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Xena Gehring
SIT Study Abroad

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Xena Gehring

Colorado State University

Submitted in partial fulfillment of the requirements for Panamá: Tropical Ecology, Marine Ecosystems, and Biodiversity Conservation, SIT Study Abroad, Spring 2023

Table of Contents

Acknowledgments

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Abstract

Panama is an incredibly biodiverse country that houses 230 species of amphibians including 188 species of frogs, 35 of which are endemic to Panama. Commonly found in terrestrial and aquatic habitats, frogs play a very important role in the regulation of insect populations and serve as a valuable food resource to other predators. The greatest threat to amphibian species at this time is Chytridiomycosis, a fungal pathogen that infects the skin and often results in death. Many species of amphibians have already gone completely extinct or are extinct in the wild due to the proliferation of this disease. The skin of amphibians is semi-permeable and therefore may be susceptible to pollutants as well. Pesticides have been proven to cause death or malformations in all life stages among different frog species. To study the impacts of suspected pesticides on frog species diversity and abundance, twenty-four riparian transects were conducted in three different locations in El Valle de Antón that were believed to have varying levels of pesticides present. A total of 969 frogs were observed, representing 18 different species, and Shannon-Weiner diversity (H') was calculated for each location. The location expected to be entirely lacking in pesticide presence had a Shannon-Weiner index value of 1.81 while the other two locations, in which pesticides were present, had values less than 1. The Shannon-Weiner index value was much higher in the location believed to have no pesticides present. Both locations in which pesticides were believed to be present had a higher overall abundance of frogs though the species richness and evenness were lower. The data support the idea that pesticide presence may negatively impact frog species diversity, though further research must be conducted to identify if pesticide presence is directly correlated to a decrease in frog species diversity.

Resumen

Panamá es un país increíblemente biodiverso en donde viven 230 especies de anfibios, incluyendo 188 especies de ranas, 35 de las cuales son endémicas de Panamá. Comúnmente encontradas en hábitats terrestres y acuáticos, las ranas juegan un papel muy importante en la regulación de las poblaciones de insectos y sirven como un valioso recurso alimenticio para otros depredadores. La mayor amenaza para las especies de anfibios que se reconocen actualmente es la quitridiomicosis, un patógeno fúngico que infecta la piel y a menudo resulta en la muerte. Muchas especies de anfibios ya se han extinguido por completo o se han extinguido en la naturaleza debido a la proliferación de esta enfermedad. La piel de los anfibios es semipermeable y, por lo tanto, también puede ser susceptible a los contaminantes. Se ha demostrado que los pesticidas causan muerte o malformaciones en todas las etapas de la vida entre diferentes especies de ranas. Para estudiar los impactos de los pesticidas presuntos en la diversidad y abundancia de especies de ranas, se realizaron 24 transectos ribereños en tres lugares diferentes en El Valle de Antón que se creía que tenían diferentes niveles de pesticidas presentes. Se observó un total de 969 ranas representando 18 especies diferentes, y se calculó la diversidad de Shannon-Weiner (H') para cada localización. La ubicación que se espera que carezca completamente de presencia de pesticidas tenía un valor de índice de Shannon-Weiner de 1.81, mientras que las otras dos ubicaciones en las que estaban presentes las pesticidas tenían valores inferiores a 1. El valor del índice de Shannon-Weiner fue mucho más alto en la ubicación que se cree que no tiene pesticidas presentes. Ambos lugares en los que se creía que las pesticidas

estaban presentes tenían una mayor abundancia general de ranas, aunque la riqueza y la uniformidad de las especies fueron menores. Los datos respaldan la idea de que la presencia de plaguicidas puede afectar la diversidad de especies de ranas, aunque se deben realizar más investigaciones para identificar si la presencia de plaguicidas está directamente correlacionada con una disminución en la diversidad de especies de ranas.

Introduction

Current status of frogs and their role

Amphibians are currently undergoing a mass extinction, primarily due to the proliferation of Chytridiomycosis, a fungal disease. While this is one of the driving forces behind the extinction of frogs, there are several other factors that could contribute to the rapid decline of amphibian populations. One hypothesis for the decline in amphibian populations is the increase in agricultural land and pesticide use, which in turn exposes amphibian species to toxic chemicals (Brühl et al., 2013).

Abundant in both terrestrial and aquatic habitats, frogs play numerous important ecological roles. They have been proposed to be used as indicators of environmental changes due to their complex life cycles, and they have also been shown to be capable of controlling insect populations (Hopkins 2007; Raghavendra et al., 2008). This is a very important service to human populations, as insects are common vectors of disease, so as frog populations continue to decline there is concern that insect populations could increase (Raghavendra et al., 2008). This could result in greater transmission of disease (Purcell et al., 2005). Frogs play an important role in the predation of insects, but they also serve as a valuable food source to many larger predators (Zug & Duellman, 2022). Changes in the ecological role of frogs are occurring as frog species composition is changing due to factors such as Chytridiomycosis, habitat loss, pesticide use, and light pollution (Lips, 2016).

Chytridiomycosis overview

Chytridiomycosis is currently considered the greatest threat to amphibian populations among researchers worldwide (Webb & Waddle, 2022). It is a disease caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). This disease was detected in samples from Africa in 1938 and is believed to have occurred as a stable endemic infection in Africa for 23 years before it was detected elsewhere (Weldon et al., 2004).

The disease is known to infect 700 species and is present on all continents where amphibians occur (Lips, 2016). Mass declines in amphibian species were detected during the 1980s, and by 1988 widespread and rapid changes had occurred in amphibian populations with 43% in decline and 32% threatened (Lips, 2016). Many die-offs that occurred around this time were later identified to be a result of the chytrid fungus (Berger et al., 2016). The disease can spread through direct contact as well as through contact with motile aquatic zoospores (Berger et al., 2016). Bd has been found to be many genotypically and phenotypically distinct lineages that vary in distribution and virulence which contributes to the varying level of responses among infected populations (Lips, 2016). Frog species are affected by the fungus with various intensities. Some species move quickly toward extinction, some slowly decline, some remain stable, and others increase in the absence of other species (Kilpatrick et al., 2010). These differing responses to infection by the fungus have resulted in changes in the community composition of amphibians around the world (Lips, 2016).

