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ARE TADPOLES OF THE BROMELIAD DWELLING-FROG (*Phyllodytes luteolus*) EFFICIENT MOSQUITO LARVAE PREDATORS?

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Área de concentração: Zoologia.

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Ilhéus, 10 de março de 2017.

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“We keep moving forward, opening new doors, and doing new things, because we're curious and curiosity keeps leading us down new paths.”

Walt Disney

Sumário

Are tadpoles of the bromeliad dwelling-frog (*Phyllodytes luteolus*) efficient mosquito larvae predators?

RESUMO

Phyllodytes luteolus provavelmente representa um complexo de espécies pertencentes à família Hylidae, que vivem em reservatórios de água formados pelas axilas das bromélias de restinga. É uma espécie considerada bromelígena, por usar bromélias para reprodução, desenvolvimento larval e forrageio. Os hábitos alimentares de *P. luteolus* são conhecidos apenas para indivíduos adultos, que possuem uma dieta composta exclusivamente por artrópodes coloniais, que vivem no interior das bromélias. No entanto, os hábitos alimentares e nível trófico dos girinos permanecem desconhecidos. Embora se saiba que haja oofagia em algumas espécies do gênero, os girinos de algumas espécies podem ser predadores, se alimentando inclusive de larvas de mosquitos. Doenças virais como Dengue, Zika e Chikungunya são transmitidas por mosquitos da família Culicidae, e em 2015 mais de 1,5 milhão de casos de Dengue foram reportados no Brasil. A predação das larvas de mosquito por girinos pode ajudar a reduzir os surtos dessas doenças. A relação entre esses dois grupos de organismos já tem sido estudada em outros tipos de corpos d'água, porém este é o primeiro estudo a ser realizado em bromélias. A eficácia dos girinos como predadores pode depender de seu tamanho corporal, neste contexto, primeiramente contatamos em um estudo de laboratório se os girinos de tamanho grande, médio e pequeno de *P. luteolus* eram capazes de predar larvas de Culicidae de diferentes tamanhos. Nosso objetivo foi avaliar se os girinos de *P. luteolus* podem, de fato, ser considerados predadores de larvas de mosquito, e em seguida, em um estudo de campo verificamos se os girinos podem controlar a abundância das larvas de Culicidae nas bromélias. Em bromélias com girinos maiores foi encontramos uma menor abundância de larvas de Culicidae enquanto que em bromélias com girinos menores encontramos uma maior abundância de larvas de mosquito.

Palavras-chave: Doenças virais. Girinos. Controle biológico. Dengue. Bromélias.

Are tadpoles of the bromeliad dwelling-frog (*Phyllodytes luteolus*) efficient mosquito larvae predators?

ABSTRACT

Phyllodytes luteolus is probably a complex of species belonging to the family Hylidae, which live in water tanks formed by the axils of the bromeliads from restinga. They are considered bromeligenous, using bromeliads for reproduction, larval development and foraging. The dietary habits of *P. luteolus* are known only for adult individuals, who have a diet composed exclusively of colonial arthropods that live inside the bromeliads. However, the feeding habits and trophic level of tadpoles remain unknown. Although tadpoles of some species have been reported to have oophagous tadpoles others can be predators, feeding even on mosquito larvae. Viral diseases like Dengue, Zika and Chikungunya are transmitted by mosquitoes of the Culicidae family, and in 2015 more than 1.5 million cases of Dengue were reported in Brazil. Predation of mosquito larvae by tadpoles can help reducing outbreaks of these diseases. The relation between these two groups of organisms has already been studied in other types of water bodies, but this is the first study to be carried out in bromeliads. The effectiveness of tadpoles as predators may depend on their body size. In this context we first observed in a laboratory study if large, medium and small tadpoles of *P. luteolus* were able to prey on Culicidae larvae of different sizes. Our objective was to evaluate if *P. luteolus* tadpoles can in fact be considered predators of mosquito larvae, and then we observed in a field study whether tadpoles can control the abundance of Culicidae larvae in bromeliads. We observed that bromeliads with larger tadpoles had a smaller abundance of Culicidae larvae, whereas in bromeliads with smaller tadpoles we found an increased abundance of mosquito larvae.

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Key-words: Phytotelmata. Culicidae. Mosquito-borne disease. Biocontrol

1 INTRODUÇÃO GERAL

No início do século XX o *Aedes aegypti* já era um problema, mas não por transmitir a Dengue, e sim a febre amarela. Em 1955 o mosquito foi erradicado devido às medidas para o controle da febre amarela. No final de 1960, o relaxamento dessas medidas resultou no ressurgimento do mosquito em território nacional. Hoje o mosquito está presente em todos os estados brasileiros (IOC, 2017). No Brasil, os primeiros relatos de dengue são do final do século XIX, na cidade de Curitiba (PR), e de acordo com o ministério da saúde a primeira ocorrência do vírus documentada laboratorialmente aconteceu em 1981-1982, em Boa Vista (RR). Em 1986 houve epidemias no Rio de Janeiro e em alguns locais da região Nordeste, desde então o vírus vem se espalhando por todo o país (BRASIL, 2017).

