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## Comparing amphibian species diversity and abundance in natural forest and cacao agroforest at Finca La Magnita, Changuinola, Bocas del Toro, Panamá Lake Barrett

Lake Barrett SIT Study Abroad

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# Comparing amphibian species diversity and abundance in natural forest and cacao agroforest at Finca La Magnita, Changuinola, Bocas del Toro, Panamá

Lake Barrett SIT Panama: Spring 2022

#### Abstract

Amphibians are currently undergoing rapid and drastic biodiversity loss worldwide, largely due to the disease Chytridiomycosis. Because of this, efforts to conserve amphibian biodiversity are urgent and have been given increasing importance. However, studies investigating the effectiveness of agroforestry systems, a commonly used agricultural method in which trees and other elements of forests are retained on land used for agriculture, for amphibian conservation are uncommon. As a result, the capacity of agroforests to serve as a tool for amphibian conservation is unclear. To determine if agroforests can serve as a habitat for amphibians and a tool for their conservation, this study examines amphibian species diversity, evenness, and richness, as well as overall abundance in a natural forest and a cacao agroforest in Changuinola, Bocas del Toro, Panama. Results show significantly higher amphibian species diversity, evenness, richness, and overall abundance in natural forest compared to agroforest. This suggests that agroforests are not a substitute for natural forests for terrestrial amphibians. However, the cacao agroforest does demonstrate the capacity to harbor some amphibians, which supports the idea that agroforestry is able to conserve biodiversity that traditional agriculture cannot. Despite this, natural forest is still shown to be overwhelmingly the better habitat for terrestrial amphibians. Therefore, conservation of natural forests and expansion of protected areas is recommended as the ideal course of action for amphibian conservation moving forward. Further investigation into amphibians and agroforestry systems and their integration worldwide is recommended to continue to examine their conservation potential for amphibians.

#### Resumen

Los anfibios están disminuyendo rápida y drásticamente en todo el mundo, en gran parte por la enfermedad Chyrtidiomycosis. Por eso, esfuerzos para conservar la biodiversidad de anfibios son urgentes y recientemente se le han dado más importancia. Sin embargo, investigaciones sobre la habilidad de los bosques agroforestales, un método de agricultura en cual árboles y otros elementos del bosque se mantienen en las parcelas de tierras utilizadas para agricultura, para conservar la biodiversidad de anfibios son pocos. El resultado es que el potencial de bosques agroforestales como herramienta para la conservación de anfibios es desconocida. Para determinar si bosques agroforestales pueden ser un hábitat para anfibios, esta investigación examina la diversidad, la equitabilidad, la riqueza, y la abundancia de anfibios en un bosque natural y un bosque agroforestal de cacao en Changuinola, Bocas del Toro, Panamá. Los resultados muestran que hay significativamente más diversidad, equitabilidad, riqueza, y abundancia de anfibios en el bosque natural. Esto sugiere que los bosques agroforestales no son sustitutos para los bosques naturales para anfibios terrestres. Sin embargo, el bosque agroforestal de cacao muestra la habilidad de contener ciertas especies de anfibios, lo cual apoya la idea de que bosques agroforestales pueden contribuir en conservar la biodiversidad en un sentido que agricultura tradicional no puede. A pesar de eso, bosques naturales se muestran a ser el hábitat mucho mejor para anfibios terrestres. Por eso, la conservación de bosques naturales y la expansión de áreas protegidas es la acción más apropiada para la conservación de anfibios en el futuro. Más investigaciones sobre anfibios y sistemas agroforestales se recomiendan para continuar a examinar el potencial de conservación de bosques agroforestales para comunidades de anfibios.

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También, me gustaría dar las gracias a Antonio Lozada por guiarme por los bosques en el día y la noche. Si yo no tuviera su ayuda no podría haber hecho la investigación. Yo habría estado perdido muchos tiempos y seguramente no podría haber encontrado ni hecho los transectos. También le doy gracias por indicar las dos serpientes equis (*Bothrops asper*) que vimos para que yo no estuviera presentado a ellos de una manera menos agradable. Antonio conoce los bosques muy bien y soy muy agradecido por su ayuda y experiencia.

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## Introduction

#### Amphibian Biodiversity Loss

The loss of biodiversity is a growing problem in the industrializing world that is causing alarm among conservationists. This is mainly attributed to habitat loss, pollution, climate change, invasive species, and infectious diseases. These drivers, and the subsequent biodiversity loss, are not limited to any one region of the globe, though they are worst in the tropics. While biodiversity loss is affecting many different types of organisms, it is worst in amphibians, because they are most sensitive to the stressors of biodiversity due to their need for aquatic and terrestrial habitat (Hussain & Pandit, 2012). Amphibians are an integral part of the ecosystems they live in and the loss of their diversity and abundance can have large, detrimental impacts on their ecosystems (Whiles et al., 2013). For example, the disappearance of amphibians from Parque Nacional G. D. Omar Torríjos Herrera in Panama has been shown to cause a parallel collapse in snake abundance (Zipkin et al., 2020). This makes understanding global amphibian declines important for predicting ecosystem health. Therefore, the causes of amphibian biodiversity loss have been well studied.

