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Anuran diversity and abundance across two sites with varying levels of light pollution in El Valle de Antón, Coclé, Panamá

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SIT Study Abroad

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Abstract

The impact of artificial light at night (ALAN) on anuran populations has been a subject of growing concern in ecological research. Anurans are particularly susceptible to the impacts of artificial light due to a nocturnal nature and an extensive exposure throughout various stages of life histories. Exposure to artificial light has been shown to impact the larval development and reproductive behavior of anurans. The impacts on anurans juvenile populations suggest a relationship between light pollution and anuran abundance. This study aimed to investigate the hypothesis that ALAN negatively affects the abundance and diversity of anurans. Field surveys were conducted in areas with and without ALAN, and lux levels were measured across various anuran species. Contrary to expectations, comparisons between areas with and without ALAN revealed no significant difference in anuran abundance, with ALAN habitats showing a higher diversity index. However, the preference of anurans for areas with 0 lux may still suggest a relationship between light pollution and anuran habitat choice. While average lux comparisons across anuran species supported differential effects of light pollution, statistical significance was only observed for one species. Nevertheless, the observed light tolerance of heavily pigmented eggs, such as those of *S. albomaculata*, to ALAN suggests paths for further investigation. Overall, this study contributes to the understanding of the impact of ALAN on anuran communities and emphasizes the importance of further investigation in this emerging field of study.

Acknowledgements

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Introduction

El Valle de Antón Background

This study observes how light pollution impacts anurans in El Valle de Antón, a town located in Coclé, Panamá. El Valle is west of Panama City and has an elevation of 600 - 700 meters (Bermúdez et al., 2011). The annual average temperature for the area is 23.4 °C (*El Valle climate*, n.d.). Due to Panama's dry and wet seasons, the region's climate fluctuates greatly depending on the time of year. The dry season, characterized by sunny and windy conditions, lasts from December through April (Gehring, 2023). The area has high humidity with monthly averages ranging from 77% to 83% from January to April (Grace-Martin, 2023). El Valle averages 2000 mm of rainfall per year and an average temperature of 21°C (Bermúdez et al., 2011). In the month of April, El Valle has an average temperature of 24.1°C with an average humidity of 81% and an average rainfall of 93 mm (*El Valle climate*, n.d.). El Valle de Antón has a relatively contained habitat due to its location in the crater of the inactive El Valle Volcano. This crater is over 1 million years old but is relatively small with a radius of only 6 km or 3.73 miles (Ramirez, 1988). An important water way in El Valle is the Rio de Anton and has a mean annual flow of 600 l/s as of 1988 (Ramirez, 1988).

The amphibians of El Valle de Antón are particularly interesting, and the region is home to many anuran species (Gagliardo et al., 2008). The crater is known as the home of the Panamanian gold frog, a species that is critically endangered and effectively extinct in the wild due to the Chytrid fungus (Bustamante at al., 2010). The most common species of frogs found in El Valle de Antón are *Smilisca sila* and *Lithobates warszewitschii*. *S. sila and L. warszewitschii* species typically mate during the transition between Panama's wet and dry seasons, and their larvae take approximately three months to develop. This indicates the potential for a high abundance of juvenile *S. sila* and *L. warszewitschi* in April and May (Gehring, 2023).

Status of Panamanian Anurans

The current populations of anurans in Panama are devastated by the proliferation of a fungal disease called Chytridiomycosis, caused by the *Batrachochytrium dendrobatidis* fungus, also referred to as Bd (Rodríguez-Brenes et al., 2016). Chytridiomycosis is a disease that affects over 700 species of amphibians across the world but has had a particularly pronounced effect on the Neotropics. The Neotropics' moist climate supports the propagation of the fungus, and its high rates of amphibian endemism result in disproportionately severe impacts on its populations (Lips, 2016). Panama has been no exception, and the chytrid fungus has quickly spread throughout the country. Bd has been present in Panama for over 25 years, when it was observed by a group of researchers studying rainforest frogs in 1997 (Berger at al., 1998). Bd is typically most prevalent at cooler temperature with high humidity and elevation (Rodríguez-Brenes et al., 2016). As a region filled with montane forest and cooler temperatures, El Valle de Antón is overrun with Bd. Rodríguez-Brenes et al. (2009) found that this disease was very common in El Valle and was considered enzootic to the region, regularly affecting the amphibian populations. The study aimed to investigate the dispersion of Bd within the region but failed due to the disease's widespread prevalence.

Chytridiomycosis Effect on Anurans

Chytridiomycosis is a skin infection that can be fatal in adult anurans. While Bd also effects larval frogs, tadpoles typically only carry the infection. The larvae aren't generally affected by the infection until they grow and metamorphose into their adult stages where the infection may turn fatal (Berger et al., 2016). The reason the impact of Bd changes between larval and mature stages is thought to be due to the increased amounts of keratin found in adult frog skin. The Bd fungus is a member of the Chytridiomycota phylum, which is known to use and decompose materials like chitin and keratin (Berger et al., 1998). With the varying impact of Bd at different life stages in mind, it makes sense that the chytrid fungus effects species of anurans differently based on their life histories (Lips, 2016). While many species of anuran have a larval stage, many others do not, and they develop from an egg stage directly into an adult stage (Buchanan, 2013). The impact of the keratinised skin on frog mortality also explains why Bd affects species differently based on phenotypic skin (Lips, 2016). In the lab, the mortality rate of Bd has been seen to differ between species. For some frogs it is very deadly with a mortality rate of up to 100%. Other species seem less drastically effected, with mortality rates as low as 0% (Berger et al., 2016). The genotype of the fungus and the physical factors of the environment also play a role in determining the impact of Bd on a species and population (Lips, 2016). Species living in warm dry microclimates within an ecosystem are less heavily impacted by Bd. Accordingly, the greatest population-wide impacts on anurans were seen with species that are more highly dependent on water sources (Berger et al., 2016). Even though prevalence varies by species, the effect of the chytrid fungus on the amphibians of an ecosystem cannot be overlooked. Ecosystems infected by Bd show significantly lower amphibian species diversity and abundance, which must be acknowledged when working with anuran populations in Chytridiomycosis affected regions (Kilburn et al., 2010).

