small-holder agriculture tends to be a more common practice in many parts of Peru (Socolar 2019). The technique of slash and burn is frequently employed as a means of clearing land to create pasture and arable farmland, which can lead to high rates of forest loss (Ravikumar 2016, Socolar 2019). Amazonian soils tend to be poor in nutrients, and fields are often abandoned or allowed to lie fallow once they have been depleted, which can lead to the generation of secondary forest (Castro 2012). Monitoring the biodiversity in these regenerative forests and comparing it to the biodiversity in primary forests is important for understanding how deforested agricultural areas recover, and it can help inform policy decisions and reforestation efforts.

Wasps are one of the most diverse group of animals in the world and a useful tool for studying biodiversity. They are a part of the order Hymenoptera and the clade Apocrita, which has 20 extant superfamilies and 97 families to date (Nastasi et al. 2023). Most wasp species are parasitoids that have co-evolved to be specific not just to a particular host species but to a specific developmental stage of their host as well (Nastaasi et al. 2023). A conservative estimate by Forbes et al. 2018 put the total number of extant species at just over 883,000, but many estimates put the number of species well over one million (Nastasi et al. 2023). Because of their role in regulating the populations of their host species and their sensitivity to ecosystem shifts, wasps have been proposed to be indicators of biological diversity and change, which makes them a good study species for comparing primary and secondary forest richness and diversity (Shoeninger et al. 2019, Rodriguez et al. 2022).

One of the most common ways of assessing non-Formicidae Hymenoptera biodiversity is through passive sampling with colored pan traps, also called bowl traps or Moericke traps. The main principle behind pan traps is that color is the main attractant for wasps and bees (Gonçalves & Oliveira 2013, Droege et al. 2016). The bowls are filled with water and some kind of detergent, which breaks the surface tension and allows for the capture of even the smallest insects. The size, amount, and arrangement of traps varies by research goals and habitat type, but generally the most effective sampling techniques involve grouping the bowls in some kind of transect or array so that each bowl is at least 5m from its nearest neighbor (Droege et al. 2015, Prado et al. 2017, Prendergast et al. 2020). Droege et al. 2016 recommends that each transect, or array have between 15 and 30 bowls to achieve the highest sampling density. Blue, yellow, and white are the most commonly used colors, and blue and yellow traps have generally been found to be the most effective (Abrahamczyk et al. 2010, Gollan 2011, Bashir 2013, Gonçalves & Oliveira 2013, Droege et al. 2016, Moreira 2016, Buffington et al. 2020). Pan trap size has not been correlated with capture rate and the majority of studies referenced here used traps ranging in size from 13 to 16 cm long, 4 to 8 cm deep, and 100 to 600 mL in volume(Cane et al. 2000, Abrahamczyk et al. 2010, Gollan 2011, Gonçalves & Oliveira 2013, Droege et al. 2016, Moreira 2016, Buffington et al. 2020, Wilson et al. 2016). The hours of the day and the length of time that pan traps are left out tend to vary, and guidelines developed by the U.S. Fish and Wildlife Service and the U.S. Forest Service advise that traps be left out for between eight and twenty-four hours to maximize capture rates.

Pan trapping is a long-established and

widely used practice for capturing Hymenoptera, however it is not without its issues. Pan traps have the advantage of being able to passively capture a large number of species in a short amount of time (Droege et al. 2016, USFS). They require less energy and training compared to hand netting, and can be a cheaper and more accessible method compared to malaise and vane traps (Droege et al. 2016, USFS). However, more active sampling methods like hand netting can allow the collector to target specific or rare species that might otherwise be difficult to capture, and it can potentially lead to a more species rich assemblage (Droege et al. 2016, USFS). Additionally, pan traps tend to bias towards smaller bodied species and often yield lower results in dense forest, where it can be difficult for Hymenoptera to see them (Droege et al 2016, McCravy & Ruholl 2017).

Despite its pitfalls, pan trapping was selected as the sampling method for this study due to its ease, affordability, and high rates of capture. Pan trapping also has lower collector bias and is easier to replicate compared to hand netting which makes it a better standard procedure for collection. Pan traps were used to assess wasp superfamilies in a neotropical rainforest in Madre de Dios Peru with the goal of seeing if there is a difference in wasp superfamily abundance, composition, and diversity between primary and secondary forest. Additionally, because few studies have been done to investigate pan trapping effectiveness in neotropical forests (Gonçalves et al 2012, Gonçalves & Oliveira 2013, De Souza Da Silva 2016, Prado 2017), this study also investigated whether colored pan traps are effective for assessing wasp superfamilies in a neotropical rainforest and if there is a preference for a specific trap color.

