The Effects of an Introduced Breeding Pond on Frog Populations in the Talamanca Mountains

Research Location:
Cloudbridge Nature Reserve, 2 km Este de Escuela, San Gerardo de Rivas, Pérez Zeledón, San José, Costa Rica, 11904

Conservation Area: ACLAP

Permit Number: Resolución Nº R-SINAC-PNI-ACLAP-014-2023

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Abstract

The cryptic behavior of amphibians has led to a chronic lack of information surrounding their population sizes and health. As some of the most sensitive organisms to disease and habitat fragmentation, extensive and continuous research is necessary to monitor their trends; specifically at high elevations. Cloudbridge Nature Reserve in Costa Rica serves as an exemplary study site to examine behaviors regarding reproduction activity. The research put forth was to determine if frogs would respond positively to an introduced, artificial breeding pond located in a natural cloud forest environment. A six month study period resulted in two separate egg clutches being present in the introduced pond, with one clutch successfully hatching into tadpoles. Such behavior indicates adaptive traits in frogs by being willing to deposit eggs in an artificial pond. This result is emblematic of the future conservation possibilities for frog and amphibian populations, as perpetual efforts will be necessary to rebuild and protect healthy communities.

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Introduction

The climatic changes exhibited on a global scale have the potential to negatively impact the populations of various vertebrates and invertebrates alike, as the environmental transitions could exceed their capacity to adapt to the shifts (Finch, 2012). While the main factor we think of when discussing climate change is habitat loss or degradation, changes in biological events (timing of reproduction, or when an animal migrates) are equally as important to take into consideration. Alterations in the timeframe of when biological events occur has significant impacts on the future success of a species, as it can lead to a faltering population size if adaptations are not prosperous (Radchuk et al., 2019).

While there are direct consequences of climate change such as increased air temperatures or rising sea levels (Walker, 1991), the indirect effects are often overlooked. Though there are many, these indirect influences can be narrowed down in order to explore certain parameters and the effects it may have on a particular animal. For example, changes in climate have been shown to strongly influence the behavior of the parasitic fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*); yet this fungus is directly related to the decline of amphibian populations (Rodder et al., 2010). So, though the *Bd* fungus acts as a secondary influence on amphibian populations, the effect has been profound. Worldwide, 42% of amphibian populations sampled have reported interactions with *Bd* (Olson et al., 2013).

Amphibian populations are not only susceptible to pathogens like *Bd*, but also have variable sensitivity to habitat modification (Nowakowski et al., 2017). As land use has increased for humans in the last century, this inevitably causes exhaustive habitat loss for animals. Although not all adult amphibian populations show an adverse response to habitat change, there still remains unknown impacts on larval success, which could have negative repercussions for the future (Nowakowski et al., 2017).

In Latin America, amphibian population declines have been drastic and chronically understudied. According to Young et al. (2008), an estimated 13 countries within Latin America have experienced these significant declines; in some cases, this has ended in species extinction or being extirpated from their native location. Specifically in Central America many of these declines have been in elevations above 500m (Young et al., 2008), like in the Talamanca mountains of Costa Rica, for instance.

Amphibian decline in Costa Rica has been extensive (Puschendorf et al., 2006), with a high percentage of populations showing a decrease in numbers all over the country. Thus, it's essential that amphibian studies are continuously conducted in an attempt to maintain an accurate understanding of what their population trends are. Because mountainous regions above 500m are seemingly more susceptible to population declines (Young et al., 2008), supporting frog and other amphibian communities at these elevations could serve as a crucial step towards limiting further reductions. In Cloudbridge Nature Reserve, located in the Talamanca mountains of Costa Rica, the elevational gradient reaches 2600 masl (Cloudbridge, n.d.); well above the 500m threshold. While not overly abundant, the frog and other amphibian populations that exist within the reserve are at potential risk of the aforementioned dangers.

In an effort to provide support to the already limited frog populations within Cloudbridge Nature Reserve, the purpose of the research reported here is to construct an artificial frog breeding pond with the anticipation that it could have a long-term, effective impact on their population size and reproduction rates. Frogs in particular are known to be semi-aquatic animals, in which many species require a body of water in order to have a successful clutch of eggs (Hazell et al., 2004). Some studies have shown that frogs have little preference when it comes to natural ponds versus artificially made ponds, as long as the surrounding vegetation and number of predators is sufficient for viable breeding (Hazell et al., 2004; Semlitsch et al., 2015). Therefore, the core objective is to determine whether the newly placed pond would be used as a breeding site for any of the water-breeding species of frog found on the reserve. Additionally, if the pond supports a clutch of eggs, the survivability of the clutch is to be monitored. It's hypothesized that the introduction of a new breeding pond would serve as a positive influence on reproductive behaviors and success of the frog populations found in the reserve.