Since chytrid was first identified in the 1980s, there have been very high rates of amphibian biodiversity loss in the tropics. This is likely due to the high diversity and endemism in these areas (Lips, 2016). The Harlequin frogs of Central America, like *Atelopus zeteki* also known as the Panamanian Golden Frog, are famous for their susceptibility to the fungus and rapid declines upon exposure (Kilpatrick et al., 2010). The different species of frogs that are infected can act as hosts, vectors, and reservoirs of the disease depending upon the strain of fungus with which they are infected and their susceptibility to the fungus (Lips et al., 2016).

Impact of pesticide use

Land used for agriculture has increased in past decades, occupying 40% of Earth's land surface (Brühl et al., 2013). As agricultural land use increases, pesticide use increases as well. Tropical climates tend to require greater amounts of pesticides, putting tropical organisms at greater risk of environmental exposure to pesticides (Ghose et al., 2003). In the tropics, specific risk assessment for pesticide exposure is not conducted for amphibians, despite being required for all other vertebrate groups (Brühl et al., 2013). The impacts of chemical pollutants should be of greater concern for amphibians due to their permeable skin and their lifestyles resulting in exposure to these chemicals in both aquatic and terrestrial habitats (Brühl et al., 2023).

A few studies have focused on the impact of nearby agricultural areas on amphibian species. A study on banana plantations in Costa Rica found that pesticides that are banned in the European Union due to health and environmental hazards are commonly used in the tropics (Brühl et al., 2023). The study also found that 100 m away from the plantations, pesticide concentrations were 18% of the application concentration (Brühl et al., 2023). This could be of great concern as another study found a 40% mortality rate for juvenile European common frogs upon exposure to 3 different pesticides at 10% of the label-recommended application concentration (Brühl et al., 2013). A similar idea was found in a study on the declines of the California red-legged frogs, in which they discovered that upwind agricultural land use was associated with greater declines in the species (Davidson et al., 2001). The presence of agricultural areas that use pesticides is also linked to decreased species richness and evenness (Bach, 2000). A comparison of amphibian species on organic and non-organic banana plantations in Costa Rica found that the organic farms had greater species richness and evenness, with some species only present on the organic plantations (Bach, 2000). Another discovered that herbicide application rapidly decreased species richness at the plot scale (Wanger et al., 2023).

There have been many studies on the impacts of specific pesticides on tadpoles and juvenile frogs, however, there have been very few that address the influence under more realistic community conditions. These studies are often conducted in laboratories that do not perfectly replicate the ecological conditions of the wild. In a study using mesocosms, pesticides were found to have differing impacts on tadpoles depending on what predators were present. In this study when beetle larvae were the main predators, tadpole survival increased during exposure to a pesticide, whereas when salamanders were the primary predator there was little change (Relyea et al., 2005). The study also found that the impact of the different pesticides varied for different species of tadpoles and depended upon the concentration to which they were exposed (Relyea et al., 2005). These findings expose the importance of pesticide impact being studied in natural communities. In a different study, Roundup, one of the most used herbicides worldwide, was tested on tadpoles and juveniles resulting in drastic reductions in survival. The Roundup treatment resulted in 79% mortality in juveniles after only one day and 98% mortality in tadpoles after three weeks (Relyea, 2005). While these two studies suggest that amphibians are highly susceptible to pesticides, a review study on the subject claimed that amphibians are no more susceptible than any other taxon (Kerby et al., 2009). The study did mention that there is a possibility that the impact of other stressors could heighten the response of amphibians to these chemical pollutants (Kerby et al., 2009).

While pesticides have been linked to mortality in amphibian species in larval, juvenile, and adult stages, they have also been linked to malformations and extinctions. A study exploring breeding sites near areas known to apply herbicides found frogs with missing toes and small eyes (Ferrante & Fearside, 2020). Another study found individuals with deformed limbs in intensely sprayed plots (Wanger et al., 2023). The use of pesticides was also associated with species that had once been abundant in an area, no longer being found, and was the main factor explaining the decline of a *Scinax caldarum* in Brazil's Atlantic Forest (Ferrante et al., 2019; Ferrante & Fearside, 2020).

There have been many studies exploring the impact of pesticides on amphibian species in labs, however, there have been very few that explore the impact on species diversity in the community context. Pesticide runoff has been shown to have varying impacts that depend upon the organisms in the community, so a study exploring the impact in this context is justifiable.

Frogs of El Valle

Frogs are easily recognizable vertebrates despite their variability in size, coloration, and morphology. They are generally classified into two groups, frogs and toads; however, this is not representative of true frogs and true toads which are separated based on the presence of the bidder's organ (Zug & Duellman, 2022). Frogs tend to display elongated and powerful hindlimbs that allow them to move by leaping, hopping, and swimming (Zug & Duellman, 2022). These vertebrates have colonized 6 of 7 continents and can be found in almost all habitats except for the arctic and some islands (Lips, 2016). They feed primarily on insects and other small invertebrates while occasionally eating larger prey like small mammals, reptiles, or other frogs (Zug & Duellman, 2022). They are variable in appearance from camouflaged colors like brown, green, and grey, to bright warning colors in the poison dart frogs (Leenders, 2016). Frogs display a wide variety of reproductive modes. They commonly breed through a process known as amplexus that can last for several days and they deposit eggs in a variety of locations and substrates (Nunes-de-Almeida, 2021). They also display varying levels of parental care across species, ranging from no parental care to those that carry the eggs on their back until they hatch (Nunes-de-Almeida, 2021). Many frog species have a mobile larval phase commonly known as tadpoles. These are organisms that are essentially a bundle of organs with a mouth and tail. This phase generally lasts for two to three months during which the larvae are able to take advantage of the almost unlimited food resources in the aquatic habitat (Wassersug, 1975). The tadpoles undergo a process of metamorphosis in which they grow limbs, absorb their tail and gills, and rebuild the skull (Wassersug, 1975). The posterior end of the body is extended to allow space for the changes in organ and body structure (Wassersug, 1975). The tadpoles then emerge to the terrestrial habitat. There are also some species of direct developers that hatch directly into froglets (Zug & Duellman, 2022).

