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Butterfly Bait Preference in Cloudbridge Nature Reserve, Costa Rica

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Opsiphanes cassina chiriquensis

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CONTENTS

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1 ABSTRACT

The main objective of this research was to see if different baits other than fermented banana would perform better and/or attract a greater diversity of butterflies (in particular butterflies not in the *Nymphalidae* family) to baited live traps. The study was carried out over a 4-week period in Cloudbridge Nature Reserve, pacific slope of the Talamanca mountains, Costa Rica. Traps were set along 4 transects, each with 4 bait traps, each containing 1 of the 4 bait types (fermented banana, fermented papaya, sweet mud, and cow dung). The fruit baits performed the best, with the banana capturing the most butterflies, and the papaya capturing the largest species richness, although only 1 species more than the banana. Both the sweet mud and cow dung baits performed poorly with only a handful of individuals captured in either bait type. Papaya, followed closely by banana, had the greatest number of unique species. However, when compared with previous baited trap research at the reserve, all of the butterflies captured in all bait types had been captured in banana baited traps before. No butterflies outside of the *Nymphalidae* family were captured by any bait type. Based on a literature review, further trials are recommended using fermented banana, fermented pineapple, egg (albumin), and carnivore dung.

2 INTRODUCTION

At Cloudbridge Nature Reserve, an ongoing butterfly survey (periodic since 2016) has mostly used banana bait in the live traps to capture butterflies and few new species have been caught in the traps in the past year, but new species are still being caught whilst sweep netting. As well, the butterfly bait has exclusively attracted butterflies in the *Nympahlidae* family, while sweep netting has collected butterflies in the *Hesperiidae*, *Papilionidae*, *Pieridae*, *Riodinidae* families as well as *Nymphalidae* (Appendix A). As sweep netting is difficult to standardize as a collection method, requiring high levels of skill and subject to collection bias from the netters, finding a bait type that would attract these different families would improve the objectivity of the data. As different species of butterflies are attracted to different kinds of foods, different baits were trialled to attempt to attract a greater diversity of butterfly species and families, and to determine if another bait besides fermented banana would attract a greater abundance or species richness of butterflies.

Other butterfly studies have used a variety of different baits including fruits, rotting fish, liver, feces, and nutrient mixtures and have found variable levels of appeal to butterflies (Holloway et al. 2013; Freitas et al. 2014; Molleman et al. 2005; and Fucilini 2014). Most fermented juicy fruits will attract fruit feeding butterflies (Freitas et al. 2014). Fruit-feeding butterflies are typical of tropical and subtropical habitats, comprising of 50-75% of all neotropical *Nymphalidae* butterflies (Brown 2005). Banana bait is the most common bait used to attract butterflies (Freitas et al. 2014): it is easy to prepare and attracts all fruit feeding butterflies, but not nectar feeding butterflies. Feces are visited by both fruit feeding and nectar feeding butterflies. In Cloudbridge, cow dung, horse manure, and dog feces are readily available. Bird feces is attractive to use, but not as easy to collect and keep consistent. Rotting fish baits have been tried at Cloudbridge in the past, but traps baited with meat tended to be destroyed by other animals, so were excluded from this study. In addition to the above food sources, butterflies also engage in a behaviour commonly referred to as 'mud-puddling'. Mud-puddling is a fairly common behaviour in butterflies, particularly tropical butterflies, where butterflies visit moist ground to suck up water and dissolved nutrients. It has been found that it is largely male butterflies that frequent mud puddles as they are attracted to the nitrogen rich resources (Beck et al. 1999). The sodium gained from mud puddling is essential for the adults, which the males transfer to the females when mating to help in egg production (Sculley and Boggs 1996).

For this research we chose to use banana, papaya, cow dung, and sweet mud. We were interested in seeing if another fruit would attract different butterflies or influence the number of butterflies caught when compared to banana. Papaya was chosen as it is cheap, readily available, and is grown in the area. Cow dung, over other

types of dung, was chosen as it was the most readily available and abundant. A recipe for sweet mud was found online (Smith 2012) and was used as an analog for a mud-puddling source. The dung and sweet mud were chosen to test as they are very different from the fruit baits, but remain attractive to butterflies so may attract butterflies from a different family.

