Difference in Abundance of Hemoparasites Between Bats in Anthropogenically Impacted and Natural Areas in Monteverde, Costa Rica

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ABSTRACT

The unique evolutionary features of bats allow them to harbor parasites, and a larger accumulation of bats means a larger accumulation of blood parasites. I caught 10 nectar bats at the Hummingbird Gallery in Monteverde by using a butterfly net, which represented my anthropogenically impacted population. At the Crandell Trail in Monteverde and by the Estación in San Gerardo, I captured eight bats total in a mist net, which represented my natural population. I collected thin blood smear samples from each bat. I compared the number of parasites I counted from samples from the anthropogenically impacted area to the samples from the natural areas. Although there is a higher average number of parasites found in bats from anthropogenically impacted areas, where bats are given an artificially provided food source, than in natural areas, there was not a statistically significant correlation between blood parasite abundance between the two populations of bats. There was no clear correlation between number of ectoparasites and number of hemoparasites, and no clear correlation between sex of the bat and number of parasites.

Diferencias en la abundancia de hemoparásitos entre murciélagos en áreas naturales y de impacto antropogénico en Monteverde, Costa Rica

RESUMEN

Las características evolutivas únicas de los murciélagos les permiten albergar parásitos, y una mayor acumulación de murciélagos implica una mayor acumulación de parásitos sanguíneos. Capturé 10 murciélagos nectarívoros en la Galería de Colibríes en Monteverde utilizando una red para mariposas, lo que representó mi población afectada por el impacto antropogénico. En el sendero Crandell en Monteverde y cerca de la Estación en San Gerardo, capturé un total de ocho murciélagos en una red de niebla, lo que representó mi población natural. Tomé muestras de frotis sanguíneos finos de cada murciélago. Comparé el número de parásitos que conté en las muestras del área impactada por el ser humano con las muestras de las áreas naturales. Aunque se encontró un mayor número promedio de parásitos en los murciélagos de áreas impactadas por el ser humano, donde los murciélagos tienen una fuente de alimento proporcionada artificialmente, en comparación con las áreas naturales, no hubo una correlación estadísticamente significativa entre la abundancia de parásitos sanguíneos entre las dos poblaciones de murciélagos. No hubo una correlación clara entre el número de ectoparásitos y el número de hemoparásitos, ni una correlación clara entre el sexo del murciélago y el número de parásitos.

Hemoparasite abundance in bat populations

Bats, due to their unique, evolutionarily advanced features, are successful disease vectors and reservoirs– they can carry a disease without showing symptoms of infection (Mackenzie et al., 2016). Their species diversity indicates that the types of hemoparasites they harbor also display diversity, which likely differ from habitat to habitat. Costa Rica is home to 120 species of bats, making Chiroptera the most abundant order of mammals in the country (York et al., 2019). With bats being 25% of all mammalian species worldwide (Han et al., 2015), Costa Rica contains 10% of these species. A Monteverde field key classifies 15 species of bat as "common" (Timm & LaVal, 2018), and three species as "abundant" (Appendix 1).

Bats remain resilient to disease due their body form, variation in metabolic strategies, genetic and species diversity, breadth of niches, and wide distribution (Han et al., 2015). Cross-contaminating socialization habits, flight abilities, feeding tendencies, echolocation aerosols, and long life spans allow them to spread these diseases (Han et al., 2015). These factors are impacted by the life zones they inhabit, so human landscape permutation would alter the way bats naturally exist; therefore, the abundance of blood parasites in these areas would also change.

In places where more bats accumulate, such as at anthropogenically provided food sources (Webber & Willis, 2016), disease spreads more easily among individuals. This could be due to their shared disease vectors, such as mites, flies, and other ectoparasites, which transfer hemoparasites across individuals. With a constantly available food source even during times of environmental stress, bats are less likely to migrate to scope out new food sources (Webber & Willis, 2016). They are exposed to each other in the same area for longer periods of time.

Bats that stay in continuous forests, who rely on their own food sources, are not subjected to the same level of cross-contamination. This means that natural populations are overall expected to have a lower abundance of blood parasites and may exhibit a different level of diversity in these parasites.

I made the assumption that comparing bats with unrelated niches would not impact my conclusions; my study contains nectar bats and fruit bats, which rely on different food sources. Nesting behaviors of fruit bats can increase the number of parasites present in each bat (Zacks 2008), so the direct sharing of food sources between nectar bats may mean that the feeding niches aren't a problem during a comparison.