Methods

Study Site and Field Methods

This study was carried out in Finca Las Piedras, a 54-ha biological research station located in southeastern Peru's Madre de Dios department about 48 km north of Puerto Maldonado and 2km from the Interoceanic Highway (-12.22789, -69.11119; ca. 240 m). The property is composed of selectively logged terra firme rainforest that stretches in an unbroken tract into Bolivia's Manuripi National Amazonian Wildlife Reserve, regenerative secondary forest, Mauritia palm swamps, and agricultural land (Ccahuana et al. 2021, Baine et al. 2019, See et al. 2018).

Apocrita not belonging to the Formicoidea superfamily or Anthophila clade were surveyed with pan traps along six 50 m long transects in Finca Las Piedras. Three transects were in the terra firme primary forest and three were in the regenerative secondary forest. In the primary forest, areas with less dense vegetation were selected for transect placement in order to maximize trap visibility, and transects were set at least 150m from the forest edge to avoid edge effect. Each transect had twelve pan traps (six blue and six yellow) for a total of 72 pan traps across the study site. The pan traps were placed on the forest floor along the transect in pairs consisting of one bowl of each color. The bowls in a pair were separated by a minimum of 5 m as recommended by Droege et al. 2015. Each pair was separated from the next by 10 m (Fig. 1). Plastic blue and yellow 1000 mL square containers with a length of 14.5 cm and a depth of 6 cm were used for trapping. The traps were filled with between 250mL and 500mL of water and two drops of Ayudín citrus scented dish washing detergent were added. Unscented dish washing detergent is preferable but was unavailable at the time of this survey.

The study was conducted on the 18th, 19th,

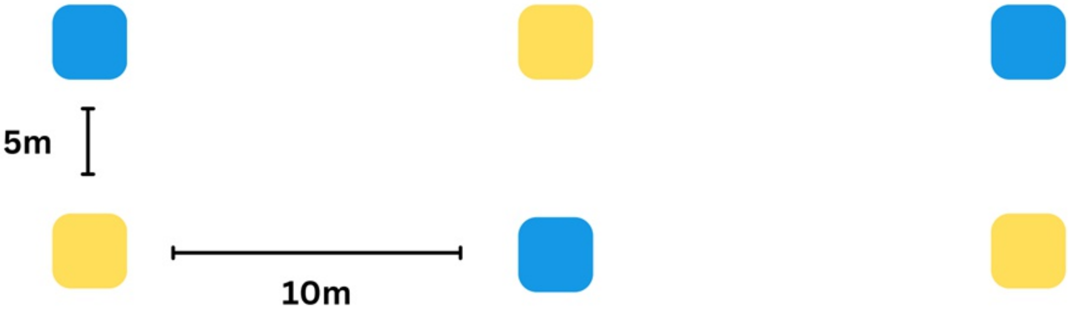


Figure 1. Transect configuration for the pan traps. Each of the 6 transects had 12 bowls of alternating colors. Colored pairs were separated from each other by 5m and from the next pair by 10m.

24th, and 25th of October, 2023. The traps were filled with soapy water between 5:30 and 8:30 AM and left out for 24-hours. Collections were done for all transects between 6:00 and 10:00 AM on the following day. The total number of insects in each bowl was counted before Apocrita species were removed with forceps and placed in a jar of 70% ethyl-alcohol that was separated by transect and bowl color. The soapy water was then strained and replaced in the bowl to be surveyed again the following day. Sampling was done on warm, sunny days.

Identification

Specimens were stored in jars filled with 70% ethyl-alcohol and separated by transect and color until they could be identified. Identification was conducted with a hand lens and headlamp. Wasps were identified to superfamily using the morphological characteristics described in Nastasi et al. 2023. After identification, the ID number of the specimen, date, transect, forest type, bowl color, superfamily, and morphospecies were recorded.

