Differences in the Diversity of Frog Species between Sierra Llorona and El Valle, Panama

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Differences in the Diversity of Frog Species between Sierra Llorona and El Valle, Panama

By Kei Thurber
SIT Panama, Fall 2014
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Abstract

Panama is home to the greatest diversity of species in all of Central America. It is home to 174 species of frogs, 35 of which are endemic to Panama. Frogs are a vital part of many ecosystems. They maintain insect populations and act as food sources for larger predators. Their job of maintaining insect populations is essential to curbing the spread of diseases. Additionally, scientists have found chemical compounds in the skin of frogs that can be used to treat pain and prevent infections. The main threat to the majority of frogs is the deadly fungus *Batrachochytrium dendrobatidis* (Bd), or the chytrid fungus. The chytrid fungus has driven many frog species completely extinct, and others can no longer exist in the wild due to its presence. The threat the fungus poses to the frogs of Panama is high, and at this point it has spread throughout the whole country. Therefore, it is important that knowledge about the statuses and distributions of Panamanian frog populations is kept up-to-date. In this paper, I examine two different areas of Panama for their diversity of different frog species. I conducted ten transects in each of the locations, and performed a Shannon-Weiner index calculation on individuals and species seen at each site to make a comparison of relative diversity. Between both sites, 16 total frog species were observed, six of which are known to be chytrid resistant, along with an additional 10 that are persevering through Bd’s invasion. The final index values of 2.19 for Sierra Llorona and 1.92 for El Valle were too close to show that there was a significant difference in the diversity of frog species between the two locations. The similar Shannon-Wiener index values suggest that the anthropogenic development of El Valle as well as chytrid are having the same level of impact on frog diversity, as the fungus alone is having on the species of Sierra Llorona, which is relatively untouched by humans.

Resumen

Panamá tiene de la mayor biodiversidad de especies en toda Centroamérica. Es el hogar de cerca de 200 anfibios, 174 de los cuales son ranas y 35 de esas son endémicas de Panamá. Las ranas son una parte vital de muchos ecosistemas. Se mantienen las poblaciones de insectos de zonas tropicales y a su vez son las fuentes alimenticias de los depredadores más grandes. Su trabajo de mantenimiento de las poblaciones de insectos es importante para detener la propagación de enfermedades. Además, los científicos han descubierto que los compuestos químicos en la piel de ranas pueden ser utilizados para tratar el dolor y prevenir las infecciones. La principal amenaza para la mayoría de las ranas es el mortal hongo *Batrachochytrium dendrobatidis* (Bd), o el hongo quítrido. El hongo quítrido ha llevado a muchas especies de anfibios completamente extintos, y otros, como la Rana Dorada de Panamá, ya no pueden existir en la naturaleza debido a su presencia. La amenaza que el hongo representa para las ranas de Panamá es alta, y se ha extendido por todo el país. Por lo tanto, es importante que el conocimiento sobre el estado y la distribución de las poblaciones de ranas panameñas se mantiene actualizada. Este artículo examina dos áreas diferentes de Panamá, El Valle de Anton y Sierra Llorona, Colón, por su diversidad de las diferentes especies de ranas. Llevé a cabo diez transectos en cada uno de los lugares, y realicé un cálculo del índice de Shannon-Weiner con especies vistas en cada lugar para poder lograr una comparación de la biodiversidad
relativa. Los valores del índice final de 2,19 para Sierra Llorona y 1,96 para El Valle, resultaron muy parecidos, lo que significa que no hubo una diferencia significativa en la biodiversidad de las especies de ranas entre los dos lugares. Esto sugiere que el hongo quítrido podría tener el mismo impacto en las especies de El Valle como las poblaciones de Sierra Llorona. Dentro de los transectos realizados en ambos sitios, se observaron 16 especies de ranas en total, seis de los cuales son conocidos por ser resistentes al quítrido, junto con otros 10 que están perseverando a través de la invasión del letal hongo.
Introduction

Panama is home to the greatest diversity of species in all of Central America. It has over 10,000 species of plants, 900 species of birds and 200 species of amphibians. Of those 200 amphibians, 174 species are frogs and 35 of those are endemic to Panama alone. Though the general geographic distributions of these 174 species are known, knowledge on the current frog diversity of different areas of the country is lacking. Amphibians are the most endangered class of animals on the planet and frog species are being driven extinct at an alarming rate due to a variety of threats. Research is constantly becoming outdated as more and more individuals vanish from the wild with every passing day. On top of this, 13 of the 174 frog species are listed as data deficient, meaning their status and potential threats to their status are completely unknown.

