Pitfall trap research on amphibian and reptile density and diversity around artificial ponds in a Cloud forest

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Golden-spotted rain frog, Pristimantis cruentus

RESERVA CLOUDBRIDGE NATURE RESERVE

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Abstract

Over the last 40 years, amphibian populations have heavily declined, influencing reptile species as well. In this paper, a potential temporary solution in the form of human made ponds is researched. This was done with pitfall traps, altered in design to be suitable for a cloud forest environment. Low data lead to no significance in herpetofauna abundance and diversity between a pond and pondless environment (t(502)=1.003, p=.16). Other recorded animals, however, did show a significance between these locations (t(502)=-3.28, p=.00056). Mice, shrews, tarantula's and more, have a predator/prey relationship with herpetofauna, giving an indication of difference in abundance after all. *Isthmohyla pseudopuma* was observed in and around the pond in large numbers (15+), proving the usage of the handmade pond. Additionally, a pitfall trap evaluation of the updated methodology, proved useful for an area with high precipitation but proved not to work well as a method for data collection in herpetofauna.

Introduction

Since 1980, amphibian populations have severely declined, with over 120 species globally going extinct between 1980 and 2007. At La Selva biological station, data shows rates of terrestrial amphibians decreasing with approximately 75% since 1970, with reptile trends showing similar declines (Whitfield et. al, 2007).
In the Talamancan mountains as well as the rest of the world, a deadly fungus named *Batrachochytrium dendrobatidis* (Bd) was mainly responsible for the decrease in amphibian numbers (De Léon et al. 2019).

It is expected that due to climate change alone, by 2050, 15-37% of all species will be 'committed to extinction', including reptile and amphibian species (Thomas et al. 2004).

Because of this extreme decline, research on this subject is essential, to minimize and prevent further extinction as much as possible. On top of this, concrete data collection of amphibian and reptile species is very difficult due to irregular breeding cycles, quick movement and sensitivity to observer bias. In addition, decline of species makes the collection process even more difficult (Zhu et al. 2005). Thus, effective research on such a time sensitive matter is essential to boost the process of getting to know more about the species.

A "solution" to the problems of decline of herpetofauna has been to produce artificial breeding locations for anurans. Most anurans are known to breed around water and deposit eggs in or around them (Britannica, 1999). Therefore the "production" of ponds and slow moving streams should increase the number of breeding locations and lead to an increase in frog numbers. As a result of more frogs, predators of these, such as snakes as well as invertebrates, birds, mammals and other anurans (Toledo et al. 2011) will also increase overtime, possibly raising numbers of rare species which have diminished over the last years. However, research on the use of ponds in relation to herpetofauna diversity and abundance is lacking and as a possible solution to reptile and amphibian decline, these should be investigated.

On the Cloudbridge property, an artificial pond has already existed for the past 2 years. This raises an opportunity to research the effects this pond has been having on herpetofauna over the past time. This was done by comparing reptile and amphibian species and numbers around a pond, to species and numbers on a location without a pond (further referred to as pondless), helping the process of understanding how useful the handmade ponds are in biodiversity growth. Having information on reptile and amphibian abundance and diversity, can help better understand this forest and its inhabitants. This broadens current knowledge about caught species and can boost other research at another point in time. It also gives an evaluation on herpetofauna populations in this point in time (March – June, 2023), that can be looked back on and if necessary, compared to in the future.

Study objectives

- Comparing reptile and amphibian abundance and diversity in proximity to and in absence of ponds.
- Comparing reptile and amphibian abundance and diversity between naturally regenerated and primary forests.
- Determining the effectiveness and assessing methodology of pitfalls traps as a data collection process for herpetofauna

Material and methods

Study site

The location of the research is at Cloudbridge Nature Reserve in Costa Rica, located in the Talamanca mountain range. The reserve contains 283 hectares of land, ranging in altitudes from 1600 meters (5249 feet) to 2600 meters (8530 feet). Due to high variation in altitude and various reforestation programs, Cloudbridge Nature Reserve has different types of forest. It contains the following; Primary forests/old growth (70-150+ years old), naturally regenerated forests (30-70 years old) and replanted forests (<20 years old).

Cloudbridge, being a tropical montane cloud forest (TMCF), has a temperature ranging between 12.32 and 22.60 degrees Celsius and an average rainfall of ~2000 mm a year (Jarvis & Mulligan, 2011). The temperature and precipitation depend on time of year. With a heavier rainfall and warmer temperatures from May to November (rain season) and a lighter rainfall and cooler temperatures from December to April (dry season).