Analysis

The data collected from each transect was pooled by forest type and color for the purpose of the analysis. The number of individuals in each superfamily was calculated and a Wilcoxon signed rank test was conducted to compare the abundances between the two forest types and the two pan trap colors. Superfamily richness was calculated for the primary and secondary forest and the Chao1 estimator was used to estimate sample coverage (Chao et al. 2014, Chao & Hsieh 2016). The Shannon-Wiener Diversity Index was used to measure diversity and Hutcheson's t-test was calculated to compare significance.

Results

A total of 562 individuals across 13 superfamilies and 130 morphospecies were captured. A total of 40 individuals could not be identified to superfamily and were not included in the analysis. Ichneumonoidea/Trigonaloidea and Diaprioidea had the highest overall abundances at 225 (43%) and 56 (11%) respectively (Table 1, Fig. 1).

Between the two forest types surveyed, 385

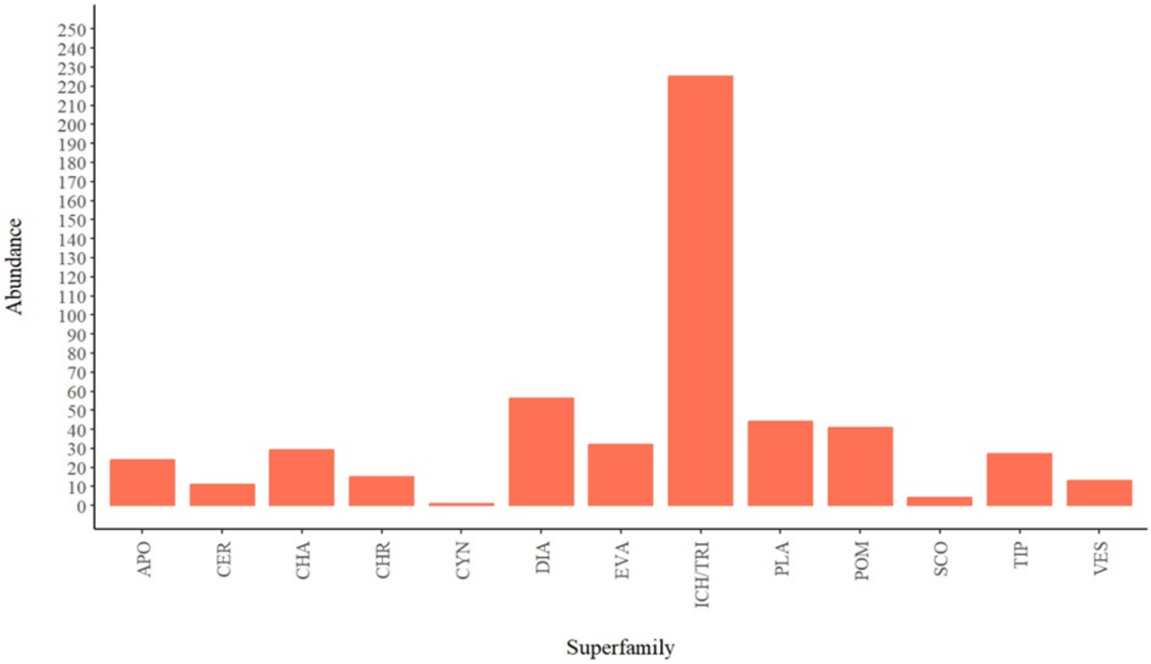
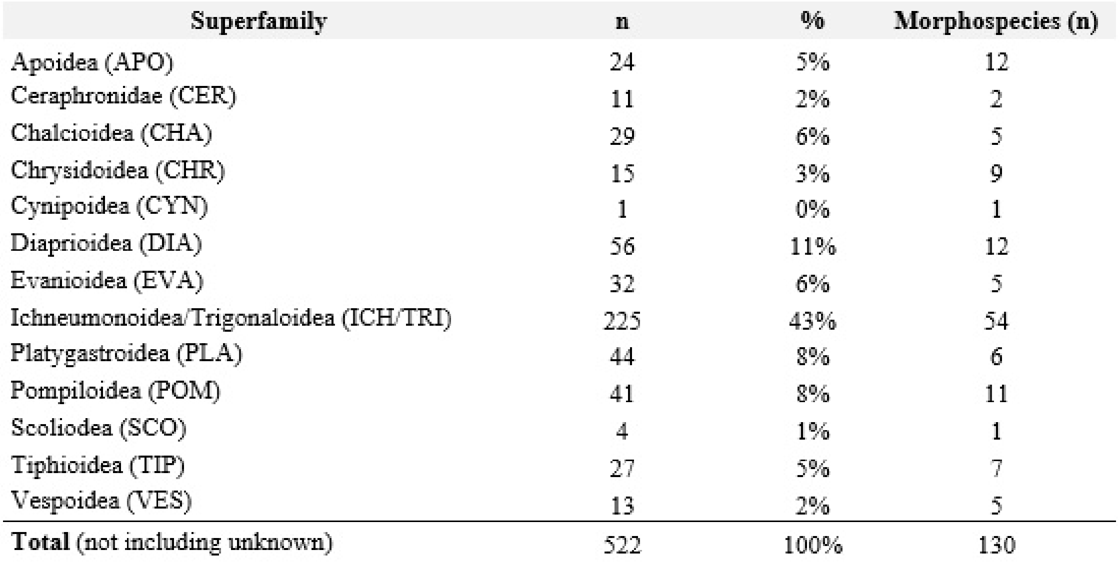


