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Oophaga pumilio

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Abstract

The tropical wetlands near Tortuguero have a high biodiversity. The organisms are well adjusted to their environment. The *Oophaga pumilio* also known as strawberry dart-poison frog has a healthy population in these tropical wetlands. The *O. pumilio* reproduces in a way that fits in his environment. The female lays eggs in the leaf litter and the male fertilizes them. After the eggs are hatched the female carries them to natural water bodies in trees. Because of the risk of flooding in the research areas. The female frogs can experience difficulties to get to cups to deposit or feed their tadpoles. The placement of the natural water bodies have an important role in the tadpole development and even the survival rate. The waterbodies can also differ in size. The different sizes of waterbodies causes that waterbodies act different by the influence of their environment such as weather. This study tests hypotheses related to discrimination of natural waterbodies by the female *O. pumilio*, influence of flooding on tadpole deposition and effect of waterbody size on the tadpole growth. The data is collected by placing a total of 338 artificial waterbodies ("cups"). These cups are placed on three trials spread over a high and a low flood risk area. There are four cups per plot a big low, small low, big high and a small low cup. The cups are checked twice a week with an interval of three to four days. Once a week there were photo's made of the tadpoles to measure them. The results show that the tadpole deposition is affected by cup height. There is no significant difference between high and low cups, big and small cups nor the interaction between these. The result also show that there is no significant difference between the total number of tadpole deposition on low and high flood risk areas. Flooding has an influence on the tadpole deposition the results show that during floods the tadpole deposition falls or stops rising. The tadpole growth does not show a difference between different cups. There should be done further research focused on the effect of flooding on tadpole growth and tadpole deposition in a longer period of time.

Acknowledgements

This study would not have been possible without the assistance of a number of people. First of all I would like to thank the Caño palma biological station staff, volunteers and interns for their support and help in gathering the data needed for this study. I would like to give a special thanks to Emily Khazan for advising me during my whole research and assist me in practical and writing work. I also want to give a big thank you to Sander van Huijzen for giving me good feedback and supporting my study. For the great guidance and this opportunity I want to thank the HAS university of applied sciences. As last I want to thank my family for making this study possible and their support throughout my study.

Contents

1 Introduction

Tropical rainforests are highly biodiverse habitats, harboring a huge number of species. Consistent climatic conditions of both high temperatures and high humidity might contribute to the development and maintenance of these high levels biodiversity. The tropical lowlands of Costa Rica represent one highly diverse habitat within the Neotropics. In the northern part of the province of Limon around the area of Tortuguero there are four major groups of forest structures. In the area around Caño Palma biological station (about eight kilometers north of Tortuguero) there are three different forest structures; herbaceous vegetation, low forests and medium forest (Myers, 1981). In the tropical lowlands around Caño Palma biological station there are a lot of different mammals, insects and amphibians. These animals are well adapted to their habitat. Although many animals have been studied, there are a lot of examples of animals that we don't have a lot of knowledge of. A group of these animals are the small dart-poison frogs.

Small dart-poison frogs are of the family Dendrobatidea. The Dendrobatidea family contains three genera; Dendrobates, Phyllobates and Colostethus. Only Dendrobates and Phyllobates contain poisoned frogs including the *Oophaga* family (Savage, 2014). Species in the *Oophaga* family subsist on unfertilized eggs as food for the tadpoles. This project will focus on the tadpole deposition of *Oophaga pumilio* species also known as strawberry dart-poison frog. *O. pumilio* occurs below 960 meters of the tropical lowlands and subtropical zone of Central America (Savage, 2014). The *Oophaga* genus of the family Dendrobatidea has a recognizable reproduction method. Female frogs lay their eggs (4-15 eggs) in the leaf litter after which the male fertilizes them. When the eggs are fertilized the male frog will keep them moist for about a week until they hatch (Stynoski, *2012*). The female will carry one or two tadpoles at a time on their backs and place them into small terrestrial waterbodies (Stynoski, *2012*). One waterbody usually contains one tadpole. It is believed that if there is more than one tadpole per waterbody, most of the time only one tadpole will survive (Brown., Morales., Summer., *2008*). Once all tadpoles are placed into a waterbody the female will deposit a clutch of unfertilized eggs into the waterbody which contains a tadpole every 1-8 days (Brust 1993). The unfertilized eggs serve as nutrition for the tadpole. Besides the unfertilized eggs the female will also deposit alkaloids stored within the eggs. *O. pumilio* adults get there alkaloids by eating ants and other small alkaloid containing insects (Stynoski, *2012*). The *O. pumilio* stores the alkaloids in their skin. Because the tadpoles of *O. pumilio* do not eat insects but only unfertilized eggs, they do not have this defense mechanism against predators. By feeding the tadpole alkaloids the tadpoles become toxic for predators (Stynoski, *2012*).