Outras doenças virais que tem alarmado o Brasil nos últimos anos são o Zika vírus e o Chikungunya, ambas transmitidas por mosquitos da família Culicídae. Em 2015 mais de 1,5 milhão de casos de Dengue foram reportados (WHO, 2015). A predação das larvas de mosquito pelas larvas de anfíbios (girinos) pode ajudar a reduzir os surtos dessas doenças (HOCKING; BABBITT 2014). MOKANI e SHINE (2003) provaram experimentalmente que os girinos podem controlar populações de larvas de mosquitos. No entanto, esses estudos trataram de comunidades de larvas de mosquitos e girinos que habitam poças, mas a relação entre esses dois grupos de organismos em outros corpos d'água como as bromélias ainda não foi estudada.

As bromélias são o tipo de fitotelmata mais representativo na região costeira do Brasil (LANTYER-SILVA et al., 2014). São estruturalmente complexas devido ao seu formato de tanque, que coleta água e detritos, fornecendo recursos e refúgio para suas comunidades (SILVA et al., 2010).

Phyllodytes luteolus é provavelmente um complexo de espécies pertencentes à família Hylidae (FROST, 2017) que vivem em reservatórios de água formados pelas axilas das bromélias de restinga nas regiões costeiras (SILVANO; ANDRADE, 2004). *Phyllodytes luteolus* tem sua distribuição na região costeira do Brasil, indo desde o estado da Paraíba até o norte do estado do Rio de Janeiro (FROST, 2016). É uma espécie considerada bromelígena, usando as bromélias para reprodução, deposição de ovos, desenvolvimento larval e forrageio (PEIXOTO, 1995).

Os hábitos alimentares de *P. luteolus* são conhecidos apenas para indivíduos adultos, que possuem uma dieta composta exclusivamente por artrópodes coloniais que vivem no interior das bromélias (FERREIRA et al., 2012; MOTA-TAVARES et al., 2016). No entanto, os hábitos alimentares e nível trófico dos girinos permanecem desconhecidos. Os girinos de muitos anuros são macrófagos suspensívoros, que se alimentam de algas, fungos, detritos, e microrganismos como bactérias e protozoários. Seus hábitos alimentares estão associados ao modo como vivem e ao local que habitam (MCDIARMID; ALTIG, 1999; ROSSA-FERES et al., 2004). Os girinos de algumas espécies podem ser predadores (e.g. WASSERSSUG et al., 1981; PETRANKA et al., 1998), alimentando-se até mesmo de larvas de mosquitos (e.g. BABBIT; TANNER, 1998; SABAGH et al., 2012; MURUGAN et al., 2015). Sabe-se, no entanto que os girinos de algumas espécies de *Phyllodytes* são oofagas (e.g. *P. gyrinaethes*, PEIXOTO et al., 2003), mas não se sabe se esta oofagia é transitória dentro da fase larval ou se a dieta da larva muda de acordo com a idade.

A eficácia dos girinos como predadores pode depender de seu tamanho corporal (MURUGAN et al., 2015). Neste contexto, primeiro observamos em um estudo de laboratório se os girinos grandes, médios e pequenos de *P. luteolus* eram capazes de predar larvas de Culicidae de diferentes tamanhos (Capítulo 1). Nosso objetivo foi avaliar se os girinos de *P. luteolus* podem, de fato, ser considerados predadores de larvas de mosquito. Em seguida, observamos em um estudo de campo se os girinos podem controlar a abundância de larvas de Culicidae em bromélias (Capítulo 2): Esperamos encontrar uma menor abundância de larvas de Culicidae em axilas de bromélias com girinos de *P. luteolus* e maior abundância de larvas de mosquito em axilas sem girinos de *P. luteolus.*

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2 Are tadpoles of the bromeliad-dwelling frog *Phyllodytes luteolus* able to prey on mosquito larvae?

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RESUMO

As doenças transmitidas por mosquitos vêm se espalhando rapidamente nos últimos anos. Embora os *Aedes* sp. prefiram os habitats feitos pelo homem para a reprodução, suas larvas podem ser encontradas na água das axilas das bromélias em ambientes antropizados. O controle biológico é considerado a melhor maneira de reduzir as populações de mosquitos. Estas populações podem ser controladas através de predação e/ou competição por outros animais que vivem nos mesmos habitats, prevenindo a transmissão de doenças. Através de experimentos laboratoriais, pudemos confirmar que girinos de *Phyllodytes luteolus* foram capazes de predar ativamente larvas de mosquitos da família Culicidae. Em ambientes naturais, o tamanho do corpo do predador é fundamental nas relações interespecíficas de predação. Nossos resultados demonstraram que girinos de tamanho médio e grande podem predar mais eficientemente larvas de mosquito do que girinos de pequeno tamanho.

Palavras-chave: Fitotelmata. Culicidae. Doenças virais. Controle biológico.

ABSTRACT

Mosquito-borne diseases have been rapidly spreading in recent years. Although *Aedes* sp. is believed to prefer man made habitats for reproduction, its larvae can be found in the water of bromeliad axils in anthropic environments. Biological control is considered the best way to reduce mosquito populations. These populations can be controlled through predation and competition, preventing the transmission of diseases. Through laboratory experiments we were able to confirm that tadpoles of *Phyllodytes luteolus* were able to prey on mosquito larvae. In natural environments, predator body size is fundamental in interspecific relations. Our results demonstrated that medium and large tadpoles can prey mosquito larvae more efficiently than small tadpoles.