Figure 2. The number of individuals captured for each superfamily across all study sites. The most abundant superfamilies overall were ICH/TRI and DIA, and the least abundant were CYN and SCO.



Superfamily	n	%	Morphospecies (n)
Apoidea (APO)	24	5%	12
Ceraphronidae (CER)	11	2%	2
Chalcioidea (CHA)	29	6%	5
Chrysidoidea (CHR)	15	3%	9
Cynipoidea (CYN)	1	0%	1
Diaprioidea (DIA)	56	11%	12
Evanioidea (EVA)	32	6%	5
Ichneumonoidea/Trigonaloidea (ICH/TRI)	225	43%	54
Platygastroidea (PLA)	44	8%	6
Pompiloidea (POM)	41	8%	11
Scoliodea (SCO)	4	1%	1
Tiphioidea (TIP)	27	5%	7
Vespoidea (VES)	13	2%	5
Total (not including unknown)	522	100%	130

Table 1. Total superfamily and morphospecies abundance across all sites.

Superfamily	Forest Type				Color			
	Primary		Secondary		Blue		Yellow	
	n	%	n	%	n	%	n	%
Apoidea (APO)	13	3%	11	8%	4	5%	20	4%
Ceraphronidae (CER)	1	0%	10	7%	1	1%	11	2%
Chalcioidea (CHA)	13	3%	16	12%	1	1%	28	6%
Chrysidoidea (CHR)	12	3%	3	2%	1	1%	14	3%
Cynipoidea (CYN)	1	0%	0	0%	1	1%	0	0%
Diaprioidea (DIA)	54	14%	2	1%	14	19%	42	9%
Evanioidea (EVA)	16	4%	16	12%	3	4%	29	6%
Ichneumonoidea/Trigonaloidea (ICH/TRI)	204	53%	21	15%	16	22%	208	46%
Platygastroidea (PLA)	21	5%	23	17%	8	11%	36	8%
Pompiloidea (POM)	25	7%	16	12%	8	11%	33	7%
Scoliodea (SCO)	4	1%	0	0%	3	4%	1	0%
Tiphioidea (TIP)	16	4%	11	8%	7	10%	20	4%
Vespoidea (VES)	5	1%	8	6%	7	10%	6	1%
Total	385	100%	137	100%	74	100%	448	100%

Table 2. Total superfamily and morphospecies abundance by forest type and color.

Forest type	Color	n	%
Primary	Blue	55	86
Primary	Yellow	330	14
Total	385		
Secondary	Blue	19	86
Secondary	Yellow	118	14
Total	137		

Table 3. Total superfamily and morphospecies abundance by forest type and color combined.