Frogs are a vital part of many ecosystems. They keep insect populations of tropical areas in check and in turn are the food sources of larger predators. Their job of maintaining insect populations is incredibly important for curbing the spread of diseases. As humans who suffer from many diseases transmitted by insects, we need to conserve frogs not only to maintain healthy ecosystems and conserve the environment, but to ensure our own health as well. On top of this, scientists have found chemical compounds in the skin of frogs that can be used to treat pain and prevent infections. Some of these chemicals are even being investigated as possible HIV treatments. Many opportunities for the discovery of future medicines rest on the existence of these creatures.

The main threat to the majority of frogs is a deadly fungus that has caused a worldwide epidemic crisis for herpetologists. The chytrid fungus has driven many frog species completely extinct. Others, such as the Panamanian Golden Frog, are being bred in captivity because they can no longer exist in the wild due to its presence. It is still unknown why some species are more or less susceptible to the fungus, but it affects different frog populations with different intensities. The threat it poses to the frogs of Panama is high, and at this point it has spread throughout the whole country. Therefore, it is important that knowledge about the status and distribution of Panamanian frog populations is kept up-to-date. This would aid in determining which species are being heavily impacted by the fungus and are in need conservation aid, much like the Golden Frog. Frogs are very important species for maintaining healthy and functional ecosystems, and their conservation is essential if the forests of Panama are to continue to flourish. Thus, it is imperative that research is constantly conducted quantifying and defining their statuses.

In this paper, I examine two different areas of Panama and compare their diversity of different frog species. To study the diversity, I conducted transects during the day and night in each of the locations. I then performed a Shannon-Weiner index calculation with species seen at each site to make a comparison of relative diversity. I predict that there will be a significantly higher relative diversity in Sierra Llorona than there will be in El Valle due to the fact that it has undergone less anthropogenic development. My theory is that the combination of chytrid and human development in El Valle will have caused greater reductions in diversity in El Valle, than just the fungus in Sierra Llorona. The null hypothesis for this alternative hypothesis is that there will not be a significant difference in observed diversity between the two locations. Furthermore, knowing that the chytrid fungus is present in both locations, I aim to propose hypotheses as to why certain frog
species have been able to persevere through the presence of the fungus, and why one location might show more diversity than the other.

**Literature Review**

*Chytrid in Panama*

*Batrachochytrium dendrobatidis* (Bd) is the fungus that causes chytridiomycosis in amphibians. There are two existing hypotheses for how the fungus kills frogs. The first is that the fungus lives in the skin of the frog and releases toxic enzymes. The second is that the fungus causes a loss of electrolytes, which affects a frog’s osmoregulation and/or oxygen uptake through their skin (Kriger 2008). Frogs that contract Bd exhibit post-metamorphic death syndrome, or death only after they grow out of the tadpole stage into frog metamorphs. This is because this stage of their life is when they begin to grow chytrid affected keratin in their skin (Kriger 2008). Bd has put approximately one-third of the world’s amphibian species on the IUCN Red List and has endangered nearly 40% of the amphibians of the Americas (Norris, 2007). The fungus is decimating amphibian populations globally, affecting any frogs it comes into direct contact with. It is the most threatening invasive species, possibly ever, in terms of its lethality and has reached global crisis recognition among biologists and herpetologists everywhere (Forrest and Schlaepfer, 2011). According to PBS’s Nature, a film series the channel runs on ecology around the world, as of 2009 the only area of Panama left untouched by the fungus was the Burbayar Forest on the edge of Darien (Argo 2009). Andrew Crawford et al. (2010) performed a study in El Copé mapping the amphibian diversity before and after Bd spread throughout the forest. The results of the study approximated that 33% of phylogenetic diversity had been lost there over a nine-year period. If this type of loss is consistent in all areas of the country that have been exposed to the chytrid fungus, then Panama, over this same period of time, could have lost up to 66% of total phylogenetic diversity. Studies like these show how intensely lethal the chytrid fungus can be and just how at risk the frogs of Panama actually are.