3 STUDY LOCATION

Cloudbridge is a private nature reserve that stretches from 1550 m to 2600 m (5085-8530 ft) in the Talamanca mountains of Costa Rica. Since 2002, 255 hectares (630 acres) of cattle pasture or cultivated land with a further 28 hectares (70 acres) of primary forest has been purchased by the reserve. Since its beginning, Cloudbridge has been dedicated to the conservation and reforestation of the cloudforest (Cloudbridge 2018b).

The lower part of the El Jilguero trail was chosen as the survey location for several reasons. One, the survey area had a large number of butterflies when previously surveyed with bait traps and sweep netting. Two, the whole area consists of planted secondary forest. And third, the area was close enough to the buildings to be able to check the traps twice a day.

Traps were set-up along 4, 150 m transects on the main El Jilguero trail, as well as the El Jilguero loop trail (Figure 1). One transect had already been used for bait trapping studies (called 'Planted' in Figure 1), and 3 new transects were set up on the path to the Jilguero Loop (Path), Lower Loop, and the Upper Loop.

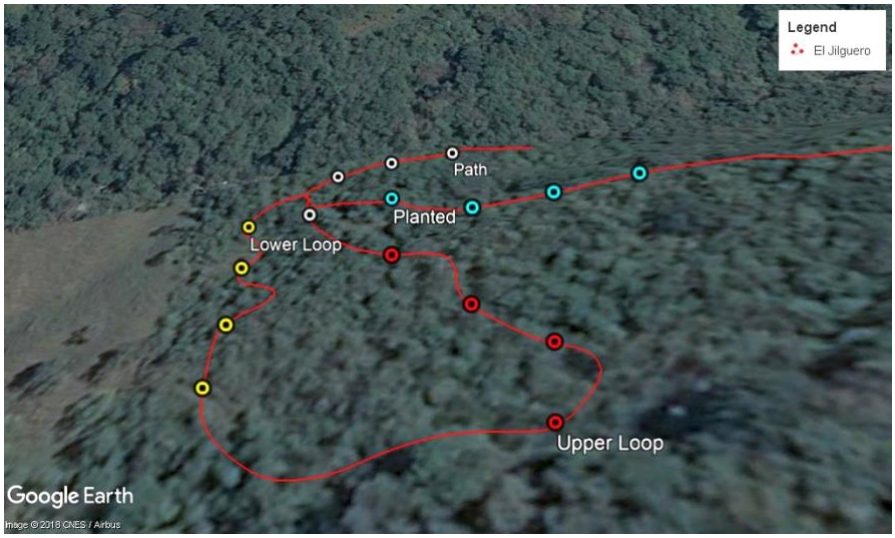

Figure 1. Location of transects on El Jilguero trail.

4 MATERIALS & METHODS

The study occurred over 4 weeks from 20th May to 15th June, 2018. Four traps were set up 50 m apart along each of the 4 transects. Each trap on a transect was baited with a different type of bait, either banana, papaya, dung, or sweet mud. Traps were set-up on Mondays, checked twice a day in the morning and afternoon, and then taken down on Fridays, for a total of 4 days of collection each week. Each week, the type of bait at each trap location was changed, so that over the 4-week period each trap location was baited with each of the 4 bait types.

4.1 TRAPS

General trap set up and checking procedures followed those presented in Cloudbridge's Butterfly Survey Protocol (Powell and Spooner 2018). The traps consist of a circular mesh tube closed on the top end with a rain cover, with bait suspended on a plate 5 cm centimetres below the bottom opening of the tube (Figure 2). The bait is placed in a small cup, filled near to the brim (<1 cm) so that the butterflies can reach the bait, and placed in the centre of the plate. The butterflies fly into the trap to feed from the bait, after which they take off, flying up into the mesh tube, where they become trapped (Figure 3). Traps were hung with the top of the trap approximately 2 m above the ground, and approximately 1-2 m away from the trunk of a tree (to help prevent other animals stealing the bait).