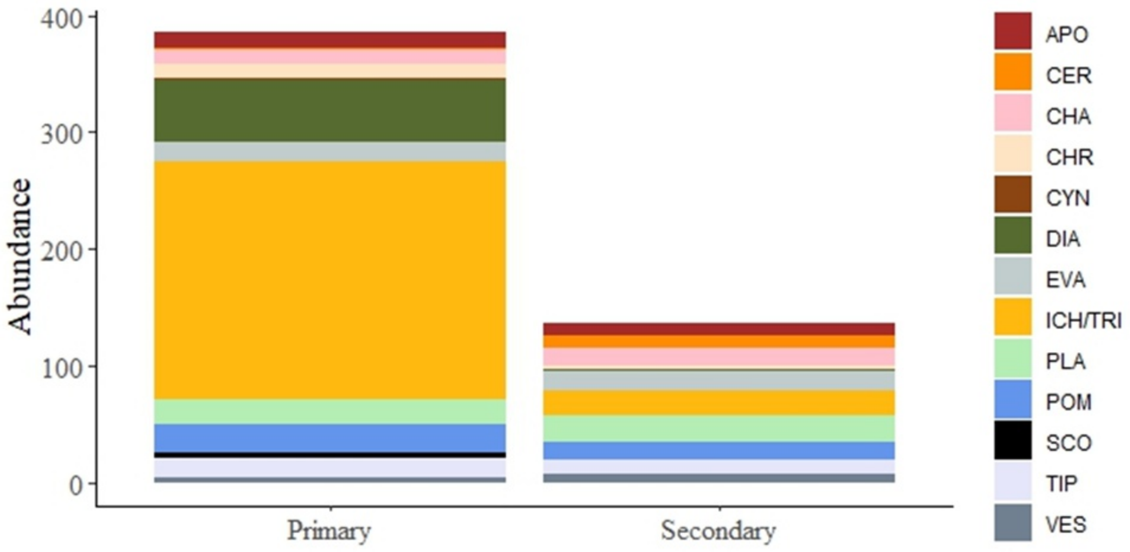


Figure 3. Superfamily abundance by forest type. ICH/TRI was the most abundant superfamily in the primary forest followed by Diaproidea. The secondary forest had a higher superfamily evenness compared to the primary forest.

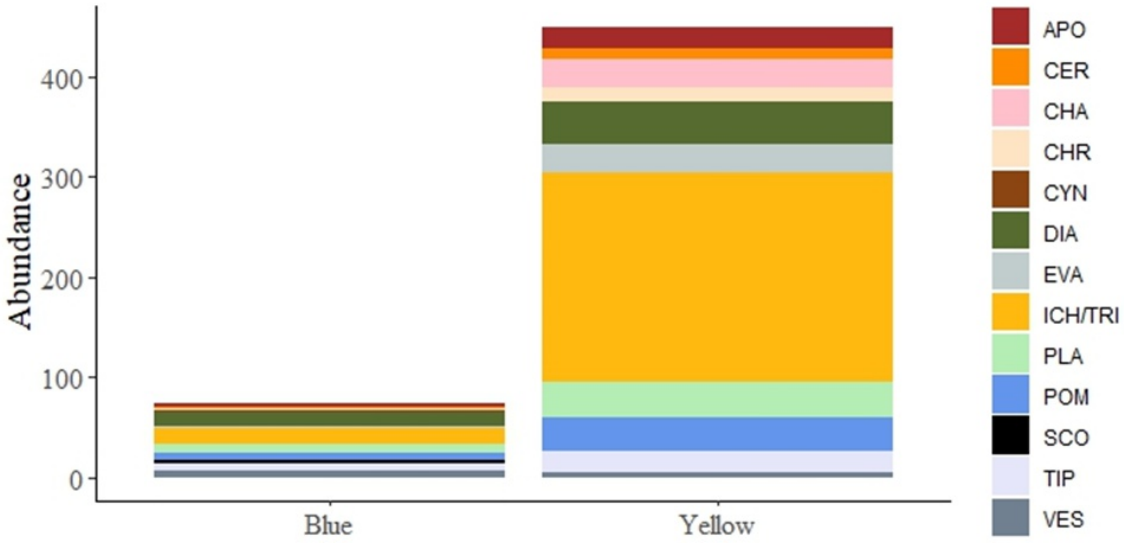


Figure 4. Superfamily abundance by pan trap color. ICH/TRI was the most abundant superfamily in the yellow traps which caught a significantly greater number of individuals (p = 0.006371) overall compared to the blue traps.