This study is important because blood parasites in *Hylonycteris underwoodi*, my most captured species of bat, are highly understudied. As humans infringe more on the natural world and purposely or accidentally provide accessible food sources to wild mammals, the amount of parasites found in their blood will likely increase. Even if the purpose of providing the nectar food source is to feed hummingbirds or increase ecotourism, it is still impacting the other species inhabiting the area. In times of stress with poor weather, bats are more likely to concentrate at the easily accessible, human provided sources of food instead of flowers. The large collection of them creates grounds for parasites to easily spread, so as the number of bats in an area increases, so does the number of parasites.

In this study, I am discovering if the abundance of blood parasites in bats differs between anthropogenically impacted areas, where bats can access artificially provided sources of food, and natural areas, where bats exist mostly unaffected by humans.

METHODS

I captured bats in natural areas and anthropogenically impacted areas, where they either needed to locate their own sources of food or could rely on artificially provided sources. I gathered information from three sites, two natural areas and one anthropogenically impacted

area. I attempted to capture bats from 19 November 2024 to 28 November 2024, between the hours of 5:45-9:15. My natural areas were Crandell Trail and the Estación in San Gerardo, where I mist netted and captured eight bats. These two sites were similar because the mist nets were set up on trails in continuous forests. Although the trails are man-made, they are mostly untouched by human infrastructure, and devoid of artificially available sources of food. The anthropogenically impacted area was the Hummingbird Gallery, where the bats acquired nectar from the feeders, and we used butterfly nets to catch 10 bats.

Mist netting at Crandell Trail and the Estación in San Gerardo

We mist netted for a total of six nights. On 19 November 2024 and 20 November 2024 we caught no bats, but we were successful on 22 November 2024, 25 November 2024, 26 November 2024, and 28 November 2024 between the hours of 5:45-8:15. To set up the net, a member of my team held all the loops on one side of the net in one hand, untied the white loop, and added it to the rest of the loops in his hand. Then, making sure the loops were in the right order by stretching the net, I helped add them to the pole with the white loop on the top. We maintained tension in the net as we stretched it out. After stretching out the full 12 meter length of the net, we repeated the same procedure with the loops for the second side, being sure to keep the tension we created. We used a stick to push the top loop up the pole to make sure it reached high up enough.

After opening the mist nets, we checked every 15-20 minutes to see if we captured any bats. If the net captured a bat, then I wore thick gloves to carefully untangle the bat. I freed its feet, legs, and body with the help of a team member. We placed the bat in a fabric bag for observation.

To remove the net, we took the pole out of the ground, and grabbed all of the loops together, sliding them off the bottom of the pole. We tied the white loop around the other loops, and carefully, still maintaining tension, took down the net. We repeated these steps for the second pole.

Hummingbird Gallery captures

With the help of some assistants on 20 November 2024 and 21 November 2024, we stood at the Hummingbird Gallery next to feeders with large butterfly nets. We made sure the lights were off, and waited until the bats approached the hummingbird feeder. With a swift motion, we caught nectar bats for sampling. We went after sunset, between 6:30-9:15.

Blood samples

A member of my team retrieved the bat from the fabric bag and held it to prepare for my sampling. I gently held one foot of the bat, sterilized it with alcohol, and dried it. I punctured the vein on the foot with a fine needle. As the blood welled, I brought a sterilized slide close to the bat's foot to collect a drop of blood. I use a second sterilized slide to smear the sample, holding it at 45 degrees and feathering, creating one even layer of cells. While smearing, a second person used cotton to apply pressure to the puncture on the bat's foot, assisting with blood clotting. I stored the slide in a slide box to stain the next day. When done bleeding, I took videos and photos of each specimen. I gave them a noticeably shaggy haircut to mark them, ensuring that I wouldn't collect a sample from the same bat twice. After blood sampling, we identified the species of each bat by using a field key, measuring certain body parts and judging by characteristics (York et al., 2019).