Traps were checked in the morning, arriving at the first trap by 8 am, and again in the afternoon, arriving at the first traps by noon. Any butterflies caught in the traps were carefully removed, photographed on both the dorsal and ventral sides, and then released.

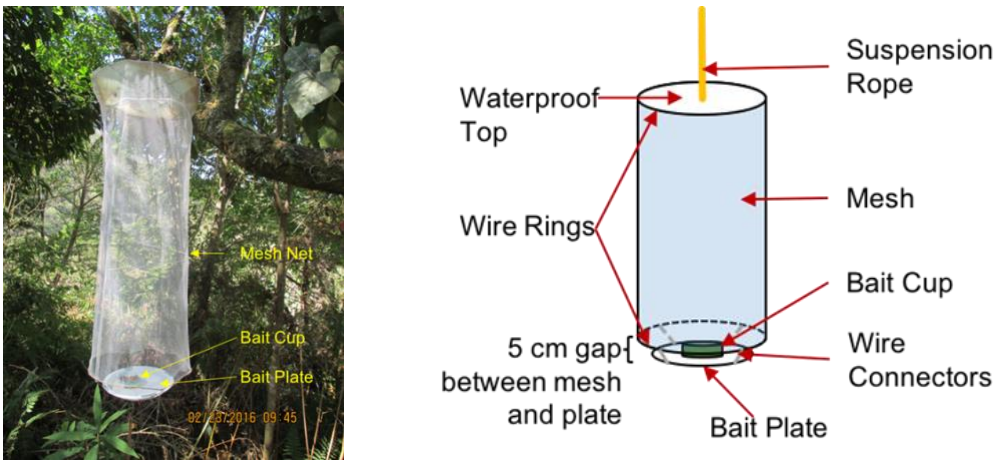

Figure 2. Butterfly live trap.

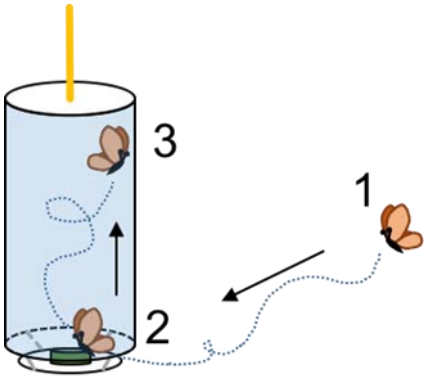

Figure 3. Diagram of how live traps capture butterflies. 1. Butterfly smells the bait. 2: Butterfly enters trap to feed on bait. 3: Butterfly leaves bait, flying upward, becoming trapped in mesh tube.

4.2 BAITS

The fruit bait for the traps was made 7-8 days prior to usage in order to give the bait time to adequately ferment. The sweet mud bait was made the day before or the morning of set-up, and the dung was collected fresh either the night before or the day of set-up. The bait recipes are provided below.

Banana and Papaya Bait (sufficient for one trap):
- ½ ripe banana (yellow with black spots to completely black) or ½ small ripe papaya,
- 1 teaspoon sugar,
- ¼ teaspoon yeast, and
- ½ cup of water.

1. Chop bananas/ papaya into small pieces and mash into a pulp.
2. Add the sugar, yeast, and warm (not hot) water and mix well.
3. Divide the mixture into several glass jars with tight fitting lids, leaving ½ to ¼ of the jar empty to give the mixture room to expand.
4. Tightly cap each jar and leave in a sunny place.
5. In order to keep the gases in the jar from building up too much, once a day:
    a. Open the jar,
    b. Cap the jar,
    c. Shake vigorously,
    d. Open the jar, and
    e. Recap the jar.

