Strawberry poison dart frog breeding habitat

A study on Oophaga pumilio and their tadpole rearing sites

Fabian Helsloot

January 12th, 2017, Barra del Colorado, Costa Rica

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Cover photo: *Oophaga pumilio 'Tortuguero'* and her tadpole – Fabian Helsloot, October 2016.

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COTERC - Canadian Organization for Tropical Education and Rainforest Conservation, Pickering Ontario

This study was conducted as part of an internship by a third year student, following an Applied Biology Bachelor course at the HAS University of Applied Sciences in the city of 's-Hertogenbosch, the Netherlands. The study was conducted at the Canadian Organization for Tropical Education and Rainforest Conservation (COTERC), at Caño Palma Biological Station, Costa Rica.

Abstract

Oophaga pumilio is a dentrobatid frog which occurs from north-western Panama throughout eastern Costa Rica and south-eastern Nicaragua. This highly colorful frog is charismatic and can be an ecotourism attraction. Literature suggests that by adding bromeliads, a commonly known tadpole rearing site for dentrobatids into an area known to be *O. pumilio* habitat, the population size will increase. This lead to the hypothesis that a population of *O. pumilio* within a certain area would be larger a with a higher number of bromeliads and species in an area with lower amount and diversity. In total ten plots were sampled to prove this hypothesis separated around two areas, Caño Palma and El Cerro Tortuguero. These ten plots were surveyed to compare differences in population sizes per location and per plot and to compare these population sizes with the amounts and different types of tadpole rearing sites. To calculate the population sizes of *O. pumilio,* the photo variant of the repeat capture mark recapture methodology was used, to be as least invasive to the frogs. It was noted down when a frog was caught from a rearing site or any other type of substrate, to get an idea of the choices for substrates within this frog species.. The *O. pumilio* populations within the plots around El Cerro Tortuguero were significantly bigger than the populations around Caño Palma. No correlation between the number or species of bromeliads was found with the *O. pumilio* around the Tortuguero area. A new type of tadpole rearing site has been discovered, the *Calathea gymnocarpa*. Like bromeliads this plant species has water filled axils, which can be used as a tadpole rearing site by *O. pumilio.* The numbers of *C. gymnocarpa* correlates with the population sizes of *O. pumilio* per plot and of all the tadpole rearing sites where frogs have been encountered, most were encountered on this plant species This might indicate that this plant species is a way of increasing the population of these frogs within a certain area. This can be of value for the local management authority (MINAE) in management decisions concerning the population sizes of this frog species.

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1. Introduction

Worldwide amphibians are experiencing decreases in their populations (Chanson J. C. et. al., 2004) Some of these problems are caused by habitat fragmentation(Cushman S. A., 2015) or diseases such as *Chytridiomycosis, Ranavirus* and *Batrachochytrium dendrobatidis* (Bell., et. al., 2006; Chacon et. al., 2013; Green et. al. 2003). For *Oophaga pumilio,* the Costa Rican populations are still stable (IUCN, 2015). This brightly colored frog can be found in Panama, Nicaragua and Costa Rica (Frost, 2014). The *O. pumilio* is known for its widespread variation in coloration, boasting 15 to 30 color morphs in some locations (Summers, 2003). The strawberry poison dart frog is well studied for the alkaloids in its body (Torres-Mendoza, 2013) and the parental breeding care of its tadpoles (Strynoski, 2012), but less is known about the specific habitat requirements of this frog.

However, it is known that *O. pumilio* depends on bromeliad species (*Bromeliaceae*) and leaf litter in its life cycle. The female frog lays her eggs on leaf litter and the male guards these clutches for approximately one week. When hatched the tadpoles will be brought to small terrestrial water pools such as the water filled axils of bromeliads. For about six weeks the female keeps visiting its offspring to feed them with unfertilized eggs. These eggs are the principal source of food for the tadpoles (Strynoski, 2012). Previous studies have looked at resource supplementation by adding leaf litter and bromeliads to various plots in Costa Rica, and showed that adding bromeliads into an area increases the population of *O. pumilio* (Donnelly, 1989). However, the addition of leaf litter in the same study did not have a significant effect on the population size (Donnelly, 1989).

Habitat destruction and fragmentation can be a problem for arboreal species (Turner, 1996). Deforestation is an ongoing problem in Costa Rica, as illegal logging occurs in some areas (Miller, 2011). For the *O. pumilio*, removal of bromeliads in particular could have a negative impact on population sizes. Bromeliads also serve as critical habitat for the larvae of some mosquito species, such as *Aedes aegypti,* which can be a vector of malaria, yellow fever and dengue (Schaperi, 1999). As such, there may be motivation for inhabitants of arboreal areas to remove bromeliads from canopies close to human habitations.

Because *O. pumilio* are charismatic species, they are often a subject of ecotourism related activities, and as such there is a great incentive for managers of protected areas open to ecotourism to monitor their population sizes. Studies on habitat requirements of *O. pumilio* are thus of utmost importance to managers interested in maintaining *O. pumilio* as a long-term sustainable ecotourism attraction. In the Tortuguero region of Costa Rica the only local high elevation habitat, el Cerro Tortuguero, has been re-opened to eco-tourism on December 19th, 2016 (SINAC, 2016). In the interest of increasing visible wildlife for tourists to witness in their natural environment, the local management authority (MINAE) has reached out to local researchers in order to determine the most responsible way to increase the population of *O. pumilio* on the Cerro.