Methodology

A. Study area

This research was conducted at Cloudbridge Nature Reserve (hereafter Cloudbridge), a 283 hectares private reserve located in a cloud forest within the Talamanca mountains, outside of San Gerardo de Rivas, Costa Rica. Cloudbridge is enveloped by the large protected area of Cerro Chirripó National Park, and contains the Río Chirripó Pacífico that begins from within the park and runs through the heart of Cloudbridge. The reserve supports various levels of forest succession, ranging from mature forest (80+ years), naturally regenerated forest (20-80 years), planted forest (<20 years), and small sections of primary forest, as shown in figure 1. Its location in the Talamanca mountains causes a large altitudinal gradient throughout the reserve, which at its lowest point is 1550 masl, and reaches 2600 masl at its highest. This habitat experiences a vastly notable dry season and rainy season, as the dry season (January-May) produces very little precipitation, while the rainy season (June-December) causes consistent heavy rainfall.

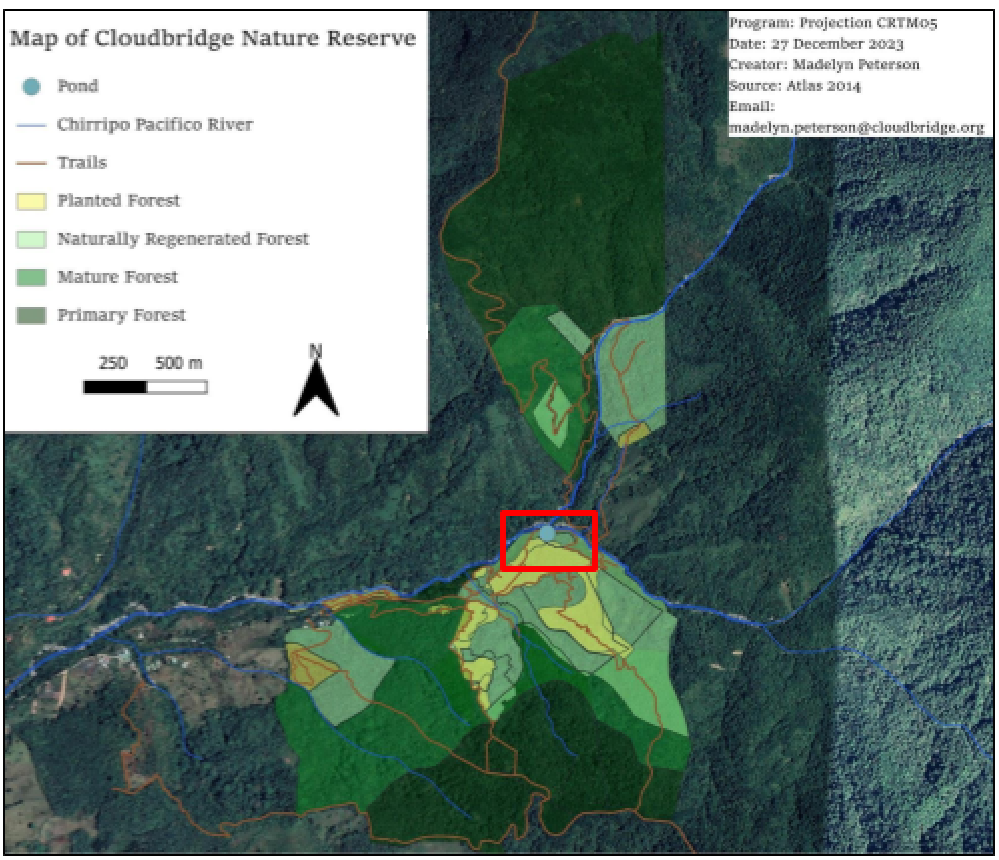

Figure 1: Map of Cloudbridge Nature Reserve, with indication of the Río trail location (in red).

Though there are several trails spread across the reserve, this study took place within the Río trail, evident in figure 2, at coordinates 09 28.470'N, 083 34.194'W; 1646 meters in elevation. The relevant forest type for this study can be classified as secondary, naturally regenerated forest. This type of vegetation supports trees ranging from 20 to 80 years of age, in addition to an abundance of epiphytic plants such as bromeliads and bryophytes. Due to the proximity of the trail shoreline to the Río Chirripó Pacífico river, several smaller streams exist on this trail. One such stream is located directly next to the pond, where this study took place.