Artificial Light at Night

Artificial light at night, also known as ALAN, is a relatively recent modern invention used across the world to make the lives of humans easier and safer. However, lights are foreign to the natural world and their use can have unexpected consequences for both humans and wildlife (Mander et al., 2023). In humans, artificial light at night disturbs circadian rhythms, upsetting the body's natural balance and increasing susceptibility to diseases like cancer or mental health conditions such as depression (Hatori et al., 2017). For the natural world, ALAN is a source of ecological light pollution, which is deemed as a type of light pollution that changes the normal patterns of light in an ecosystem (Longcore & Rich, 2004). Light pollution has a significant effect on animal behavior and has been known to change the foraging habits of animals like bats, birds, and rodents. The loggerhead sea turtle hatchlings illustrate how artificial light alters behavior, as they are drawn toward it instead of migrating toward the ocean for protection. (Feuka et al., 2017). In general, ALAN disrupts natural light cycles by creating light at times and environments where it is naturally absent. The flora and fauna have adapted and evolved to operate under the natural cycle of light daily and seasonally. The disturbance of these cycles' effects a species both directly and indirectly (Gaston et al., 2014). ALAN causes changes in species' reproductive behaviors. It can further affect their foraging and survivorship by changing the reproductive behavior of that species' predators and prey. The changes ALAN causes to the interspecific interactions within an ecosystem affect the abundance and distribution of the ecosystem's species. The effect of artificial light at night on ecosystems has not been studied, but its effects on the community's abundance and trophic structure suggest a larger ecological impact exists (Gaston et al., 2014).

Effect of ALAN on Anurans

Amphibians are particularly susceptible to light pollution, and anurans even more so (Buchanan, 2013). ALAN is expected to harm frogs more than other animals because anurans rely on moisture environments and have complex life histories. Many frog species lay aquatic eggs which then develop into tadpole larvae before metamorphosing into adults. This life history means that most frogs spend a significant portion of their lives contained in shallow bodies of water. These bodies of water frequently include man-made bodies, often located closely to artificial light sources. A frog's inability to relocate during embryonic and larval stages prevent anurans from moving away from sources of artificial light, potentially leaving frogs more exposed to ALAN during susceptible developmental periods (Buchanan, 2013). ALAN can reach levels of illumination that can change the photophase and scotophase durations. Scotophase duration changes can affect the development of embryonic frogs, potentially impacting the timing of important developmental mechanisms like gene expression and cell division (Buchanan, 2013). Gutierrez et al. (1984) found that *Discoglossus pictus* larvae were developmentally inhibited when raised with smaller scotophase durations. A similar effect was found in *Xenopus laevis* larvae, which developed slower when raised with a smaller scotophase duration (Edwards and Pivorun, 1991). The manipulation of the scotophase in these studies did not specifically mimic the effects of ALAN on frog development, but they do demonstrate the potential harm ALAN can cause by messing with the natural cycle of light and darkness. Anurans' reliance on sources of water for oviposition and moisture accumulation for hibernation in some species also limit frogs' habitats. The need to be located near water sources limits the distribution of adult frogs, minimizing anurans' ability to relocate and escape artificial lighting (Buchanan, 2013).

Artificial light at night is also considered to be especially impactful on anurans because frogs are primarily nocturnal organisms. Frogs' nocturnal nature can increase exposure to ALAN and change how ecological light affects their behavior. Frogs forage for food visually and have evolved to have larger retinas and more photoreceptors to allow them to capture the low-light usually present at night (Buchanan, 2013). Frogs' high light sensitivity makes the changes in light patterns caused by light pollution even more impactful and are changing anurans' foraging and mating behaviors (Buchanan, 2013). Baker and Richardson (2006) found that *Rana clamitans melanota* made less frequent reproductive calls when exposed to artificial light. A similar behavior was found in *Hyla squirella* and *Hyla leucophyllata* males, which were observed to retreat from the light to make their calls (Buchanan, 2013). Artificial light also impacts female reproductive behavior. A study by Tárano in 1998 showed that female *Physalaemus pustulosus* frogs moved their nesting sites, hiding them deliberately, when exposed to artificial light. Changes in reproductive behaviors indicate that the presence of ALAN could influence the population dynamics of many anuran species (Baker & Richardson, 2006). The effect of artificial light at night differs across anuran species and even though some specific species have been studied, there is still much that is unknown (Feuka et al., 2017). In general, the study of light pollution is new and while research looking at how it affects ecosystems and species is ongoing, the discipline is relatively young. Existing studies suggest anurans are affected by ALAN, but long-term effects on larval development, habitat choice and behaviors like mating and forging are still unknown (Buchanan, 2013).