Sweet Mud Bait (sufficient for one trap):
- ¾ cup of soil,
- 1 teaspoon sugar,
- ½ teaspoon of salt, and
- ¼ cup of water.

4.3 DATA ANALYSIS

A chi-square goodness-of-fit test was used to test for significant difference in the abundance and species richness of butterflies caught with the different bait types. The expected values used for both tests were calculated as the average of the sum of all categories.

Species assemblages were compared using Morisita's Index (MI) as described in Kwak and Peterson (2007), where the similarity, *C*, between assemblages *j* and *k* is calculated as:

$$C_{jk} = \frac{2\sum K_{ij}X_{ik}}{(\lambda_j + \lambda_k)N_j N_k}$$

where

$$\lambda_j = \frac{\sum[X_{ij}(X_{ij}-1)]}{N_j(N_j-1)} \quad \text{and} \quad \lambda_k = \frac{\sum[X_{ik}(X_{ik}-1)]}{N_k(N_k-1)}$$

MI varies from 0 to 1, with 0 indicating no species in common, and 1 indicating completely identical composition, and is not significantly influenced by the number of individuals in an assemblage sample (unless the total is very small) and is insensitive to size displacements.

Using the MI values, hierarchical cluster analysis using average-linkage clustering as described in Kwak and Peterson (2007) was used to examine the relationship between the community similarity of the different bait types.

The species captured during this study was compared to the species collected during previous butterfly live-trap studies at Cloudbridge Nature Reserve (Appendix A) to determine if any new species were collected that were not collected in banana baited traps previously.

5 RESULTS

Overall, 63 butterflies of 20 confirmed species were caught in the traps during the study (Table 1). Three butterflies were caught that were not identified. Unidentified butterflies were used in abundance analyses but in none of the other analyses. Banana bait traps caught the largest number of butterflies (31), followed by papaya (22). Both the cow dung and sweet mud traps caught far fewer at 4 and 6, respectively.

Table 1. Species collected with different bait types.

Species	Banana	Papaya	Cow Dung	Sweet mud
Archaeoprepona amphimachus amphiktion	1	2		
Cissia gigas		1		
Cissia hermes	4			
Cissia sp.		1		
Consul electra electra	1	2		
Consul panariste jansoni		1		
Cyllopsis argentella				1
Cyllopsis rogersi	1			
Drucina leonata	3	2	1	2
Eryphanis bubocula		2		
Memphis elara		1		
Opoptera staudingeri staudingeri		1		
Opsiphanes cassina chiriquensis		1		
Opsiphanes quiteria quirinus	1			
Oxeoschistus cothon	1	4		
Pedaliodes dejecta dejecta	7	1	2	1
Praepronophila perperna	1			
Satyrotaygetis satyrina	5	3		2
Smyrna blomfildia datis	1			
Yphthimoides renata	3			
Unknown	2		1	
Total	31	22	4	6
Species Richness	12	13	2	4
Unique Species	6	7	0	1

Butterfly abundance across the bait types differed significantly from expected (X^2(df = 3, N = 63) = 32.05, p < .001). The banana bait attracted more butterflies than expected (X^2(df = 1) = 19.69, p < .001), while the cow dung and sweet mud baits attracted less butterflies than expected (dung, X^2(df = 1) = 11.69, p < .001; sweet mud, X^2(df = 1) = 8.05, p = .005), and papaya did not differ significantly from expected (X^2(df = 1) = 3.31, p = .069) (Figure 4).

Butterfly species richness across the bait types differed significantly from expected (X^2(df = 3, N = 31) = 11.97, p = .008). The papaya bait attracted more species than expected with 13 species (X^2(df = 1) = 4.74, p = .03), while the cow dung bait attracted less species than expected with 2 species (X^2(df = 1) = 5.69, p = .017), while banana and sweet mud did not differ significantly from expected with 12 and 4 species, respectively (banana, X^2(df = 1) = 3.11, p = .078; sweet mud, X^2(df = 1) = 2.42, p = .120) (Figure 5).