The most common species found during the research conducted in El Valle were *Smilisca sila* and *Lithobates warszewitschii*. They were prevalent in very large numbers, especially as juveniles. *S. sila* tend to reproduce in the dry season, December through March (Leenders, 2016). Tadpoles generally take between two and three months to develop into juvenile frogs, so it is not surprising that there were many juveniles of the species emerging from the water during the month of April. *L. warszewitschii* reproduce year-round. It is possible that their reproduction is also triggered by the changing of the seasons (Heidi Ross, personal communication, April 17, 2023). This could be one reason that so many juveniles and meta morphs of this species were seen as well. Both of these species are known to survive well in areas that have undergone habitat alteration (Leenders, 2016). Other common leaf litter species in the area are *Craugastor crassidigitus* and *Craugastor fitzingeri*. These are both direct developers that reproduce primarily during the rainy season (Leenders, 2016). Both species are present in pristine forests but can thrive in most areas with tree cover and abundant leaf litter (Leenders, 2016). They have tested positive for the chytrid fungal pathogen Bd but tend to exhibit relatively minimal effects (Leenders, 2016). Many other species were observed, however, in lower numbers.

Background on El Valle de Antón

This study explores the impact of pesticide runoff on the diversity of frog species in El Valle de Antón, Panama. El Valle is a stratovolcano about 120 km from Panama City (About El Valle, n.d.). It is the second largest inhabited volcano crater, which has a radius of 8 km and was formed 1.1 – 1.3 million years ago (About El Valle, n.d.). It is 600 m above sea level and has a year-round spring-like climate with an average daily high temperature of 28° C (About El Valle, n.d.). The dry season begins in December and lasts until April, and during this time the area is sunny and very windy, whereas the rainy season is known as “the green season” (About El Valle, n.d.). The average yearly precipitation is 2273 mm with March generally being the driest month and October generally the wettest. (Weather and climate, n.d.) Río Anton is 53 km long with 32% forest cover and an average flow of 3.21 m^3/s (Anton, n.d.). There is a watercress plantation along the river that is known to use pesticides. El Chorro Macho is a larger stream lying to the East of El Valle and is expected to have little pesticide runoff (Raul Arias, personal communication, April 8, 2023).

In this paper, three different locations in El Valle de Antón are examined and the frog species diversity along each are compared. Each transect was examined 8 times, 4 times during the day and 4 times at night. A Shannon-Weiner diversity index calculation was used to compare the diversity of species found in the different locations. It was hypothesized that Transects A and B would have lower species diversity due to the presence of pesticides while Transect C would have greater species diversity. The semi-permeable skin of frogs, which plays an important role in the regulation of osmosis, gas exchange, and electrolytes, was hypothesized to be impacted by the pollutants that are introduced by the pesticides in the area, resulting in lower overall species diversity.

Methods

A variation of the methods used by Sapsford et. al. (2013) were used to collect data on frog species diversity and abundance. Three 100-meter transects were set up (Figure 1). The first transect (Transect A) was set up at the corner of a watercress plantation in Río Antón in an area known as Los Berrales, which was just northeast of El Valle Amphibian Conservation Center (EVACC). This plantation was known to use pesticides, and two discarded bottles of the pesticides were recovered from the vegetation surrounding the plantation. The second transect (Transect B) was set up along Río Antón in an easily accessible area behind EVACC along the Square Tree Trail. The third transect (Transect C) was set up along El Chorro Macho, running through Canopy Lodge. This river is known to contain very few upriver pollutants as most of the land on which it runs is owned by Canopy Lodge. Each transect was set up and marked with flagging tape at least 12 hours before the first collection to reduce the effects of any disturbance. The flagging tape was used in 10-meter increments for ease of recording the location of any frogs found. Each transect was examined 4 times during the day and 4 times during the night. In total 24 sets of data were captured, 8 for each 100-meter transect.

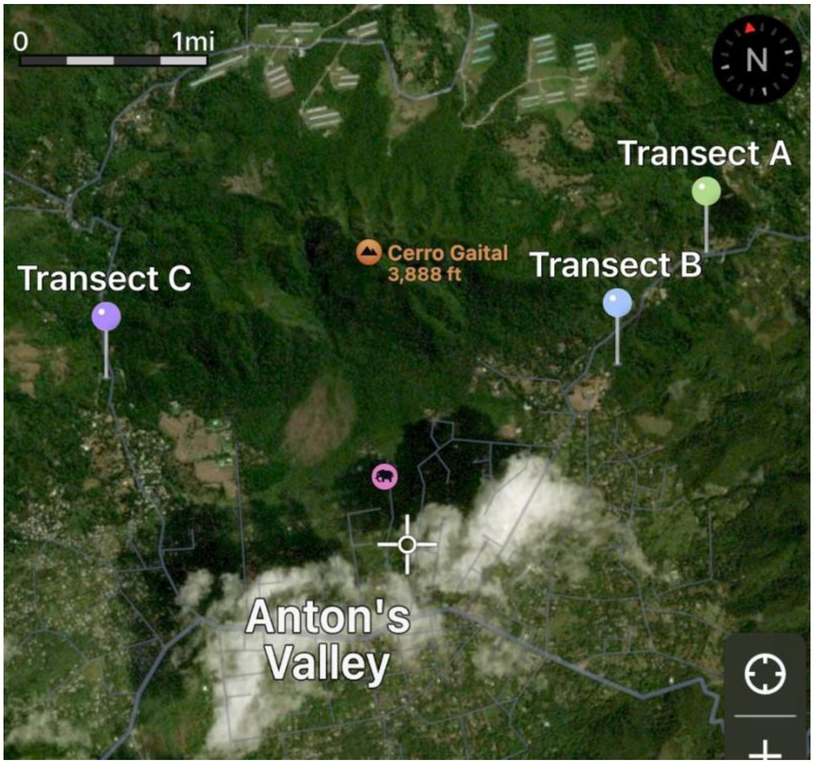

Figure 1. Map of the three transect locations where research was conducted. Transect A is represented by the green pin. It is approximately 1 km northeast of EVACC and is the location suspected to have the highest pesticide presence. Transect B is represented by the blue pin. This transect is directly behind EVACC along the Square Tree Trail. This location was expected to contain some pesticide run-off, though less than Transect A due to being further downriver. The purple pin represents Transect C which is along El Chorro Macho on the opposite side of El Valle. This stream was suspected to contain little to no pesticide run-off.