Research Question

Does Anuran species diversity and abundance change based on the presence of artificial light at night in El Valle de Antón, Panamá?

Methods

Anuran species diversity and abundance data were collected using a variation of the methods implemented by Sapsford et al. (2013). Two 100 meter transects were created, one on the Río Antón, closely located to EVACC, and the other on El Chorro Macho on the grounds of the Canopy Lodge. The transect located on El Chorro Macho was not chosen randomly. Artificial light at night was only present at part of the river and for the data collection to be done successfully, the presence of ALAN had to be maximized. The transect on the Río Antón was chosen randomly within the portion of forest-covered river a month prior, and the same transect was used once again.

Figure 1. Map of Chorro Macho and Rio Antón study transects.

The starting point of each transect was flagged using flagging tape and recorded as 0 meters. The transects travelled upstream and a new flagging tape was placed every ten meters using a tape measure. Each 10 meters was flagged with the appropriate meter mark (10, 20, 30…). Once the 100-meter mark had been placed and tagged, the GPS was used to find the UTM at the end of the transect. This process was repeated to create both 100-meter transects at both study sites. Data collection did not begin the same day the transects were created to minimize the effect of any disturbance on the data. The data was collected five times at each transect and occurred at night between 7-11 pm. The study sites were alternated to allow anurans to return after any disturbance. During data collection, three researchers moved slowly up the transect as a group, one researcher on each side and a third walking up the middle. Flashlights and headlamps were used to visually locate anurans. When an individual was located, it was recorded using both visual observation and detailed photography. Latex gloves and the techniques taught by Professor Edgardo Griffith were used to catch certain anuran individuals to take more in-depth photos of frogs' leg and underbelly markings. The photos and Köhler (2011) were used to identify species present.

The amount of light pollution at the two sites was determined during every data collection period. ALAN was determined using a variation of the methods used by Dzul-Cauich et al. (2022). A digital illuminance meter was used to record the lux, or one lumen of light dispersed equally across one square meter of area. Illumination data was recorded by keeping the illumination meter horizontal and steady 5 cm above the ground. The lux measurement at every 10 meters for each transect was recorded. During each data collection, the lux was also measured at the time and location of every anuran sighting. A lux measurement associated with each anuran observed allowed the calculation of the average lux level each species was present in. The average lux for each species was analyzed using an ANOVA test as well as a Tukey-Kramer post-HOC test to compare between species. Seven ranges of lux values were also created based on the recordings taken. Those ranges were 0, 0.1-0.5, 0.6-1.0, 1.1-5.0, 5.1-10.0, 10.1-20.0, and 20.1-35.0. The abundance of anurans within each range was calculated, and an ANOVA test was used to compare the lux ranges. A Tukey-Kramer post-HOC test was also used to compare each lux range after the ANOVA was found to be statistically significant. The Shannon-Weiner Species Diversity index was also calculated for each lux range.

The lux measurements from each anuran observation and the measurements taken every ten meters on the transects were used to create ALAN and No ALAN groups. The portions of transect without any artificial light at night present were grouped together, while the portions with artificial light at night were grouped separately. ALAN area was determined as the parts of both transects in which a lux measurement over 0 was taken. The ALAN area was determined to be meters 0 – 60 of the Chorro Macho transect. The No ALAN area was determined to be meters 61– 100 of the Chorro macho transect and meters 0 – 100 of the Rio Antón transect. The Shannon diversity index was calculated for each group as well as the overall anuran abundance. The diversity index and abundance were analyzed using at Two Sample t-test. The lux measurements correlated with the individual anurans were also used to find the average lux measurements of the ALAN and No ALAN areas. The lux measurements of all anurans located in each ALAN and No ALAN area were averaged respectively. These averages were analyzed using a Two Sample t-test to determine if there is a significant difference in the average illuminosity across the two study areas.

Ethics

To conduct this research ethically, an IRB research form was filled out detailing the proposed research. In this form, the research topic and methods used in this paper were included. This form was approved by the SIT review board and no research was conducted before approval. The research in the field prioritized the health and well-being of all anurans above data collection. Anurans were first be identified based on physical appearance, and photos were taken of each individual in ways that were unobtrusive and attempt to minimize any impact made on the specimen. If these photos were not sufficient to differentiate between species, frogs were carefully caught using latex gloves and the techniques taught by Edgardo and Sam during the Amphibian unit. This training included the ways to properly catch and hold a frog to prevent bodily harm, as well as how to manipulate a caught frog to take species-distinguishing photographs. All caught frogs were released in the same spot they were found to limit disturbance.

Results

Population

A total of 333 anurans and 14 species were observed across the Chorro Macho and Rio Antón transects. These individuals were separated by the transect they were present in and the presence of artificial light in the location they were found. The Rio Antón transect consisted of 183 individuals and 12 species. The Chorro Macho transect consisted of 150 individuals and 11 species. The group found in an area with ALAN consisted of 95 individuals and 7 species. The group found in an area without ALAN consisted of 238 individuals and 12 species. The most common species found were *L. warszewitschii and S. sila*.