The papaya bait captured the most number of unique species (not captured in any other bait type) with 7 species, followed closely by banana at 6 species. Cow dung attracted no unique species, while sweet mud captured only 1 unique species. One new species was captured during the study, *Opsiphanes quiteria quirinus*, however, it was captured with the banana bait (Table 1). When compared with previously butterfly data

collected at Cloudbridge (Appendix A), all of the species collected during this study were also collected in banana baited live-traps previously, frequently in large numbers. Therefore, the 'unique' species collected during the study are not solely attracted to a single bait type and did not capture species previously unseen in the traps.

Overall, the community similarity between the different bait types was highest between cow dung and sweet mud (MI = 0.95); and lowest between banana and sweet mud (MI = 0.30), and papaya and sweet mud (MI = 0.31) (Table 2). Hierarchical cluster analysis found two clusters, with cow dung and sweet mud being the most similar at 0.95, and banana and papaya being moderately similar at 0.70 (Figure 6). The two clusters had a MI value of 0.60 when compared to each other.

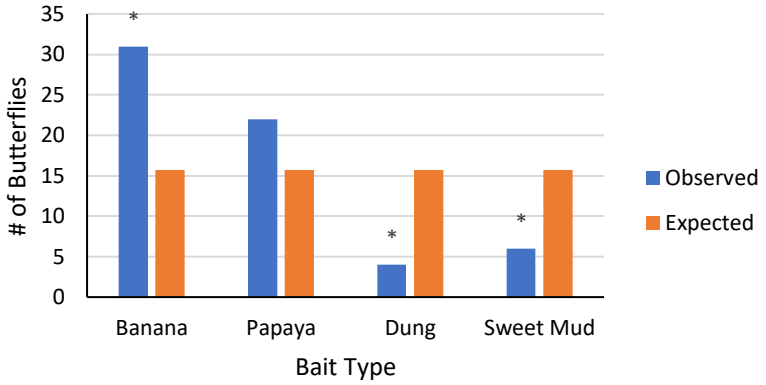

Figure 4. Chi-square goodness-of-fit analysis of butterfly abundance across different bait types. * indicates significant difference from expected.

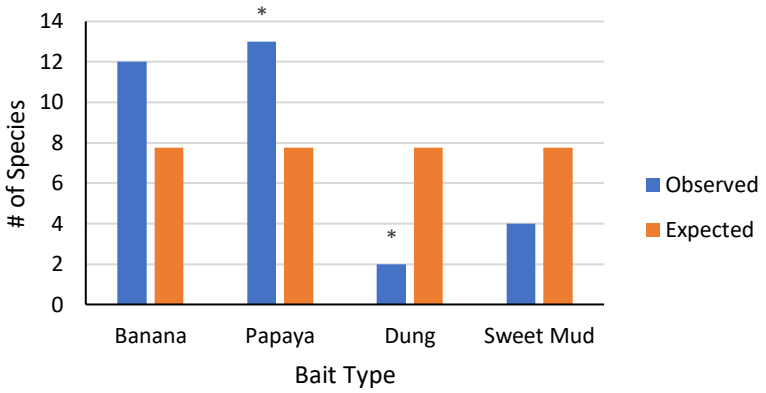

Figure 5. Chi-square goodness-of-fit analysis of butterfly species richness across different bait types. * indicates significant difference from expected.

Table 2. Similarity of species assemblages in each bait type using Morisita's Index.