The purpose of this study was to examine and compare specific tadpole rearing site preferences of *O. pumilio* populations, in order to best inform management authorities on how to responsibly manage population sizes of *O. pumilio* in Barra del Colorado National Wildlife Refuge. Bromeliads have been shown to have a generally positive impact on the population size of *O. pumilio* (Donnelley, 1989). By comparing populations of *O*. *pumilio* based on the amount and types of bromeliads around Cano Palma Biological Station, and El Cerro Tortuguero it was possible to identify and test differences between populations due to the presence of this plant species and other rearing sites.

2. Materials and Methods

2.1. Locations of Sampling

Two main locations were monitored to find differences in frog populations, species and number of bromeliads and other rearing sites. This took place around two main locations: Caño Palma and el Cerro Tortuguero, these areas are both located in the Barra Del Colorado wildlife refuge in the province of Limón, Costa Rica. The plots around Caño Palma labeled with A and around the Cerro plots were labeled with B. The two location were divided by the Caño Palma (canal) and were separated by approximately 750 meters. El Cerro is an old small volcanic cone 119 meters above sea level (SINAC, 2016), this area of approximately 25 hectares is located about 6 kilometers north of the Tortuguero Village. El Cerro Tortuguero and Caño Palma are both wet neotropical lowland rainforest habitat. Around each location, five plots were set up on different locations with a size of 15 by 15 meters (figure 2.1.), All coordinates of the plot coordinates were noted down. (Appendix 1.).

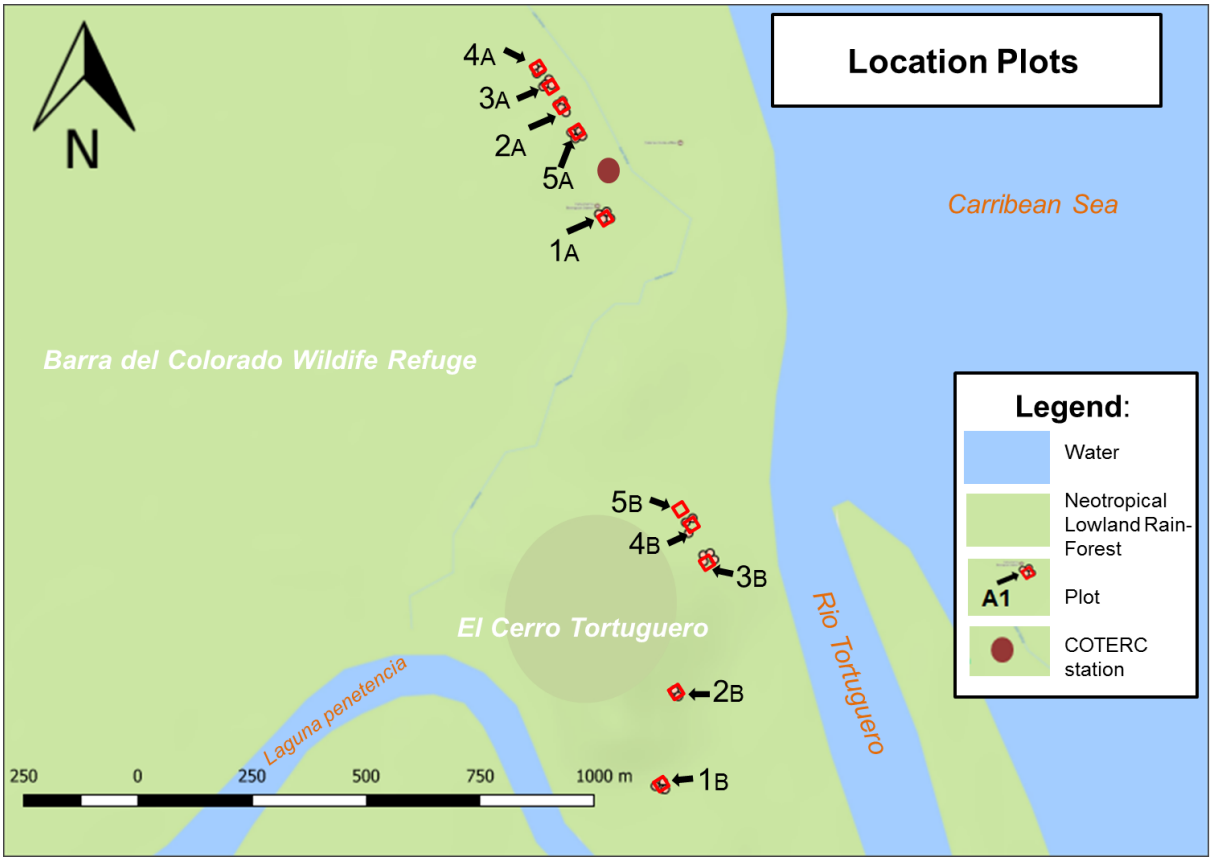

Figure 2.1., Locations of all the plots around the Caño Palma and El Cerro Tortuguero area.

2.2. Data Collection

Data inside the plots were collected in two runs: one for the capture and identification of the frogs, according the methods described in 2.2.1, and all the labeled possible rearing sites within the plots were checked following protocol (2.2.2). A zig-zag pattern was walked in every plot so that every square meter was searched for presence of *O. pumilio*. Every plot survey started in the north west plot corner and it ended at every south-east plot corner. The gender of the frogs was determined by throat coloration and calling. Males have a dark throat

and are the only ones able to call. Juveniles were distinguished by a snout to vent length (SVL) smaller than 20 millimeters (Donnelly,1989).