Keywords: Phytotelmata; Culicidae; mosquito-borne disease*;* Biocontrol

2.1 INTRODUCTION

Several epidemic diseases, such as Dengue, Chikungunya, Yellow fever and Zika are mosquito-borne viral diseases that have rapidly spread globally in the last few years. These illnesses are transmitted by female mosquitoes mainly of the Culicidae family. Due to suitable climatic conditions for the mosquito reproduction, tropical countries are more susceptible to these diseases (WHO, 2015). Brazil, for example, reported over 1.5 million cases of Dengue in 2015, approximately twice the number reported in 2014 (WHO, 2015). In 2016, 91.387 cases of Zika (BRASIL, 2016), and 13.236 cases of Chikungunya were reported, most of the latter in the Northeast region (FIOCRUZ, 2016). The mosquito *Aedes aegypti* is the primary vector of diseases and prefers man-made larval habitats for reproduction. However, its larvae can also be found in rain water contained in axils of bromeliads that grow in modified or semi modified environments (FORATTINI; MARQUES, 2000; MARQUES et al., 2001; VARJÃO et al., 2005; GONÇALVES; MESSIAS, 2008; MOCELIN et al., 2009).

Disease prevention depends on effective vector control measures (WHO, 2015). Several mechanical, chemical and biological methods have been used to control mosquito population all over the world. Among them, the mostly applied methods are mechanical (through traps—that are often proved to be inefficient) and chemical (through pesticide use), but mosquitoes are becoming more and more resistant to pesticides (MACORIS et al., 1999). Approaches using genetically modified mosquitoes are also increasing (WILKE et al., 2009).

Biological control often happens naturally and is considered the best way to control mosquito populations because it can reduce ecological impacts and side effects to humans

(BOWATE et al., 2013). The introduction of larvivorous predators can be the most economic, social and environmental cost-effective measure of mosquito control (MARIAN et al., 1983). Several fish species (e.g., paradise fish *Macropodus operculatis*, Mozambique tilapia *Oreochromis mossambica,* Siamese fighting fish *Betta splendens,* goldfish *Carassius auratus*) have been introduced as predators of mosquito larvae (NAM et al., 2000; CHANDRA et al., 2008) and in some cases the efficiency of different fish species as *Gambusia affinis* and *Aphanius dispar* as mosquito control agents has already been shown (HOMSKI et al., 1994).

However, the introduction of exotic larvivorous fishes can affect several groups of native species, such as fishes, amphibians and invertebrates (GOODSELL; KATS, 1999). This change in the habitat might also negatively impact many different trophic levels and even trigger cascade effects (WEBB; JOSS, 1997; PYKE, 2008). That was the case when the Nile Tilapia (*Oreochromis niloticus*) was introduced in Brazilian rivers affecting the water transparency and inhibiting recruitment of other fish species that feed on zooplankton and need clear water to search for their prey (DIANA et al., 1991; ATTAYDE et al., 2007). Thus, prospecting and identifying biological control measures using native species would be a big step forward for the epidemics control.

Amphibians, especially during larval stage, can offer a regulatory service by altering disease transmission and pest outbreaks because predatory species can help to reduce the spread of mosquito-borne illnesses through predation and competition with mosquitoes (HOCKING; BABBITT, 2014). Mosquito larvae and tadpoles can co-occur in the same place and they may compete for scarce resources, mainly in epiphytic plants such as bromeliads (CALDWELL, 1993, CALDWELL; ARAÚJO, 1998). Experimental studies have shown that some tadpole species are effective in regulating mosquito larvae populations by predation or competition (e.g. MARIAN et al., 1983; MOKANI; SHINE 2003).

Despite the knowledge on the controlling effect of mosquito larvae populations by tadpoles, any species have been studied in Brazil, which is the country with the largest worldwide amphibian diversity with 1080 species (SEGALLA et al., 2016). This is the first study about the relationship between mosquito larvae populations and tadpoles of different size classes in tank bromeliads.