Staining procedure and observations

I retrieved a slide from the slide box to observe. Using freshly cleaned glassware, I placed the slide into 100% ethanol for two minutes to fix the material in the sample. I prepared

the Giemsa staining solution, which consisted of one part Giemsa stain (four mL) and four parts buffer (16 mL). After thoroughly mixing, I moved the slide directly from the ethanol into a petri dish containing the 20 mL of Giemsa solution. I let the stain sit for 11-12 minutes, making sure the slide was completely covered. When removing, I gently washed each slide with distilled water, being careful to not disturb the smear. I allowed the slides to air dry completely. Keeping a 100x magnification standardized for my samples, I examined every part of the slide under a compound microscope, and took photos through the lens to document my results. I did an additional 400x magnification observation for slides heavily populated by parasites for a closer view, but did not change my data based on this.

RESULTS

I collected a total of 10 blood samples from anthropogenically impacted areas, and eight samples from natural areas. I caught a total of six species, consisting of 18 individuals (Appendix 2). Bats one through ten, the bats from the anthropogenically impacted area, were all from the same species, *Hylonycteris underwoodi*. The bats collected from Crandell Trail mist nets (first natural area, bats 11 and 12) were both from the species *Carollia sowelli*, and the bats collected from San Gerardo mist nets (second natural area, bats 13-18) were from the species *Anoura geoffroyi*, *Carollia sowelli*, *Sturnira hondurensis*, two *Sturnira mordax*, and *Carollia perspicillata*. Mostly male bats were caught (13), but four female bats were caught at the Hummingbird Gallery and one was caught in the Crandell Trail mist nets.

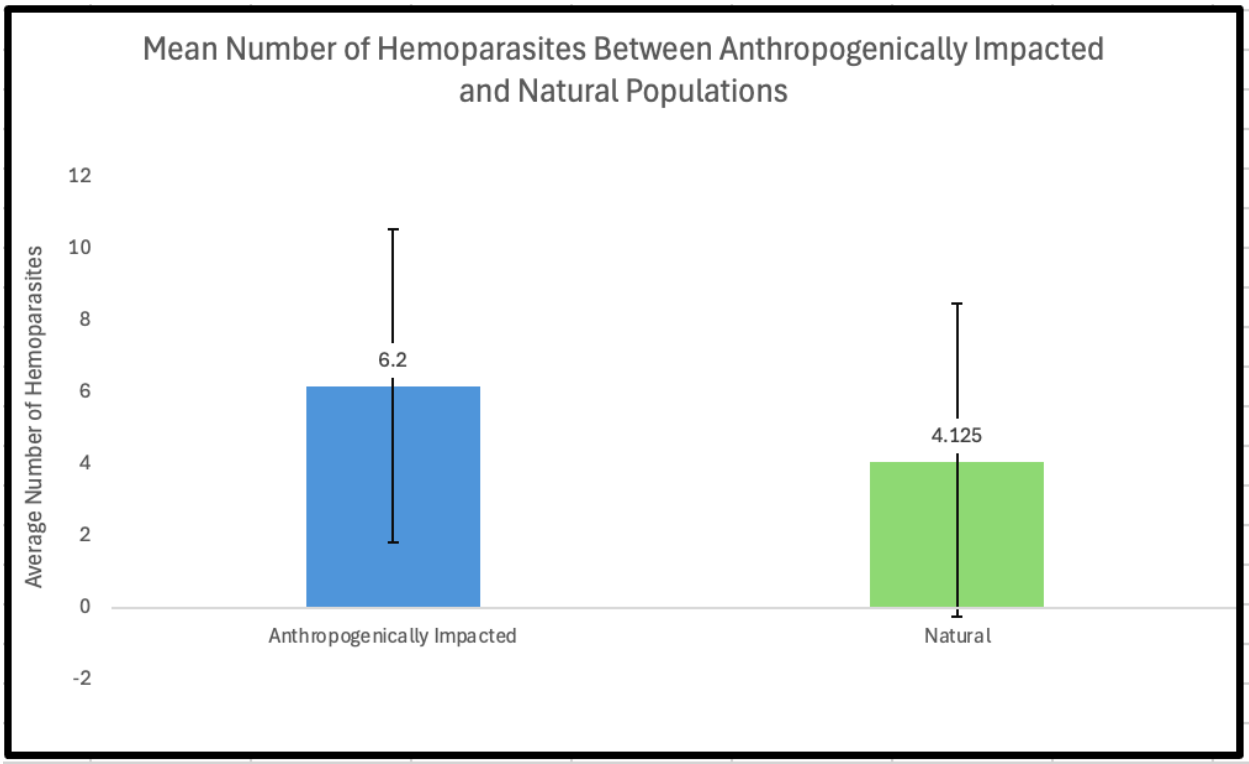

Figure 1: The average number of hemoparasites was higher for anthropogenically impacted areas (6.2) than natural areas (4.125). I used this data to run a *t*-test (t=0.9455, df=16, p=0.3585). The vertical error bars indicate the standard error in the number of parasites.