*The Frogs of Sierra Llorona*

Sierra Llorona is situated a short ways from Colón, in central Panama. The lodge where I will conduct my research is isolated in the mountains there, away from the nearest city, Sabanita. It is situated at only 37 meters above sea level, and remains fairly hot throughout the day and night there. During the rainy season, rain is heavy and almost daily (Küng et al. 2014). Though the Sierra Llorona Lodge has been the site of many research projects about the frogs of Panama, very few published papers exist about the frog populations that exist within the reservation. One paper by Küng et al. (2014), notes that a population of *Colostethus panamensis* lived within the reserve, coexisting with the chytrid fungus there. I was also advised by Dr. Roberto Ibañez that a population of *Atelopus limosus* once lived and thrived within the park’s borders but had not been seen for the last couple of years. The Lodge’s website features a number of nature photos of different frog species found on the property that include pictures of *Agalychnis callidryas*, *Dendrobates auratus*, and *Craugastor gollmeri* (Sierra Llorona, 2014).
Thurber 8

The Frogs of El Valle

El Valle de Antón is located in the crater of an inactive volcano close to the Pacific coast of Panama, west of Panama City. It is situated at 600 meters above sea level, but is still fairly hot during both the day and the night. It is comprised of cloud forests, and rains almost every day during the rainy season (La Guardia Publicidad, S.A. 2009). John Sullivan is a herpetologist that runs a travel blog about his herpetology trips around the world. His blog post about El Valle de Antón is perhaps the only published article that gives any sort of list of frog species that can be seen around the area. In his post, he notes that he saw Silverstonia nubicola, Craugastor crassidigitus, and Pristimantis cerasinus in the crater in El Valle, as well as Lithobates warszewitschii, Smilisca sila, and Sachatamia albomaculata behind Hotel Campestre where I conducted most of my transects in El Valle. He mentioned seeing only one species of frog up at Cerro Gaital where I conducted one of my transects, the species being Pristimantis ridens. The only other species he mentions seeing is Pristimantis gaigei on someone’s private property (Sullivan, 2014). Given that he took his trip less than a year before this, it is likely that all of these species are still able to be found in the area and have not yet been driven into extinction by Bd. Additionally upon talking with the frog experts that work at the El Valle Amphibian Conservation Center (EVACC), I was advised that in the area I would be able to see Agalychnis callidryas, Dendropsophus ebraccatus, and Sachatamia ilex.

Differences in Host Species Mortality and Morbidity

A study was conducted by Anna Peterson and Valerie McKenzie of University of Colorado at Boulder regarding the differences across frog host species in terms of Bd. What is currently known about Bd is that it is transmitted via direct contact with the fungus or individuals infected with the fungus. There is a clear difference in susceptibility to the fungus in frog species, with certain species showing rapid and widespread mortality upon infection and other species showing a very low morbidity both in the lab and in the field (Peterson and McKenzie, 2014). Their study, conducted in the wetlands of Colorado, proved that certain species that show low mortality to the fungus can act as carriers for Bd. These carriers may end up spreading Bd to the more susceptible species in the area. It also showed that the higher the diversity of frog species in an area, the lower the disease risk is for all frogs via the dilution effect. The dilution effect is where the increased number of species and number of frog individuals lowers a single individual’s likelihood of contracting disease. From this, it can be conjectured that those species of frogs that were driven extinct by the fungus must have been a) very susceptible to the lethality of Bd, and b) more likely to have existed in areas of low amphibian diversity. One question then, is why areas with high diversity of frog are also experiencing decimations of their frog populations.

Another study conducted on frog species of North America by Gahl et al. in 2011 showed that, even when infected with foreign strains of Bd, frogs that were resistant to the local strain were often not affected by the new form of the pathogen either. While not all species proved to survive through the subjection of a new strain of chytrid, only metamorphic green frogs suffered fatalities, meaning that the majority of the locally
resistant species survived. The individuals that survived were found to be carrying the fungus heavily in their skin and were able to resist it’s lethal effects by shedding it, making them potential vectors of both types of chytrid. Consequently, it can be concluded that heavy regulations should be placed on the intercontinental transport of frogs, because multiple forms of Bd can clearly be spread through the transport of a single species (Gahl et al. 2011).

Further research has shown that populations of the same species that live in different environments will exhibit differing levels of mortality when presented with Bd. The population of Colostethus panamensis that lived within the borders of Omar Torrijos National Park in El Copé declined critically upon Bd site infection. The population of the same species of frog living in Sierra Llorona showed virtually no population decline and appeared to be living in coexistence with the fungus, with Bd showing only an 11.4% prevalence (Küng et al. 2014). The coexistence of C. panamensis with the chytrid fungus in Sierra Llorona could possibly be due to the fact that it is a generally warmer and at a lower altitude than Omar Torrijos National Park. The higher temperatures should be less conducive to the existence of the fungus, and it probably exists in Sierra Llorona at a much lower zoospore count than it does in Omar Torrijos. Given this, it is likely that there are many other species of frogs that show resistance to Bd in alternative environments. Research investigating this possibility has not been conducted as of yet. Perhaps, if species thought to be highly susceptible to Bd were raised in slightly different habitats from the ones they are currently living in, or the ones they have already gone extinct in, they might fare better in the presence of the fungus. This holds huge implications for how relocation efforts could foreseeably have a positive outcome in terms of species conservation.