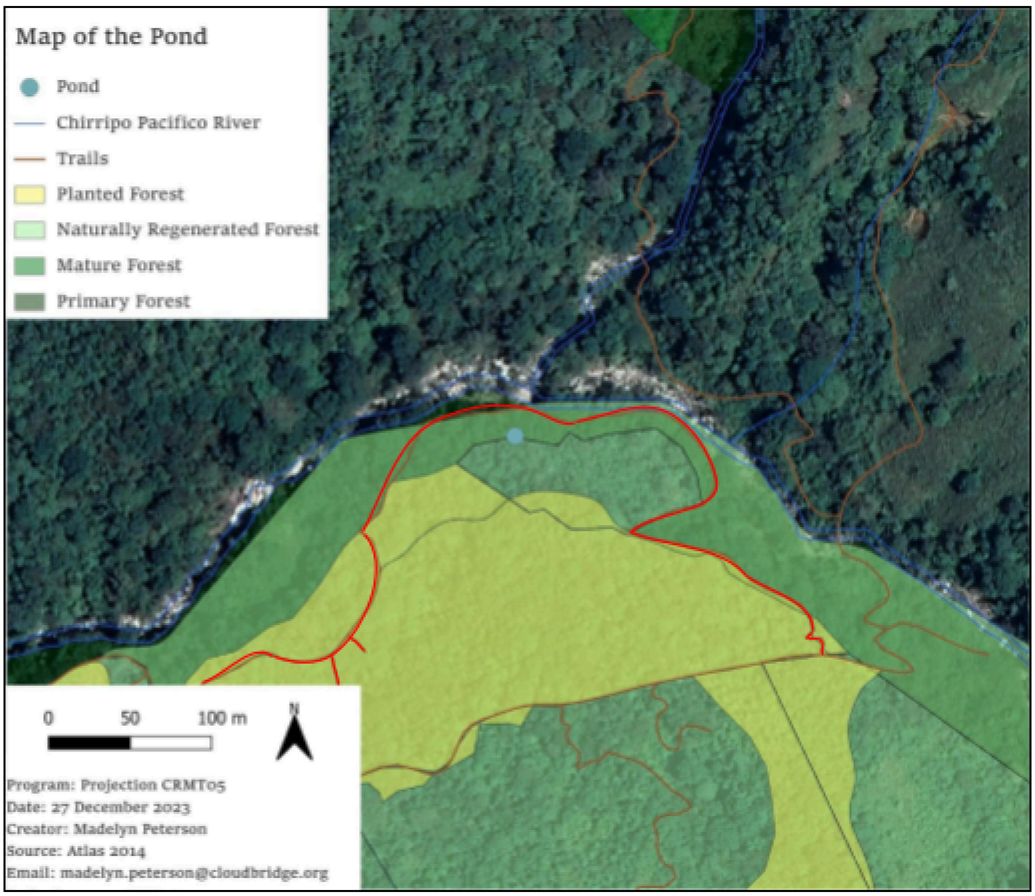

Figure 2: Map of the Río trail (indicated in red) with the location of the pond.

B. Pond Construction

Building the pond served as the first step in this research process. An important distinction when choosing the pond placement is whether the accompanying stream would remain active during the dry season, as the stream would be the water source for the pond year round. Various smaller streams run dry when the rain ceases, directly impacting the long term practicality and usefulness of a breeding pond. The chosen stream was selected at the conclusion of the dry season, and still had flowing water, rendering it unlikely that it will become dry in the future dry seasons. The stream runs in a general direction from south to north, with a water velocity of $0.6cm^3$/second and width of 60cm (July 2023). The stream has an unknown beginning or end point, as these are hidden either underground or in an inaccessible part of the forest.

To construct the pond, a 2m:2m:50cm hole was dug three meters west from the original route of water flow. To cooperate with the wet terrain, it was necessary to use rocks and plant materials to maintain the integrity of the hole. The stream was then diverged to flow into the hole on the south-east end via a small channel, which then a secondary channel on the north-east end of the hole was created to allow the stream to flow back into itself. This allows the pond to be continuously and sustainably filled year round with no human intervention for water maintenance.