Hemoparasite abundance in bat populations

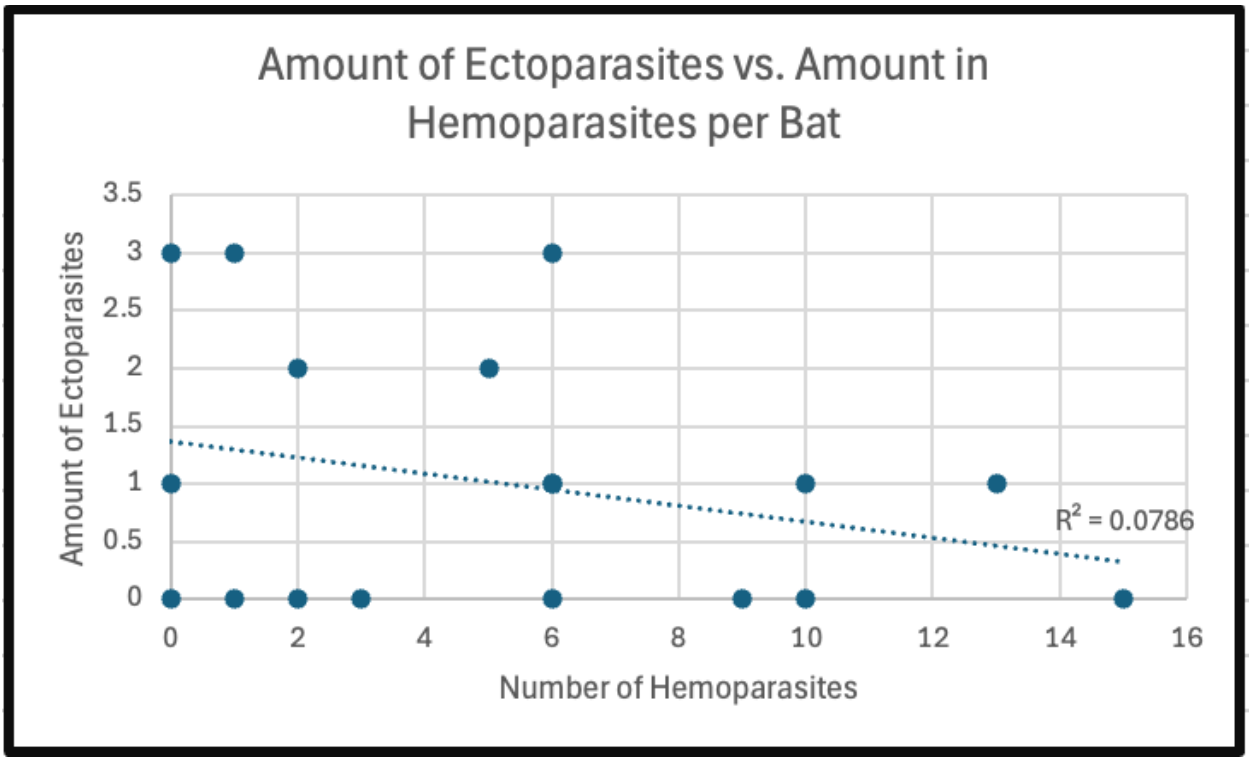

Figure 2: Each point on the scatterplot represents one bat. The amount of ectoparasites was collected and divided into four categories: zero, one, some (two), and many (three). Those with zero were bats 2, 3, 7, 8, 9, 14, 15, and 18. Those with one were 1, 4, 10, 12, and 16. Those with some were 13 and 17. Those with many were 5, 6, and 11. When plotted on a graph together, with each plot point representing one bat from the study, the R^2=0.079.