Environmental Factors that Influence Bd

It is known that the environment plays a strong determining factor in the prevalence of Bd. The fungus thrives in cooler, damper climates and is normally found at higher altitudes and at latitudes further from the equator. In a study conducted by Forrest and Schlaepfer in 2011, adult individuals of five different frog species were given short-term exposure to temperatures above 25 degrees Celsius. This exposure to heat actually cleared their Bd infections (Forrest and Schlaepfer, 2011). This means that Bd cannot survive in nature at temperatures of 25 degrees Celsius or higher. This also presents a potential cure for frogs infected with the chytrid fungus. Meanwhile, research conducted by Sapsford et al. 2013 in Australia showed that the fungus was much more present at high-altitude stream sites, and low-altitude streams contiguous with the high-altitude sites than it was at low altitude stream sites that were not connected to high-altitude streams (Sapsford et al. 2013). Sierra Llorona, however, is both a warm and low altitude environment. It is only 37 m, 123 ft., above sea level and is usually very warm during the day and even at night. Why then, is Chytrid able to flourish within the boundaries of the reserve, so much so that it has potentially managed to wipe out a species of Atelopus that once resided there?

Though temperature and weather dictate the presence and intensity of Bd, studies show that once Bd invades a site, seasonality is never dramatic enough to allow for the complete elimination of the fungus from that site. A study conducted by Chestnut et al. in
2014 proved that chytrid exists in the environment year round. Their study noted that chytrid varied in intensity with seasonality, showing heterogeneous spatial and temporal density throughout the year. Once a site was infected with chytrid, the zoospores never completely disappeared (Chestnut et al. 2014). Though this study was conducted solely in the US, Bd was tested at a variety of sites with different environments, ranging from the cool waters of Maine, to the hot, dry landscapes of Southern California. This was done to ensure that the study could be applicable to a variety of environments, and so that the results could be applied more generally. Given that Panama has low seasonality and stable annual temperatures, we can conclude two things: 1) that Bd intensity at any given site does not change much throughout the year, and 2) that once a site is infected with Bd, it stays infected.

**Chytrid Resistance**

In their studies on how Bd influences different species, Gahl and his associates concluded that different factors might affect the susceptibility of individual frogs to the chytrid fungus. They theorized that, as is commonly posited, different species were more susceptible than others. They also proposed that chytrid may affect individuals differently based on age and that younger frogs, tadpoles, and eggs were more susceptible to Bd than adult frogs. Lastly, they hypothesized that the higher the density of Bd or Bd hosts, the more mortalities would be seen in individuals (Gahl et al. 2011). If what is hypothesized is true then it is vital to protect young populations of frogs susceptible to the disease. One alternative method of conservation could be to spread the population out in order to prevent density related mortalities.

In another study conducted in Florida by McMahon et al. in 2014, it was found that in three frog species, individuals that had been cured of Bd via temperature-induced clearance showed avoidance of the fungus. This means that frogs that had been infected with the chytrid fungus and then cured of it, avoided the water inoculated with Bd between 65% and 70% of the time in favor of the non-inoculated water (McMahon et al. 2014). The implications of this study are incredibly positive. If chytrid-infected frog communities in the wild can be cured, we know that more likely than not, these frogs will not become re-infected with the fungus because they will choose to avoid it. Furthermore, the spread of Bd from individual to individual would be greatly reduced if frogs were to show avoidance in its presence. This could keep a lot of frog populations protected from the fungus via herd immunity, which could contribute greatly to the conservation of susceptible species.

**Atelopus: Plight of the Panamanian Golden Frog**

The frog genus Atelopus is one of the genera of frogs that has been hit the hardest by the spread of the chytrid fungus. It is genus that the Panamanian Golden Frog (*Atelopus zeteki*) belongs to. In addition to the Golden Frog, many of its close relatives have been completely eradicated from the wild. There are 113 known Atelopus species, and to date, as many as 30 species have been confirmed to be extinct in the wild. According to the IUCN, the status of 80% of Atelopus species has been declared Critically Endangered and 70% have clearly declining populations. Atelopus experience
rapid and extensive population declines upon Bd site invasion, demonstrating incredibly high chytrid susceptibility (DiRenzo et al. 2014).