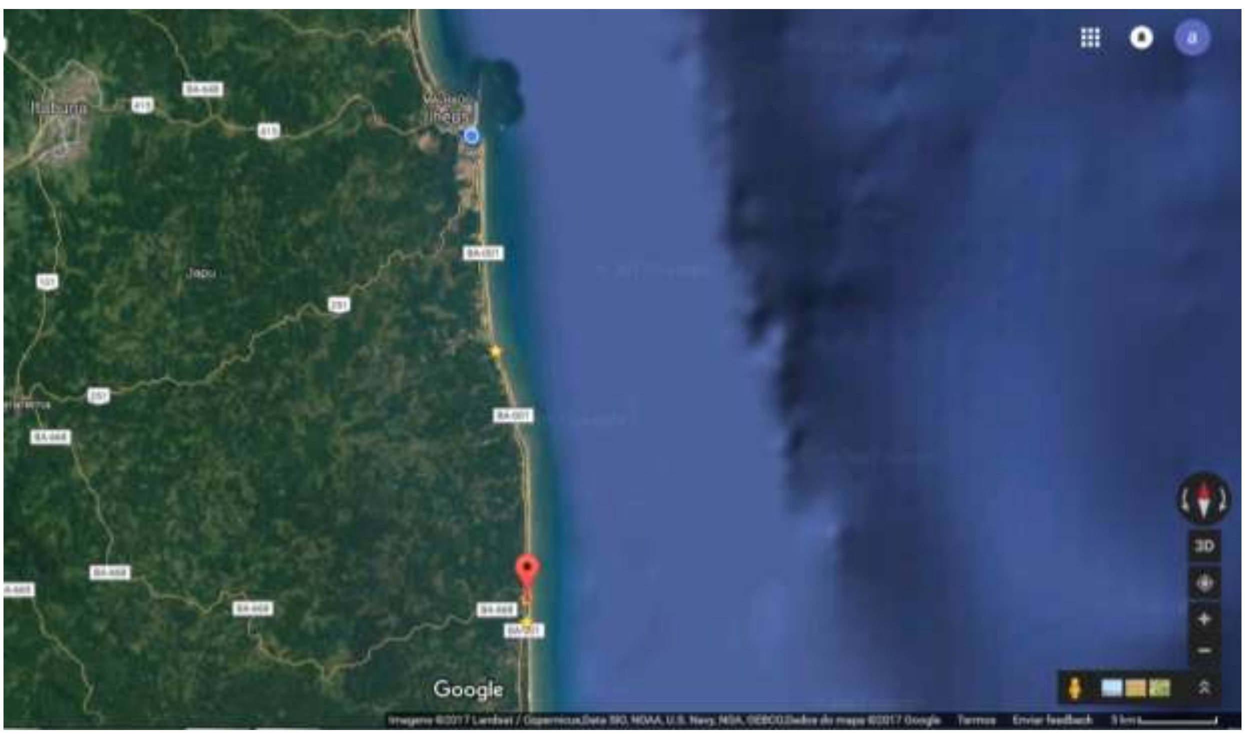
Furthermore, assumed that in case of larval predation, the feeding rate could be dependent of the size or age of tadpoles, but predation of mosquito larvae was not dependent of it. In this context, our main aim is to assess if tadpoles of *Phyllodytes luteolus* are physically able to prey on mosquito larvae, and if the predation rate on mosquito larvae differs according to the size of the predator tadpoles and mosquito larvae size. We expect that tadpoles of any size should be physically able to prey on mosquito larvae, and we also expect that larger tadpoles will prey on a larger number of mosquito larvae and will be more efficient to prey on smaller mosquito larvae, while small tadpoles may experience difficulties when preying on large larvae.

2.2 MATERIALS AND METHODS

2.2.1 *Field work and laboratory experiment*

We collected *Phyllodytes luteolus* tadpoles and mosquito larvae from bromeliads (*Aechmea blanchetiana* (Baker) L.B.Sm.) found in two restinga areas located in Ilhéus, Bahia, Brazil (S 15°37´09.2”, W 95 039°51´55.5”; S 15º05´13.2”, W038º59’55.1”). The two places (Figure 1) are areas with anthropogenic influence from tourists and locals, and in both environments bromeliads were planted forming clusters (Figure 2).

Figure 1- Location of study areas.



Source: Google Maps.

Figure 2. Artificial Bromeliads clusters

The tadpoles and mosquito larvae were collected by sucking water from the bromeliads using a PVC tube of 1 cm of diameter and were taken to the laboratory at the Universidade Estadual de Santa Cruz – UESC (Ilhéus, Brazil).

The laboratory was maintained under controlled temperature (25°C) and natural photoperiod. We separated mosquito larvae from tadpoles and placed each in different containers. The mosquito larvae were identified with a common optical microscope, using an identification key (MERRIT et al., 2008). Before starting the experiment, tadpoles and mosquito larvae were acclimatized for 48 h in independent storage tanks (11 L plastic boxes) filled with 3 L of mineral water and water taken from bromeliads. Apart from detritus found in the bromeliads and collected along with them, no food was provided to the tadpoles before the experiment.

As experimental units, we used 50 ml conical centrifuge tubes filled with 35 ml of mineral water. We placed the tubes on a holder constructed on eight plastic boxes with 3L of water (Figure 2). This water was added to control the water temperature of the tubes in the same plastic box in 25ºC. The water temperature of all plastic boxes was measured every day with a digital thermometer.

Figure 2. Styrofoam construction to hold the centrifuge tubes

After acclimation, the total length of tadpoles was measured with a digital caliper and for mosquitos' larvae we used the ImageJ® software and separated them into three size categories (Table 1). We also evaluated the stage of larval development of tadpoles in each category according to Gosner (1960), to compare each treatment, and to access if tadpoles at each stage are more or less developed than others according to the Gosner scale

After that we randomly selected the order and arrangement of experimental units applying nine treatments composed by a combination of tadpole size—small (Sm), medium (Me) and large (La) —and mosquitos larvae size (Table 2).

We replicated each treatment 12 times, totaling 108 experimental units. In each treatment, we added one tadpole (that was acclimatized before for 48h) in each experimental unit , and after it we added five mosquito larvae into each experimental unit. The experiment was conducted during four days and we checked the predation of mosquito larvae every 24 h. The tubes were reviewed daily, the amount of mosquito larvae preyed was checked and at the end of the experiment we defined the value of the predation rate.

2.2.2 Statistical analyses

We performed a One-way ANOVA to evaluate the difference of predation rates of mosquito larvae among treatments. We used the predation rates of mosquito larvae as a response variable and the treatments (i.e. size classes) as a predictor variable. Following, we applied a Post-Hoc Tukey test to verify if bigger tadpoles were able to prey on different sizes of mosquito larvae. We also performed a One-way ANOVA to evaluate if there was a significant difference among the water temperature values of the plastic boxes during the experiments.