Hemoparasite abundance in bat populations

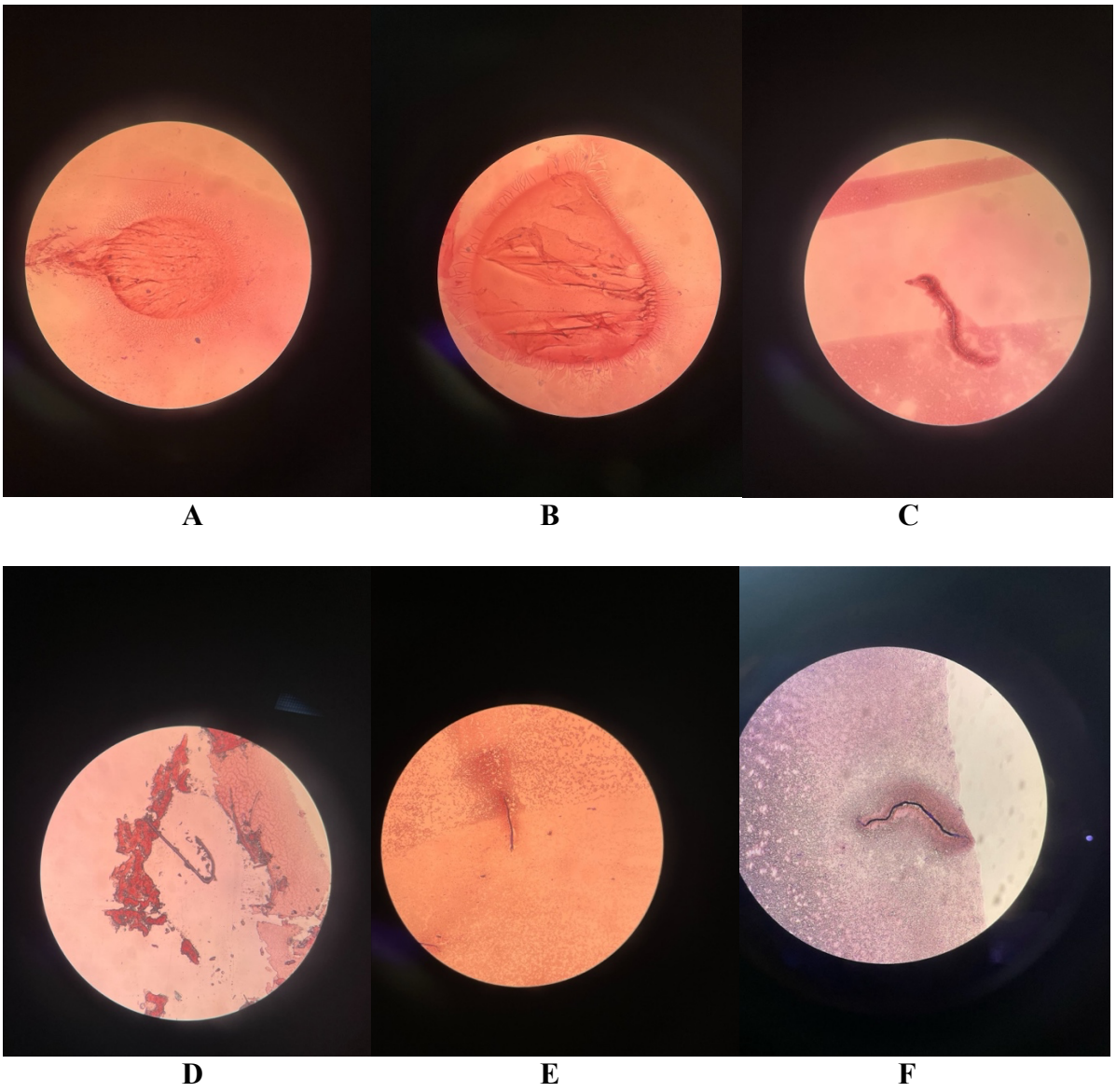

Figure 3: Parasite observations: A and B are parasites from Bat 1, a male *Hylonycteris underwoodi* captured with a butterfly net at the Hummingbird Gallery (anthropogenically impacted) on 20 November 2024. This bat had 13 parasites, one of the most abundant parasite loads. These are the largest parasites I observed. C is a parasite from Bat 2, a male *Hylonycteris underwoodi* caught at the Hummingbird Gallery with a butterfly net on 20 November 2024. It had nine hemoparasites and zero ectoparasites. D is a parasite from Bat 3, a male *Hylonycteris underwoodi* caught with a butterfly net at the Hummingbird Gallery on 20 November 2024. With 15 hemoparasites, this bat had the most abundant parasite load in the study, although it had zero ectoparasites. E is a parasite from Bat 12, a female *Carollia sowelli* caught at the Crandell Trail (natural area) mist nests at 7:10 PM on 22 November 2024. This natural area bat had 10 hemoparasites and one ectoparasite. F is a parasite from Bat 18, a male *Sturnira mordax* caught in the San Gerardo (natural area) mist nets at 6:15 PM on 28 November 2024. This bat had 10 hemoparasites and zero ectoparasites.