While they suffer from many stressors, one of the largest factors driving amphibian loss globally is Chyrtidiomycosis (Scheele et al., 2019). Chyrtidiomycosis is a disease caused by the fungi *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* that has been spread worldwide by humans (Scheele et al., 2019). Estimates suggest it has caused the decline of at least 501 amphibian species worldwide (Scheele et al., 2019), though some have argued this estimate to be inflated (Lambert et al., 2020). While an issue worldwide, this effect has been concentrated in certain regions. The disease has had the greatest effect on the amphibians of Australia and Central and South America (Scheele et al., 2019). Within those regions, the species most affected are typically those in the genera *Atelopus, Craugastor*, and *Telmatobius* (Scheele et al., 2019), though all species can be affected by the disease. The situation with Chytridiomycosis truly makes amphibian declines unique from those of other taxa, since it is so linked to one specific disease. No other taxa are threatened by a singular disease the way amphibians are by Chytridiomycosis (Scheele et al., 2019). Because of this, Chytridiomycosis is well studied (ex. Lips, 1999; McCaffery & Lips, 2013; Perez et al., 2014; McCaffery et al., 2015).

The damage the disease has done can attract the spotlight and draw attention away from other threats, however other threats still add to the problem as well. For example, habitat loss has been shown to harm amphibian communities greatly (Fulgence et al., 2021; Greenberg et al., 2018). These other effects are not separate from Chytridiomycosis and, in fact, other

drivers of biodiversity loss, like habitat loss, can work in tandem with Chytridiomycosis, enabling the disease (Scheele et al., 2019). Despite efforts to understand all aspects of decline, the true extent of amphibian decline is elusive and remains undetermined. Because of that, understanding, monitoring, and preventing the decline of amphibians worldwide is of the utmost importance for conservationists.

#### Amphibians in Panama

Panama is located in one of the areas hardest hit by Chytridiomycosis: Central America. The effects of the disease were first reported in the area in Costa Rica in the 1980s by anecdotal reports (Hussain & Pandit, 2012). However, the disease was not given enough attention until nearly 20 years later (Scheele et al., 2019) when in the 1990s, serious amphibian declines had begun to be widely reported (McCaffery et al., 2015). Since then, mass amphibian declines have spread like a wave from Costa Rica west through Panama, leaving a trail of decimated amphibian communities (Perez et al., 2014). Because of this, amphibian declines caused by Chytridiomycosis have left an unignorable mark on the country's biodiversity.

Because of this, much of the research on amphibians done in the country today revolves around how populations have changed because of the disease (ex. Perez et al., 2014; McCaffery et al., 2015; McCaffery & Lips, 2013). Extensive research has been done on the frogs of the genus Atelopus in Panama (ex. Perez et al., 2014; McCaffery et al., 2015) for multiple reasons. One reason is the dire state that Atelopus species find themselves in. At least 76 of 99 species are endangered or critically endangered (IUCN, 2022), and the ranges of species are steadily declining (McCaffery et al., 2015). Therefore, understanding their characteristics is critical for being able to help them. The other reason is the cultural importance of these frogs. Many species in this genus are locally beloved, but no more so than la Rana Dorada (Atelopus zeteki), which is a national symbol in Panama. A. zeteki is now presumed extinct in the wild and its survival hinges on the work of the El Valle Amphibian Conservation Center and the Panama Amphibian Rescue and Conservation Project preserving them in captivity. This has led many researchers to investigate A. zeteki's relationship with Chytridiomycosis to determine if and how the species can be restored to its natural habitat (ex. Perez et al., 2014; Bustamante et al., 2010; Gagliardo et al., 2008). Atelopus are certainly one of the most studied groups of amphibians in the country, but research is done into other amphibians as well.

For example, *Oophaga pumilio* has been intensively studied in the Bocas del Toro region of western Panama because of the color polymorphism exhibited in the region (ex. Cummings and Crothers, 2013; Dugas et al., 2015; Maan & Cummings, 2012; Richards-Zawacki et al., 2012). Researchers have tested many different variables aiming to determine what has driven the evolution of different color morphs. The response to the aposematic color by predators (Cummings and Crothers, 2013; Maan & Cummings, 2009), the influence of female choice and sexual selection (Richards-Zawacki et al., 2013), and behavioral differences (Dugas et al., 2015) are all variables that have been examined, though the true driver could be any combination of factors and remains undetermined. Through this work, extensive physiological, behavioral, and ecological data have been collected on this species. This puts *O. pumilio* among the most well studied species in the country alongside *A. zeteki*.

Aside from these popular study species, research on amphibians can be slim in Panama. Central Panama is typically the most well studied (Batista et al., 2020) and because of this, researchers that work in the east and west of the country suggest more effort be placed in those areas (Medina et al., 2019; Batista et al., 2020). Moving forward, filling these gaps can play an important role in providing the insight necessary for conservationists to be able to support amphibian communities that remain in Panama after the destructive influence of Chytridiomycosis.