This research will be done to get a better understanding of the reproduction behavior of egg eating small dart-poison frogs, *Oophaga pumilio*. To get a better understanding of the reproduction method of the *O. pumilio*, it is important to know what environmental requirements the female *O. pumilio* has for selecting rearing sites. The environmental requirements of the *O. pumilio* female rearing sites and tadpoles aren't well known (May., Medena-Müller., Donelly., Summers., *2009*). In this research I will aim to understand

tadpole deposition patterns, testing what pool size and heights are preferred by *O. pumilio* females and what consequences these different characteristics have on tadpole development. In other words; Do *O. pumilo* prefer big waterbodies over small waterbodies, high placed waterbodies over low placed waterbodies, and does this preference change in different seasons or conditions?

2 Materials and methods.

2.1 Study site, species

The data is collected from the three forest trails near the Caño Palma biological station. The forest consist out of a tropical wet forest. The trails are referred to as Raphia, Snake and Cerro. The trails in the study area are chosen because of their accessibility, abundance of *Oophaga pumilio* and risk of floods. The Snake and Raphia trails experience regular floods, while Cerro has a low flood risk. The floods are caused by extensive and heavy rain. In between July-august and November-December is the risk of floods higher.

To collect the number of *Oophaga pumilio* tadpoles and deposition rate of the *Oophaga pumilio*, I placed a total of 338 artificial waterbodies spread over the three trails. There is a total of 85 plots that contains four cups each. The plots have a buffer zone of 20 meters. The waterbodies are checked for *Oophaga pumilio* tadpoles twice a week with an interval of three and four days. The surveys are done between 13th of April and 13th of August.

2.2 Rearing site preference

To quantify the number of *Oophaga pumilio* tadpoles per artificial waterbodie, I placed four cups per plot. In order to find out which height and size of waterbodies the female *O. pumilio* prefers to deposit her tadpoles in I placed a big high, big low, small high and a small low cup in every plot. For the high cups I used a height of 1.80 meters and the low cups are placed at 70 centimeters from the base of the tree. The total amount of 85 plots is spread over the three trails. Raphia and Snake trial consists out of 30 plots per trail and Cero consists out of 25 plots. All cups are placed on individual trees so that they will not influence each other.

Big cups are made by cutting off the tops of 500mL bottles and drilling holes below the rim so that the cup will not contain more than 150mL of water. After creating drainage holes the small cups can contain up to 25mL. These drainage holes also prevent the cups from overflowing during extreme rain events. All cups are placed on a different random tree that is at least three centimeters in diameter. All the cups are filled with fresh rainwater, if a cup is dried out during a survey I refilled it with fresh rainwater.

To survey the artificial waterbodies I used a pipette. With the pipette I was able to slowly move the dirt and check if there was a tadpole inside the cup. If there was a tadpole found I recorded if the trail, date, cup number and if it was big, medium or small.