DISCUSSION

In other studies exploring a similar question to mine, hemoparasite prevalence appeared to be larger in bat species living in anthropogenically impacted areas than bats dwelling in natural areas. A larger group of bats generally indicates larger parasite abundance (Cote & Poulin, 1995), and anthropogenically provided food sources allow space and motivation for groups of bats to accumulate. The food at these sites (the hummingbird feeders in my study) are not scarce, drawing a large crowd of bats in search of extra energy during a time of environmental stress. Nectar bats rely on echolocation to sense food sources (Gonzalez-Terrazas et al., 2016), and can spread pathogens orally from the aerosols they emit during this process (Mackenzie et al., 2016).

Statistically speaking, there are no differences between hemoparasites in the two populations when comparing anthropogenically impacted bat blood parasite abundance and the number found in bats residing in natural areas. Despite the apparent differences in the average number of hemoparasites found in an individual for each population (Fig. 1), the information from the two populations overlaps too much for there to show significant contrasts in blood parasite prevalence.

Bat 1 had the biggest parasites observed, and it's unclear why I only observed that size of parasite in one sample (Fig. 3, A & B). The most parasites were found first in Bat 3 (Fig. 3, C), then in Bat 1. These bats may have had the most abundant parasite load because they are from the anthropogenically impacted population (Webber & Willis, 2016). Bats 12 (Fig. 3, E) and 18 (Fig. 3, F) had the same number of parasites, with Bat 2 (Fig. 3, D) almost having the same amount. Bats 12 and 18 had the most abundant parasite loads of the natural population, and a comparable amount to the anthropogenically impacted populations. This may be due to the fact that fruit bats tend to have a higher abundance of blood parasites during the wet season than the dry season (Thiombiano et al., 2023). Regardless of the area they are from, the heavy rainfall may have caused overall parasite abundance to be prevalent because of the body condition of the bats at the time of the rainy season (Tai et al., 2022). Weather can have an impact on the prevalence of blood parasites, and the movement of their potential vectors (Pawelczyk et al., 2004).

Bats from both populations, anthropogenically impacted and natural, are both coming from continuous forests. Contagions that nectar bats at the Hummingbird Gallery spread to each other are also likely being spread to other bats in the continuous forest. Fruit bats from natural areas may be leaving those areas and seeking out anthropogenic food sources that were provided on accident. I would not have a way of detecting or controlling this for my study. The two populations are likely not as isolated from each other as I originally thought.

According to my study, there does not appear to be a correlation between the number of ectoparasites and the number hemoparasites (Fig. 2). A former EAP student also could not find a clear correlation between ectoparasite and hemoparasite presence.This indicates that ectoparasites may not always be a vector for hemoparasites, and that bats may contract hemoparasites by a different medium. However, ectoparasites often contain hemoparasites, so the more that are present and biting a bat, the more hemoparasites that host should harbor (Szentiványi et al., 2019). I would have expected to find a more direct correlation.

There does not appear to be a correlation between the prevalence of parasites in male and female bats, although more males were caught than females. Libet also could not establish a link between sexes of bats and abundance of parasites. Only five out of the 18 bats in the sample were females, but the unevenness may be due to my data collection limitations. Females

theoretically have more hemoparasites due to their roosting behaviors (Frank et al., 2016), but my study did not match these results. However, although there can be differences in the number of parasites between sexes of bats, these differences may not be statistically significant (Okeke et al., 2020).

Possible factors that explain the some of the disagreement between my data and the conclusions of past studies include limited sample size, overlapping populations, unexpected modes of transmission, and weather. If I had a larger sample size with more bats, the source of error would decrease because I would have gained more experience in collecting and treating samples. I would have more data to analyze to draw more definitive, statistically significant conclusions. Overlapping populations would mean that there is a lot of cross-contamination between bats that visit the hummingbird feeders and other bats that exist in the continuous forest. It is also quite possible that the hummingbird feeders are consistently cleaned because they are at an ecotourism site, so they are not as contaminated as I previously thought. Although microbats transfer parasites through aerosols during echolocation that can spread disease, hemoparasites may have a different mode of transmission. Lastly, the weather impacted the data collection because there were less bats to test for parasites. It's possible that the ones that I caught had less parasites overall, and were metabolically stronger so parasites were less detectable.