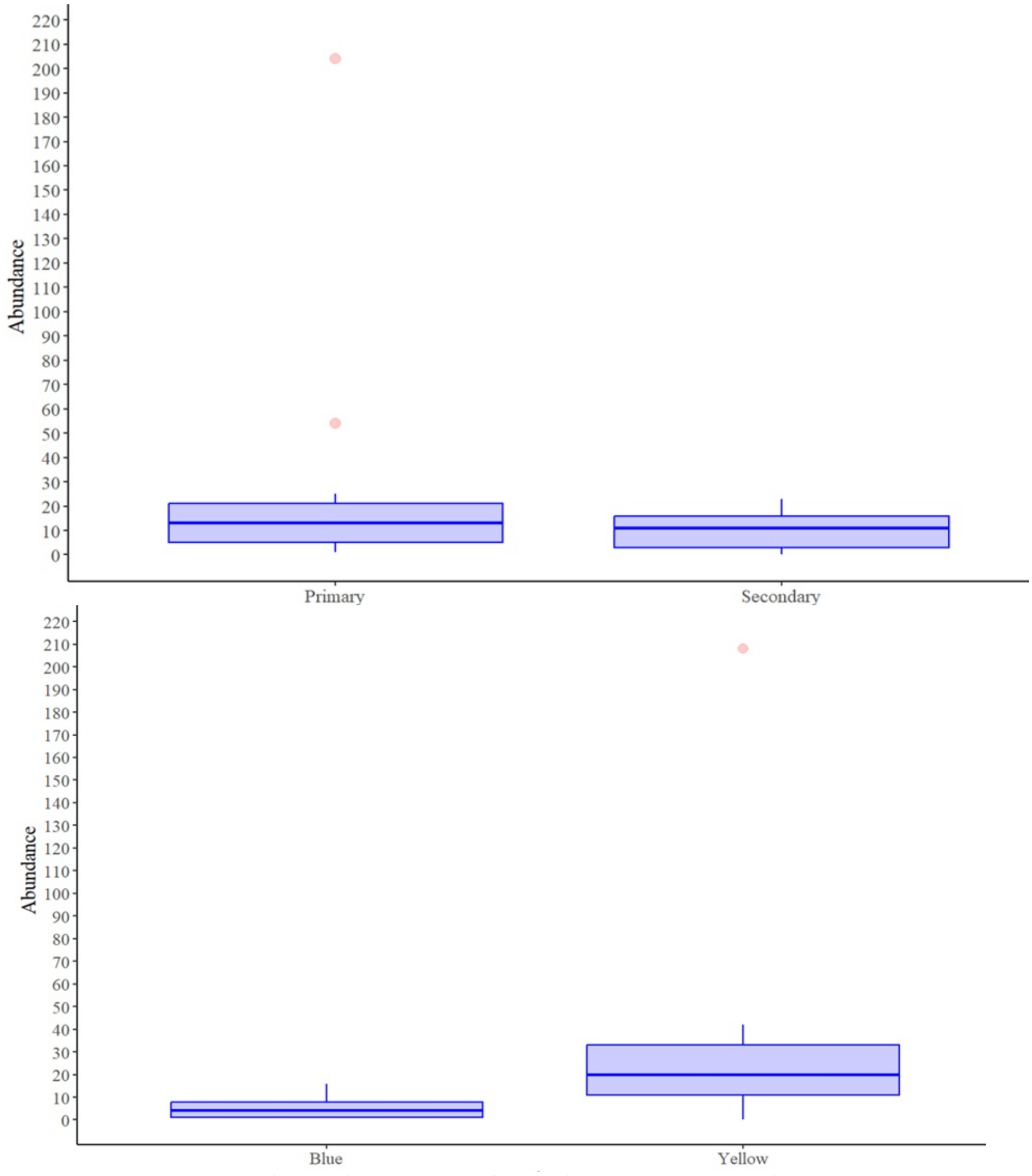


Figure 5. Box plot comparison of each superfamily's abundance by forest type (a) and pan trap color (b). There was a similar median abundance between the primary and secondary forest (a) and a greater median abundance in yellow traps versus blue traps (b). ICH/TRI and DIA were the outliers in the primary forest (a) and ICH/TRI was the outlier in the yellow traps (b)