2.3 RESULTS

All the mosquito larvae collected from the bromeliads belonged to the Culicidae family, and tadpoles measures are presented in the tables below (Tables 1 and 2)

Table 1. Size categories for tadpoles (TL: Total Length; SD: Standard Deviation)

Size categories	Mean of TL (mm)	SD	Range (mm)	Individuals
Small	9.24	1.38	6.05 - 10.09	35
Medium	14.56	1.55	12.10 – 17.01	36
Large	20.03	2.66	19.17 – 29.28	38

Source: research data.

Table 2. Size categories for mosquito larvae.

Size categories	Mean (mm)	SD	Range (mm)	Individuals
Small	0.32	0.02	0.25 - 0.38	15
Medium	0.48	0.02	0.42 – 0.50	15
Large	0.59	0.04	0.53 – 0.59	15

Source: research data.

All collected tadpoles were in Gosner stages between 24 and 37 (Table 3).

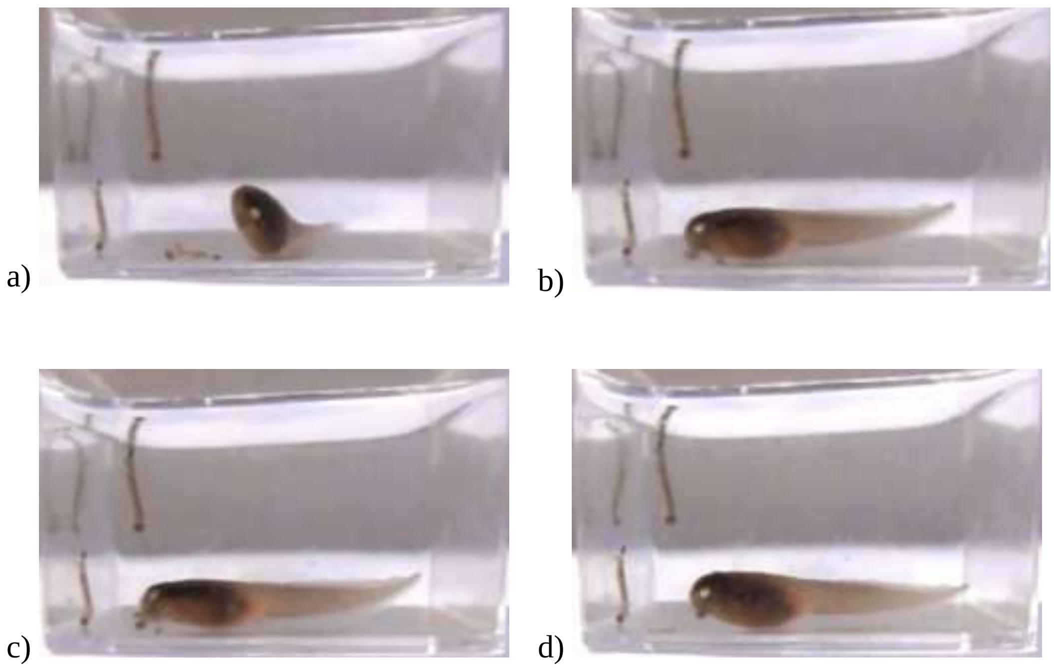
Table 3- Tadpoles class, mouth length and Gosner stages of development.

Class	Mouth Length (mm)	Gosner Stage	Class	Mouth Length (mm)	Gosner Stage
P	1.935	25	M	1.500	24
P	1.602	27	M	0.978	26
P	0.950	26	M	1.031	28
P	0.828	25	M	1.284	28
P	1.113	25	M	1.598	29
P	1.383	25	M	1.525	28
P	1.122	25	M	1.807	29
P	1.166	26	M	1.185	28
P	1.083	25	M	1.330	29
P	0.958	25	M	1.217	29
P	0.819	26	M	1.000	28
P	1.021	25	M	1.807	27
P	0.741	25	M	1.294	29
P	1.229	26	M	0.992	28
P	1.176	25	M	1.011	27
P	0.859	25	M	1.258	26
P	0.859	25	M	1.338	27
P	0.956	26	M	1.021	28
P	1.043	26	M	0.955	28
P	1.222	26	M	1.477	27
P	0.530	25	M	1.655	28
P	0.598	25	M	1.619	28
P	0.945	26	M	2.740	29
P	0.698	26	M	2.060	28
M	0.696	31	M	1.931	27
M	1.798	26	G	2.647	35
M	1.459	26	G	2.474	37
M	1.586	27	G	1.884	31
M	1.192	26	G	2.241	32
M	1.867	27	G	2.458	35
M	1.468	28	G	1.503	37

Source: research data.