	Banana	Papaya	Cow Dung
Papaya	0.70		
Dung	0.89	0.31	
Sweet Mud	0.30	0.88	0.95

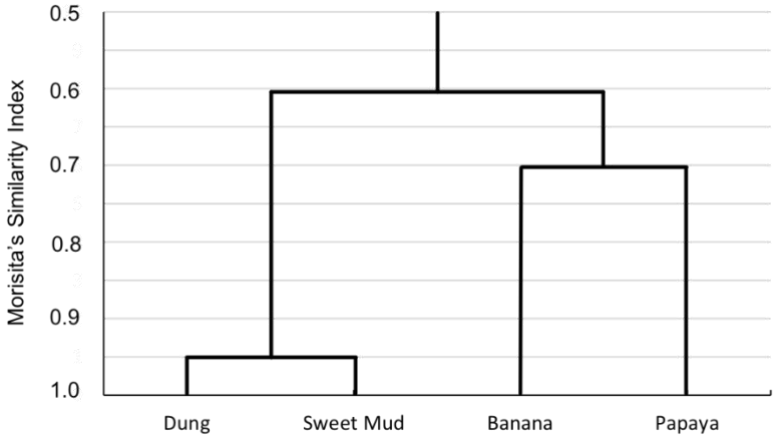

Figure 6. Hierarchical cluster analysis of community similarity between the bait types.

6 DISCUSSION

While the fruit baits performed much better for attracting butterflies than cow dung or sweet mud, none of the baits performed better or caught new species when compared to the banana bait. The cow dung and sweet mud baits performed similarly poorly and did not attract any species not also caught with the fruit baits. The only unique species caught between the two baits, *Cyllopsis argentella*, has been caught in large numbers in the banana baited traps in the past (Appendix A).

Boggs and Dau (2004) found in a study in Colorado in the United States that when comparing mud, and herbivore and carnivore dung baits, butterflies tended to be most attracted to the carnivore dung, followed by the herbivore dung, and finally the mud, although there were some species specific variations. This general preference also coincided with the sodium content of the bait types, with carnivore having the highest and mud the lowest sodium content. The carnivore dung also attracted the highest numbers of butterflies in the *Lycaenidae* and *Hesperiidae* families, which are two families that have been poorly studied at Cloudbridge. As such, a repeat of this study using a carnivore or omnivore dung may prove beneficial.

Beck et al. (1999) also found sodium content to be an important attractant for baits. In a study conducted in Borneo, they tested a variety of salts and a protein solution of albumin, and found sodium (NaCl) and albumin had by far the highest rates of acceptance across all butterfly species. In their study, they used a 1.0 M NaCl solution, which is the equivalent of 58.44 g NaCl / 1 L of water. The table salt content in the sweet mud used in this study was around 20 g salt / 1 L water, a much lower concentration. If a mud (or salt and water solution) were to be trialled again as a bait, the salt content should be increased to a level similar to the Beck et al. (1999) study in order to hopefully improve its effectiveness.

While sodium content is an important attractant in mud-puddling, Beck et al. (1999) also found that albumin was accepted by all butterfly families and proved more attractive than sodium (NaCl) for some families. They found that *Papilionidae* and *Pieridae* visited NaCl solutions more than the albumin, while *Nymphalidae*, *Hesperiidae*, and *Lycaenidae* preferred the albumin solutions. *Lycaenidae* butterflies in particular preferred the albumin solutions, which is of particular interest for Cloudbridge as only one species of the *Lycaenidae* has been identified in the reserve to date (Cloudbridge 2018a). Egg whites are a good natural source of albumin, and, as such, it may be worthwhile trialling an egg white solution as a bait type.

Within the fruit baits, banana generally out performed papaya. Although the papaya bait had lower abundance, the papaya traps caught one more species than did the banana traps. However, all the species caught within the papaya traps that were not caught in the banana traps during this study, have been caught in banana traps in the past. Lapkratok and Suwanwaree (2014) also tested banana and papaya as a butterfly bait and found that banana baits attracted more individuals and species than papaya. Therefore, the introduction of papaya as a new bait would not seem to provide any additional value to future butterfly live-trapping studies when compared to using banana bait.

Beyond just testing banana and papaya, Lapkratok and Suwanwaree (2014) tested a wide variety of baits in Thailand including (listed in order of effectiveness): fermented fish and pineapple, fish sauce, fermented fish, pineapple, banana, papaya, watermelon, and beer. As they found that pineapple captured both more individuals and species than the banana, it would be worthwhile to trial pineapple as a bait in Cloudbridge, as it is a readily available fruit in Costa Rica.