Data was collected between April 7th and April 21st. Data collection took place every morning and night during this time period, except for April 12th and 13th. Transects conducted during the day occurred between 8 and 11 AM and at night between 7 and 11 PM. The transects in each location were first conducted at night and then the following morning. There were two days before returning to the same transect which allowed time for any disturbed frogs to return and resettle.

Data collection at each transect began at the 0 m mark, which was always the most downriver part of the transect. The collection proceeded by moving upriver to the 100-meter mark. Visual cues were used to locate any easily visible frogs along both banks and a stick was used to minutely disturb the leaf litter and any frogs that jumped were also recorded. Auditory cues were also used to locate any calling or jumping frogs (Sapsford et al., 2013). All frogs that were spotted within 2 m to the left and right of the riverbank and up to 2 m high were recorded, including meta morphs. Meta morphs are individuals that have not yet reached the stage of juvenile, after emerging from the water, often recognized by the presence of a partial tail. When a frog was spotted a photo was taken. If the species was not immediately recognized, the frog was captured while wearing latex gloves, and photos of the dorsal, ventral, and laterals were taken (Sapsford et al., 2013). *Amphibians of Central America* by Gunther Köhler (2011) and *Amphibians of Costa Rica* by Twan Leenders (2016) were used to identify the species. Help from professionals at EVACC was also utilized in identifying species.

With the data collected, Shannon-Weiner Index calculations were performed to compare the relative diversity of the three locations. This index takes into account the number of species living in a habitat, the species richness, the species evenness, and their relative abundances to compare the organismal diversity in different areas.

Ethics

In order to minimize any impact on the frogs observed, training was received by professionals at EVACC on handling practices to ensure no physical harm occurred. If the frog was easily identified a photo was taken with no manipulation to minimize stress on the organisms. If more photos were needed for a later identification, the frog was carefully captured while wearing latex gloves. Photos of all sides were taken, and the frog was then released directly to the same location where it was found. Looking for frogs during day collections required some disturbance of the leaf litter habitats in which most frogs were found, however, movements were very intentional to minimize any change to habitats. During all collections, caution was taken to minimize disturbance to any and all flora and fauna in the area. To prevent the spread of any contaminants from one location to the next, equipment was cleaned between sites (Sapsford et al., 2013).

Results

A total of 969 frogs were observed throughout the 3 transects during the research period (Table 1). These frogs represented a total of 18 species, with *Smilisca sila* and *Lithobates warszewitschii* being the most abundant. The abundance of frogs was greatest in Transect A, the location with the greatest suspected pesticide influence, which had a total of 383. Frog abundance was second greatest in Transect B, the location suspected to experience slightly less pesticide influence, which had a total of 342. Frog abundance was lowest in Transect C, the location suspected to experience little to no pesticide influence, which had a total of 244. There were 9 species observed along Transect A, 12 species observed along Transect B, and 14 species observed along Transect C. *Espadarana prosoblepon* and *Hyalinobatrichium tataytoi* were only observed along Transect B (Figure 2). *Boana Rufitela*, a *Diasporus diastema complex*, *Leptodactylus savagei*, and *Pristimantis taeniatus* were only observed along Transect C (Figure 3).

Table 1. Frog species and abundances observed in 3 locations in El Valle. The star serves as a reminder that Transect C was the location that was suspected to experience little to no pesticide run-off.

#	Species	# Observed in Transect A	# Observed in Transect B	# ★ Observed in Transect C	Total # Observed
1	*Allobates talamancae*	-	1	2	3
2	*Boana rufitela*	-	-	1	1
3	*Craugastor crassidigitus*	5	15	66	86
4	*Craugastor fitzingeri*	4	5	14	23
5	*Diasporus diastema complex*	-	-	1	1
6	*Espadarana prosoblepon*	-	1	-	1
7	*Hyalinobatrachium tatayoi*	-	1	-	1
8	*Leptodactylus savagei*	-	-	2	2
9	*Lithobates warszewitschii*	96	254	64	414
10	*Pristimantis gaigei*	1	-	4	5
11	*Pristimantis ridens*	-	1	11	12
12	*Pristimantis taeniatus*	-	-	3	3
13	*Rhaebo haematiticus*	-	5	10	15
14	*Rhinella horribilis*	1	-	-	1
15	*Sachatamia albomaculata*	3	1	1	5
16	*Silverstoneia flotator*	1	1	-	2
17	*Smilisca sila*	271	55	62	388
18	*Teratohyla spinosa*	1	2	3	6
	Totals	383	342	244	969

Figure 2. Three species of glass frogs observed in El Valle. a) *Teratohyla spinosa* observed along Transect A. b) *Espadarana prosoblepon* observed only along Transect B. c) *Sachatamia albomaculata* observed along Transect C.

Figure 3. a) *Diasporus diastema complex* observed only along Transect C. b) *Pristimantis gaigei* observed along Transect A.