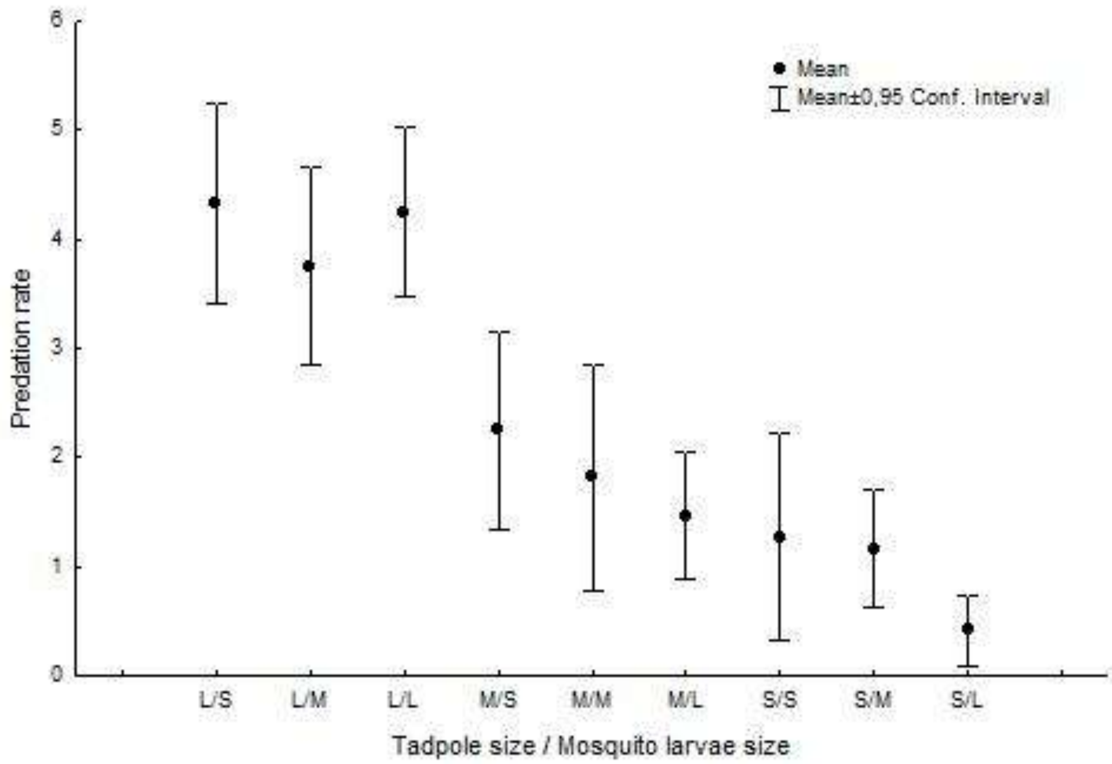
We observed that tadpoles of *Phyllodytes luteolus* of any size were physically able to prey on mosquito larvae (Figure 3), and there were differences in predation rates according to tadpoles size ($F(8) = 16{,}550$; $P<0{,}001$ – Figure 4) (See supplementary material).

Figure 3. Tadpole of *Phyllodytes luteolus* actively preying on mosquito larvae.



In a post-hoc Tukey test, we observed that larger (La) tadpoles preyed a larger number of mosquito larvae than medium (Me) and small (Sm) tadpoles (Figure 4).

Figure 4. Predation rates in each group (Large/Small; Large/Medium; Large/Large; Medium/Small; Medium/Medium; Medium/Large; Small/Small; Small/Medium; Small/Large).



Source: research data.

Furthermore, there were no differences in the predation rate of different sizes of mosquito larvae in each size class of tadpoles; i.e. regardless of their size, tadpoles feed on mosquito larvae of different sizes (Table 3).

Table 3. Post- hoc Tukey test results. (Tadpole size/Larvae size. La: large; Me: medium; Sm: small.)

Treatment	La/Sm	La/Me	La/La	Me/Sm	Me/Me	Me/La	Sm/Sm	Sm/Me	Sm/La
F	4.333	3.750	4.250	2.250	1.818	1.461	1.272	1.166	4.166
La/Sm									
La/Me	0.963								
La/La	1.000	0.985							
Me/Sm	**0.002**	0.083	0.004						
Me/Me	**0.000**	0.008	**0.000**	0.995					
Me/La	**0.000**	**0.000**	**0.000**	0.804	0.998				
Sm/Sm	**0.000**	**0.000**	**0.000**	0.616	0.981	0.999			
Sm/Me	**0.000**	**0.000**	**0.000**	0.445	0.938	0.999	1.000		
Sm/La	**0.000**	**0.000**	**0.000**	**0.012**	0.153	0.468	0.767	0.857	

Source: research data.

Note: bold values: $p<0.05$.

There were no differences among water temperature (mean = 24.8 °C ± 0.10, range: 24.6 – 24.9 °C; 8 boxes) values in the plastic boxes during the experiment ($F(7) = 0, 56$; p= 0,782). Thus, the water temperature of the plastic boxes did not affect the observed predation rates.

2.4 DISCUSSION

We observed that the tadpoles of *Phyllodytes luteolus* were physically able to prey on mosquito larvae. This is the first time that this interaction for bromeliad phytotelmata amphibians was recorded experimentally, although predatory behavior had already been observed before in several tadpole species: Caldwell (1998) observed, under field conditions, that *Adelphobates casteneoticus* and *Dendrobates auratus* tadpoles attacked and killed many other heterospecific tadpoles. Many species have carnivore/cannibal tadpoles (e.g. *Spea bombifrons*; *S. hammondii*; *S. multiplicata*). Some representatives of Asian-African genera (e.g. *Hoplobatrachus occipitalis*; *H. tigerinus*; *H. chinensis*; *H. crassus*) have reversed the main ecophysiological adaptation of tadpoles (filter-feeding) and became secondary carnivores (POLIS; MYERS, 1985). Tadpoles also can be opportunistic predators when food is scarce, *Lithobates sylvaticus* tadpoles were observed preying on *Ambystoma maculatum* embryos that contained abundant yolk reserves (PETRANKA et al., 1998). Studies have shown that some tadpoles were reported to prey on mosquito larvae where they are the only food resource available (RAGHVENDRA et al., 2008).