Fermented fish is a common bait to use in butterfly trap studies (Eger et al. 2015, Freitas et al. 2014, Sourakov and Emmel 1995) as well as other entomological traps. Cloudbridge had previously attempted using tuna as a bait for beetle pitfall traps as well as butterfly traps, only to have other animals raid and/or destroy the traps. As well, as the smell of rotting fish is extremely unpleasant to humans it causes issues both with the volunteers

and researchers baiting and checking the traps and with the public who come to visit the reserve. While Lapkratok and Suwanwaree (2014) did not provide a breakdown of the species or families attracted to the different bait types, given their findings that the fermented fish baits were by far the most effective, it may be worth trialling another study with a fish bait, although some modifications to the traps may need to be done to help prevent theft of the baits. However, the issue of the smell may prove too large to overcome.

Of note during the study was that the number of butterflies captured increased every week. The majority of those caught in the first week were caught in the traps on the 'planted' transect which is the transect that has been used previously for an ongoing butterfly study. It may be possible that it took time for the butterflies to get used to, or find the traps on the other transects, which were newly created for this project.

Going into wet season during this study, there was an increase in rain and it started to rain earlier in the day, meaning the butterflies were less active than in the dry season and the plates of the bait traps were often found with water inside during the morning checks. Adjustment to the trap design to help prevent the water accumulation could help improve data collection in future studies.

7 CONCLUSION & RECOMMENDATIONS

The data showed that there was no added benefit in terms of butterfly numbers or new species to using papaya, sweet mud, or cow dung in addition to, or instead of banana bait. For future bait studies, it is recommended to trial baits of: carnivore or omnivore dung, fermented fish, pineapple, egg, and a high concentration salt solution. As different butterflies can be present at different times of year, it may be beneficial to conduct the study in different seasons, to see if the results vary. As well, it would be interesting to repeat the study using both understory and canopy traps to see if the same results occur with high canopy species.

8 ACKNOWLEDGEMENTS

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Appendix A: Butterflies captured in previous butterfly studies between 2016-2018 at Cloudbridge Nature Reserve.

Family	Species	Capture Method		
		Photo	Sweep Net	Live-Trap
Hesperiidae	Autochton vectilucis		3	
	Halotus angellus		1	
	Synapte salenus salenus		1	
	Talides alternata		1	
	Urbanus pronus		4	
Nymphalidae	Actinote anteas	2		
	Actinote ozomene nox	5	16	1
	Adelpha demialba		4	
	Adelpha tracta		2	
	Anartia fatima fatima		1	
	Anthanassa ardys	1	3	
	Anthanassa crithona	4	4	10
	Anthanassa otanes fulviplaga		7	8
	Anthanassa otanes otanes		1	1
	Anthanassa sosis		3	22
	Archaeoprepona amphimachus amphiktion			31
	Archaeoprepona demophon centralis			1
	Caligo atreus dionysos		1	3
	Caligo brasiliensis sulanus	1		8
	Castilia fulgora		1	
	Catonephele chromis godmani		1	4
	Cissia confusa			1
	Cissia gigas			2
	Cissia hermes	1	1	4
	Cissia labe			1
	Cissia sp.			1
	Colobura dirce dirce			5
	Consul electra electra	1	2	35
	Consul panariste jansoni			4
	Cyllopsis argentella	3	22	189
	Cyllopsis rogersi		1	1
	Danaus plexippus plexippus		1	
	Diaethria astala astala	1	1	1
	Diaethria clymena marchalii			2
	Diaethria gabaza eupepla	1	4	1
	Diaethria pandama	1	5	
	Dione moneta poeyii	3	4	
	Dircenna klugii	1	9	
	Doxocopa cyane mexicana	1	1	
	Doxocopa laurentia cherubina	1		
	Drucina leonata	3	17	76
	Dryadula phaetusa	1		

Appendix A: Butterflies captured in previous butterfly studies between 2016-2018 at Cloudbridge Nature Reserve.