Species:	# Observed in Rio Anton	# Observed in Chorro Macho	# Observed in ALAN	# Observed in No ALAN	Total
L. warszewitschii	29	20	12	37	49
L. warszewitschii juvenile	119	47	27	139	166
S. sila	3	37	23	17	40
R. haematiticus	5	21	18	7	26
C. fitzingeri	5	9	5	9	14
P. gaigei	1	6	1	6	7
R. horribilis	12	1	1	12	13
E. prosoblepon	5	0	0	5	5
S. albomaculata	1	5	5	1	6
H. fleischmanni	0	3	3	0	3
L. savagei	1	0	0	1	1
P. taeniatus	0	1	0	1	1
A. talamancae	1	0	0	1	1
L. melanonotus	1	0	0	1	1
S. flotator	0	1	0	1	1
Total	**183**	**150**	**95**	**238**	**333**

Table 1. Anuran species observed abundance at the Rio Antón transect, the Chorro Macho transect, the ALAN area, the No ALAN area, and total abundance.

Abundance

Anuran abundance was recorded for areas with and without artificial light at night over five sets of data collection. The abundances were then divided by 1.4 and 0.6 for No ALAN and ALAN respectively to standardize the abundances to 100 meters of studied area. The average abundance for 100 meters of No ALAN area was calculated as 35.43. The average abundance for 100 meters of ALAN area was calculated as 31.67. The difference between the average abundance for the No ALAN and ALAN areas was not statistically significant (df = 7, t-statistic = 0.44, p = 0.672).

Data collection:	No ALAN	No ALAN/100 m	ALAN	ALAN/100 m
1	34	24.29	9	15

2	24	17.14	17	28.33
3	74	52.86	24	40
4	49	35	18	30
5	67	47.86	27	45
Average:	49.6	**35.43**	19	**31.67**

Table 2. Anuran abundance of ALAN and No ALAN areas per data collection. Hundred-meter adjusted abundance for ALAN and No ALAN areas per data collection.

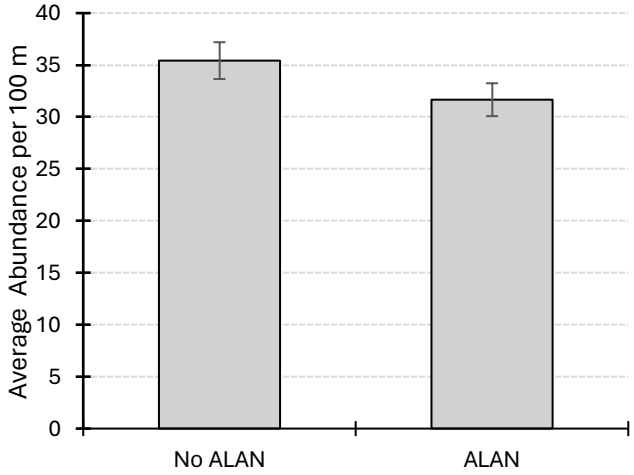

Figure 2. Average Anuran Abundance of No ALAN area and ALAN area ($p > 0.05$).

Diversity

The Shannon-Weiner Species Diversity index was calculated for both the ALAN and No ALAN area. The Shannon diversity index of the ALAN area had an H value of 1.545with a species richness of 8. The Shannon diversity index of the No ALAN area had an H value of 1.10 with a species richness of 13. The difference in Shannon diversity indices between the ALAN and No ALAN area was statistically significant ($df = 280$, t-statistic = 3.50, $p < 0.001$). The Shannon evenness of the No ALAN area was 0.43. The Shannon evenness of the ALAN area was 0.74.

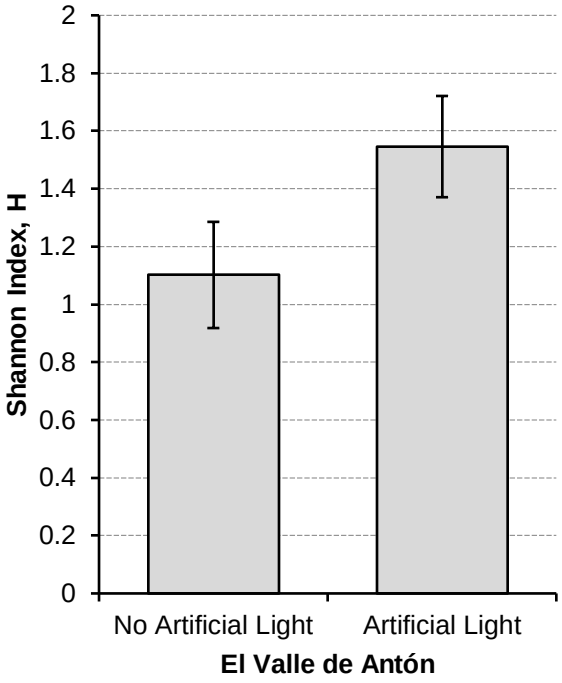

Figure 3. Comparison of Shannon-Weiner Species Diversity Indices (H) of No ALAN area and ALAN area ($p < 0.001$).

Artificial Light

The artificial light present at each anuran observation was recorded and used to calculate the average lux measurement of the ALAN area and the No ALAN area. The average lux measurement of the ALAN area was 2.9. The average lux measurement of the ALAN area was 0. The difference in average lux measurement between ALAN and No ALAN area was statistically significant ($df = 94$, t-statistic = 5.08, $p < 0.001$).