#### Agriculture as a Driver of Biodiversity Loss

While Chytridiomycosis takes the spotlight for amphibian biodiversity loss, agriculture is currently the biggest threat to all IUCN red list species around the world (Kehoe et al., 2017). Agriculture typically converts natural habitat into monocultures, which are known to greatly decrease the biodiversity of the area (Udawatta et al., 2019). Agriculture is currently converting land at a scale that makes it the most prominent cause of biodiversity loss on the planet (Newbold, 2018; Chaudhary et al., 2016; Udawatta et al., 2019). Further, the rate of deforestation for agriculture is estimated to have only increased in the 21<sup>st</sup> century (Bhagwat et al., 2008). This trend of increase and expansion is the most concerning aspect of agriculture for biodiversity. The human population continues to grow and with it demand for agricultural products (Chaudhary et al., 2016). One study predicts a 70% increase in food demand by 2050 (Chaudhary et al., 2016), while another predicts it will double by 2050 (Kehoe et al., 2017). Though estimates differ, demand is sure to rise and an increase in land use is likely in order to reach this need (Kehoe et al., 2017). The amount of increased land use predicted varies depending on estimates of how much yields can be increased using current land, but one moderate prediction estimates a 10-25% increase in land use by 2050 (Lanz et al., 2018). This would mean biodiversity loss beyond what is currently being observed. One model estimates land use change has already been responsible for 20% of local species richness loss and predicts that this number could increase up to 30% (Newbold, 2018). This is particularly concerning considering that ecosystem functions can begin to be greatly diminished when as little as 20% of species are lost (Newbold, 2018).

This effect of biodiversity loss will be most concerning in the tropics, where agriculture is expanding the most (Kehoe et al., 2017). Within the tropics, the rich ecosystems of tropical forests and grasslands stand to lose the most biodiversity if the predicted agricultural expansions occur (Newbold, 2018; Kehoe et al., 2017). Agricultural growth in these environments is worrying due to the amount of biodiversity they harbor. Of this biodiversity lost due to agricultural expansion, reptiles and amphibians are the most vulnerable and a predictive model expects that they will be disproportionately lost, with amphibians slightly

more so than reptiles (Newbold, 2018). Among amphibians, members of evolutionarily distinct lineages of amphibians are most affected by the conversion of natural habitat to human use and will be lost more than other lineages (Greenberg et al., 2018). Therefore, agriculture poses a great threat to the world's already vulnerable amphibian biodiversity. The future of biodiversity conservation will depend on human agricultural methods (Mendenhall et al., 2014), meaning that the way agriculture is practiced will directly affect conservation outcomes. Because of this, preventing the loss of more amphibians than what has already been lost due to Chytridiomycosis requires agricultural solutions that can avoid the destruction of habitat that is ongoing and predicted for the future.

#### Agroforestry Systems

One such solution that has been used to accommodate biodiversity and agriculture is agroforestry. Agroforestry is the retention of trees and other elements of forests on land used for agriculture (Atangana et al., 2014), and the most prominent examples are cacao and coffee farms (Murrieta-Galindo et al., 2013; Van Bael et al., 2007). The best agroforestry systems allow for significant canopy cover, involve minimal management, and use no chemicals (Bhagwat et al., 2008; Deheuvels et al., 2014). The land is able to provide local livelihoods and produce crops while retaining aspects that are beneficial for the local environment (Bhagwat et al., 2008; Deheuvels et al., 2014). This allows the land to serve multiple functions and fill ecological, social, and economic needs (Nair et al., 2017). Because of that, agroforestry systems have many benefits that traditional agriculture does not.

First, agroforests maintain the ability to provide ecosystem services that agricultural monocultures lose (Nair et al., 2017). Agroforests also retain much more biodiversity than typical agricultural monocultures (Atangana et al., 2014). Agroforests have been shown to provide ample habitat for bird communities (Van Bael et al., 2007; Van der Wal et al., 2012) and can do the same for insect communities (Perry et al., 2016). It is estimated that they can maintain up to around 60% of the biodiversity found in the true forests (Bhagwat et al., 2008). Beyond conserving what lives within the agroforest, agroforests also play an important role in agricultural landscapes because agroforestry systems can function as biological corridors (Atangana et al., 2014). Agricultural landscapes, especially in the tropics, are typically very fragmented and agroforests can provide opportunities for mobile organisms to travel between separated forests through the agroforests (Brüning et al., 2018). Agroforests can also function as buffer zones to aid protected areas by providing areas with forest cover around them (Brüning et al., 2018; Bhagwat et al., 2008). While not natural forests, the benefits agroforests provide mean they are an important part of maintaining biodiversity in fractured landscapes (Bhagwat et al., 2008; Brüning et al., 2018; Mendenhall et al., 2014).