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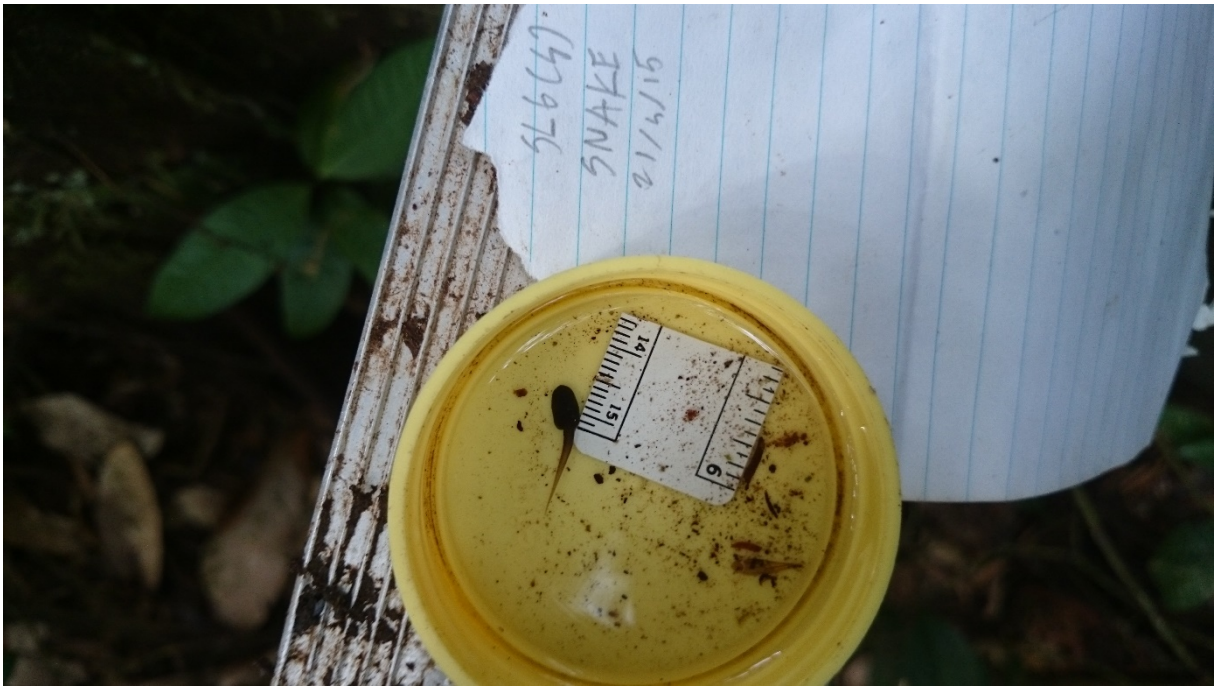
2.3 Waterbody variation and measurements

From each individual tadpole I recorded the size and height of the cup. If the survey area was flooded I noted down the height of the flood. The data collected about the different heights and sizes of cups will be used to determine if there is a difference in the use of cups between different seasons and during floods. Because of the different seasons it is expected that the female *O. pumilio* will deposit there tadpoles more often in high cups in the wet season then in the dry season. Because of the low competition in the area it is expected that the number of *O. pumilio* tadpoles will not differ between small and big waterbodies. To get the difference of growth rate between different tadpoles I took a photo of every individual tadpole that is present on the first survey of the week. To take the picture the tadpole will be removed from its cup and placed into a small lid with a ruler inside, see picture 1. A photo will be made and the tadpole will be measured afterwards using imageJ (version 1.48) software. After I made the picture the tadpole will be placed back in the cup. It is believed that if there is more than one tadpole in a cup only one tadpole will survive. By measuring the tadpoles and follow the individual growth rate we can follow the development of tadpoles in different environments and with singletons and doubletons.



Picture 1; The design of the picture that will be used to measure the tadpole

2.4 Statistical analysis

The number of tadpoles in each cup will be compared between the height and size of the cups. The statistical analysis will be done with a two way ANOVA Test to compare the differences between height and cups sizes. The difference between sizes and height will also be done by a Friedman test. The null-hypothesis of the Friedman test and the two way ANOVA test is that big cups will not be used more than small cups and high cups will not be used more than low cups. The difference between heights will be tested with a T-test. The T-test will also be used for the difference between cup sizes. The null-hypothesis of the T-test is that there will not be a difference between the cup sizes and cup heights. To test between the different seasons there will be looked at the differences between the three trails (raphia, snake and Cero) and the difference between the different heights. The difference between the different seasons will be analyzed with a two way ANOVA test. The null-hypothesis of this two way ANOVA is that there will be no difference between the different trails and the different seasons. All the statistical analysis will be done in SPSS (version 21).

3 Results

More tadpoles are found in big cups than in small cups in total I found 191 tadpoles which of 115 were found in the big cups and 76 tadpoles in small cups. In total I found 115 tadpoles in big cups 76 in small cups 73 in high cups and 118 in low cups as you can see in table 1.

Tabel 1; number and percentage of tadpoles per flood risk area and cup size or height; BH=big high, BL=big low, SH=small high, SL=small low

area	number of tadpoles	number morphed	Cup	total number of tadpoles	percentage of tadpoles
low flood risk	114	10	B	68	60%
			S	46	40%
			H	39	34%
			L	75	66%
high flood risk	77	13	B	47	61%
			S	30	39%
			H	35	45%
			L	42	55%
total	191	23	B	115	60%
			S	76	40%
			H	73	38%
			L	118	62%

The t-test to compare the total number of tadpoles in high and low flood risk areas shows that there is no significant difference between these areas (p=0.079).