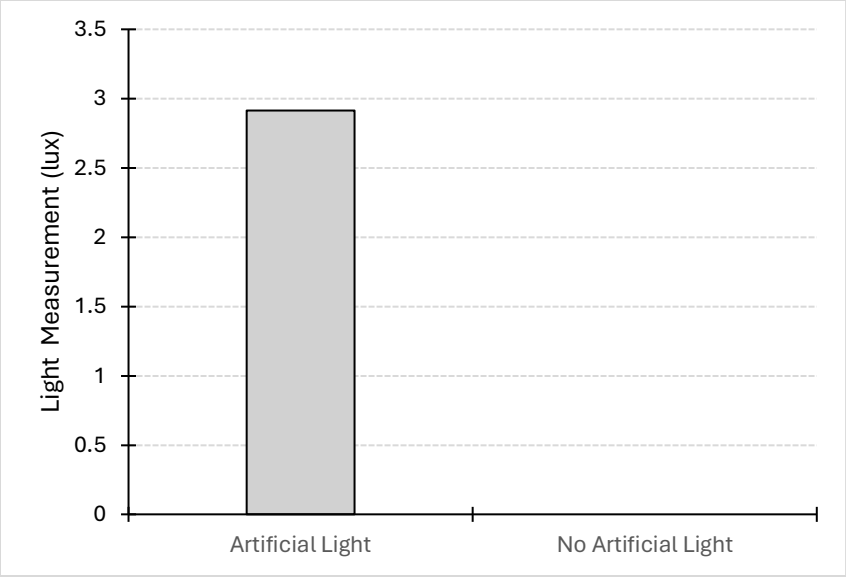

Figure 4. Comparison of average lux measurement of ALAN area and No ALAN area ($p < 0.001$).

The artificial light present at each anuran observation was recorded and used to calculate the average lux measurement of each anuran species. The difference in average lux measurements between species was statistically significant, but only for *S. albomaculata* ($df = 14$, F statistic = 4.91, $p < 0.001$). The average lux of *S. albomaculata* was statistically significant when compared to all other species. No other species average lux comparison was statistically significant. The species with the highest average light measurement was *S. albomaculata* with an average lux of 10.55. The species with the lowest average light measurement were *A. talamancae, C. fitzingeri, E. prosoblepon, L. melanonotus, L. savagei, P. taeniatus,* and *S. flotator* with an average lux of 0.

Species:	***Average Lux:***
L. warszewitschii	0.757
L. warszewitschii juvenile	0.681
S. sila	0.318
R. haematiticus	1.623
C. fitzingeri	0
P. gaigei	0.023
R. horribilis	0.292
E. prosoblepon	0
S. albomaculata	10.55
H. fleischmanni	1.767
L. savagei	0
P. taeniatus	0
A. talamancae	0
L. melanonotus	0
S. flotator	0

Table 3. The average light measured (lux) for each anuran species observed.

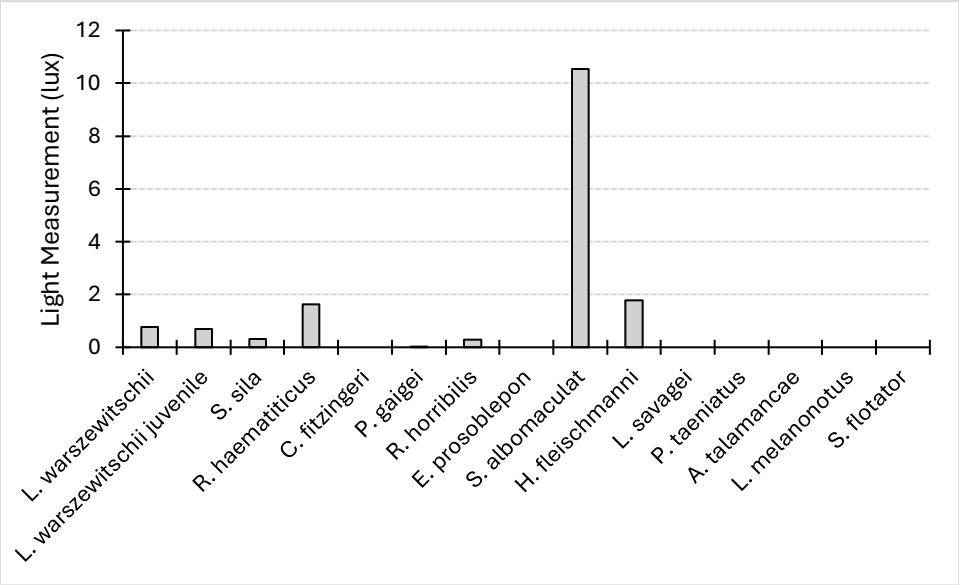

Figure 5. Comparison of average lux measurement by anuran species ($p < 0.001$).

The average abundance of anurans was calculated for seven different illumination ranges. The difference between the average abundances of the lux ranges was statistically significant (df = , 6, F statistic = 40.91, $p < 0.001$), but only for the 0 lux range. The abundance of the 0 lux range was statistically significant when compared to all other lux ranges. No other lux range comparison was statistically significant. The lux range with the highest abundance was 0 lux with an average abundance of 54.8. The lux range with the lowest abundance was 20.1-35.0 lux with an average abundance of 0.4. The lux range of 0.1-0.5 lux had an average abundance of 5. The lux range of 0.6-1.0 lux had an average abundance of 3. The lux range of 1.1-5.0 lux had an average abundance of 1.6. The lux range of 5.1-10.0 lux had an average abundance of 1.2. The lux range of 10.1-20.0 lux had an average abundance of 0.6.