The Shannon-Weiner diversity index value for Transect C, 1.81, was significantly higher than those of Transects A and B, 0.796 and 0.908 respectively (Figure 4). The abundance of frogs was higher for Transects A and B, however, the species evenness was much lower for both. Transects A and B had very similar species evenness values, being 0.362 and 0.365 respectively. Transect C had a species evenness value of 0.686, almost double that of the other two locations (Figure 6). In Transect A, 95% of the individuals observed were *Smilisca sila* or *Lithobates warszewitschii. S. sila* was the most observed species, with many juveniles being found resting on the leaves of low vegetation (Figure 5). The species with the next most individuals in transect A was *Craugastor crassidigitus* with 5 observed. In Transect B, 90% of the individuals observed were also *S. Sila* and *L. warszewitschii*, with *L. warszewitschii* being the most common in this location (Figure 5). *C. crassidigitus* was the third most observed species in this location as well with 15 individuals observed. All other species observed included 5 or fewer individuals. Transect C exhibited the most species evenness with the same 3 most common species, however, all had abundances within the 60s. Three other species were observed with an abundance of 10 or greater.

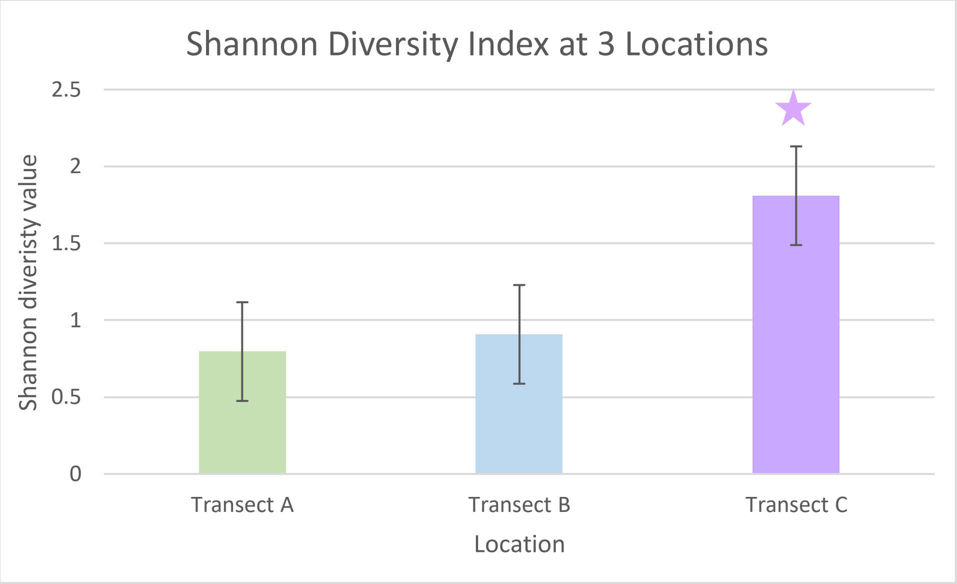

Figure 4. The Shannon-Weiner index values were much lower for Transects A and B, the sites that are suspected to experience pesticide run-off, while the value for Transect C is much higher. The star serves as a reminder that Transect C was the location that was suspected to exhibit little to no pesticide run-off.

Figure 5. a) A *Smilisca sila* juvenile observed on low vegetation on Transect A. b) a *Lithobates warszewitschii* meta morph observed along Transect B.

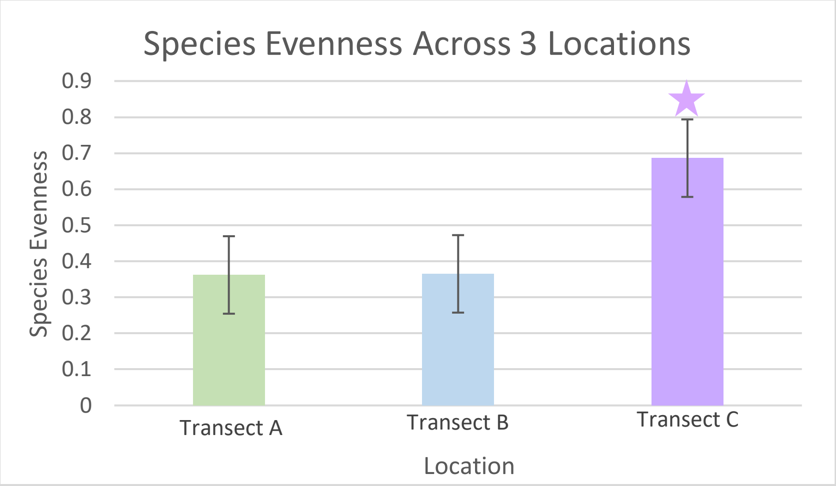

Figure 6. The species evenness was much higher along Transect C. The star serves as a reminder that Transect C was the location that was suspected to exhibit little to no pesticide run-off.

The number of frogs found each night in Transects A and B increased almost linearly (Figure 7). During the first night collection in both locations, approximately 20 frogs were observed and by the last night collection in each of these locations between 140 and 185 frogs were observed. The abundance of frogs found increased steadily for Transects A and B. The number of frogs found for Transect A was 22 during collection 1, 67 during collection 2, 88 during collection 3, and 181 during collection 4. For Transect B 23 were found during collection 1, 52 during collection 2, 97 during collection 3, and 144 during collection 4. Many of these frogs were juveniles or meta morphs, and large numbers of tadpoles were observed throughout the stream in these locations. Transect C had a much more consistent number of frogs found throughout, with 47 during collection 1, 53 during collection 2, 54 during collection 3, and 58 during collection 4.

The trend of increasing abundance as collections continued was not observed during the morning collections. There were very few frogs observed each morning with a minimum of 3 and a maximum of 12. All locations varied similarly in the number of frogs found during morning collections. Along Transect A, only *Smilisca sila*, *Lithobates warszewitschii*, and *Craugastor crassidigitus* were found during morning collections. Along Transect B these same 3 species plus two others were observed for a total of 5 species. Along Transect C those same 3 species were found, and 5 others were also observed for a total of 8 species. While differing numbers of species were observed at the different locations, the total number of individuals observed was very similar for all collections.