In low risk areas I found more tadpoles in low cups (mean = 1.0125) than in the high risk area (mean = 0.525) the T-Test shows that there is no significant difference (p=0.88). The T-Test showed no difference (p=0.768) in the deposition in high cups between the high and low risk areas. The mean deposition in high cups of the high risk area is 0.3846 and the mean deposition in the high cups of the low risk area is 0.4359.

On the Raphia trail I found a total of 102 tadpoles of which 57 tadpoles are found in big cups and 45 tadpoles in small cups. On the Snake trail I found a total of 69 tadpoles. In the big cups I found 43 tadpoles and 26 Tadpoles in small cups. The total number of tadpoles on Cerro is 20. 15 tadpoles were found in the big cups and five were found in small cups. Between the total number of tadpoles in big cups and the total number of tadpoles in small cups.

In total I found 73 tadpoles in high cups and 118 tadpoles in low cups. On Raphia trail I found a total of 32 tadpoles in high cups and 70 tadpoles in small cups. On the Snake trail there

were 33 tadpoles in high cups and 36 tadpoles in low cups. A total of 8 Tadpoles in high cups and a total of 12 tadpoles are found in low cups on Cerro. There is a significant difference between the total tadpoles in high and total tadpoles in low cups Overall there is no significant difference in size for the deposition (p=0.150) the height is important (p=0.019) and the flood risk is important (p=0.040). There are no interactions between any of the fixed affects.

The two way ANOVA test for the low flood risk area showed that deposition is affected by cup height (p=0.031) and not by its size (p=0.394) or interaction between those (p=0.581) the mean high cup deposition was 0.435 and the mean low cup deposition is 1.012. The two way ANOVA test for high flood risk showed that deposition in not affected by cup height (p=0.438) by cup size (p=0.179) or by the interaction between height and size (p=0.289).

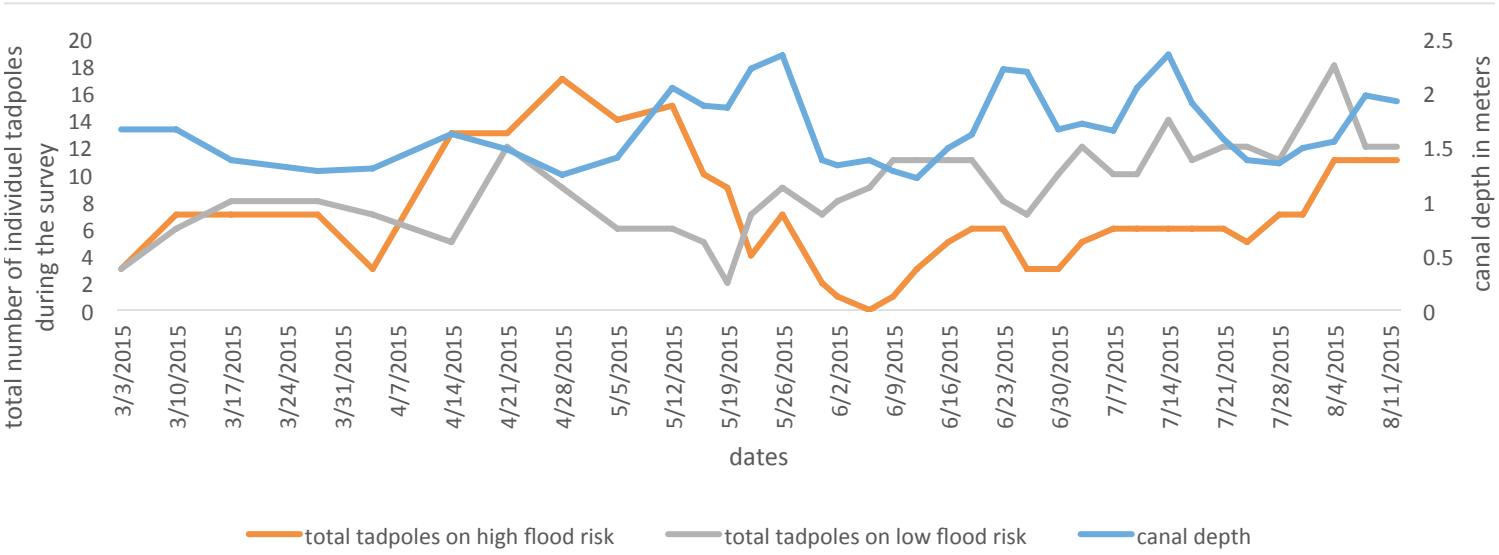
Tabel 2; number and percentage of tadpoles per flood risk and cup type; BH=big high, BL=big low, SH=small high, SL=small low

area	cup type	total number of tadpoles	number morphed	total used cups	percentage used cups	percentage of tadpoles	number of days flooded
high flood risk	BH	26	2	14	31%	34%	37
	BL	21	3	8	18%	27%	
	SH	9	2	5	11%	12%	
	SL	21	6	14	31%	27%	
low flood risk	BH	23	3	13	33%	20%	26
	BL	45	3	16	40%	39%	
	SH	15	0	9	23%	13%	
	SL	31	4	12	30%	27%	