The pond that was researched is on one of Cloudbridge's trails, the Rio trail (figure 1). This location is part of the naturally regenerated forest and this is the location of the first trap (figure 1, point 2)(appendix A, location 2). The trap was placed around 5 meters away from the pond. The second pitfall trap location was placed in the same forest type, on the same trail but without a pond (figure 1, point 3) (appendix A, location 3). As a control group, the last pitfall trap location was placed in the old growth forest. The traps in the old growth forest were placed on the Heliconia trail (figure 1, point 1) (appendix A, location 1).

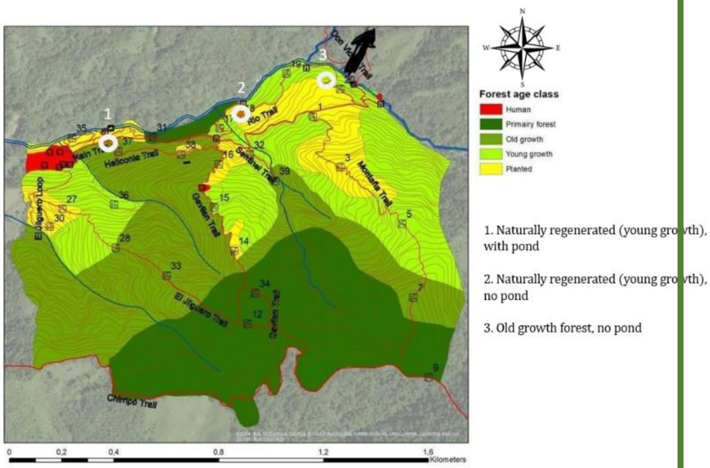


Figure 1, pitfall trap locations on Cloudbridge map

A table with coordinates, elevation, trail and forest type can be found in appendix A.

Data collection

Every trap location contained 4 buckets placed around an ~8 meter tarp that served as a drift fence to lead the herpetofauna into the buckets (figure 2). The tarp was made of green plastic and blended in with the colors of the forest. Plastic buckets (26.15 liters) were dug into the ground completely, leaving only the opening of the bucket exposed. The inside of the bucket contained a layer of natural materials to increase survival chances of the animals falling in (figure 3). The lid on top of the bucket was installed to prevent dead leaves, plant matter rain water and predators to enter the pitfall traps while open. The buckets additionally contained a smaller lid which was put on when data collection was paused.

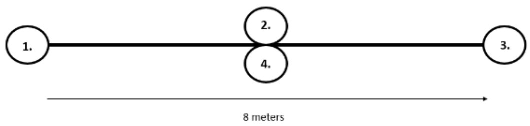


Figure 2, pitfall trap location design

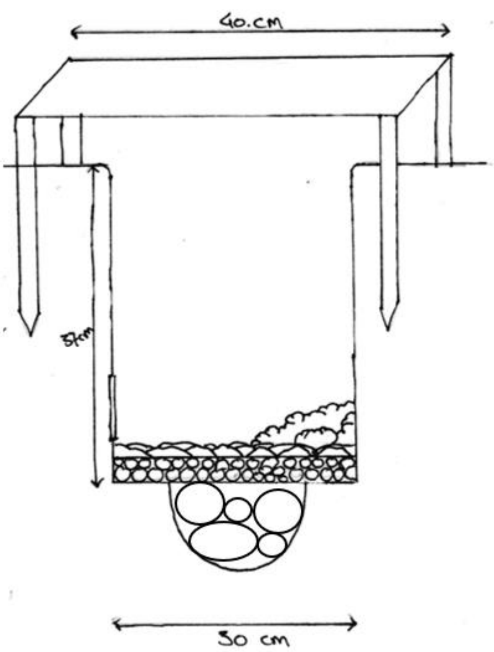


Figure 3, pitfall trap design

The inside of the pitfall trap consists of the following 3 layers:

- Moss was added to keep humidity on a level and provide hydration for water dependent species.
- Leaf matter served as hiding places for species to reduce stress while being in the trap.
- Tiny stones were added as a drainage layer for rainwater to pass through and avoid buckets filling up with water and causing caught animals to drown. The amount of stones was enough, so the bottom of the bucket was not visible anymore.

The bottom of the bucket contains holes big enough for water to escape but tiny enough to keep all animals in.

On the bottom of the bucket, a second, smaller hole, was dug and filled with rocks. This creates air holes that facilitate water drainage into the soil and further minimizes chances of drowning.