C. Data Collection and Methodology

Surveys were conducted once a week at 19:00. Additional survey days were removed in an attempt to reduce the amount of erosion and disturbance around the delicate habitat. Prior to beginning the survey, data was collected for: temperature; humidity levels; daily rainfall prior to/at time of survey; lunar phase. Any weather irregularities occurring at time of survey were recorded (eg. continuous rainfall, heavy winds, etc). A camera trap was deployed one meter west of the pond to monitor any additional animal activity, checked weekly.

A standardized procedure for data collection was executed. Upon arrival at the pond the camera trap is turned off and the SD card is changed. While standing at the pond, a 15 minute time allotment is given to allow for a visual encounter survey directly in the pond and the immediate area. Aspects taken into account were presence of frogs or frog eggs, or other vertebrate activity.

The depth of the pond at the deepest and most shallow points were taken. Subsequently, a second visual encounter survey was conducted for 30 minutes in a 10 meter radius from the pond in all four cardinal directions. The factors of importance in this secondary survey included physical frog presence, the presence of frog vocalizations (even if not seen physically), and other organism activity. Invertebrates were not included in any part of this study.

Results

During the six month research period, 22 surveys were completed. The main variables taken into consideration for the results were: daytime rainfall prior to beginning the survey; moon phase; physical frog presence; auditory frog presence; frog egg presence. Physical frog presence is broken into two groups; being physically at or in the pond, or being within a 10 meter radius of the pond (denoted as "general presence"). Rain was measured by the length of time the rain occurred, counted in hours. A table expressing the rain class can be found in Appendix B, table 1. A simple linear regression was used to measure the correlation between both frog presence and frog vocalizations with corresponding variables ($y = \beta 0 + \beta 1x$). The Simpson's Diversity Index was utilized for determining the level of animal diversity captured by the camera trap [$D = \Sigma n_i(n_i\text{-}1) / N(N\text{-}1)$]. Although humidity, temperature, and pond depth data was recorded, the changes of these variables during this study were negligible and therefore not considered as significant factors to calculate into results.

Result A: Pond use for reproduction purposes.
Two sets of frog egg clutches were deposited in the pond on two separate occasions, first in July 2023 and secondly in December 2023. One set of eggs hatched into tadpoles, demonstrating a 50% success rate. The first set of eggs are from an unknown species, while the second set of eggs are from *Lithobates warszewitschii*, or Warszewitsch's frog. A photo of the eggs and the laying individual from the successful clutch can be found in Appendix C, photographs 1 and 2.

Result B: Physical frog presence at pond.
Of the 22 surveys conducted, a frog was seen at or inside the pond twice, or 9% of the time. This is taken independently from the other variables measured during this research period, as frog presence is denoted as a simple 'yes' or 'no'.

Result C.1: General frog presence.

Within the 10 meter radius limit, 15 frogs from five species were seen over the study period of 22 surveys. The majority of the surveys (50%) had no frog sightings, shown in figure 3. A table depicting the species of frogs observed throughout the study can be seen in Appendix D, table 1.

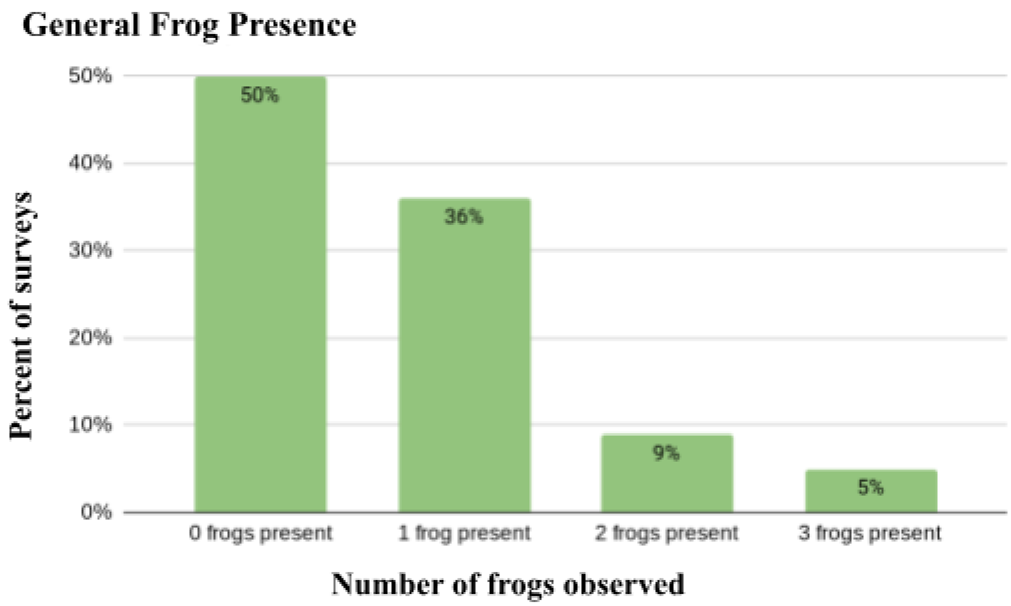

Figure 3: Number of frogs observed in relation to the percent of surveys that quantity of frogs was seen.