2.2.1. Capturing and photographing Dart frogs

A photo identification method variant of the capture and recapture methodology was used to get insight in *O. pumilio* population sizes inside the 5 different plots on each location. Every caught frog was photographed dorsal, ventral and both lateral sides (Appendix 3.). These photos were used to recognize individual frogs within the plots and count the amount of recaptures later. A photo box (Appendix 2.) was constructed so photographing of the frogs could take place when it was raining. On top of the photo box there was a waterproof light to improve the photo quality in dark forest floor conditions. The frogs were captured by hand, and a latex glove was mandatory due to the toxins that can be released from the skin of the frogs. After a frog was processed it was put back in the exact same place where it was caught. Locations of where the frogs were encountered before they were caught were written down in one of the categories (Figure 2.2.). If a frog was encountered on a possible rearing site this was written down in following categories (Figure 2.3.) Plastic 0,01L cups were found in in plot 2A, these were found attached on a tree as a leftover from former frog research. Each plot was sampled for a minimum of 30 minutes to standardize effort.

2.2.2 Collecting tadpole rearing site data

As very few bromeliads were found within the plots around both areas, other possible rearing sites were tested for their ability to hold water, and when able counted in addition to bromeliads so that they could be monitored as well for any possible rearing activity. These potential tadpole rearing sites were labeled with flagging tape and checked every time for the presence of frogs within 30 centimeters, amount of eggs and amount of tadpoles. The types of rearing sites were in the following categories (Figure 2.3.). Different types of rearing sites were compared to get insight in what type of rearing sited was used most.

Location of the Frog	Abbreviation
Leaf Litter	**LL**
Log	**LO**
Tree Trunk	**TT**
Tree Root	**TR**
Plant*	**PL**
Calathea gymnocarpa	**CG**
Bromeliad sp.	**BR**
Cup	**CU**
Branch	**BR**
Palm	**PA**
Tree Puddle**	**TP**
**Random plant species in the undergrowth* ***Natural occurring puddle in a tree*	

Figure 2.2. Location classes on what substrate frogs were encountered.

Type of Rearing site	Abbreviation
Guzmania monostachia	**GM**
Aechmea diclamydea	**AD**
Bromelia pinguin	**BP**
Werauhia gladioliflora	**WG**
Aechmea mexicana	**AM**
Cup**	**CU**
Tree Puddle**	**TP**
Calathea gymnocarpa	**CG**
**Natural occurring puddle in a tree*	

Figure 2.3. Different types of tadpole rearing sites.

3.3. Data processing and statistics

All frog photos were sorted and placed in different folders, so that each individual frog got its own folder to keep track of the amount of recaptures. In each folder subfolders were made, these were named with the dates of when this individual frog was caught. The population sizes per plot were calculated with the capture and recapture methodology (Appendix 4.).

IBM SPSS 22 was used as statistics program. All significance was based on a 95% confidence interval, indicated by an alpha (α) value of less than 0.05.

Descriptive statistics were used to compare the *O. pumilio* population sizes between the plots and Caño Palma and El Cerro. A T-test was used to find a difference in the populations of *O. pumilio* per main location. A linear regression was used to find associations between the numbers and types of total rearing sites per plot and the populations of *O. pumilio per* plot. This test was also used to find a correlation between the amount of bromeliads per plot and the amount of frogs per plot.

A linear regression was used to find associations between the populations of *O. pumilio* and the number of bromeliads and other rearing sites per plot. The most preferred rearing site in general was tested by comparing the amount of frogs to this specific type of rearing site by using a one way ANOVA. The favorite substrate of this frog to be located was found with descriptive statistics. The same test was used to analyze whether there were differences between the number of frogs found on different types of rearing sites.

3. Results

3.1. *O. pumilio* populations sizes of Caño Palma and the Cerro

A significant (P=0,0015<α0,05) difference has been found in the mean population size of *O. pumilio* per plot between the locations of Caño Palma and El Cerro Tortuguero.

The population size of *O. pumilio* within all five plots around Caño Palma was 143 frogs. On the Cerro this number was 340 (Figure 3.1.1.). Within all five plots around Caño Palma five rearing sites were found in total, and around the Cerro 87 rearing sites have been found (Figure 3.1.1.)

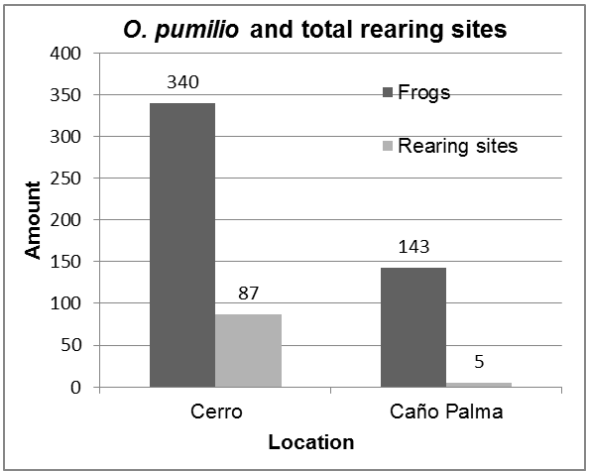

Figure 3.1.1. *O .pumilio* populations and rearing sites around Caño Palma and El Cerro Tortuguero.