We observed that large tadpoles were able to prey on larvae of any size, while small tadpoles predated only on small larvae. Additionally, predation was slower and less efficient in Small tadpoles than in Medium and Large tadpoles. Thus, body size of predator and prey played an important role in natural interspecific relationships, as observed by Marian (1983): Smaller tadpoles prefer to prey on smaller preys, while larger tadpoles prefer to prey on larger preys. Spielman and Sullivan (1974) reported that larger tadpoles are able to destroy greater number of mosquito larvae while smaller tadpoles only are able to prey on small mosquito larvae. Our results suggest that medium and large tadpoles are able to prey on mosquito larvae with more efficiency than small tadpoles. Body size indirectly affects the outcome of predator-prey events. According to the results found by Marian (1983) the number of prey consumed increased according to the body size of the predator. For example, in the interaction between dragonfly naiads and tadpoles, the size of naiads positively affects predation rates on small tadpoles, while as tadpole sizes increase, the number of preyed tadpoles decreases (TRAVIS et al., 1985; BABBIT; TANNER, 1998). For Dendrobatidae and Toxorhynchitinae body size determines who will be the prey and the predator: smaller tadpoles were killed and eaten by Toxorhynchitinae while larger tadpoles killed mosquito larvae (CALDWELL, 1993).

Tadpoles of some species, as *Pseudacris maculata*, did not exhibit active predation in laboratory experiments. Notwithstanding, in natural habitats where *P. maculata* tadpoles were present mosquito larvae were never abundant (SPIELMAN; SULLIVAN, 1974) while *Hoplobatrachus tigerinus*, for example, are considered active predators of all larval instars of *A. aegypti*, each individual consuming approximately 29 mosquito larvae per day (MURUGAN et al., 2015). Another good example is that *Spea hammondii* tadpoles exhibit active predation in shallow borrow pits and in a laboratory experiment preyed 75 Culicinae larvae in five and one-half hours (BARBER; KING, 1927), thus, highlighting the importance of biologic control. These concordant findings suggest that tadpoles of different species, which use different habitats around the world, may significantly control mosquito larvae populations and avoid mosquito-borne disease spread, but the effectiveness of these interactions depends on the body sizes of the tadpoles involved. Tadpoles and mosquito larvae feed on various types of organic matter, debris, bacteria and protozoa. With all these concordant findings we would like to emphasize the need for further studies on the ecological services rendered by tadpoles, and the necessity to know more about the biology and ecology of *Phyllodytes luteolus* tadpoles.

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3 Can tadpoles of *Phyllodytes luteolus* act as biologic control agents of mosquito larvae in tank bromeliads?

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RESUMO

No Brasil, os mosquitos da família Culicidae transmitem várias doenças infecciosas, tais como Dengue, Chikungunya, Zika e Malária. O método mais utilizado para o controle dos mosquitos é o químico. Por serem grandes reguladores de larvas de mosquito eles podem ser usado como controle biológico, como alternativa ao método químico, já que os pesticidas também podem causar danos à saúde humana e ao meio ambiente. A presença de predadores dentro das comunidades mantém as populações de presas em níveis baixos. Mostrando que os girinos podem ser predadores ativos e reguladores de comunidades de presas, como larvas de mosquito que são vetores de doenças virais. Nesta pesquisa, verificamos que girinos com maior tamanho corporal podem atuar como controladores das populações de larvas de mosquitos em bromélias de habitats seminaturais.

Palavras-chave: Predação. Competição, Fitotelmata. Dieta. Comunidades.

ABSTRACT

In Brazil, Culicidae mosquitoes spread several infectious diseases as Dengue Fever, Chikungunya, Zika and Malaria. The mostly applied method for mosquitoes control is chemical, although tadpoles can be great mosquito larvae regulators, and can be used as biocontrol, as alternative to the most applied chemical method. While pesticides can also cause damages to human health and to the environment the presence of predators within communities maintains prey populations at low levels. This means that tadpoles can be active predators and regulators of prey communities such as mosquito larvae which are vectors of viral diseases. In this research we show that tadpoles with larger body size can act as biocontrol of mosquito larvae population in bromeliads in semi natural habitat.

Keywords: Predation; competition; phytotelmata, diet; community

3.1 INTRODUCTION

Traditional theories on population dynamics suggest two kinds of interspecific interactions that structure communities: Competition and Predation. Their combination produces the community structure and species equilibrium (MGUIRE JR, 1971; KITCHING, 2000). Predation significantly affects population dynamics in prey community, especially in aquatic systems (FINCKE et al., 1997).