A study conducted by DiRenzo et al. in 2014 showed that *A. zeteki* individuals reach Bd infection levels several levels of magnitude greater than most other frog species. This causes Bd infected *A. zeteki* individuals to die usually within a month after infection. This also holds the serious implication that the Golden Frog is a supershedder of chytrid zoospores, or that it has a large potential to spread the chytrid fungus many individuals and water sources that it comes in contact with (DiRenzo et al. 2014). This means that *A. zeteki* may well have contributed to the downfall of other species of frogs in El Valle in the midst of its own decline. This also implies that any remaining frog species in El Valle must be hyper-tolerant if not resistant to the chytrid fungus and limits the possibility that they have survived through avoidance.

**Methods and Materials**

I performed a variation of the methods used by Sapsford et al. in 2013, and established five, 50 meter transects at a variety of different sites in both Sierra Llorona and El Valle. I based the transect locations off of the likelihood of seeing frogs in that location, i.e. areas with still water or low-flow streams nearby. Due to the fact that I only had one measuring tape, I established multiple transects the night before, marking some with flagging tape along the 50 meter line, and leaving the measuring tape along the last one established. I established transects the night before so that any frogs that were disturbed in the area would have time to return and resettle. Each transect was examined once during the day and once during the night so that in all, 10 sets of data were captured, two for each 50 meter transect. Transects conducted during the day were done between 9 and 10 AM. Transects conducted in the night were done between 7 and 8 PM and were named 1-2, 2-2, 3-2, etc., to signify that they were the second transect conducted at that site. Transects were conducted regardless of rain or shine. I spent six nights in Sierra Llorona, where I conducted three transects in the deep woods, one transect along a stream and one transect along the trail. I spent five nights in El Valle, where I conducted three transects along streams, and two transects along trails. Due to safety concerns and lack of a proper guide, I did not conduct any deep woods transects in El Valle.

For each transect, I started at the 0 meter mark, and walked along the line to the 50 meter mark. I walked with a camera in hand looking for movement, and photographed any frog I found within a meter on each side of the transect line. I later looked at the photographs I had taken, and used the book *Amphibians of Central America*, by Gunther Köhler, to identify the different species of frogs that I had seen. I enlisted the help of professionals at the El Valle Amphibian Conservation Center (EVACC) as well in identifying species. To add supplementary data to the data obtained from the transects, I also took pictures of any other frog species I saw outside the transects at each location. After I identified the total number of different species seen in each transect and location, I performed a Shannon-Wiener calculation for each to compare relative diversity between the two locations.

**Results**
In Sierra Llorona, 19 individual frogs were observed within the 10 transects conducted. Transects 1, 2 and 3 were conducted on November 13th at 9:14 AM, 9:20 AM and 9:31 AM respectively, and the weather was clear and sunny for all. Transects 1-2, 2-2 and 3-2 were conducted at 7:08 PM, 7:19 PM and 7:31 PM respectively, and the weather was clear for all of these as well. One frog was observed in Transect 1, a *Craugastor fitzingeri*. Four frogs were observed in Transect 1-2, two *Craugastor fitzingeri*, one *Craugastor gollmeri* (Figure 1), and one *Craugastor podiciferous*. Two frogs were observed in Transect 2, a *Silverstonia flotator* and a *Craugastor podiciferous*. Two frogs were observed in Transect 2-2, one *Craugastor gollmeri*, and one *Craugastor fitzingeri*. One frog was observed in Transect 3, a *Pristimantis cerasinus*. Two frogs were observed in Transect 3-2, one *Craugastor crassidigitus*, and one *Craugastor fitzingeri*. Transects 4 and 5 were conducted on November 14th at 9:42 AM and 9:58 AM. The weather was cloudy with grey skies during Transect 4, and raining heavily during Transect 5. Transects 4-2 and 5-2 were conducted at 7:02 PM and 7:20 PM, and it was raining lightly during both. Two frogs were observed in Transect 4, one *Craugastor opimus* (Figure 1), and one *Silverstonia flotator*. Three frogs were observed in Transect 4-2, one *Rhaebo haematiticus*, and two *Smilisca phaeota*. One frog was observed in Transect 5, a *Craugastor podiciferous*. One frog was observed in Transect 5-2, a *Silverstonia flotator*. Outside of the transects however, an additional 52 individuals were observed, making 70 total observed individuals in Sierra Llorona (Figure 3). Within the transects, a total of nine different species were observed (Figure 2), and an additional three species were observed outside of the transects, making 12 total observed species.