However, agroforestry systems are not natural forests, and therefore there are certainly drawbacks to them as well. To start, agroforests only have value as an alternative to traditional agriculture, not an alternative to forests. If agroforests become too profitable and begin

expanding, agroforests will only hurt conservation of biodiversity by becoming the newest agricultural driver of deforestation (Atangana et al., 2014). Further, agroforestry systems need to be managed properly to provide the benefits to biodiversity, as agroforests that are under intense management, such as chemical treatments, are less likely to provide habitat to biodiversity (Mendenhall et al., 2014; Atangana et al., 2014). Another shortcoming is that agroforests mainly provide habitat to species that are generalists (Angarita et al., 2015) and rare, specialist species are unlikely to inhabit them (Bhagwat et al., 2008). Because of this, many endangered and critically endangered species are unable to use them. As a whole, however, these limitations of agroforests do not make them invaluable. Though there is a stigma against agroforests due to the human manipulation of the environment, they will have to be an important part of conservation, especially in the tropics (Bhagwat et al., 2008). Because of this, agroforests will inevitably also need to play a big role in the conservation of amphibians. Understanding the way agroforestry systems interact with amphibians is therefore necessary to be able to maximize agroforestry's ability to aid in amphibian conservation.

## Effectiveness of Agroforestry for Amphibians

Despite the benefit investigations into the relationship between amphibians and agroforests would provide, the relationship remains under-researched (Fulgence et al., 2021). Since amphibians are very sensitive to changes in their environment (Deheuvels et al., 2014), it is typically expected that they would respond negatively to a large change such as the conversion of forest to agroforest (Fulgence et al., 2021). However, because there is a lack of research on the subject, there have not been enough studies performed to arrive at a consensus and conclusions vary. Generally, studies have found results that would suggest that amphibian diversity and abundance is decreased in agroforestry environments compared to natural forests. For example, studies in Colombia, Costa Rica, and Indonesia found decreased diversity and abundance in agroforestry systems compared to natural forests (Angarita et al., 2015; Deheuvels et al., 2014; Wanger et al., 2010). However, two other studies in Colombia and Mexico have demonstrated agroforests retain a significant amount of amphibian species, suggesting that agroforests are suitable habitat for amphibians (Brüning et al., 2018; Murrieta-Galindo et al., 2013). A review of literature published on the subject also found inconsistent results (Palacios et al., 2013). In that review, the majority of studies comparing forests before and after conversion to agroforestry found decreased amphibian diversity but higher abundance after conversion (Palacios et al., 2013). However, the same review found that in most studies comparing local areas of agroforest and natural forest, amphibian diversity did not change between the environments, though the species found there differed (Palacios et al., 2013).

One likely reason that study results vary is due to different regions, ecosystems, and species having different responses to agroforests (Fulgence et al., 2021). However, because the literature on the subject is limited, regional effects and whether or not agroforests can harbor levels of amphibian biodiversity and abundance comparable to natural forests remain unclear.

One thing that studies do appear to agree on is that the species found in agroforestry systems represent a different community than those found in natural forests. Many studies demonstrate different communities of amphibians in forest and agroforest environments (Palacios et al., 2013; Brüning et al., 2018; Murrieta-Galindo et al., 2013). One particular change in composition that has been observed is the dominance of generalists and species of low conservation concern in agroforest environments (Wanger et al., 2010). In contrast, specialists and endangered species are unlikely to be found in agroforestry environments (Palacios et al., 2013). This fact, along with the inconsistency in which it has been found that agroforests maintain amphibian diversity, lead some to question their effectiveness as a conservation tool for amphibians (Fulgence et al., 2021). However, agroforests do not need to harbor identical amounts of biodiversity as natural forests to serve as a tool for conservation. Due to the fragmented nature of the tropics (Brüning et al., 2018), every area that conserves habitat counts. Because of this, though evidence suggests they are not the same as natural forests for amphibians, agroforests will have to be a part of conserving amphibian biodiversity moving forward (Palacios et al., 2013).

#### Agroforestry in Panama

Research on agroforestry in the Panama is generally slim. Studies that have been done focus on the Bocas del Toro province, where cacao agroforestry is common. Social studies have surveyed small farm owners in an attempt to understand motives behind implementation of agroforestry and optimize adoption of the method (Gosling et al., 2020; Fischer and Vasseur, 2002). From an ecological view, studies have examined the birds and insects of cacao agroforests as well (Van Bael et al., 2007; Córdoba et al., 2013). However, there is still a large amount of uncertainty around agroforestry systems in the country. Amphibians in agroforestry systems are no exception and in Panama and remain completely unstudied. However, one study done in Costa Rica examined biodiversity of cacao agroforestry systems in the Talamanca region of the country (Deheuvels et al., 2014). This region happens to border Panama on the Caribbean coast and is in close proximity to the Bocas del Toro province of Panama, where cacao agroforestry is also common. Because of its proximity, it is the most applicable study of amphibians and agroforestry systems in Panama. This study found that the agroforestry systems of the region were able to preserve the diversity of all study organisms except amphibians (Deheuvels et al., 2014). The proximity of the study would suggest that a similar pattern would be found in Bocas del Toro, Panama. However, since no studies have been performed in the area, the relationship between amphibians and agroforestry in Panama remains unknown.

Due to the precarious state of many of Panama's amphibian populations, this is an area that needs investigation. This study intends to compare amphibian species diversity and abundance in natural forest and cacao agroforest in Bocas del Toro to fill this research gap.