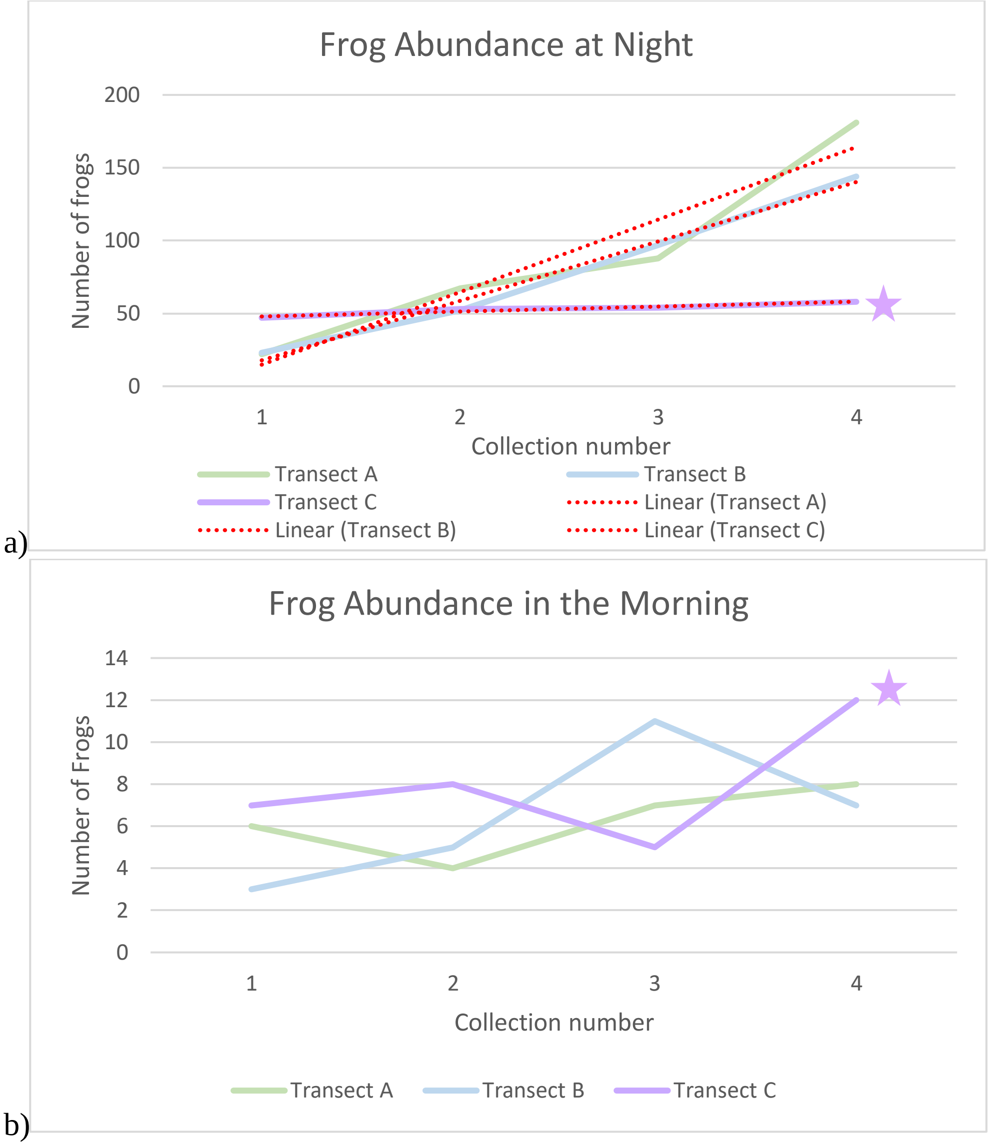

Figure 7. a) Trend lines show the almost linear increase in the number of frogs found during each collection with Transect B showing a very linear relationship. b) There is no trend within or across locations for morning collections. The star serves as a reminder that Transect C was the location that was suspected to exhibit little to no pesticide run-off.

Frog collections were completed during morning and night as frogs are expected to be most active during these times. There were far more frogs observed during night collections than day collections in all three locations. The average number of frogs observed during the morning collections was between 6 and 8 for all locations while the average number observed during night collections was above 50 for all locations (Figure 8). The lowest number of frogs found during any morning collection was 3 and the highest was 12. During night collections the lowest number of frogs found was 22 and the highest was 181.

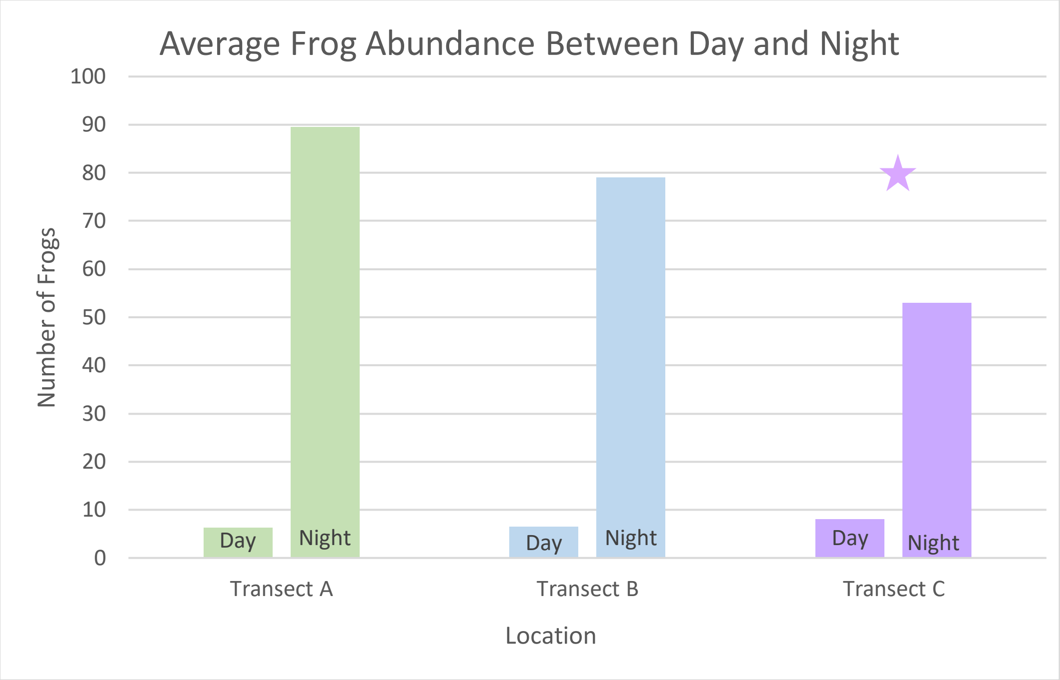

Figure 8. There were many more frogs found during night collections than day collections. The star serves as a reminder that Transect C was the location that was suspected to exhibit little to no pesticide run-off.