Each location was checked twice daily, in the morning between 07:00 and 09:00, and in the afternoon between 16:00 and 18:00. Data was collected between March 15th and June 8th 2023, during weekends traps were closed. During data collection, trapped animals were carefully taken out using plastic bags, gloved hands or leaves and branches, depending on the animal. The animals were photographed, identified, noted and released 50 meters from the trap. Insects and some arachnids, which were caught most of the time were not noted and were not analyzed. Mammals (mostly rodents), crustaceans, and tarantula's were noted as these animals have a predator or prey relationship with herpetofauna and can be an indication of herpetofauna presence or absence.

Ethics

Though the negatives of this methodology are arguably significant, the minimization of these have been a priority in the research. The main risks were amphibian, reptile and rodent death after being caught in the pitfall traps. As the animals were not stuck in the buckets for longer than 15 hours each time, the risk of starvation was not probable. Interactions of prey and predator species being stuck in the same bucket and causing death of prey species is inevitable. This was kept to a minimum by regularly checking the traps and installing lids on top of the traps so the caught animals were not visible from above. Animals drowning in the traps from heavy rainfall was also minimized in the design of the traps. Holes in the bottom of the bucket allowed water to flow out without letting animals escape.

Though animal death occurred, collected data from the pitfall traps is not limited to this research. Data of amphibian, reptile and even rodent presence in the Cloudbridge reserve can open up many possibilities for other researchers looking to know more about diversity.

Data analysis

Abundance

An independent t-test was done, to find significance of caught herpetofauna between each combination of locations (OG - NR-P, OG - NR-PL, NR-P – NR-PL). This test will compare the means of two locations, which are unrelated to each other. To find significance in abundance, the catches from each species, is unimportant. Only number of herpetofauna caught is of importance.

Diversity

To measure diversity of herpetofauna on each location the Simpson's Diversity Index was used. This was then applied to all three locations simultaneously to learn about the overall diversity among all locations.

Pitfall trap evaluation

The success rate of the pitfall traps was calculated with the following formula;

(amount of times a bucket could have caught something/total number of animals caught) x 100%.

Results

Over the course of 3 months of data collection, 12 traps have been open during 30 days and 33 nights. A total amount of 51 individuals were caught in the traps. Ten of these consisted of herpetofaunal species. two (snakes), five (anoles), two (frogs) and one (salamander)(appendix C1). Additionally a total of 21 rodents, 10 crabs and 10 tarantulas were caught (appendix C2).

Abundance

There was no significant influence for location, $t(502)=1.003$, $p=.16$, on the abundance of herpetofauna between the pond (NR-P) ($M=0.012$, $SD=0.108$ and the pondless (NR-PL) ($M=0.004$, $SD=0.063$) environment.

There was no significant influence for location, $t(502)=1.008$, $p=.16$, on the abundance of herpetofauna between the pond (NR-P) ($M=0.012$, $SD=0.108$ and the old growth (OG) ($M=0.024$, $SD=0.152$) environment.

There was no significant influence for location, $t(502)=1.003$, $p=.16$, on the abundance of herpetofauna the old growth (OG) ($M=0.024$, $SD=0.152$) and the pondless (NR-PL) ($M=0.004$, $SD=0.063$) environment.

Diversity

The Simpson's Diversity Index (SDI) of herpetofaunal catches per location is as follows;

Location	N	n(n-1)	SDI
OG	6	4	0.87
NR-P	3	0	1
NR-PL	1	0	1
All locations	10	36	0.6

Figure 4, Simpson's Diversity Index of herpetofauna diversity in each forest

Pitfall trap evaluation

Out of the 756 times that a bucket could have caught something, herpetofauna was caught 10 times, other animals were caught 41 times. A total of 51 noted animals have fallen in. The success rate of the pitfall traps for herpetofauna was 1.32%, for other animals this percentage was 5.42%. This gives a total success rate of 6.75%. This is further seen in appendix D.

Conclusion and discussion

According to the results, there is no significant difference in herpetofauna abundance based on the presence of the pond.

As seen before, there is a significant lack of data in this research. Especially in a t-test where abundance is researched, the results are not representative.