## **Research Question**

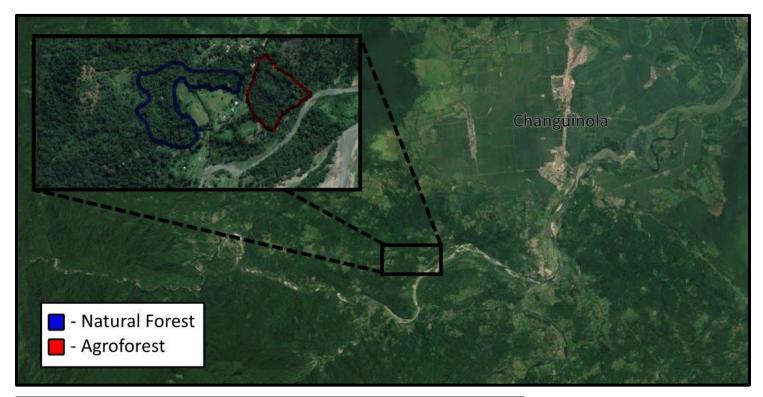
Is there a difference in amphibian species diversity and abundance in natural forest and cacao agroforest at Finca La Magnita, Changuinola, Bocas del Toro, Panama?

## Methods

## Study Site

The Bocas del Toro province, located in the north-west corner of Panama on the Caribbean coast, has good conditions for agroforestry. Most of the region sits at low elevation and the makeup of the land consists of lowland tropical humid forests (Collin, 2005). The region is very wet, receiving 3-5 meters of rainfall per year with less pronounced seasonality in rainfall compared to Panama's Pacific coast (Collin, 2005). These features create prime habitat for amphibian communities. Problematically, they also create prime land for banana plantations and, because of this, deforestation in the region is common (Collin et al., 2009). The creation of these banana plantations dates back all the way to the late 1800's when the United Fruit Company first began operations in the area (Pleasant & Spalding, 2021) and, as a result, banana plantations cover vast areas of land in the province (Collin, 2005). Agriculture therefore poses a threat to these amphibian communities in the form of habitat loss. Despite this threat, there are still many areas of the province that remain forested. In addition to those forests, agroforestry is widespread in the area (Córdoba et al., 2013). Cacao forests, which are the most prominent form of agroforestry in the region, are estimated to cover 4,500 hectares in the province (Córdoba et al., 2013). Because of this, Bocas del Toro is still home to great biodiversity.

Finca La Magnita, in Changuinola, Bocas del Toro, helps to preserve this biodiversity. Finca La Magnita is a family-owned, organic farm that grows cacao in an agroforestry system, as well as protecting areas of natural forest. The property borders the Rio Teribe and is 42 hectares in total. In the agroforestry system, Sr. Orlando Lozada maintains a diverse array of cacao varieties and banana trees and has hardwood trees providing natural forest cover. The understory is made up of the cacao and banana trees and other brush is typically absent, though leaf litter is abundant. The area is flat and builds up small puddles after strong rains. It is easily accessible and has frequent human activity. In a separate area, the natural forest is maintained on the property to allow for the provision of ecosystem services. This area contains a small creek and is more sloped than the agroforest. Brush is thicker but leaf litter coverage is comparable to the agroforest. The terrain of the natural forest makes it much more difficult to access. However, Antonio, Sr. Orlando's son and knowledgeable guide to the property, reports that poachers do sometimes enter the property without permission to hunt. (For a visual representation of the two environments, see Figure 8 in the appendix). Figure 1 shows the locations of the forests on the land. Due to the presence of both types of forest, this is an ideal study site to compare the amphibian diversity of the two types of forest.



Finca la Magnita		Gulf of Darien
> ***	Panama City	
PANAMÁ	Gulf of Panama	R

#### Figure 1 – Map of Finca La Magnita

These maps show the general and exact location of Finca la Magnita. In the enlarged box, the layout of the farm is shown. Natural forest is outlined in blue, and agroforest is outlined in red. Extensive banana plantations are visible to either side of Changuinola.

Map one (above) was created using Google Earth<sup>®</sup> (Google, 2022). Map two (below) was created using ArcGIS Earth<sup>®</sup> software by Esri (ESRI, 2022).

#### Methods

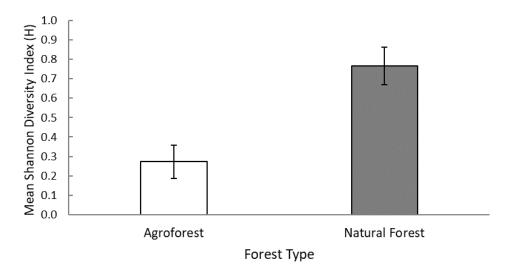
The study was conducted at the end of the dry season from April 7 through April 21, 2022. The method used to measure amphibian diversity and abundance for this project was transect surveys (described in depth in Rödel & Ernst, 2004). This study used linear transects of 50m, with flagging tape marking every 10m (similar to those from Perez et al., 2014). This method involved slowly, vigilantly, and at a consistent pace walking the transect line in an effort to identify all amphibians (Lips, 1999). The location of the transects in the cacao forest were randomly selected by assigning numbers to locations on a map of the study site and allowing a random number generator to choose the locations. The direction of each transect was then determined by spinning a stick in the field and directing the transect in the direction it pointed. The transects in the natural forest presented extra challenges since the terrain was more difficult to traverse. There, transects needed to be selected based on accessibility. The locations and directions were chosen based on the available space to be able to fit transects. In both locations, day transects were run between 8 a.m. and 10:30 a.m. and night transects were run between 7:30 p.m. and 9:30 p.m. Each transect was walked at a consistent pace optimal for detection of amphibians (average pace 4.2 m/min). Measurements of air temperature, barometric pressure, and humidity were taken at the beginning of each transect. The start and end coordinates and start and end time were recorded as well. Each amphibian seen was recorded including the meter mark and the time of observation. Then, if necessary, each amphibian was photographed for identification. The species were afterwards identified using Amphibians of Central America and Amphibians of Costa Rica (Köhler, 2011; Leenders, 2016). In total, 20 transects were created in each environment, (natural forest and agroforest), and run both during the day and at night to document diurnal and nocturnal species. For each transect in each environment the Shannon Weiner diversity index, species evenness, species richness, and overall abundance was calculated. Then, at test was run for the resulting values to determine if the results of the two forest types were statistically different.