Because my data does not agree with my existing knowledge or some past studies, this study should be conducted again with a larger sample size and more isolated populations, in conditions of less environmental stress. The impacts of climate change and a longer rainy season have been apparent this year, and impacted my data collection.

Because of the high number of parasites that I discovered in many of the bats from my sample, it is clear that bats are resilient to disease due to their evolutionary adaptations. They are not only hosts of parasites, but also reservoirs of zoonotic diseases (Chomel et al., 2014). Exposure to humans will continue to worsen as we provide food sources that draw bats from their natural habitats, even though the diseases present in these bats may be the same as those that consistently reside in continuous forests. As human contact with the natural wild populations increases, risk of disease spillover for both humans and animals increases (Hayman et al., 2012). Further research should explore a similar study with more isolated populations, solely focusing on nectar bats. This research could expand into how to prevent destruction of bat habitats that causes them to pursue anthropogenically provided sources of food, and how to mitigate disease spread at these sites when bats are under environmental stress.

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APPENDICES

Appendix 1

Species identified and classified in a field key (Timm & Laval, 2018)

LATIN NAME	COMMON NAME	CLASSIFICATION
Saccopteryx bilineata	Greater White-lined Bats	Common
Phyllostomus discolor	Pale Spear-nosed Bat	Common
*Anoura geoffroyi**	Geoffroy's Tailless Bat	Common
Glossophaga commissarisi	Commissaris' Long-tongued Bat	Common
Glossophaga soricina	Pallas' Long-tongued Bat	Common
*Hylonycteris underwoodi**	Underwood's Long-tongued Bat	Common
*Carollia perspiculata**	Short-tailed Fruit Bat	Uncommon
*Carollia sowelli**	Silky Short-tailed Bat	Common
Artibeaus jamaicensis	Jamaican Fruit Bat	Common
Artibeus lituratus	Big Fruit Bat	Common
Platyrrhinus vittatus	Greater Broad-nosed Bat	Common
Thyroptera tricolor	Spix's Disk-winged Bat	Common
Eptesicus brasiliensis	Brazilian Brown Bat	Common
Lasurus ega	Southern Yellow Bat	Common
Myotis nigricans	Black Myotis	Common
Molossus molossus	Little Mastiff Bat	Common
Molossus sinaloae	Sinaloan Mastiff Bat	Common
Dermanura tolteca	Toltec Fruit-eating Bat	Abundant
*Sturnira hondurensis**	Highland Yellow-shouldered Bat	Abundant
*Sturnira mordax**	Talamancan Bat	Uncommon
Myotis pilosatibiales	Hairy-legged Myotis	Abundant

*Species I caught

Hemoparasite abundance in bat populations

Appendix 2

Data Entries for Each Individual

BAT NUMBER	DATE	TIME	METHOD	LOCATION	SPECIES	GENDER	ECTOS	HEMOS
1	20 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	M	1	13
2	20 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	M	0	9
3	20 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	M	0	15
4	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	F	1	6
5	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	F	Many	1
6	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	M	Many	6
7	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	M	0	1
8	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	M	0	3
9	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	F	0	2
10	21 November 2024	-	Butterfly net	Hummingbird Gallery	*H. underwoodi*	F	1	6
11	22 November 2024	7:10	Mist net	Crandell Trail	*C. sowelli*	M	Many	0
12	22 November 2024	7:10	Mist net	Crandell Trail	*C. sowelli*	F	1	10
13	25 November 2024	7:30	Mist net	San Gerardo	*A. geoffroyi*	M	Some	5
14	26 November 2024	6:45	Mist net	San Gerardo	*C. sowelli*	M	0	6
15	26 November 2024	6:45	Mist net	San Gerardo	*S. hondurensis*	M	0	0
16	26 November 2024	6:45	Mist net	San Gerardo	*S. mordax*	M	1	0
17	28 November 2024	6:15	Mist net	San Gerardo	*C. perspicillata*	M	Some	2
18	28 November 2024	6:15	Mist net	San Gerardo	*S. mordax*	M	0	10