Result C.2: General frog presence in relation to moon phase.

Moon phases ranged from 0.01% illumination to 99.95% illumination. There was no significant relationship between a particular moon phase and whether a frog was present in the general area ($r^2 = 0.04$, $F(1,18) = 0.76$, $p = 0.395$). However, a secondary linear regression was run with the moon phases split into two groups, when the moon is less than 50% illuminated or greater than 50% illuminated, shown in figure 4. While still an insignificant result, greater than 50% moon illumination ($r^2 = 0.36$, $F(1,7) = 3.91$, $p = 0.088$) had a much stronger correlation to frog presence than a moon illumination of less than 50% ($r^2 = 0.02$, $F(1,9) = 0.15$, $p = 0.706$).

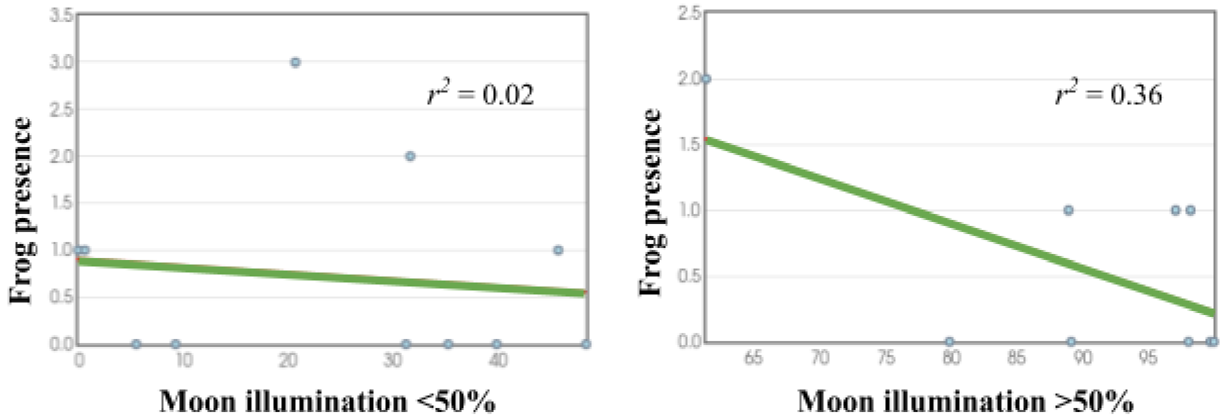

Figure 4: Number of frogs seen with respect to the phase of the moon, divided into <50% and >50% illumination.

Result C.3: General frog presence in relation to daily rainfall.

There was no significant correlation between rainfall prior to the survey beginning and the general presence of frogs (r^2 = 0.042, $F(1,18)$ = 0.79, p = 0.386).

Result D.1: Auditory frog presence within 10 meter radius in relation to moon phase.

The phase of the moon did not have a significant effect on the presence of frog vocalizations during a survey, seen in figure 5 (r^2 = 0.09, $F(1,18)$ = 1.89, p = 0.186).

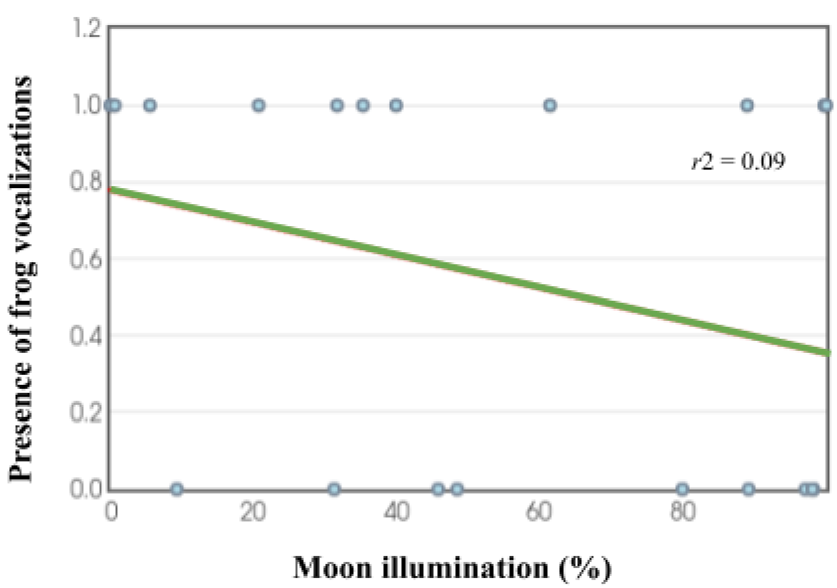

Figure 5: The phase of the moon in relation to the detected vocalizations of frogs. The vocalizations are measured as 1 for present or 0 for not present.