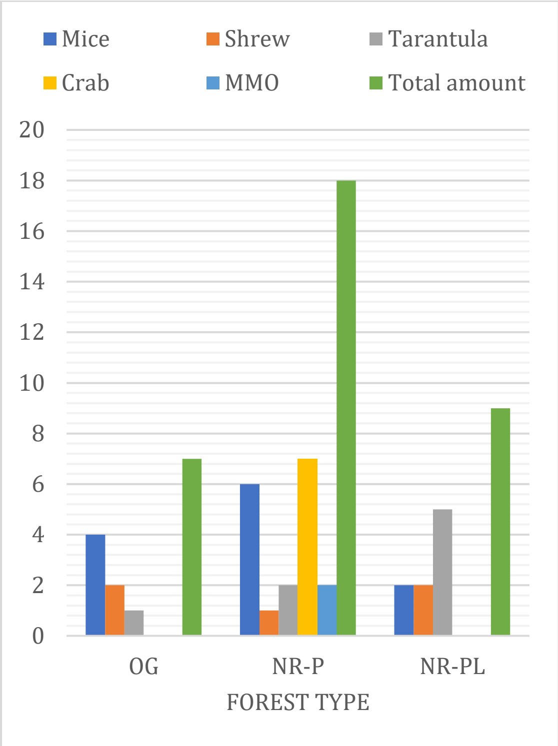
Simpson's diversity index shows that there is a relatively high diversity per location. Low data can be the cause of this strong diversity index. On the locations NR-P and NR-PL, of each species found, only one individual was caught. This translates into a perfect diversity but is not representative. The diversity among all locations is 0.6. This relatively high diversity is also not representative.

Though not many herpetofaunal species were found, the other animals that were noted, can give an indication of reptile and amphibian presence.

Anurans, for example, are known to be preyed on by tarantulas, crabs, other spiders, ants, horseflies and more (Luis & Toledo, 2023). Though not all recorded, all of the named species have been observed around the pitfall trap near the pond, in higher numbers than in the other environments (figure 5). Tarantulas and crab presence, being recorded, can be an indication of anurans inhabiting these environments. Snakes, besides rodents, are also known to predate on anurans (Toledo et al. 2011). Higher presence of anurans as prey animals can be correlated with a higher number of snakes in the environment of the pond.

Significant difference in abundance of other animals was observed among the three locations. The independent t-test shows a significant influence of location on "other animal" abundance between a pond and pondless environment. A significant influence was also found between the traps near the pond and old growth forest. This significance of other animals in the different forest types can be made more accurate with more data.

In the case of more other animals and a true significance, this still does not necessarily prove herpetofaunal species presence but does give an indication.



Figurre 5, Number of non herpetofaunal species caught in pitfall traps. Mexican mouse opossum abbreviated by MMO

There was a significant influence of location, t(502)=-3.28, p=.00056, on the abundance of other animals in the pond (NR-P) (M=0.040, SD=0.063) and the pondless (NR-PL) (M=0.052, SD=0.221) environment.

There was a significant influence of location, t(502)=2.14, p=.016 on the abundance of other animals the old growth (OG) (M=0.028, SD=0.000) and the pond (NR-P) (M=0.004, SD=0.063) environment.

There was no significant influence of location, t(502)=1.37, p=.086, on the abundance of other animals the old growth (OG) (M=0.028, SD=0.000) and the pondless (NR-PL) (M=0.052, SD=0.221) environment.

Additionally, during data collection, the researched environment was seen firsthand. On April 18th 2023, over 15 individuals of the meadow tree frog, *Isthmohyla pseudopuma*, were seen in and around the researched pond. *I. pseudopuma*, being a relatively rare frog in the reserve, was a valuable find. *I. pseudopuma* are known for breeding in both temporal and permanent ponds (Stamper, n.d.). The pond was most likely being used as breeding grounds for the species, since being observed multiple nights after the first find. With the pitfall trap location placed 5 meters away from the pond, none have ever been recorded inside the trap. This could be because of the fact that the (relatively large) frogs are capable of escaping the traps. Regardless, the usage of the pond, though not significant, observations proves it to be a suitable environment for *I. pseudopuma*.

Frogs jumping out of the trap before the arrival of the researcher, may have happened more frequently. This is difficult to determine in this research because apart from data collection moments, there was no recording of the traps. Tree frogs have adhesive mechanisms with which they can stick to flat surfaces (Drotlef et. al., 2013). Many frogs that live slightly or far above the ground have a similar function that could enable them to escape from the traps. Capability of leaving the trap is also the case for large snakes, who are long enough to go inside the trap and get out with ease.