Discussion

The highest abundance of frogs was found along Transect A, the site expected to experience the most pesticide run-off. The second highest abundance was along Transect B which was expected to experience pesticide run-off though, slightly less. The lowest abundance was found along Transect C, the site expected to experience little to no pesticide run-off. All species observed were designated as least concern by the IUCN red list; however, 6 of the observed species are believed to have declining populations (The IUCN Red List of Threatened Species, 2022). The results show that despite a higher abundance of frogs found along Transects A and B, Transect C had the greatest species diversity. Greater species richness and species evenness were observed in this location. The significantly higher Shannon-Weiner Index value for Transect C supports the hypothesis that pesticide presence reduces frog species diversity. While this supports the hypothesis, there are many other factors that could have contributed to the higher Shannon-Weiner index value in Transect C and there is no specific evidence that this was due to pesticide presence.

Species abundance and richness

Transect A exhibited high anthropogenic disturbance related to the watercress plantation. There were man-made dams throughout the area and a road running through the river. The 10 meters surrounding this road were excluded in an effort to minimize its impact on the data. This location had significantly more low leafy vegetation surrounding the riverbank which appeared to be the preferred location of *Smilisca sila* juveniles. In all three locations over 40% of *S. sila* individuals were found on low leafy vegetation, but this was true for 73% of individuals of this species along Transect A. Not only were the vast majority of them spotted on low leafy vegetation, but large numbers of them could be heard calling from the watercress growing approximately 10 m from the riverbank. This species is known to persist in areas that have undergone considerable alteration, so this area which has experienced large changes to support the plantation seemed to continue to provide a habitat for this species (Leenders, 2016). A lower abundance of frogs was expected to be found so close to pesticide usage, but the habitat created by the low vegetation surrounding the river and the easily accessible watercress seemed to produce a preferred habitat for this species specifically. There were some tadpoles present in the stream, although far fewer than were seen in the river along Transect B. Despite fewer tadpoles being seen, based on the steady increase in individuals found throughout the study, it is likely that these juveniles were emerging from the river daily. Many very small juveniles and meta morphs were observed of both *S. sila* and *Lithobates warszewitschii*.

Transect B likely had the lowest anthropogenic effects as it was not easily accessible by the general public. There was a hiking trail along parts of the river, but the foot traffic on the trail was not particularly high. There were no lights in the area, but it was only about 800 m from where the watercress plantation run-off entered the river. This area was expected to be impacted by pesticide usage, but less so than Transect A. There was a very similar total abundance of frogs in Transect B to Transect A, with *Lithobates warszewitschii* being the most abundant. There were very large amounts of *L. warszewitschii* juveniles and meta morphs in this area. This was not surprising as there were areas along the transect in which the bottom of the river was completely covered in tadpoles of this species. There was an almost linear increase in the number of frogs found during each night collection at this location. More *L. warszewitschii* meta morphs were seen in each collection which serves as evidence that they were emerging from the river daily during the study period. This species is known to reproduce year-round so further research could be done on what triggered such large quantities of breeding during this time period (Leenders, 2016). Some of the professionals at EVACC suggest that breeding is triggered by the change in seasons which would have occurred around December or January and lines up well for so many individuals to be emerging in April (Heidi Ross, personal communication, April 17, 2023; Leenders, 2016). Three more species were observed along Transect B than Transect A, however, the overall species evenness was similar for both locations. The species richness and evenness were slightly higher in this area than in Transect A. Transect B was downriver from Transect A, so it was expected to still experience pesticide run-off, however, it was expected to be less concentrated. The slightly higher species richness and evenness in this location supports that idea.

Transect C was suspected to experience very little to no pesticide run-off, though the presence of the lodge resulted in some anthropogenic effects in the area. There was a bridge going across one segment of the river and during the day the grounds crew contributed to some noise pollution. Despite the anthropogenic effects, no chemicals were used in the area and the most fauna was seen here of the three locations. Agoutis ran around the lodge and there were many bird and butterfly species spotted during each visit. This location had the lowest total abundance, 98 frogs less than Transect B; however, it had the greatest species richness and evenness of the three locations. Both *Boana rufitela* and *Diasporus diastema* are species that, while not considered uncommon, are not commonly observed (Leenders, 2016). These two species were only seen in this location. A *Pristimantis taenniatus* individual was also only seen in this location but its population and distribution range are currently unknown (Leenders, 2016).

One possible reason for the lower total abundance of frogs in this location is the presence of bright lights between 21m and 69m along the transect. The lodge common area had seating above the river in this area with floodlights shining over it. Artificial lighting has been proven to impact the calling behavior of male frogs and may impact their behavior in other ways as well (Baker et al., 2006). Of the 244 total frogs observed, only 72 were observed in the middle segment in which these lights were present. On each side of the lights, a total of 172 frogs were observed. If these lights had not been present or were of a different style that had a lesser impact on nocturnal amphibians it is possible that close to 100 more individuals could have been observed in this location. If this is true, then the abundance would be very similar across all three locations. It is unknown how this would impact the species richness and evenness in this location. Future research could be done in which the lights are used for a year and then are not used for a year and researchers may be able to identify if the lights truly have an impact. It is also possible that motion-activated lights or dimmer lights could be used to reduce the impact they have on nocturnal animals (Gaston et al., 2012). A similar study could be done to see if this has a positive impact on frog abundance.