Result D.2: Auditorial frog presence within 10 meter radius in relation to daily rainfall.

The number of hours it rained prior to the survey had a significant effect on the presence of frog vocalizations, as increased rain caused a dramatic decrease in the vocalizations present during a survey, shown in figure 6 ($r^2 = 0.27$, $F(1,18) = 6.71$, $p = 0.018$).

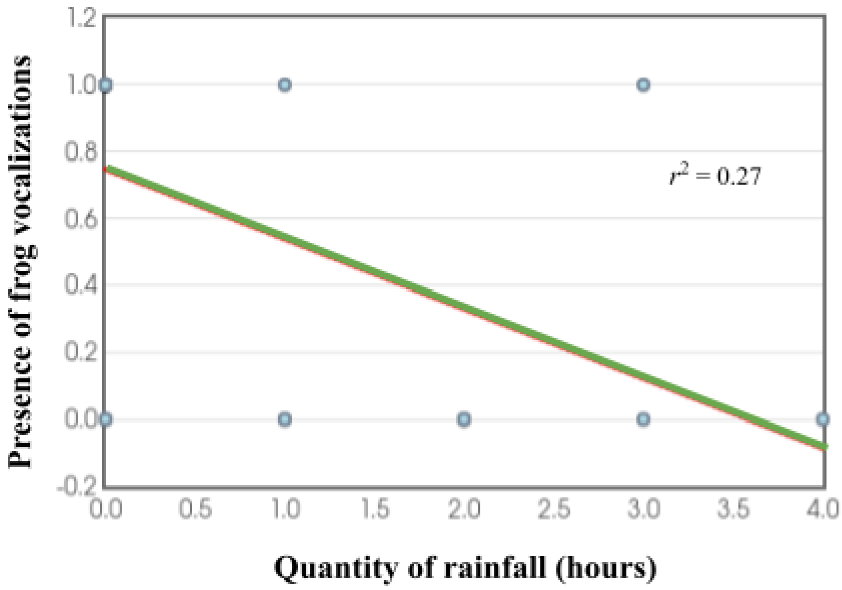

Figure 6: Presence of frog vocalizations with respect to the amount of rainfall prior to the survey. Rain is measured in hours (see rain class; Appendix B, table 1). The vocalizations are measured as 1 for present or 0 for not present.

Result E: Organisms discovered by camera trap.

A Simpson's Diversity Index test was used to measure the relative diversity of the species seen. Within the study period 11 different species were captured on the camera ($N = 11$; $D = 0.88$). Two species were seen walking into the pond, the gray four-eyed opossum (*Philander opossum*) and the tayra (*Eira barbara*). The collared peccary (*Pecari tajacu*) was seen drinking from the pond. Dice's cottontail (*Sylvilagus dicei*) was observed eating plants from around the pond, and was the animal with the highest presence at the pond. The remaining seven species only interacted with the greater pond area or passed through with no interaction. The full list of the species seen on the camera trap can be found in Appendix E, table 1.

Discussion

A. Summary on Reproductive Use

Although the raw number of frogs seen in and around the pond appears low, the main purpose of this study was to determine if the presence of an additional frog breeding pond would lead to reproductive use. Due to the discovery of two separate frog egg clutches, this study is deemed successful given that the frogs used an artificial pond to deposit their eggs while in a natural environment. The proposed reasoning for the first clutch of eggs being unsuccessful is due to insufficient hiding places available in the pond at that time (Hazell et al., 2004; Dorcas & Gibbons, 2011). Upon creation, there was a lack of living flora growing inside the pond, and the placement of the rocks surrounding the pond did not provide structures where a clutch of eggs could be hidden. When the second set of eggs were laid five months later, the pond had been acclimated to the environment, allowing time for plants to have grown in the pond. This provided much needed protection, as mortality rates for frogs are high, consequently making secure egg deposit sites crucial (Dorcas & Gibbons, 2011). Additionally, an intentional effort was made to re-shape the structure of the supporting rocks halfway through the study. These supplemental rocks were carefully placed to mimic a protective cave-like structure, where ultimately the successful set of eggs were deposited, shown in Appendix C, photograph 2. It's believed that these variables of plant acclimation and rock formation is what caused the successful hatching of this second set of eggs, as they were well hidden from predators (Hazell et al., 2004).