On the transect areas where there is a high flood risk I found that there is a higher percentage of tadpoles in high cups than in low flood risk areas. Between the cup sizes there is only a one percent difference (see figure 1). A t-test to compare the total number of tadpoles found in high and low risk areas shows that there is no significant difference (p=0.094). The low risk area has a mean of 158 cups and the high risk area a mean of 180 cups. The mean number of tadpole depositions in the high risk area is 0.44 in the low risk area the mean number of tadpole deposition is 0.73.

There is no significant difference (p=0.094) found with a one way ANOVA between the four different cups. The posthoc analysis showed a marginal significance (p=…) between big low cups and small high cups.

During this study there are only 2 reported floods in which low cups were completely flooded. These were at 16-5-15 and 23-6-15. Figure 1 displays that after a flood the number of tadpoles stop rising or even drop.



e 1; Interaction between the total number of tadpoles and the canal depth. Total tadpoles is measured in number of individuals per survey and the canal h is measured in meters.

The difference in tadpole length per cup is displayed in figure 2. The tadpoles in the high cups are smaller than the tadpoles in the other cups. These tadpoles are just a few millimeters smaller and the other tadpole lengths in the other three cups are more similar.

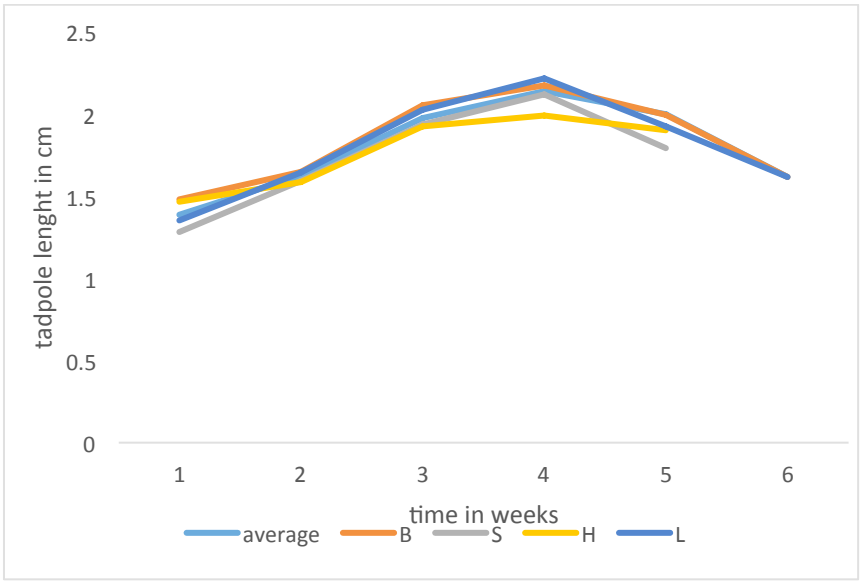


Figure 2; average tadpole length per cup size and height; BH=big high, BL=big low, SH=small high, SL=small low

4 Discussion and conclusion

In the end of the Raphia, snake and whole of cerro trail I found a lower number of tadpoles. This can be caused because of the differences in *O. pumilio* density. A good explanation for this difference is the fact that these plots were set later then the first 15 plots of Raphia and Snake. The beginning of Raphia and Snake were set up at 3-3-15 and the rest was set up at 12-5-15. This can explain the difference between the number of tadpoles between the three different trails. This does not affect the number of tadpoles of low and high flood risk areas as much. This is because the low flood risk area consist partly out of the first 15 plots of the Raphia trail and the high flood risk area consist partly out of the first 15 plots of the Snake trail. The high flood risk area contains a total of 45 plots and the low flood risk consist out of 40 plots. This means that there is a difference in the percentage of cups that I placed at 3-3-15 between high and low flood risk areas. Low flood risk areas have a higher percentage of old cups than low flood risk areas.