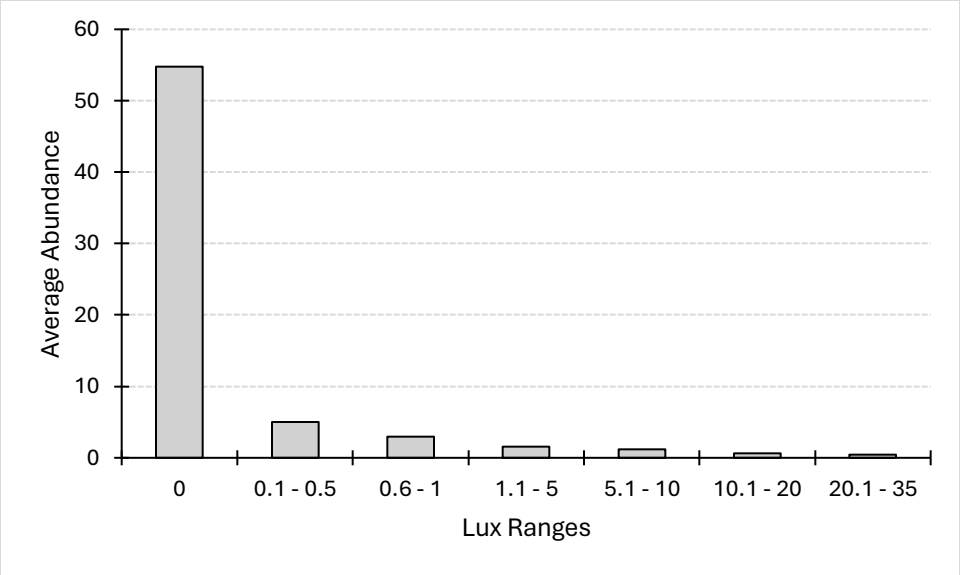

Figure 6. Comparison of average anuran abundance across illumination ranges ($p < 0.001$).

The Shannon-Weiner Species Diversity index was calculated for seven different illumination ranges. The lux range with the highest diversity index was 0 lux with an H value of 1.23 and a species richness of 14. The lux range with the lowest diversity index was 0.6–1 lux with an H value of 0 and a species richness of 1. The lux range of 0.1-0.5 lux had a diversity index of 1.20 and a species richness of 4. The lux range of 1.1-5.0 lux had a diversity index of 0 and a species richness of 3. The lux range of 5.1-10.0 lux had a diversity index of 1.05 and a species richness of 4. The lux range of 10.1-20.0 lux had a diversity index of 0.63 and a species richness of 2. The lux range of 20.1-35.0 lux had a diversity index of 0.69 and a species richness of 2.

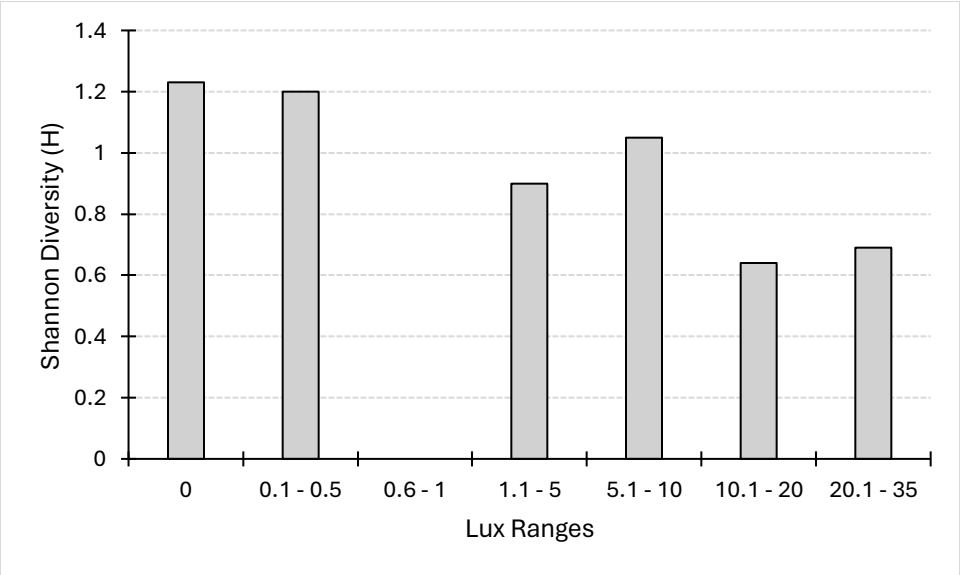

Figure 7. Shannon-Weiner Species Diversity Indices (H) of illumination ranges.

Discussion

Anuran populations

As seen in Table 1., a total of 333 anurans and 15 species were observed across two transects and five data collections per transect. This anuran abundance averages out to 33.3 anurans observed during each data collection period. Compared to a previous study by Gehring (2023), this average abundance was similar. In the Gehring study, three transects were observed, two of them being located on similar stretches of Rio Antón and Chorro Macho. The Gehring study found an average of 36.6 anurans across these same study sites. More specifically, the Gehring study found an average of 42.8 anurans at the Rio Antón transect and an average of 30.5 anurans at the Chorro Macho transect. This study found similar average abundances for both study sites, with an average of 36.6 anurans at the Rio Antón transect and an average of 30.0 anurans at the Chorro Macho transect. Comparing the abundances of this study with the abundances of the Gehring study suggests the anuran population stayed similar from April 2023 to April 2024. The species abundance difference between the two studies shows a different story. This difference can most plainly be seen in the abundances of species *C. crassidigitus, R.horribilis,* and *S. sila.* Gehring (2023) observed 117 *S. sila* across the Rio Antón and Chorro macho study sites. This study observed much fewer *S. sila* individuals, only 40 in total. A larger difference between this study and the Gehring study is the abundance of *C. crassidigitus*. The Gehring study located 81 individuals of the *C. crassidigitus* species while this study failed to observe any. An inverse pattern can be seen with the species *R. horribilis* which was not observed at the Rio Antón or Chorro macho transects in the Gehring study but was observed 13 times in this study. The species population differences may be explained by the Gehring study's use of daytime data collection. The population differences may also be explained by the presence of chytridiomycosis. The difference of a year is unlikely to cause changes in anuran populations at the levels observed across these studies. This does not necessarily remain true when considering the presence of Bd in El Valle de Antón (Rodríguez-Brenes et al., 2016). Frog populations can change dramatically over a year when chytridiomycosis is prevalent (Kriger & Hero, 2007).