Though tropical forests rarely remain stagnant, the topic of an acclimation period when new structures are introduced poses interesting explanations for the study presented here. Frogs in specific have been observed revisiting previous breeding sites and depositing eggs in the same body of water each year (Pasŭkonis et al., 2016), suggesting that habitual breeding opportunities lead to more successful broods. This is indicative that over the years, the newly constructed pond at Cloudbridge should yield continuous breeding behavior. This is especially paramount when considering the climatic impacts of rising water temperatures, as aquatic or semi-aquatic organisms like frogs could be impacted. A report on the cardiac acclimation ability of invertebrates in increased aquatic temperatures showed a significant decrease in heart function (Stillman, 2003), a phenomenon that could perhaps affect the viability of tadpoles and their food sources at the Cloudbridge study site. However, positive cases of acclimatory behavior have been demonstrated by other groups of vertebrates, such as arboreal mammals. A study conducted in Peru assessed the efficacy and acclimation period of newly constructed natural bridges over the course of a year. It was exhibited that over time, the use of the bridges increased in both quantity of individuals and variation of species (Gregory et al., 2017), representing a favorable acclimation period. While the long term behavior of the frogs is yet to be determined regarding the new pond, the results demonstrated in this Peruvian study suggest a positive relationship in the years to come.

B. Physical Frog Presence Relative to Independent Variables

The secondary variables tested in this study had no significant impact on the physical presence of the frogs in or around the pond. The phenomenon of the moon phase potentially impacting amphibian species is not a new development, however, in this study it did not show a significant effect on their presence. In a study by Bissattini et al. (2020), it was concluded that moonlight brightness, and not the lunar phase, is what has a stronger influence on amphibian presence; additionally, the effects tend to be species specific. This could help explain why the moon phase seemed relatively insignificant in this study at Cloudbridge. With a mixture of potential frog species to see at the pond, it's possible they have variable responses to the moonlight brightness, therefore maintaining a steady presence whether the moon was bright or dim. Further, the canopy cover of the forest in this study is moderately dense, which could potentially mitigate the extremeness of the moonlight as it will never be fully illuminated in the understory.

Rainfall also did not have any significant impacts on the physical presence of the frogs throughout this study period. As frog species are extremely variable in terms of water requirements (Hazell et al., 2004), it's possible that some species were more impacted than others in this study which ended in a null effect. For example, a meta-analysis by Ficetola & Maiorano (2016) focusing on the effects of temperature and precipitation changes in amphibians showed contrasting results in terms of phenology and abundance; while some species responded poorly to an increase in precipitation, other species had increased survival rates, demonstrating the variability in amphibian reactions. Because the cloud forest at Cloudbridge experiences regular precipitation during the rainy season (when this study took place), it's assumed that the amphibian population is accustomed to the temporal climate and were therefore unphased behaviorally.

C. Auditory Frog Presence Relative to Independent Variables

There was also no significant correlation between moon phase and frog vocalizations. However, frog vocalization demonstrated a prominent decrease as the moon displayed illumination above 50 percent. With frogs being a prey species, it’s possible that making noise when the moon is brighter puts them at higher risk for predation, as predator species are more active on brightly lit nights (Bissattini et al., 2020).

The significant impact rainfall had on the presence of frog vocalizations is more convoluted to explain. In one study, a controlled group of frogs were found to increase their vocal responses with increased precipitation, perhaps as a way to compete with the vibration and noise caused by the heavier rainfall (Halfwerk et al., 2016). This phenomenon was reflected in a separate study done in 2003, where moderate rainfall caused an “excitatory” effect on the frog vocalizations (Penna et al., 2005). Both of these studies illustrate opposing results to what was exhibited at Cloudbridge, perhaps due to the varying effects water has on particular species of frogs (Hazell et al., 2004). An outcome that expressed similar findings to the study at Cloudbridge was done by Lengagne & Slater (2002), where it was proposed that the reliability of acoustic information is decreased with heavier rainfall, and thus causes an animal to be outcompeted by the rain and vocalize less. Considering that the response at Cloudbridge is that the frogs vocalized significantly less after longer bouts of rainfall, it’s assumed that the noise of the rainfall surpassed the frogs' efforts.