3.2. the number of rearing sites and *O.pumilio* populations within the plots per main location

An association (P=0,002<α0,05) has been found between the mean population size of *O. pumilio* and the amounts of tadpole rearing sites per plot around the locations of Caño Palma and El Cerro Tortuguero.

Around Caño Palma, plot 5A has the largest *O. pumilio* population with 40 frogs, followed by plot 4A and 2A (Figure 3.2.1.). Around El Cerro Tortuguero, plot 3B has the highest *O. pumilio* population with 91 frogs followed by plot 1B and 4B with both 71 frogs (Figure 3.2.2.).

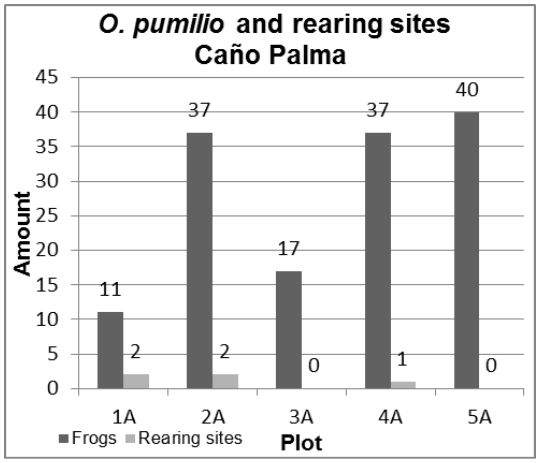

Figure 3.2.1. *O. pumilio* populations and rearing sites per plot around Caño Palma.

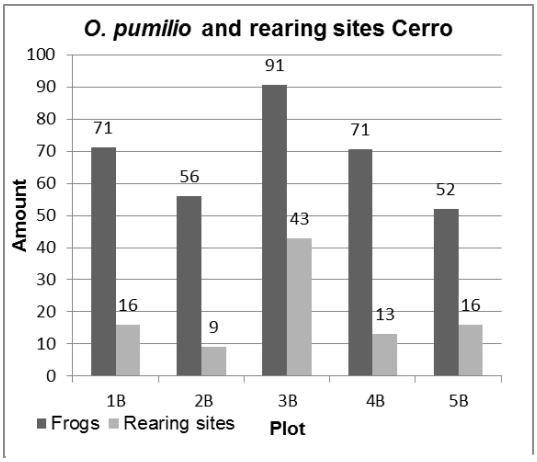

Figure 3.2.2. *O. pumilio* population and rearing sites per plot around El Cerro Tortuguero.

3.3. Different types of tadpole rearing sites on both locations

An association (P=0,001<α0,05) has been found between the mean population size of *O. pumilio* and the amounts of Calathea gymnocarpa per plot around both locations. No C. gymnocarpa has been found around Caño Palma.

Around Caño Palma three different types of tadpole rearing sites have been found (Figure 3.3.1.). Four different types of tadpole rearing sites have been found around El Cerro Tortuguero (Figure 3.3.2.).

3.3.1. Different types of tadpole rearing sites in Caño Palma

No significant (P=0,752>α0,05) association has been found in the mean population size of *O. pumilio* and the amounts of tadpole rearing sites per plot around Caño Palma.

The bromeliad species *Aechmea diclamydea* has been found two times in plot 1A and two cups were found in plot 2A(Figure 3.3.1.). One bromeliad of *Guzmania monostachia* species was found in plot 4A (Figure 3.3.1.).

3.3.2. Different types of tadpole rearing sites on El Cerro

No significant (P=0,081>α0,05) association has been found between the mean population size of *O. pumilio* and the amounts of tadpole rearing sites per plot around El Cerro Tortuguero. No significant (P=0,094< α0,05) association has been found between the mean population size of *O .pumilio* and the amounts of *C. gymnocarpa* per plot around El Cerro Tortuguero

Around El Cerro plot 3B had 33 *Calathea gymnocarpa* and ten *Aechmea diclamydea*. Plot 1B and 5B had both sixteen *C. gymnocarpa.* The only plot with a tree puddle and *Bromelia pinguin* was plot 2B (Figure 3.3.2.).

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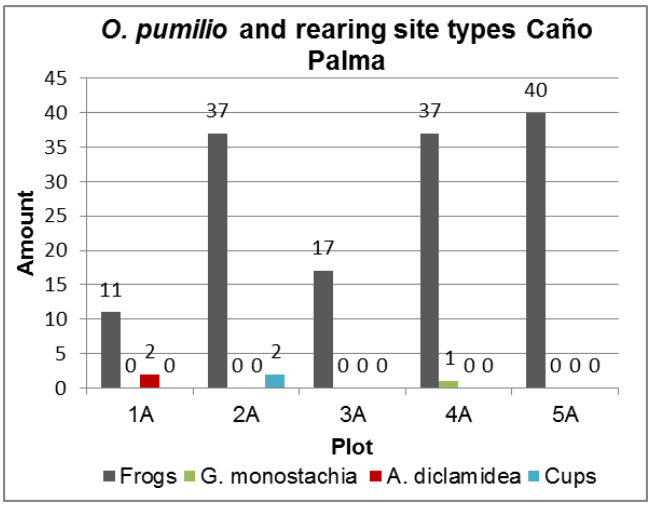

Figure 3.3.1 *O. pumilio* populations and different types of rearing sites per plot around Caño Palma.