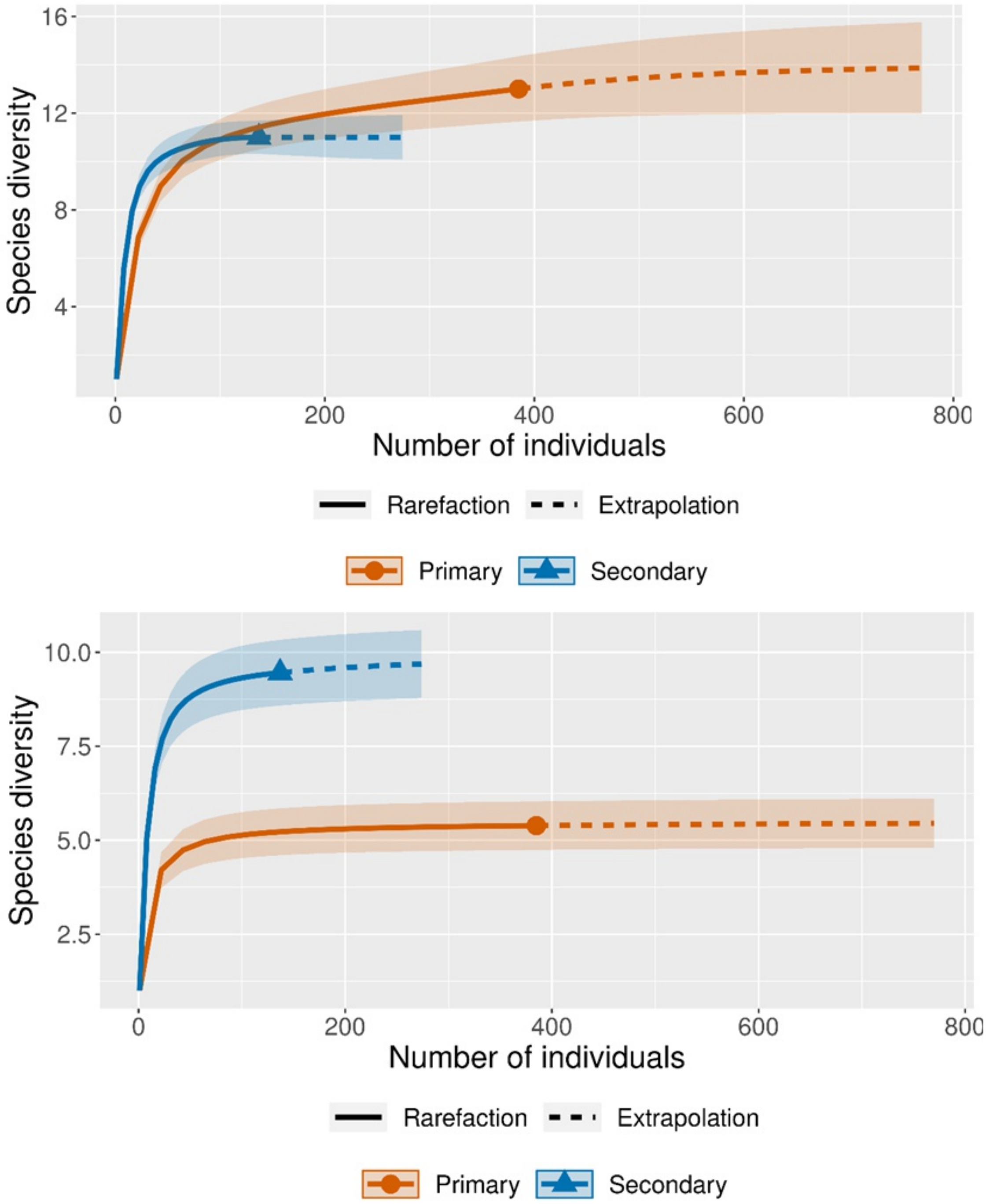


Figure 6. Forest type sample-size-based rarefaction and extrapolation sampling curves based on the Chao1 estimator for richness (a) and Shannon-Wiener Diversity Index (b). The primary forest exhibited a higher overall richness and a lower overall diversity compared to the secondary forest. The plateau in the richness curve in (a) indicates an almost 100% sampling completeness. Curves were generated using iNext (Chao & Hsieh, 2016).

(74%) individuals were identified in the primary forest and 137 (26%) in the secondary forest (Table 2, Fig. 2). Although the primary forest had a higher overall abundance compared to the secondary forest, the difference was not found to be significant (p = 0.157) (Fig. 5). In the primary forest, the three most abundant superfamilies were Ichneumonidae (53%) followed by Diaprioidea (14%) and Pompiloidea (7%). In the secondary forest, the most abundant superfamilies were Platygastroidea (17%) followed by Ichneumonidae/Trigonaloidae (15%), Chalcioidea (12%), Evanioidea (12%), and Pompiloidea (12%) (Table 2).