Family	Species	Capture Method		
		Photo	Sweep Net	Live-Trap
Nymphalidae (cont'd)	Episcada salvinia		28	2
	Eryphanis bubocula		1	20
	Eueides procula vulgiformis		4	
	Eutresis hypereia theope		1	
	Forsterinaria neonympha	1	1	11
	Fountainea glycerium glycerium	1	2	17
	Fountainea nobilis peralta			8
	Godyris nero		3	
	Greta annette		11	
	Greta polissena umbrana		10	
	Greta sp.		3	
	Heliconius clysonymus montanus	6	19	
	Heliconius doris			1
	Heliconius pachinus	2	1	
	Hermeuptychia harmonia		14	9
	Historis acheronta acheronta			1
	Hypanartia dione arcaei	5		1
	Hypanartia lethe	1	1	
	Hypanartia trimaculata autumna	1		
	Hyposcada virginiana evanides		3	
	Ithomia heraldica heralica	1	12	
	Ithomia patilla		7	1
	Ithomia xenos xenos		1	
	Manataria hercyna maculata			3
	Marpesia marcella valetta	3	7	
	Mechanitis menapis saturata		11	
	Memphis ambrosia ambrosia			5
	Memphis arginussa eubaena	1		3
	Memphis aulica	1		
	Memphis beatrix		1	13
	Memphis elara		1	8
	Memphis pithyusa pithyusa			1
	Memphis xenocles carolina			4
	Morpho helenor marinita	1		
	Morpho peleide limpida	3		
	Napeogenes cranto paedaretus		3	
	Oleria vicina	5	38	
	Olyras crathis staudingeri		1	
	Opoptera staudingeri staudingeri			64
	Opsiphanes cassina chiriquensis			43
	Opsiphanes quiteria talamancensis			1
	Oxeoschistus cothon	3	24	42

Appendix A: Butterflies captured in previous butterfly studies between 2016-2018 at Cloudbridge Nature Reserve.

Family	Species	Capture Method		
		Photo	Sweep Net	Live-Trap
Nymphalidae (cont'd)	Oxeoschistus crothonides		3	9
	Oxeoschistus hilara euriphyle		1	16
	Pareuptychia metaleuca	2		2
	Pedaliodes dejecta dejecta	3	10	48
	Pedaliodes manis	1	1	2
	Praepronophila perperna		1	2
	Pronophila timanthes		3	5
	Pseudomaniola phaselis rogersi		2	1
	Pteronymia artena		1	
	Pteronymia fulvimargo		3	
	Pteronymia simplex simplex		28	
	Pycina zamba zelys			1
	Satyrotaygetis satyrina	6	21	188
	Siproeta epaphus epaphus		3	
	Smyrna blomfildia datis	4	2	33
	Taygetis uzza	1		
	Tegosa anieta anieta		2	
	Tegosa nigrella niveonotis		1	
	Vanessa virginiensis		1	
	Yphthimoides renata	3	1	9
Papilionidae	Papilio isidorus rhodostictus	1		
	Pterourus menatius laetitia	1		
	Eurytides calliste olbius	1		
Pieridae	Catasticta ctemene actinotis		6	
	Catasticta flisa melanisa		4	
	Catasticta hegemon hegemon		6	
	Catasticta nimbice bryson		11	
	Catasticta teutila flavomaculata		2	
	Catasticta theresa	1		
	Dismorphia crisia lubina		16	
	Dismorphia zathoe pallidula	1	3	
	Eurema salome		5	
	Eurema sp.		13	
	Leodonta dysoni		1	
	Leptophobia aripa elodia		8	
	Leptophobia caesis tenuicornis	1	9	
	Lieinix nemesis atthis		1	
	Pereute charops		1	
Riodinidae	Mesosemia asa asa		6	
	Mesosemia grandis		5	