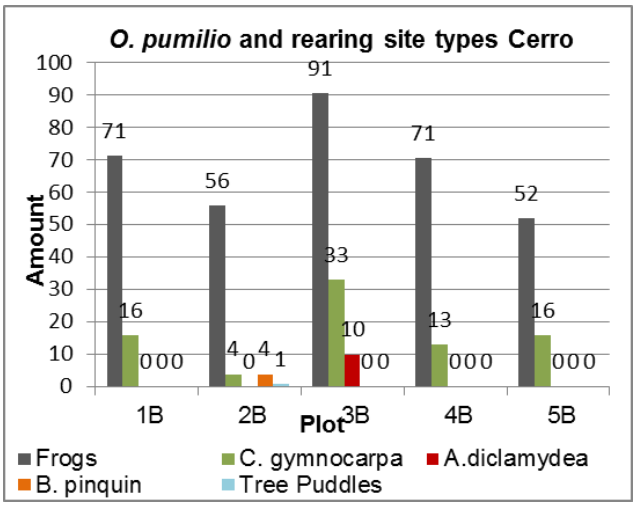

Figure 3.3.2 *O. pumilio* populations and different types of rearing sites per plot around El Cerro Tortuguero.

3.3.3. Number of bromeliads and population sizes of *O. pumilio* within the plots

No association (P=0,131>α0,05) has been found in the mean population size of *O. pumilio* and the amounts of bromeliads per plot on both locations.

Around Caño Palma the only plots which contained bromeliads were plot 1A with two bromeliads, and plot 4A with one bromeliad(Figure 3.3.3.). Plot 3B contained ten bromeliads and plot 2B contained four bromeliads, these two plots were the only plots around El Cerro Tortuguero with bromeliads(Figure 3.3.4.).

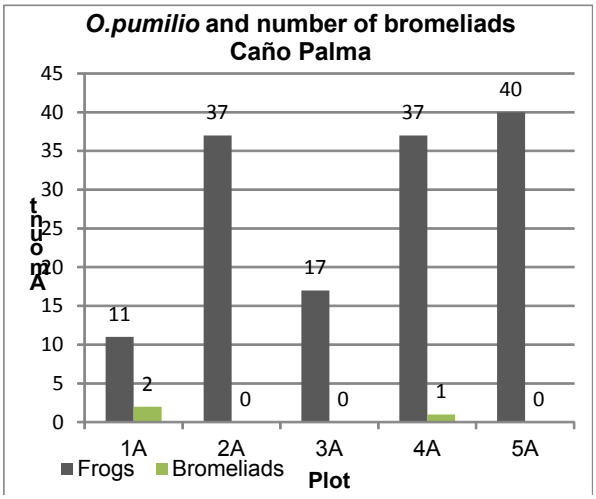

Figure 3.3.3. *O. pumilio* populations and numbers of bromeliads per plot around Caño Palma.

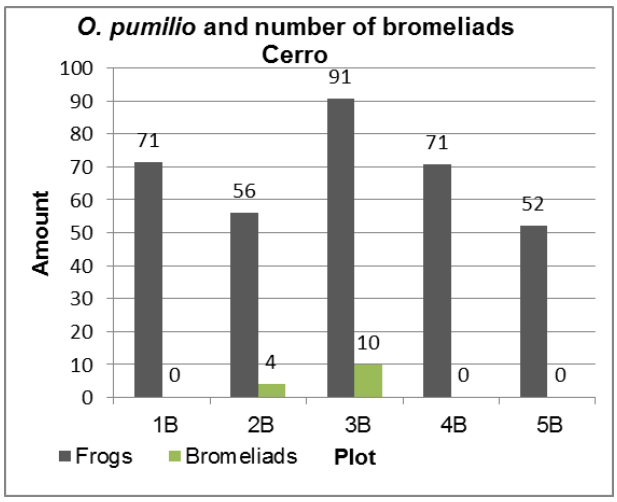

Figure 3.3.4. *O. pumilio* populations and numbers of bromeliads per plot around El Cerro Tortuguero.

3.3.4. *Bromeliaceae* species and *O. pumilio* populations within the plots

Two different bromeliad species were found in the plots around Caño Palma, *Aechmea diclamydea* in plot 1A and *Guzmania monostachia* in plot 4A (Figure 3.3.5.). Around El Cerro Tortuguero also two species of bromeliads were found, *Aechmea diclamydea* in plot 3A and *Bromelia pinguin* in plot 2B (Figure 3.3.6.).

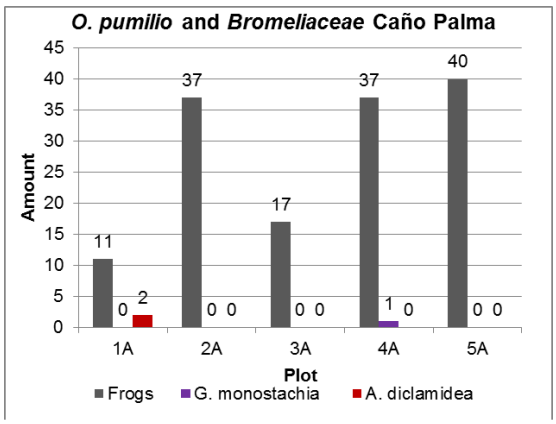

Figure 3.3.5 *O. pumilio* populations and *Bromeliaceae* per plot around Caño Palma.