#### Ethics

The S.I.T. IRB form was completed and submitted for consideration, and as the project did not involve work with human subjects, no further action was required. In the field, care was taken to not disturb flora and fauna of the forest while working. This meant being aware and attentive of surroundings, particularly where steps were taken. When observing the amphibians, effort was made to stress the individuals as little as possible. To do this, observations were kept as brief as possible and at night the exposure to bright light was minimized. Amphibians were not handled as a part of this project, and therefore no physical harm was done. Overall respect towards the forests the study was conducted in allowed for a minimally invasive study.

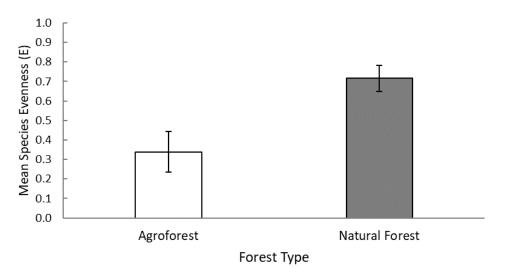
## Results

Diversity



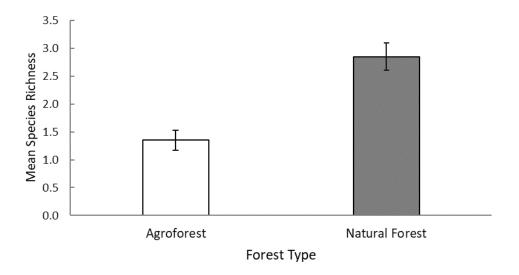
#### Figure 2 – Mean Shannon Index value per transect in agroforest and natural forest

Mean diversity values per transect in the natural forest (n = 20) were significantly higher (p = 0.0006) than mean diversity values per transect in the agroforest (n = 20). Error bars represent standard error of the mean.



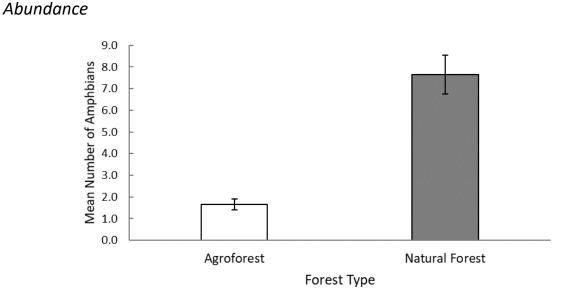
#### Figure 3 – Mean species evenness per transect in agroforest and natural forest

Mean species evenness values per transect in the natural forest (n = 20) were significantly higher (p = 0.005) than mean species evenness values per transect in the agroforest (n = 20). Error bars represent standard error of the mean.



#### Figure 4 – Mean species richness per transect in agroforest and natural forest

Mean species richness values per transect in the natural forest (n = 20) were significantly higher (p < 0.0001) than mean species richness values per transect in the agroforest (n = 20). Error bars represent standard error of the mean.



#### Figure 5 – Amphibian abundance per transect in agroforest and natural forest

Significantly more amphibians (p < 0.0001) were observed per transect in the natural forest (n = 20) than per transect in the agroforest (n = 20). Error bars represent standard error of the mean.

#### **Observed Species**

Thirteen species belonging to five families (*Dendrobatidae*, *Craugastoridae*, *Bufonidae*, *Hylidae*, and *Leptodactylidae*) were observed throughout the study (shown in Fig. 7). All observed species were Anurans. Neither salamanders nor caecilians were seen. Observations were mainly comprised of terrestrial Anurans while arboreal, aquatic, and fossorial species were typically not seen. One arboreal species (*Smilisca sordida*) was observed on one occasion. Eight species were observed in the agroforest, ten were observed in the natural forest, and the two environments shared five species (See Fig. 6).