![Figure 1](image-url). A *Craugastor opimus* (left) observed in Transect 4 in Sierra Llorona and a *Craugastor gollmeri* (right) observed in Transect 2-2 in Sierra Llorona.
Figure 2. A graphical representation of the distribution of frogs seen within the 10 transects conducted in Sierra Llorona. The most commonly observed frog was the leaf litter frog *Craugastor fitzingeri*, which was seen in four of the 10 transects giving it the highest frequency of appearance as well. In total, 19 different individuals were observed within the transects conducted in Sierra Llorona. This was representative of nine different frog species.
In El Valle, 13 individual frogs were observed within the 10 transects conducted. Transects 1 and 2 were conducted on November 18th at 9:34 AM and 9:50 AM. The weather was clear and sunny for both. Transects 1-2 and 2-2 were conducted at 7:13 PM and 7:35 PM. The weather was clear for both of these as well. Three frogs were observed in Transect 1, two Engystomops pustulosus, and one Rhinella marina. No frogs were observed in Transect 1-2. One frog was observed in Transect 2, a Lithobates warszewitschii. Two frogs were observed in Transect 2-2, two Hyalinobatrachium colymbiphylum. Transects 3, 4 and 5 were conducted on November 20th at 9:15 AM, 9:28 AM and 9:45 AM respectively. The weather was cloudy and overcast for Transects 3 and 4, and lightly drizzling for Transect 5. Transects 3-2, 4-2 and 5-2 were conducted at 7:05 PM, 7:22 PM and 7:38 PM respectively. The weather was raining while all three of these were conducted. One frog was observed in Transect 3, a Sachatamia albomaculata (Figure 4). Two frogs were observed in Transect 3-2, two Smilisca sila. Two frogs were observed in Transect 4, one Sachatamia albomaculata, and one Smilisca sila. One frog was observed in Transect 4-2, a Smilisca sila. One frog was observed in Transect 5, a Scinax boulengeri. No frogs were observed in Transect 5-2. Outside of the transects however, an additional 38 individuals were observed, making 51 total observed individuals in El Valle (Figure 6). Within the transects, a total of seven different species were observed (Figure 5), and an additional four species were observed outside of the transects, making 11 total observed species.
Figure 4. A Dendropsophus ebraccatus (left) seen in El Valle, and a Sachatamia albomaculata (right) seen in Transect 4 conducted in El Valle.

Figure 5. A graphical representation of the distribution of all of the frogs observed within the transects conducted in El Valle. The most commonly observed frog was Smilisca sila, with four individuals in three different transects. In total, 13 frogs were observed within the transects from a total of seven different species.
Figure 6. A graphical representation of the distribution of all of the frogs observed during my time in El Valle. The most commonly observed frog was *Rhinella marina*, with 13 total individuals observed. The second most commonly observed frog was *Engystomops pustulosus* at eight individuals. In total, 51 different individuals were observed in El Valle, from 11 different frog species.

Calculations for the Shannon-Wiener index values yielded 1.92 for the transects conducted in Sierra Llorona, and 1.82 for the transects conducted in El Valle. For over-all species observed in each location, the values were 1.62 for Sierra Llorona and 2.09 for El Valle. However, it was determined that the excessive number of observed *Silverstonia flotator* individuals greatly biased the Shannon-Wiener index value for Sierra Llorona, and when the species was eliminated from the index calculation for each site, the resulting index values were 2.19 for Sierra Llorona and 1.92 for El Valle (Figure 7). The performance of a two-tailed t-test of unequal variance to determine significant difference in diversity for the two sites, including the species *S. flotator*, yielded a p-value of 0.72. Excluding the species, the test yielded a p-value of 0.17. Neither of these values is low enough to show a significant difference in diversity and the null-hypothesis cannot be disproven.
Figure 7. Six different Shannon-Wiener index values graphed alongside each other for comparison. The highest Shannon-Wiener index value was calculated from total species observed in Sierra Llorona, excluding the species Silverstonia flotator. The transects conducted in Sierra Llorona had a higher Shannon-Wiener index value than the transects conducted in El Valle as well. Including the species S. flotator however, the index value for Sierra Llorona was the lowest over all, and lower relative to the index value for El Valle.