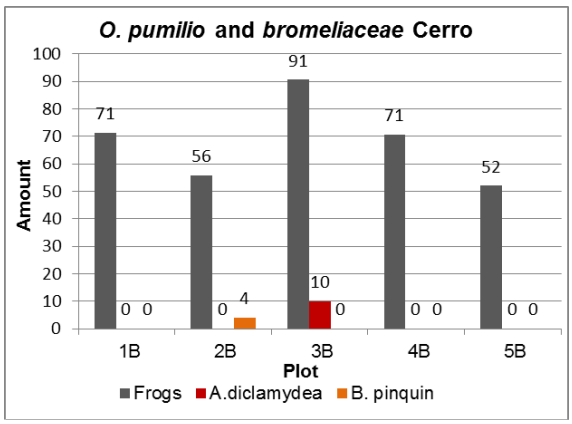

Figure 3.3.6. *O. pumilio* populations and *Bromeliaceae* per plot around El Cerro Tortuguero.

3.4. Encounters of *O. pumilio* on different rearing sites around both locations.

No significant differences (P=0,564>α0,05) have been found between the numbers of frogs and encounters on the different types of rearing sites.

Of the frogs that were caught on a possible rearing sites, 68,3 percent was located by *C. gymnocarpa.* A percentage of 22,1 percent of the frogs of frogs that were caught on a rearing site were captured on cups. A percentage of 8,7 was captures on a bromeliad (Figure 3.4.).

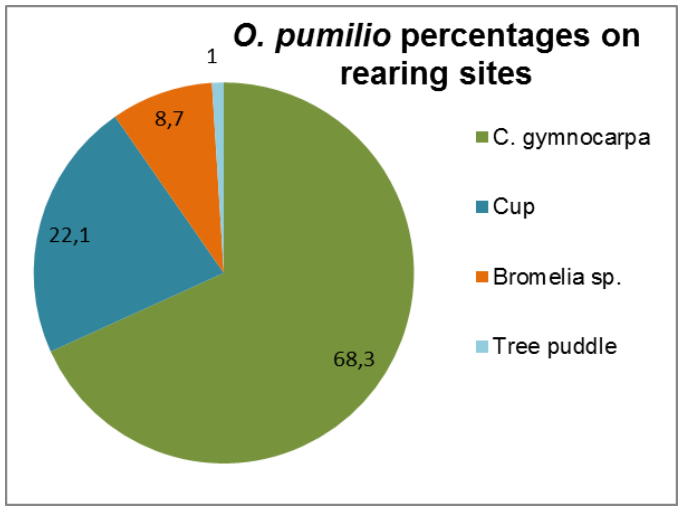

Figure 3.4. Percentages of the encounters of *O. pumilio* on different types of tadpole rearing sites in all the plots on both locations. N= 104

3.5. Encounters of *O. pumilio* on different substrates.

Significant differences (P=0,000<α0,05) have been found between the different types of substrates and the number of frogs who were first encountered on these. The encounters on leaf litter differed significant (P=0,000<α0,05) with the encounters on logs. The encounters with logs differed just significant (P=0,049<α0,05) with the encounters on trees. The smaller fractions did not show a significant difference (P> α 0,05).

From the frogs that were caught on different substrates, 42,6 percent was caught on leaf litter. Twenty percent of the caught frogs were first encountered on a log and a percentage of 14.4 of the captured frogs were first encountered on a tree trunk. A percentage of 8,6 percent was first encountered on *C. gymnocarpa* (Figure 3.5.).

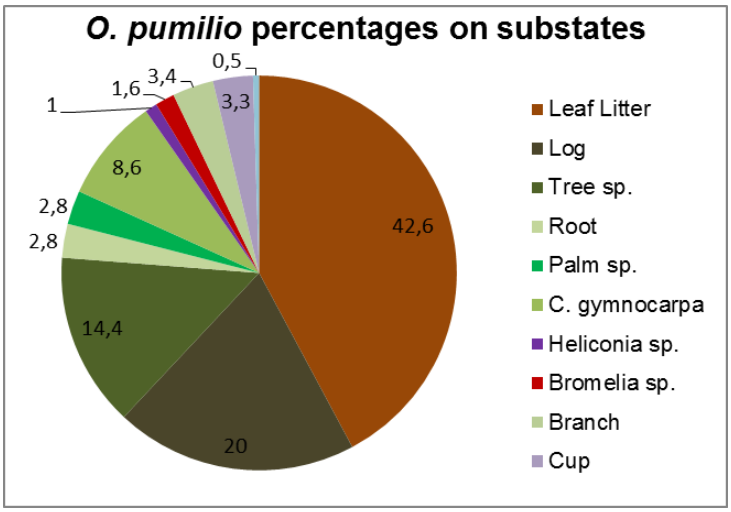

Figure 3.5. Percentages of *O. pumilio* encounters on different types of substrates in all the plots on both locations. N= 828

4. Discussion and Conclusion

4.1. *O. pumilio* population and number of rearing sites per location

In plots with the highest number of rearing sites, the *O. pumilio* population was the biggest. This suggest that the population size of the *O. pumilio* per plot depends on the total number of rearing sites per plot, as also was found by Donnelly who added rearing sites to plots (Donnelly, 1989). A association has been found between the number of tadpole rearing sites and the *O. pumilio* population. With a total population of 340 frogs and 87 rearing sites within all five plots around El Cerro and 143 frogs and five rearing sites within al five plots around Caño Palma, the *O. pumilio* populations per plot were significantly bigger around El Cerro Tortuguero. In another study around La Selva Biological station in Costa Rica, adding bromeliads as type of rearing site also made the *O. pumilio* population bigger (Donnelley M. A.,1989).