Pitfall trap evaluation

During the research, one of the main concerns was keeping rainwater out of the traps as much as possible to prevent caught animals from drowning. This was done by creating a second, smaller hole underneath the bucket in the soil. This hole was filled with 10 to15 bigger rocks that provided air pockets underneath the bucket for better drainage. Additionally, the bottom of the bucket contained tiny stones to prevent soil and leaf litter to enter and plug the holes that were drilled in. This slight change in pitfall trap designed worked throughout the entire data collection period, with one exception. On June 5th 2023, almost three months after the start of data collection, bucket 2 and 4 of the old growth forest trap, flooded with water. No animals, besides insects, were found dead in these buckets but as a measure, bucket 4 was shut for the remainder of the data collection. The reason for this was most likely a problem with the big rocks underneath the bucket which had let through too much soil and reduced the drainage. All the other buckets remained dry throughout the entire research period.

Two maintenance moments were necessary. Soil that had fallen inside of the buckets was removed to prevent plugging of the holes and reducing the effects of water drainage.

Another important part of the pitfall traps had been creating table like lids, hanging ~30 centimeters above the trap opening. This prevented falling leaves and plant material from falling in the trap and creating a bridge for the animals to escape. This method also prevented predators from seeing the inside of the trap from above and being able to catch what had fallen inside. Though this was not consistently monitored, no sign of predator interaction was recorded (s.a. bones, body remains or blood).

However, ground dwelling predators, mainly insects and parasites which had most likely fallen into the trap as well, took advantage of larger animals that had fallen in. The inability of escape for these animals, putting them in a weaker state, made for an easy prey. On three recorded occasions, this had happened. A parasite filled mouse, which suspectedly had already been infected was taken out of one of the traps. The other 2 occasions were a partly consumed pacific ground anole and a slender anole in a similar situation, which had been eaten by insects from the inside.

Other death in the traps could possibly have been a result of insects and parasite consommation, fatigue, lack of food or stress (figure 7).

A total of 7 deaths occurred during data collection, 2 of which were herpetofauna. This translates to a 13.7% death rate of all caught and recorded animals and a 20% death rate of herpetofauna. This can be caused by the low numbers of caught animals as well.

Species	Occasion
Anolis limifrons	1
Anolis marsupialis	1
Aphonopelma crinirufum	1
Mouse (various species)	4
Grand total	7

Figure 7, Table depicting death count of all animals fallen into the trap

Recommendations

The sensibility of observer bias is relatively small since most measures are absolute. The identification of the species can be debatable. In the case of doubt, the animal will be photographed and properly analyzed when returned to the base camp.

When reproducing this or doing a similar a reptile and amphibian research, pitfall traps are not recommended. The hard work in data collection is returned with low results. When using pitfall traps for herpetofauna, extending the data collection period is recommended. Regardless, this research takes a lot of dedication and effort.

If the data collection is meant for a more broad and frequent species composition, such as rodents, insects, arachnids. The pitfall traps give higher data numbers and a more valuable research can be done.

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Appendix

Appendix A

Coördinates and elevation of pitfall trap locations

Site	Forest type	Abbreviation	Trail	Laditude	Longitude	Elevation (meters)
1	Old growth	OG	Helliconia	09°28.341'N	083°34.552'W	1600
2	Naturally regenrated - pond	NR-P	Rio	09°28.418'N	083°34.280'W	1709
3	Naturally regenerated - pondless	NR-PL	Rio	09°28.468'N	083°34.113'W	1711

Appendix B

Image of one of the pitfall trap location in the field

<u>Appendix C</u>

C1; Herpetofauna caught in pitfall traps per location

Herpetofauna caught				
Row Labels	**NR-P**	**NR-PL**	**OG**	**Grand Total**
Anolis limifrons			2	2
Anolis marsupialis			1	1
Anolis pachybus			2	2
Craugastor crassidigitus	1			1
Geophis hoffmanni	1		1	2
Oedipina uniformis	1			1
Prismantis ridens		1		1
Grand Total	**3**	**1**	**6**	**10**

C2; Other animals caught in pitfall traps per location

Other animals caught				
	NR-P	**NR-PL**	**OG**	**Grand Total**
Crab	10			10
Mexican mouse opossum	2			2
Mouse	6	4	4	14
Shrew	1	2	2	5
Tarantula	2	7	1	10
Grand Total	**21**	**13**	**7**	**41**

Appendix D

Success rate of the pitfall traps for each species group

Day/Night	Succes rate (Herpetofauna caught per open bucket)	Succes rate (Other animals caught per open bucket)	Succes rate (Total animals caught per open bucket)
Night	1.01%	8.59%	9.60%
Day	1.67%	1.94%	3.61%
Day and Night	1.32%	5.42%	6.75%