ALAN vs No ALAN

The Artificial Light at Night, or ALAN area studied, consisted of the first 60 meters of the Chorro Macho transect. In this area, the river was exposed to artificial light from a nearby building of the Canopy Lodge hotel. There were four major lights illuminating the first 60 meters of the transect at night. The amount of artificial light along the 60 meters varied, with the highest average reading taking place at the 0-meter mark with a light measurement of 35.5 lux. The lowest average reading took place at the 60-meter mark with a light measurement of 0.1 lux. After the 60-meter mark there were no light measurements over 0 lux and the last 40 meters of the transect were considered part of the No ALAN area. The rest of the No Artificial Light at Night area consisted of all 100 meters of the Rio Antón transect. Despite being located on the property of another hotel, Hotel Campestre, the Rio Antón transect was set far away from the property's buildings along a forested hiking trail. The Rio Antón transect had no nearby sources of light pollution, and the light measurements recorded never registered above 0 lux. As shown in Figure 4., there was a significant difference in the average light measurements of the ALAN and No ALAN areas ($p < 0.001$). The ALAN area had a significantly higher average amount of lux with a measurement of 2.9 lux than the No ALAN area with a measurement of 0 lux.

As shown in Figure 2. and Table 2., there was no significant difference in the average anuran abundance for both the ALAN and No ALAN areas. Due to the inherently unequal length of the ALAN and No ALAN areas, the abundance of both areas' data collections had to be averaged across 100 meters. As shown in Table 2., the average length-adjusted abundance for the ALAN areas was calculated as 31.67 anurans while the average length-adjusted abundance for the No ALAN areas was calculated as 35.43 anurans. The lack of a significant difference ($p > 0.05$) between the average abundance of ALAN and No ALAN area contradicted the hypothesis that the presence of ALAN would negatively affect anuran abundance. This hypothesis was based on the idea that ALAN can inhibit species reproduction and change predator-prey relations that may limit species abundance (Gaston et al., 2014). The insignificant difference in ALAN and No ALAN abundance also contradicts what may be assumed from the past research. Studies by Baker and Richardson (2006) and Buchanan (2013) show that male anurans of the *R. clamitans melanota, H. squirella,* and *H. leucophyllata* species exhibit less frequent reproductive calling when exposed to artificial light sources. *Physalaemus pustulosus* female frogs have also been observed to have diminished reproductive activity under higher amounts of light. *P. pustulosus* females choose male reproductive partners more often under darker conditions (Rand et al., 1997). These diminished reproductive behaviors would disagree with the findings of this study and suggest an area without ALAN should have a higher anuran abundance.

Unlike anuran abundance, as seen in Figure 3., there was a significant difference in the Shannon-Weiner species diversity indices (H) of No ALAN area and ALAN area ($p < 0.001$). The ALAN area had a Shannon diversity index of 1.55 which is a moderate level of diversity. The No ALAN area had a Shannon diversity index of 1.10 which is a moderately low level of diversity. This statistically significant result directly contradicted the hypothesis that ALAN would negatively affect anuran diversity. An interesting consideration into this calculation is that fact that the No ALAN area had a species richness of 13 while the ALAN area had a species richness of 8. The cause for the low species diversity index in the No ALAN area can be explained by the No ALAN area's relatively low Shannon evenness of 0.43. The low Shannon evenness was likely due to the overwhelming number of *L. warszewitschii* observed, with a total of 176 individuals and an average of 35.2 individuals per data collection.

Lux ranges

The light measurements taken at each observed anuran were used to analyze the abundance of anurans at seven different lux ranges. These lux ranges were created after data collection, based on the relative frequency of anuran observations along the 0 – 35 lux range of the light measurements. The average abundance for each lux range was calculated based on the five data collections. As shown in Figure 6., there was a significant difference between the average abundances of the seven lux ranges, but only for the 0-lux range. No other lux range comparison was statistically significant. This supported the hypothesis that lower levels of ALAN would be paired with lower anuran abundance and suggests that anurans do prefer to be in areas without artificial light present. It is interesting to note that although there was no significant difference in the average abundances of the ALAN and No ALAN areas, the 0-lux range is statistically significant when compared to the other six lux ranges. While these data points may seem contradictory, the significant lux range finding is supported by previously observed effects of artificial light on anuran reproductive behavior (Buchanan, 2013; Rand et al., 1997; Richardson, 2006). The differences between the ALAN vs. No ALAN findings, and the lux range findings, can most likely be explained by the nature of these comparisons. In the ALAN

vs. No ALAN comparison, the ALAN group consisted of all lux ranges from 0.1-35.0. Breaking this group into six smaller groups explains why the abundance difference wasn't significant in one test but was in another.