4.1.1. Different types of rearing sites and *Calathea gymnocarpa*

More types of rearing sites were found within the plots around El Cerro then in the plots around Caño Palma and one type stood out, the plant species of *Gymnocarpa Calathea* (Gargullo M. B, 2008) a member of the Arrowroots (*Marantaceae*). This plant species has water filled axils, which can be of tadpole rearing site purpose for the *O. pumilio*. No studies have found yet that *O. pumilio* uses this plant species as a rearing site, this has been discovered while doing the frog surveys when a female frog crawled out of an axil and two eggs (Appendix 5.) were found. A correlation between the number of *C. gymnocarpa* and the *O. pumilio population* size per plot has been found which may suggest that this plant species can be used to increase the population size of these frogs within a certain area. Around Caño Palma the most used rearing site was the artificial cup. More studies suggest this is a rearing site of interest for the *O. pumilio* (Strynoski J. L., 2012 ; Strynoski J. L.,2014).

4.1.2. Numbers and species of *Bromeliaceae* within the plots.

In total three different bromeliad species were identified within all the plots around Caño Palma and El Cerro. *Aechmea diclamydea* is a bromeliad species native to Trinidad and Tobago (Downs R. J., 1979) but has been identified in plot 1A and plot 3B. Costa Rica has over three thousand different species and 58 genera of bromeliads (Cáceres González, D. A, 2013) so it is possible that this particular individual has been mis-identified, as several difficult to distinguish species may exist. Another bromeliad species found in plot 4A was *Guzmania monostachia*. This bromeliad species from the Guzmania family is a dominant species around Costa Rica (Cascante - Marín Alfredo M, 2006). *Bromelia pinguin* was a bromeliad that was found in plot 2B around El Cerro. This species of bromeliad also known as wild pineapple is common throughout Central America (Gargullo M. B, 2008). There was no association between the number of species and abundance of bromeliads per plot and the *O. pumilio* populations of a plot.

4.2. *O. pumilio* encounters on different types of rearing sites.

From all total of 828 frogs that were encountered, 104 were encountered on rearing sites. No significant differences have been found between the numbers of frogs and encounters on the different types of rearing sites. This could mean that there are no specific preferences within

this frog species for specific rearing sites. The rearing sites on which *O. pumilio* was encountered most, with 68,3 percent was the *C. gymnocarpa*. This could be because around El Cerro Tortuguero the *C. gymnocarpa* is an abundant plant species and many of these were found within the plots there. A percentage of 22 frogs were caught on the cups in plot 2A in Caño Palma and around this location this was the most popular type of rearing site. Artificial puddles can be of use to monitor frogs and tadpoles and it is proven that they are used by *O. pumilio* (Strynoski J. L., 2012 ; Strynoski J. L.,2014). A percentage of 8,7 of the frogs was found on a bromeliad species. An explanation for this fact is that the number of bromeliads per plot was rather low.

4.3. *O.pumilio* encounters on different substrates.

Significant differences were found between the different types of substrates and the number of frogs who were first encountered on these. The encounters on leaf litter differed significant with the encounters on logs. The number encounters on logs differed just significant with the encounters on trees. The lower fractions showed no significant difference. The highest percentage of the encountered frogs with 42,6 percent was caught on leaf litter. An explanation for this could be that these frogs were foraging. The diet of *O. pumilio* consists of arthropods, such as mites ants and tiny beetles (Saporito, R. A., 2011). These arthropods are highly abundant in the leaf litter in neotropical rainforests (Heinen, J. 1992). Twenty percent of the frogs were caught on logs. Dead wood in rainforest usually also contains high numbers and diversity of arthropods (Clark D. B., 2002) which could mean that the frogs were also foraging on the logs. A percentage of 8,6 of all captured frogs was encountered on *C. gymnocarpa*. This may again suggest that this plant species is of habitat importance to *O. pumilio* in the Barra del Colorado wildlife refuge area. A percentage of 14.4 percent was encountered first on a tree. As *O. pumilio* is a *dentrobatid* thus partly an arboreal frog species , trees are of importance to its habitat (Myers C.W., 1984). For *O .pumilio* it is more difficult to move around an area with few trees (Nowakowski, A. J ., 2013).

4.4. Conclusion

The biggest O. *pumilio* population sizes per plot were found in the plots with the highest number of tadpole rearing sites. The total number of total tadpole rearing sites per plot associates with the *O. pumilio* population per plot. *O. pumilio* populations per plot were significantly bigger around El Cerro Tortuguero. Within the plots around El Cerro, more different types of rearing sites were found then around Caño Palma. On El Cerro, *Calathea gymnocarpa* was found as potential tadpole rearing site and number of *C. gymnocarpa* per plot correlates with the *O. pumilio* per plot This suggest that this plant species can be used to increase the population size of these frogs within a certain area. In this study a total of three different bromeliad species were identified, *Aechmea diclamydea, Guzmania monostachia* and *Bromelia pinguin.* The total number and species of bromeliads per plot did not show associations with the *O. pumilio* populations per plot, probably due the fact that not many bromeliads were found. The possible rearing sites on which *O. pumilio* was encountered most, with 68,3 percent was the *C. gymnocarpa*. This could be because around El Cerro Tortuguero the *C. gymnocarpa* is an abundant plant species and many of these were found within the plots there. The highest percentage of the encountered frogs with 42,6 percent was captured on leaf litter. An explanation for this could be that the frogs were foraging.