Discussion

From the calculated Shannon-Wiener index values for each site we can make some general observations about the relative diversity of each site. Excluding the outlier of Silverstonia flotator, Sierra Llorona showed a slightly higher relative diversity than El Valle, both within and outside of the transects. Including the species however, the diversity index value for Sierra Llorona drops by more than 0.5, which is a very large amount in terms of this index. I will stick to using the value excluding this species however because in the end, a greater number of species and individuals were observed in Sierra Llorona and I do not believe the diversity in El Valle should be deemed greater simply because of this species’ presence. Even though there were more species observed in Sierra Llorona, statistical tests including and excluding S. flotator did not prove a significant difference in diversity between the sites. This means that though the diversity was higher in Sierra Llorona, it was not significantly different from that of El Valle, and I cannot disprove my null hypothesis.

Considering that El Valle is situated at a much higher altitude than Sierra Llorona, one would think that the fungus would be more lethal there due to its cooler average temperatures and cloud forest climate. This would be shown in a significantly lower diversity index. Since there is not a significant difference in diversity between sites we can see that the fungus is not necessarily doing more harm in El Valle than Sierra
Llorona. On top of this, El Valle features much more human development than Sierra Llorona, the former being a very touristy area and the latter being a privately owned reserve. Even with all of this anthropogenic development, the diversity of frog species in El Valle is only slightly lower than it is in Sierra Llorona. From this we can assume then that the development of the area is not necessarily having negative impacts its frog species. In fact, the clearing of some land might make it more habitable by certain frog species. For example, *Rhinella marina* are usually found in less forested, more open marshland areas. This species was the one seen with the highest number of individuals in El Valle and the human development of the land is probably why they are so populous there. This same principle can be applied to the species *Engystomops pustulosus*, also known as the Tungara frog. In Sierra Llorona, the dense forest cover of the area probably limits the types of frogs that can live there. This is why leaf litter frogs were the most frequently observed type of frog in Sierra Llorona, and why they were seen with a much lower frequency in El Valle. Additionally, considering that the diversity of Sierra Llorona is only slightly higher than El Valle without human development, one could say that Bd is comparatively more intense there. From this, we can conjecture two things: 1) a small amount of anthropogenic development might be beneficial to certain frog species, and 2) the chytrid fungus might be having a greater negative impact in Sierra Llorona.

That being said, though a similar number of species was observed in each place, only three species were present in both, *Craugastor crassidigitus*, *Silverstonia flotator* and *Rhinella marina*. None of the other observed species crossed over between sites, leaving a total of 16 remaining observed species that persevered through the presence of the chytrid fungus in their respective habitats. First I will discuss why the site-common species might be able to survive in both. Then I will go on to conjecture how the other remaining species might have survived the presence of the fungus.

First, *Rhinella marina* is a species that has been introduced in different areas all across the world for pest control purposes (Drake et al. 2014). Chytrid is known to be present in most of the world as well, on six of the seven continents, in many places where this frog species lives and thrives (Olson et al. 2013). For example, chytrid is known to be present in Australia where *R. marina* is a pervasive, invasive species (Sapsford et al. 2013). This must mean that the toad is resistant to Bd, because it is much too populous to simply be avoidant. It would not be able to breed, reproduce, and remain highly invasive simply by being avoidant of the fungus.

*Silverstonia* is a genus of frogs that recently aggregated out of the genus Colostethus, but remains closely related. Colostethus is a genus of frogs that is known to be resistant to the chytrid fungus already. This would explain why *Silverstonia flotator* was present at both sites and why more than 40 individuals were seen in Sierra Llorona. The genus *Craugastor* on the other hand, has not proven to be resistant, with some 50 endangered species in the genus. This means that the species within the genus that are not endangered must be some how avoidant of the fungus. Considering that *Craugastor* frogs lay eggs on the forest floor that develop directly into metamorphs (skipping the tadpole phase), it is likely that they never have to enter chytrid infected streams (Encyclopedia of Life). Living and developing in this manner, most of these frogs can avoid coming into contact with the fungus for their entire lives and is most likely why the species can persist in both areas of Panama.
There are a number of possible reasons as to why the remaining frogs species have persevered through the invasion of the fungus in their respective habitat. If we look at the observed glass frog species, *Sachatamia albomaculata* and *Hyalinobatrachium colymbiphyllum*, these frog species spend the majority of their time out of the water on elevated surfaces. Glass frogs lay their eggs directly on leaves overhanging ground streams in forests. These species undergo a tadpole stage meaning they have tolive submerged in water up until a certain point in their lives. I hypothesize that glass frogs must be resistant because I do not believe they could survive by only being avoidant given how much time they have to spend in the water. Another possibility is that even if they are not resistant, they could experience very low infection intensities, giving them time to reproduce before they die and perpetuate the species. However there is a high probability that glass frogs in general are resistant seeing as a study conducted in Omar-Torrijos National Park in El Copé showed that Bd infected glass frogs of a different species did not show decreasing abundance upon arrival of the fungus there (McCaffery and Lips, 2013).