On the high risk area I found less tadpoles than on the low risk area this can be explained by the fact that the cups on the high risk areas have a higher number of total days flooded. It is also possible that the density of female *O. pumilio* differs between the areas.

My test to find a preference of tadpole deposition in different sizes and heights has shown that the big low cups are used the most and small high cups are used less. The statistics show that tadpoles deposition is affected by height (p=0,034). This is probably caused by the fact that low cups cost less energy to access. Big cups are used more than small cups but there is no significant difference (p=0,150) there was also no difference in cups size deposition affected by high or low flood risk areas. The fact that big cups are used more can be explained because of the more consistent environment in the big cups. Considering evolution and competition female *O. pumilio* would choose small cups over big cups (Brown, J,L., Morales, V., Summers, K., *2009*). In the study site that is used for this research the *O. pumilio* is the only species of their family that can be found. This causes that *O. pumilio* do not have competition in deposition sites with other frog species. It would be recommended to do a study on cup size depositions in an area where they do have competition of other frogs. It is also recommended to add a cup that can contain more than 150mL of water to see if they would still use the 150mL cups that I used more.

Flooding has caused the tadpole deposition to stop and can even cause a loss of total number of tadpoles other than predation and morphing. The fact that it causes to stop tadpole deposition means that it is possible that female *O. pumilio* are not able to bring their tadpoles to the natural waterbodies in trees during a flood.

The tadpole length does not differ a lot between different cup sizes. In high cups tadpoles grow a slower or do not grow as big as in the rest of the cups. There needs to be more research done to find out how and if tadpole growth is influenced by the waterbody size and height. It is also recommended to do more research about the influence of flooding on the tadpole growing rate.

The seasonal changes cannot be tested because of the minimal changes between the different seasons. It is recommended to test the difference between different seasons. It is expected that the difference will mainly be caused by floods.

Thus *O. pumilio* will deposit their tadpoles in easier accessible waterbodies even if that means that the tadpoles are more vulnerable for floods. There is a higher percentage of depositions in high placed cups on areas with a high flood risk compared to the number of depositions in low flood risk areas, but there is still a high rate of low cup depositions. The statistical tests have shown that tadpole deposition is affected by cup height (p=0,031). The cup size has not shown a significant difference, also the interaction between flood risk and cup size does not show a difference. During floods the *O. pumilio* has more difficulties getting to the cups which makes the tadpoles grow slower because of lack of food. Apart from the lower grow rate the number of new depositions also fall especially on high flood risk areas. The size of cups do not influence the grow rate of tadpoles. In order to get a better understanding of the tadpole deposition rate in different waterbody sizes there should be a study done in an area where there is more competition between *O. pumilio* and other frogs that use natural waterbodies in trees to deposit their tadpoles. The tadpole growth rate and deposition rate difference during floods and dry periods should be monitored for a longer time period to be able to get a better understanding of how floods influence the tadpole growth and tadpole deposition rate.

Literature

- Brown, J, L., Morales, V., Summers, K., “Divergence in parental care, habitat selection and larval life history between two species of Peruvian poison frogs: an experimental analysis” (2008)

- Brown, J,L., Morales, V., Summers, K., “A Key Ecological Trait Drove the Evolution of Biparental Care and Monogamy in an Amphibian” (2009)

- Brust DG “Maternal brood care by Dendrobates pumilio: a frog that feeds its young. Journal of Herpetology 27:96-98” (1993)

- Lin, Y., Kam, Y., “Nest Choice and Breeding Phenology of an Arboreal-Breeding Frog, Kurixalus eiffingeri (Rhacophoridae), in a Bamboo Forest” (2008)

- May, Rudolf, V., Medina-Müller, M., Donnelly, M, A., Summers, K., “Breeding-site selection by the poison frog Ranitomeya biolat in Amazonian bamboo forests: an experimental approach” (2009)

- Myers, Ronald, L., “The ecology of low diversity palm swamps near Tortuguero, Costa Rica”.(University microfilms international.(1981)

- Savage, J, M., “The Dendrobatid Frogs of Central America” (2014). Copeia, Vol. 1968, No. 4, pp. 745-776 (Nov. 30, 1968)

- Schulte, L, M., Lötters, S., “The power of the seasons: rainfall triggers parental care in poison frogs” (2013)

- Stynoski, J, L., "Behavioral Ecology of Parental Care in a Dendrobatid Frog (Oophaga pumilio)". Open Access Dissertations. Paper 755. (2012)