Differences in frog abundance between morning and night

Frogs are generally expected to be most active at night and in the early morning (Leenders, 2016). The number of frogs observed varied drastically between day and night. The average number of frogs observed in the morning was between 6 and 8 for all three locations while the average number of frogs observed at night was between 50 and 90. Professionals at EVACC stated that Chytrid had a greater impact on the diurnal frogs in the area and the most common diurnal frogs remaining in El Valle currently are *Allobates talmancea* and *Silverstoneia flotator*. (Edgardo Griffith, personal communication, April 15, 2023). A total of 5 individuals of these species were found, so despite being the most common, relatively few were observed. The most commonly observed individuals during the day were juveniles of *L. warszewitschii* and *S. sila*. In the past, the most common diurnal frog in El Valle de Antón was the Panamanian Golden Frog, but it went extinct in the wild in 2009 (Warner, 2017). It is generally expected that fewer frogs will be found during day collections than night collections yet, observing so few was unexpected. Professionals at EVACC stated that there were far more diurnal frogs before the chytrid fungus spread through the area (Edgardo Griffith, personal communication, April 15, 2023). This is also true for nocturnal frogs, so it is unclear if the difference is due to a greater loss in diurnal frogs due to the chytrid fungus or an overall decrease in frog abundance.

Pesticide impact

There was no direct evidence of pesticide impact on frog populations at any of the locations in this study. Other studies have found individuals with malformities in eyes and limbs, however, nothing of this sort was found during this study (Ferrante & Fearside, 2020). One study found that pesticide presence could actually increase frog abundance when the top predator was an insect (Relyea et al., 2005). This could possibly be applied to Transect A, as the pesticide presence was suspected to be highest in this area and there were very large numbers of *S. sila* and *L. warszewitschii* juveniles in this area. They may have had higher survival rates if the pesticides present decreased predator presence. Pesticide usage has also been linked to a loss in species diversity in some areas. (Ferrante et al., 2019). It is possible that other species may have been common in the areas in which Transects A and B were conducted and are no longer present due to the pesticide presence. It would be difficult to prove this at this time as any species loss would likely overlap with population decreases due to chytrid as well. This study did not involve the measurement or close observation of individual frogs, so a future study could include these aspects to better identify if there are morphological impacts due to pesticide presence.

Role of super spreaders

There is a theory that some frogs that were strongly impacted by the outbreak of Bd could have contributed to greater losses in other species as well (DiRenzo et al., 2014). Harlequin frogs such as *Atelopus zeteki* would rapidly become ill and exhibit high Bd infection intensities upon exposure to the chytrid fungus (DiRenzo et al., 2014). In an experiment, they were shown to shed very large amounts of chytrid zoospores as they neared death (DiRenzo et al., 2014). This quantity of zoospores could be representative of *A. zeteki* serving as a super spreader of Bd. There are many researchers who have years of experience with harlequin frogs who argue there is no evidence of these frogs contributing to greater declines in other species (Edgardo Griffith, personal communication, May 4, 2023).

According to the professionals at EVACC, 20 years ago, along a 100 m transect only about 10 *Lithobates warszewitschii* individuals would be seen (Heidi Ross, personal communication, April 17, 2023). During night collection 4 at Transect B, 109 individuals of this species were observed. *L. warszewitschii* and *Smilisca sila* are both likely chytrid resistant as they have managed to have success in proliferation despite the presence of chytrid in the area. Professionals at EVACC mentioned that *L. warszewitschii* is known to be able to live in conjunction with chytrid fungus and therefore could also be considered a super spreader (Heidi Ross, personal communication, April 17, 2023). With this in mind, as reintroductions of some species that have been extinct in the wild begin to approach, conservationists should consider the impact that these super-spreading species can have. It could be better to introduce frogs into an area like Transect C, where *L. warszewitschii* and *S. sila* are less prevalent and may be less likely to immediately cause infection in the reintroduced individuals.

Potential research errors

The research methods used during the study were standardized but there was still potential for many errors. One potential error could be that, in total, there were 4 different people who aided in searching for frogs. It is likely that some of these individuals were better at finding frogs than others which could impact the total abundance of frogs that were observed. The trends of frog abundance were similar across locations, so it seems as though the impact of this was minimal. The greatest potential error in this study was the identification of the frogs found. To identify the frogs, two different books on local amphibians, photos from amphibiaweb, and expertise from professionals at EVACC were used (AmphibiaWeb Search, n.d.). Professionals at EVACC could not go through each and every photo which leaves the potential for some of the frogs to have been misidentified. To identify *Craugastor crassidigitus* and *Craugastor fitzingeri* the coloration on the inside of the legs had to be observed. Some of these frogs escaped before this could be done so there which potentially led to some misidentifications. Another potential error could be the lack of quantitative evidence of the water quality in the three areas. Based on locating a discarded bottle of pesticide on the bank of Transect A it was assumed that there was a certain concentration of pesticide in the water in that area. It is also assumed that approximately 800 m downriver where Transect B was located, the pesticide concentration was lower but still present. It was also assumed that the water flowing through Transect C was of higher quality. These were all assumptions, and no analysis of the water was done to ensure that they were accurate.

Conclusion

The results indicate that frog species diversity was greatest in Transect C, the area that had little to no pesticide presence. This supports the hypothesis, although it is possible that there are other factors correlated with the observed differences. The research objective of identifying if the presence of pesticides had an impact on frog species diversity could not be conclusively achieved as other factors could be involved, however, the results provide support that there is an impact. In order to get a better idea if there is a strong correlation specifically between pesticide presence and frog species diversity, this study would need to be conducted over at least one year. This would allow for data to be taken during all seasons, minimizing the effects of the breeding and emergence of young individuals during the research. It would also be beneficial to use some method to quantify the water quality in the different areas such as chemical analysis of water samples or macroinvertebrate sampling.

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