Tree frogs, such as the observed *Smilisca sila*, *Scinax boulengeri*, *Dendropsophus ebraccatus*, and *Smilisca phaeota*, also spend the majority of their time up in the canopy or on the leaves of ground plants. They lay their eggs directly in streams or still ponds. Their necessary contact with water once again indicates that these species are more likely to be resistant. Past research, conducted by Karen Lips et al. in 2005, has shown that *Smilisca phaeota* is in fact resistant to the fungus and a potential carrier for the disease (Lips et al. 2005). Though we know from the case of Craugastoridea, that not all frogs within the same genus are resistant to chytrid, the abundance of *Smilisca sila* in El Valle seems to suggest that this species is resistant to the fungus as well. *Scinax boulengeri* and *Dendropsophus ebraccatus* were seen with a lower frequency than Smilisca species, but their presence in El Valle suggests that they are at the very least, chytrid avoidant and experience a low-level infection intensity with regards to the fungus.

The same must be said that the remaining observed species must also be resistant or avoidant. The sheer abundance of *Engystomops pustulosus* suggests that it is resistant. A study conducted by Guillermo Velo-Antón et al. showed that Bd had caused a dramatic loss in genetic diversity of *Lithobates warszewitschii* due to a bottlenecking of the population. This means that this species is not necessarily resistant, but may feature a genetic structure that is chytrid tolerant (Velo-Antón et al. 2012). *Rhaebo haematiticus* is a toad species, in the same family as *Rhinella marina*, and most toads have proven to be resistant. *Allobates talamancae* is also likely to be resistant because it is not a direct developing frog and must spend part of its life cycle in the water. As for *Pristimantis cerasinus*, and the remaining Craugastor species, they are all new-world direct developing frogs. Like C. crassidigitus it is likely that they are chytrid avoidant, and individuals survive due to the fact that they never come into contact with Bd infected waters.

**Conclusion**

Through my conducted research, one can get a clear idea of the types of frog species that are surviving through chytrid’s invasion of Panama. Nine theoretically chytrid resistant frog species were observed as well as an additional nine species that
show avoidance or tolerance of the fungus. Though my research objectives were achieved and I was able to glean a basic idea of the diversity of frogs in both Sierra Llorona and El Valle, I was not able to prove my hypothesis that there was a significant difference in diversity between the two locations. This signifies that either the effect of anthropogenic development in El Valle is having minimal impact on the frog species there, or that the chytrid fungus is comparatively more intense in Sierra Llorona. Though I was able to observe many different frog species with only one guide for assistance, I feel that if this project were to be conducted on a larger scale with more hands in the field, more conclusive results and perhaps some data with significance could have been discovered.

For future studies I would recommend that time and distance be the parameters within which frogs are observed because frogs are very quick and can move outside the boundaries of transects in seconds once you disturb them. It is very hard to keep them within your transects in order to observe and identify them, and sometimes it is difficult to place transects in areas that would be best for finding frogs. As my total data shows, I observed many frog individuals outside of my conducted transects, and those frogs could have been very useful to my data set. A set amount of time and distance would probably be a much more successful way to document frog species. Another recommendation for future studies would be to employ the guidance of a fellow herpetologist when searching for frogs in the field. Without the guidance of an employee of EVACC who helped me conduct my transects in El Valle, I feel I would not have been able to find three or four of the species I observed there. If I had had a proper guide in Sierra Llorona, I might have seen even more species there, and my Shannon-Wiener index value for the site could have been very different.

I suggest that for follow up research, studies should be conducted on the potential Bd resistance of some, if not all of the species observed in my project. If any species are found to be resistant, follow up research should be done as to what makes these species resistant. Furthermore, research should be conducted as to whether it is some or all of the stream bodies in each location have been infected by Bd. Another possible line of study to follow up on this project would be to determine whether the species observed are resistant as a whole or if it just the populations in these two locations that are resistant. It could be that populations of the same species elsewhere are showing serious declines.

There is much more research to be done on the chytrid fungus if declining frog populations are to be saved and the genocide of the fungus on the amphibian world is to be put to an end. The survival of amphibians requires that as much research effort as possible be put towards discovering a cure for chytrid if critically endangered frog species, such as the symbolic Panamanian Golden Frog, are to be saved.
Works Cited


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