## Discussion

#### Comparing Agroforest and Natural Forest

The results show that amphibian species diversity (Fig. 2), species evenness (Fig. 3), species richness (Fig. 4), and overall abundance (Fig. 5) were all significantly lower in the agroforest than compared to the natural forest. Beyond this, five species (Allobates talamancae, Silverstoneia flotator, Rhaebo haematiticus, Craugastor gollmeri, and Pristimantis gaigei) were found only in the natural forest and one more species, Oophaga pumilio, while found in the agroforest once, was observed the natural forest 94 times (Fig. 6). This suggests that these species are dependent on the natural forest for habitat and are unable to use the agroforest. These results indicate that, in the Bocas del Toro province of Panama, cacao agroforests are not comparable to natural forests as a habitat for terrestrial amphibians. This conclusion is consistent with a previous study in the nearby Talamanca region of Costa Rica (Deheuvels et al., 2014), which also found decreased amphibian diversity and abundance in cacao agroforests. It is also consistent with other studies done in other areas of the globe in Colombia and Indonesia, which also found decreased diversity and abundance in agroforestry systems compared to natural forests (Angarita et al., 2015; Wanger et al., 2010). However, results of this study do not completely discredit cacao agroforests as a habitat for amphibians. Three species were found in both environments in similar numbers (*Craugastor crassidigitus*, Craugastor bransfordii, and Craugastor fitzingeri), and one species, Dendrobates auratus, was found only in the agroforest (Fig. 6), suggesting it has a preference for the agroforest as a habitat. A further two, Pristimantis ridens and Smilisca sordida, were only found in the agroforest as well but, as they were both only observed once, there is insufficient evidence to be able to say they prefer that habitat. These observations mean that there is evidence in these results that, at least for some species, agroforest can be a suitable habitat for amphibians. This is consistent with previous studies that found that agroforests were able to maintain certain levels of amphibian diversity (Brüning et al., 2018: Murrieta-Galindo et al., 2013). Despite this, the results overwhelmingly indicate that natural forest is a significantly better habitat than agroforest for amphibians.

This is not unexpected, since amphibians are more sensitive to environmental changes than other taxa (Deheuvels et al., 2014) and there are definitely environmental differences between the cacao agroforest and the natural forest at the study site that could be behind the observed differences. The agroforest lacked twigs, branches, and small plants that were abundant in the natural forest, which removes microhabitats for many terrestrial amphibians. The agroforest also bears many marks of human activity that the natural forest does not, such as clear walking paths and clearings. This can undoubtedly also affect resident amphibians. Finally, while the agroforest does fill with water after hard rains, the agroforest lacks a reliable source of water because the land is controlled to be uniform for the cultivation of cacao. The natural forest contains a creek and other areas that will more consistently have water for amphibians. Due to amphibians' dependence on water, this difference is a likely a large reason that the agroforest is less effective as a habitat. Each of these changes, while perhaps less important to other organisms that have been shown to be able to use agroforests as habitat such as birds or insects (Van der Wal et al., 2012; Van Bael et al., 2007; Perry et al., 2016), appear to prevent terrestrial amphibians from using the environment.

#### **Conservation Implications**

Unfortunately, this places doubt on the ability of agroforests to aid in amphibian conservation, both locally in Panama and worldwide. Both the results and the observed differences between the two environments suggest that agroforests are not an adequate habitat for amphibians compared to natural forests. This means we need to prioritize protecting natural forests for amphibian conservation moving forward and we should certainly not be converting natural forests to agroforests. The ideal course of action to take to conserve amphibian biodiversity is to increase the number of protected areas, like nearby Parque Internacional Ia Amistad, to preserve the best habitat for amphibians. Beyond preserving forests, protected areas also minimize human impact in the area which allows them to protect species that are less tolerant of human activities. Both the agroforest and the natural forest, which are affected by human activities and near human dominated landscapes, did not contain any endangered species. In fact, all 13 amphibian species found in the study are listed as of least concern by the IUCN (IUCN, 2022). This provides evidence that to protect the most vulnerable species, protected areas need to be the priority.

Though protected areas need to be the priority, agroforests will definitely need to play a role in conservation. Though it certainly contained less amphibian diversity and abundance than the natural forest, there is still evidence that agroforests can provide habitat to some amphibians. It is important to remember that agroforests do not need to be natural forests to be of conservation value. The amphibians the agroforest contained indicate that agroforests can be of use as a conservation tool for certain species. Agroforests also have value beyond amphibians and anecdotal observations in the agroforest during field work confirm previous findings that reptiles (Palacios et al., 2013), birds (Van der Wal et al., 2012; Van Bael et al., 2007), and insects (Perry et al., 2016) are able to use agroforest as habitat. Birds, insects, and

particularly reptiles were all frequently observed while collecting data in the cacao agroforest. The ability to conserve these species, alongside some amphibian diversity, supports the value of agroforests. Because of this, agroforests should definitely continue to serve as an alternative to traditional monocultures.