In regard to trap color, 448 (86% of total) individuals were identified in yellow pan traps and 74 (14%) in blue (Table 2, Fig. 3). The Wilcoxen test indicated that there was a significant difference between the rates of capture for blue and yellow traps, with yellow traps capturing a greater number of individuals (p = 0.006371) (Fig. 5). The three most abundant superfamilies in the yellow traps were Ichneumonidae/Trigonaloidae (46%), Diaprioidea (9%), and Platygastroidea (8%). The most abundant superfamilies in the blue traps were Ichneumonidae/Trigonaloidae (22%), Diaprioidea (19%), Platygastroidea (11%), and Pompiloidea (11%) (Table 2).

When forest type and color were analyzed together, it was found that the proportion of individuals captured in the two color types was the same across forest type (Table 3). In the primary forest, 55 individuals (14% of all primary forest captures) were captured in the blue traps and 330 (86%) in the yellow traps. In the secondary forest, 19 individuals (14% of all secondary forest captures) were captured in the blue bowls and 118 (86%) in the yellow.

Observed species richness was 13 superfamilies in the primary forest and 11 superfamilies in the secondary forest. According to the Chao1 estimator, the species coverage was approximately 99% for the primary forest and 100% for the secondary forest (Table 3). The Shannon-Wiener Diversity Index was 1.65 for the primary forest and 1.88 (p < 0.001) for the secondary forest, indicating that while the primary forest had a higher richness and abundance, the secondary forest had a higher evenness and overall diversity (Fig. 6).

Discussion and Conclusion

Assessing biodiversity levels as the Amazon undergoes forest loss and land use change is critical for understanding ecosystem stability. Wasps are useful bioindicators as they are sensitive to habitat shifts and can be sampled fairly easily through the method of pan trapping. In this study, neotropical wasp superfamily composition, abundance, richness, and diversity was assessed in a primary and secondary forest ecosystem in the Madre de Dios department of Peru. No significant difference in superfamily abundance was observed between the primary and secondary forest, however there was a difference in richness and overall diversity.

Though the secondary forest had a lower superfamily richness compared to the primary forest, it had a higher overall diversity. This indicates that the number of individuals captured in the secondary forest were more evenly distributed across superfamilies compared to those captured in the primary forest. So while the traps in the primary forest tended to catch high rates of a few dominant superfamilies like Ichneumonoidea/Trigonaloidea and Diaprioidea, the secondary forest tended to capture a lower rate of more evenly distributed superfamilies. This could indicate that secondary forests can harbor more diversity than is immediately obvious, however it must be noted that wasp foraging range was not taken into account in this study and it's possible that the wasps captured in the secondary forest have nests in the primary forest.

In terms of the differences between pan trap colors, there was a significant difference in the abundance between yellow and blue pan traps, with yellow pan traps catching far more individuals compared to blue traps. This is in line with other color comparison studies conducted in forests, as it is likely that yellow is more visible to Hymenoptera in the low light level of a forest ecosystem.

Overall, more work needs to be done to be done to fully compare primary and secondary Hymenoptera assemblages. While this study has some potentially interesting implications for the diversity found in primary and secondary forests, pan trapping is not a sensitive enough method to detect all of the species in an area. The Chao1 estimator calculated that the primary and secondary forest reference samples had almost 100% coverage, which indicates that increasing the number of traps or sampling days would not increase the species richness sampled. And yet there were many species that were not captured by the pan traps, which shows that another sampling method is needed in order to capture a richer assemblage. Additionally, pan trapping is not always an ideal method for sampling forest diversity as visibility is reduced in high density areas. Differences between visibility in the primary and secondary forest may have led to the traps in the less-dense, more open secondary forest sampling a higher rate of individuals compared to the more dense primary forest. Pan trapping tends to bias towards smaller and less rare species and only employs color as a means to attract a species, and so pairing it with hand-netting, which allows the collector to target larger and more rare species, could lead to a more complete inventory. Bait trapping could also be an interesting method to use in the future, as it has been found to attract unique tropical species in particular (Droege et al. 2016, Prado et al. 2017). Lastly, identifying wasps beyond the superfamily level and doing more morphospecies analysis could also reveal more information about wasp diversity between neotropical primary and secondary forest.

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