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Appendix 1. Corners of used plots at Caño Palma and Cerro.

Corners:	
NW =	North West
NE =	North East
SW =	South West
SE =	South East

Plot coded in field as: CP A1 (FH) NW
plot# : 1t/m 5 Initials on plot: FH
location: A=Caño Palma, B= Cerro Tortuguero

		GPS Coördinates		
Caño Palma (A)		X	Y	Accuracy (M)
A1	NW =	0223362	1172148	4
	NE =	0223379	1172153	6
	SW =	0223371	1172137	4
	SE =	0223387	1172138	5
Caño Palma (A)		X	Y	Accuracy (M)
A2	NW =	0223275	1172383	3
	NE =	0223286	1172391	4
	SW =	0223283	1172376	4
	SE =	0223293	1172366	4
Caño Palma (A)		X	Y	Accuracy (M)
A3	NW =	0223244	1172422	3
	NE =	0223257	1172437	4
	SW =	0223250	1172427	4
	SE =	0223263	1172425	3
Caño Palma (A)		X	Y	Accuracy (M)
A4	NW =	0223226	1172463	4
	NE =	0223238	1172671	4
	SW =	0223231	1172450	5
	SE =	0223241	1172457	4
Caño Palma (A)		X	Y	Accuracy (M)
A5	NW =	0223304	1172323	4
	NE =	0223322	1172326	4
	SW =	0223313	1172310	5
	SE =	0223328	1172315	4

		GPS Coördinates		
Cerro Tortuguero (B)		X	Y	Accuracy (M)
B1	NW =	0223474	1170922	9
	NE =	0223491	1170931	7
	SW =	0223489	1170916	7
	SE =	0223495	1170915	6
Cerro Tortuguero (B)		X	Y	Accuracy (M)
B2	NW =	0223515	1171123	7
	NE =	0223517	1171127	9
	SW =	0223525	1171114	8
	SE =	0223525	117131	9
Cerro Tortuguero (B)		X	Y	Accuracy (M)
B3	NW =	0223581	1171416	7
	NE =	0223596	1171420	7
	SW =	0223582	1171402	7
	SE =	0223605	1171406	6
Cerro Tortuguero (B)		X	Y	Accuracy (M)
B4	NW =	0223544	1171486	6
	NE =	0223559	1171495	6
	SW =	0223550	1171463	5
	SE =	0223564	1171467	4
Cerro Tortuguero (B)		X	Y	Accuracy (M)
B5	NW =	0223539	1171509	5
	NE =	0223522	1171519	5
	SW =	0223550	1171498	4
	SE =	0223563	1171503	6

Appendix 2. Photo box to take photo's when raining.

Figure 2.1. A rainproof photo box in the field.

2.1. The rainproof photo box.

As the name suggest, a rainforest can be a rainy place. When a waterproof camera is no option then it is relatively easy to construct a rainproof photo box. A clear plastic box with the measurements of at least 40 by 30 by 30 centimeter is needed with a waterproof photo light to put on top. When needed to measure a frog it is suggested to attach a measuring tape at the bottom of the rainproof box.

Appendix 3. Example for identification photos *O. pumilio*.

Figure 3.1. Dorsal side of the frog.

Figure 3.2. Left-lateral side of the frog.

Figure 3.3.Right-lateral side of the frog.

Figure 3.2.Ventral side of the frog.

3.1. Taking photos of four sides from the *O. pumilio*

For identification based on pattern is important to take images of high quality. A macro setting on the camera is a must to get all the details. Because it takes a long time for a sensor to expose in dark forest-floor conditions, a waterproof photo light can be of good use. In my experience the technique of grabbing the frog on a hind leg is the best for photographing all four sides. In this way it is relatively easy to work the frog in matter.

Appendix 4. Repeat capture mark recapture methodology.

The formula:

$$N = \frac{M_2C_2 + M_3C_3 + M_4C_4 + M_5C_5 + M_6C_6 + M_7C_7 + M_8C_8 + M_9C_9 + M_{10}C_{10} + M_{11}C_{11} + M_{12}C_{12} + M_{13}C_{13} + M_{14}C_{14} + M_{15}C_{15}}{R_2 + R_3 + R_4 + R_5 + R_6 + R_7 + R_8 + R_9 + R_{10} + R_{11} + R_{12} + R_{13} + R_{14} + R_{15}}$$

N = Estimated population
M= Number of marked individuals marked/captured within the sample
C = Individuals captured within the sample
R = Number of individuals recaptured within the sample
Lower number stands for the round/repeat.

Appendix 5. Eggs found in the *Calathea gymnocarpa.*

Figure 5.1. Eggs of *O. pumilio* attached to the inside of a *Calathea gymnocarpa* axil,

Figure 5.2. Area where the eggs were discovered in the *Calathea gymnocarpa* axil,

5.1. Location of the eggs

The eggs were discovered on October 30th 2016 in one of the *C. gymnocarpa* within plot 2B on El Cerro Tortuguero. A female *O. pumilio* was found crawling out of this axil and this is how the two eggs were discovered.

Figure 5.3. Close up of the area where the eggs were discovered in the axil,