## Limitations and Error

There are limitations to this study. For one, the study was conducted only in the dry season. Though less pronounced in Bocas del Toro, the dry season can still affect the amphibians that were seen. For a complete picture, the study may need to also be run in the rainy season. Further, the study method used does not identify every amphibian in the area. The method focuses on terrestrial amphibians that inhabit the forest floor and therefore conclusions should only be drawn regarding those amphibians. Additional methods, such as auditory surveys, stream transects, active traps, and passive traps would be useful for providing the complete picture. There is also likely the existence of detection error since some hidden amphibians were likely missed during transect surveys. For example, one species, Agalychnis callidryas, was observed in the area (See Fig. 9) while not on survey and is therefore known to be present. This species inhabits the forest canopy, and could plausibly have been present in transects, but, due to its arboreal nature, was never detected. Despite this, any cases such as this one would be equal between the two environments and would not bias the results. There is also some known error in the results. *Dendrobates auratus* was never observed in a transect in the natural forest but was observed once outside of the transects (see Fig. 9). Because of this, the result that *D. auratus* was never observed in the natural forest is an error. However, this is also a likely indication that D. auratus is very uncommon in the natural forest, meaning the error is not large enough to significantly change results. These limitations and errors should not detract from the results, but it is important that they are considered when discussing the results.

## Conclusion

The goal of this study was to determine if there is a difference in amphibian species diversity and abundance between natural forest and agroforest in Bocas del Toro, Panama to assess agroforestry's conservation potential for amphibians. The results of the study demonstrate that natural forest has significantly higher amphibian species diversity, species evenness, species richness, and overall abundance. This implies that agroforests are not a substitute to natural forests for terrestrial amphibians and that their conservation potential for them is limited. However, the agroforest was shown to harbor some species of amphibians, which supports the idea that agroforestry can be implemented as an instrument to conserve biodiversity that traditional agriculture cannot. The difference between environments observed reinforces the idea that amphibians are more sensitive to changes in their environment than other taxa and that, therefore, are more dependent on natural forests. Because of this and the limited conservation potential of agroforests for amphibians suggested by the results,

conservation of natural forests and expansion of protected areas is recommended as the ideal course of action for amphibian conservation moving forward.

However, the capacity of agroforests to provide habitat to amphibians and agroforestry in Panama remains understudied. Further research in Panama and other locations around the world is needed to be able to draw firm conclusions about the effectiveness of agroforestry for amphibian conservation. In Panama, more research into widespread cacao agroforests and their potential for biodiversity conservation is still needed. At the study site, repeated surveys, particularly in the rainy season, would provide valuable additional insight on the results gathered from this study. Further research on agroforestry and amphibians would be invaluable towards conservation planning in the coming years. Because of this, I encourage more studies in as many areas as possible looking at how agroforestry is able to conserve amphibian biodiversity.

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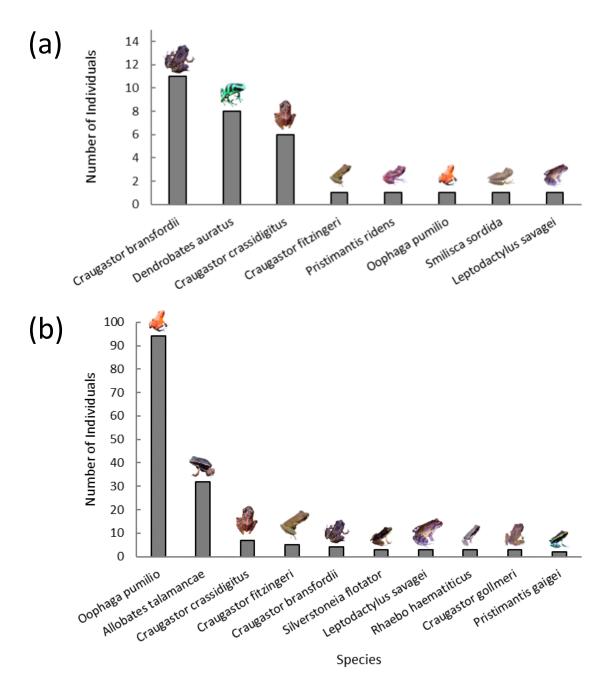
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## Appendix



### Figure 6 – Abundance by species in (a) agroforest and (b) natural forest

Overall abundance was higher (n = 156) in the natural forest than in the agroforest (n = 30). *O. pumilio* and *A. talamancae* showed a preference for natural forest. *D. auratus* showed a preference for agroforest. 5 species were found only in the natural forest (*A. talamancae, S. flotator, R. haematiticus, C. gollmeri,* and *P. gaigei*). 3 species were found only in the agroforest (*D. auratus, P. ridens,* and *S. sordida*).



Figure 7 – Images of the 13 species observed in the transects

These images show the species observed in the study. In order: [1] *Oophaga pumilio*, [2] *Allobates talamancae*, [3] *Silverstoneia flotator*, [4] *Leptodactylus savagei*, [5] *Rhaebo haematiticus*, [6] *Craugastor fitzingeri*, [7] *Dendrobates auratus*, [8] *Craugastor crassidigitus*, [9] *Craugastor bransfordii*, [10] *Pristimantis ridens*, [11] *Pristimantis gaigei*, [12] *Smilisca sordida*, [13] *Craugastor gollmeri* 



## Figure 8 – Images of typical agroforest and natural forest environment

Images were taken at the study site and demonstrate the typical conditions in each environment. Natural forest is shown above, and cacao agroforest is shown below.



## Figure 9 – Sources of error

Images of the (left) *Dendrobates auratus* individual observed in the natural forest and (right) *Agalychnis callidryas* individual observed in the study area which are indicative of known and potential sources of error in the study.