As shown in Figure 7., the data separated by light measurement ranges were also used to calculate Shannon-Weiner species diversity indices (H) for all seven lux ranges. The Shannon diversities were relatively low across all seven lux ranges. The only diversity index that stands out is that of the 0.6-1 lux range, in which only one species was ever observed. The lux range diversity index data is not particularly telling about the relationship between light pollution and anuran diversity but does seemingly contradict the relationship found between the diversity indices of the ALAN and No ALAN areas. The ALAN vs. No ALAN diversity index comparison showed that the ALAN area had a significantly higher Shannon diversity index. The difference between the ALAN vs. No ALAN findings and the lux range findings can again be associated with the division of the ALAN grouping into six ranges. The diminished ALAN group and the lack of a statistical test comparing the diversity indices of the lux ranges suggest that the findings of the ALAN vs. No ALAN comparison is likely the more useful source of information.

Average species lux

The light measurements taken at each observed anuran were used to analyze the average lux measurement in which each species was observed. This comparison across observed species was analyzed to see if the data suggested any species had different ALAN preferences. These varied life histories mean that some species are more likely to tolerate artificial light. Other species, and more specifically their embryos and larvae, are less adapted for high-light environments. As shown in Table 3. and Figure 5., the data supported the hypothesis that there would be a difference in average lux level across the 15 observed species. The 10.55 lux average light measurement of *S. albomaculata* was statistically significant when compared to all other species ($p < 0.001$). No other species average lux comparison was statistically significant. The significantly higher average lux of *S. albomaculata* could indicate that the species has a higher tolerance for artificial light. The biology of the *S. albomaculata* aligns closely with the ideas shared by Buchanan (2013). While there hasn't been research comparing how different anuran species react to light pollution, there is information that suggest a difference might be expected. One of the largest reasons anurans are thought to be disproportionately affected by artificial light at night is due to their larval stages and reliance on bodies of water. Both the presence of a larval stage and this need for water vary greatly across anuran species, however. The offspring of some species of frog are born in the form of unpigmented eggs, which are very sensitive to light (Buchanan, 2013). The eggs of the *S. albomaculata* are known to be densely pigmented (Guayasamin et al., 2020). This dense pigmentation and its associated light tolerance could help explain why *S. albomaculata* individuals were found at such significantly higher lux levels than any other anuran species.

Sources of error

The methods used in this study were standardized and followed carefully to minimize potential error in the data and the data analysis. Even so, error is impossible to completely avoid and there remain sources that may have impacted the data. One source was the reliance on multiple investigators during data collection. Having three different individuals collecting data may cause errors in data collection due to the difference in individual attention level and frog-finding ability. Some researchers may have been more adept at locating anurans than others. To

limit the impact of this potential source of error, the roles of investigators were standardized across all data collections. The researchers assigned to each side of the transect and the researcher assigned to the middle of the transect were kept the same across data collections. This would not affect larger sources of anuran location error, but the standardization of roles limits the error between data collection. Another source of potential error was the identification of anuran species. The expertise of EVACC professionals and a Central America amphibian book were used to identify frog species. The use of these resources was standard, but unlikely to be perfect. Individual anurans moved quickly at times, preventing the taking of species-distinctive pictures. Without detailed photos of every individual, researchers had to use knowledge from past identifications to identify the individuals. The limitations of the illuminance meter were also a source of error. The meter could only read light measurements to one decimal point. Many of the light reading were low-light, and the meter had trouble distinguishing true light absence from very low light. Researchers were forced to visually decide whether a reading of 0 lux was accurate, or if the meter wasn't strong enough to detect the low light. The use of a more sensitive illuminance meter would further limit this source of potential error and should be prioritized for any future research.

Conclusion

The results of the study varied significantly in their support of the hypothesis that artificial light will have a negative effect on the abundance and diversity of anurans. The results of the ALAN vs. No ALAN area comparisons did not support this argument, and even contradicted the hypothesis. There was no significant difference in the abundances of ALAN and No ALAN anuran populations, and the diversity index of the ALAN population was determined to be significantly higher than the diversity index of the No ALAN population. These comparisons suggest that the presence of artificial light has no effect on the abundance of anurans and has a positive effect on the diversity of anurans. The results of the lux range comparison did contradict these suggestions. The statistically significant average abundance of the 0 lux range suggested that there was a preference for anurans to be located in areas without ALAN. This interpretation should be evaluated with regard to the contradictory findings in the ALAN vs. No ALAN comparison, and not be taken at face value. The contradiction between the two comparisons suggests the need for additional research regarding the relationship between ALAN and anuran abundance.

The average lux comparison across the anuran species supported the idea that light pollution effects anuran species differently but only with minimal evidence. With *S. albomaculata* being the only species observed in statistically significant lux levels, the idea that light pollution effects differ by frog species can only be speculated upon. The lack of research in the topic of species-distinct light-pollution effects does suggest that this finding could be used as a basis for future research. The connection between the heavily pigmented eggs of the *S. albomaculata* and its suggested ALAN tolerance could be used to create further study. Comparing the average lux levels of glass frogs with different levels of pigment density in eggs could be an interesting way to study this relationship and the effect of ALAN at the embryonic stage. The study of light pollution and its ecological effects is young, and many gaps exist in the current knowledge base. This study demonstrates how much more there is to study about the effect of ALAN on anuran species and populations.

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