D. Limitations

Though this report is indicative of a prosperous result, the research was not impartial to limitations. The amount of planned surveys were halved after two weeks of data collection in an attempt to reduce erosion on the land, significantly diminishing the dataset. Furthermore, a hindering factor during this research period was the presence of heavy rainfall, which rendered it impossible to complete several of the surveys, again impeding how many surveys were completed. As in most biological studies, human error is taken into consideration in terms of both visually seeing and identifying the specimens reported here. Finally, a preliminary assessment of the amphibian abundance on the study site was not completed beforehand; therefore, examining any comparisons of the abundance pre and post pond creation is unobtainable.

Conclusion

Although the majority of the results of this study showed no significance regarding the measured variables, it is still demonstrative of the main goal proposed. Witnessing breeding behavior in the pond is a favorable result, as it shows the viability of supporting amphibian populations with an artificial pond. As sensitive as these populations are to climatic changes and disease (Radchuck et al., 2019; Rödder et al., 2010), the response of the frogs using the pond to breed is a positive outlook towards future adaptations. This knowledge serves as a step in what conservation could look like in the years to come. As climate change is inevitable in the foreseeable future (Finch, 2012), it’s with hope that this study is repeated in other environments in an attempt to mitigate long-term or permanent impacts on amphibian populations both in Costa Rica and globally.

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Appendix A

Photograph of the Pond

The pond (photograph 1) immediately after it was constructed (July 2023). The color of the water can be attributed to soil disturbance, however, the water ran clear throughout the duration of this study (evident in Appendix C). This photograph does not show the alterations made to the pond in the six month research period, which include: increased plant growth inside pond; changes of plant growth overhanging the pond; changes made to the rock structures to create "caves"; placement of a camera trap.

Photograph 1: The artificial pond created at Cloudbridge Nature Reserve, Costa Rica.

Appendix B

Rain Classification

This study utilized the amount of time it rained throughout the day prior to the survey in hours versus the amount of rainfall in mL. This was mainly due to the limitation of access to accurate rainfall data, as well as the unpredictability of the rain to self-collect.

Table 1: Classification of rainfall

Hours of rainfall	0 hours	1-2 hours	3-4 hours	5-6 hours	7+ hours
Rain classification	0	1	2	3	4

Note: The rain score was dependent on how many hours it rained during the day of the survey until the survey began. This does not include any rainfall that may have occurred during the survey time.

Appendix C

Frog Specimens Discovered at the Pond

Photographs of the individual providing parental care to the eggs (photograph 1), and the deposited eggs (photograph 2), taken *in situ*. The frog species and subsequent eggs are of Warszewitsch's frog (*Lithobates warszewitschii*).

Photograph 1: *L. warszewitschii*

Photograph 2: Eggs of *L. warszewitschii*

Appendix D

Table 1: Frog species observed during the research period.

Common Name	Scientific Name	Number of Observations	Number of Individuals
Slim-fingered rain frog	*Craugastor crassidigitus*	7	9
Cerro Utyum robber frog	*Craugastor podiciferus*	1	2
Underwood's litter frog	*Craugastor underwoodi*	1	1
Emerald glass frog	*Espadarana prosoblepon*	1	1
Warszewitsch's frog	*Lithobates warszewitschii*	2	2

Appendix E

Table 1: Organisms captured on the camera trap.

Common Name	Scientific Name	Number of Events	Abundance of Individuals
Chestnut-capped Brushfinch	*Arremon brunneinucha*	3	3
Slaty-backed Nightingale Thrush	*Catharus fuscater*	1	1
Common opossum	*Didelphis marsupialis*	2	2
Tayra	*Eira barbara*	3	4
Buff-rumped Warbler	*Myiothlypis fulvicauda*	2	2
White-nosed coati	*Nasua narica*	3	5
Collared peccary	*Pecari tajacu*	3	7
Gray four-eyed opossum	*Philander opossum*	2	2
Puma	*Puma concolor*	1	1
Dice's cottontail	*Sylvilagus dicei*	8	8
Unknown rat species	*N/A*	1	1

Note: List of species that were seen on the camera trap throughout the study period. The amount of individuals counted is separated from the number of camera trap events* as multiple individuals could be within a single frame.

*A camera trap event was considered only one event if the camera was triggered multiple times within the same hour, and was considered a second unique event if triggered more than an hour apart within the same day.

$N = 11$, $D = 0.88$.