Different environments can provide specific conditions and resources, which in turn will influence the structure and composition of their communities (ARMBRUSTER et al., 2002). The environments provided by phytotelmatas are aquatic habitats located in terrestrial or semi-terrestrial ecosystems as well as forests and swamps. Five major recognized classes of phytotelmata habitats are: water-filled tree holes, plant axils, pitcher plants, tank bromeliads and bamboo internodes (KITCHING, 2000).

Bromeliads are structurally complex plants that offer ideal microhabitat for their animal community (SILVA et al., 2010). The tank shaped axils of these plants collect leaf litter and rain water, thus providing both nutrient reserves and spatial refuges to a rich fauna of vertebrates and invertebrates (ARMBRUSTER et al., 2002). Among vertebrates, amphibians use bromeliads in two ways: bromeliculous species only use bromeliads for foraging or as refuge from adverse environmental conditions; bromeligenous species use bromeliads over their entire life span, thus depending on bromeliads to complete their whole life cycle (PEIXOTO, 1995). *Phyllodytes luteolus* is a bromeligenous species mainly found in resting areas inside tank bromeliads (PEIXOTO, 1995). Their feeding habits are known only for adults, its diet being composed exclusively by colonial arthropods as Formicidae and Isoptera (MOTA-TAVARES et al., 2016), however, the alimentary habits, and trophic role of their tadpoles is still unknown.

The presence of predators within communities maintains prey populations at low levels. Tadpoles may be active predators and regulators of prey communities such as mosquito larvae which are vectors of viral diseases (JULIANO, 2009). Most anuran tadpole species are herbivores, detritivores, and eat some microorganism as bacteria (ALTIG et al., 2007). Nonetheless, tadpoles of some species (e. g. *Scaphiopus*, *Ceratophrys*, *Dendrobates*, *Lithobates*) can prey upon larvae of other anurans (WASSERSSUG et al., 1981; CRUMP, 1983; PETRANKA et al., 1994; CALDWELL, 1993), embryos of salamander (PETRANKA et al., 1998), mosquito larvae and other invertebrates (TRAVIS et al., 1985; CALDWELL, 1993; BABBIT; TANNER, 1998; SABAGH et al., 2012; MURUGAN et al., 2015).

Many vertebrate and invertebrate groups can be found living inside tank bromeliads (e. g., Araneae, Oligochaeta, Odonata, Diptera, Gastropoda, Scincidae and Hylidae; ARMBRUSTER et al., 2002). Most aquatic insects (except mosquito larvae) can move between compartments by traversing the juncture between overlapping leaves (SRIVASTAVA, 2006). Other species use phoretic strategies to be carried from one bromeliad to another by amphibians (e.g. LANTYER-SILVA et al., 2016). Little is known on types of preys consumed by tadpoles in phytotelmata environments, and nothing is known for tadpoles of *P. luteolus* diets. However, it is possible that mosquito larvae are part of the diet of these organisms. Frequently, tadpoles are understudied when compared to other consumer groups as fishes or macroinvertebrates in freshwater ecosystems. Some classic tadpole diet studies provide only information about their feeding habits and functional roles, but the trophic roles of various species of tadpoles remain unknown (ALTIG et al., 2007).

In Brazil, Culicidae mosquitoes spread several infectious diseases as Dengue Fever, Chikungunya, Zika and Malaria. The mostly applied method for mosquitoes control is chemical (MACORIS et al., 1999). Studies already have shown that *Aedes aegypti* larvae can be found in bromeliads near urban areas (GONÇALVES; MESSIAS, 2008; MOCELIN et al., 2009), leading locals to destroy the bromeliads that are habitat of other species (as *P. luteolus*). Tadpoles can be great mosquito larvae regulators (BARBER; KING, 1927; MARIAN et al., 1983; MURUGAN et al., 2015; SARWAR, 2015), and can be used as biocontrol, as alternative to the most applied chemical method. By repeatedly applying insecticides mosquitoes can become more and more resistant to pesticides (MACORIS et al., 1999), while pesticides can also cause damages to human health and to the environment (HEMINGWAY; RANSON, 2000), leading predator species as *P. luteolus* to death (EGEA-SERRANO; SOLÉ, 2016). In this context, our objective is to assess if *Phyllodytes luteolus* tadpoles are able to exclude or control the mosquito population in tank bromeliads located in semi-natural habitats. We expect to find lower mosquito larvae abundances in tank bromeliads with the presence of *P. luteolus* tadpoles and larger mosquito larvae abundances in bromeliads without tadpoles.

3.2 MATERIAL AND METHODS

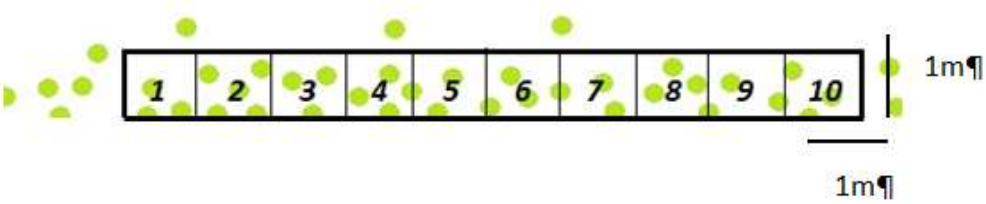
3.2.1 *Study area*

We conducted the study with bromeliads of the species *Aechmea blanchetiana* (Baker) L.B.Sm. found in three areas located in Ilhéus municipality, Bahia, Brazil (S 15°37´09.2”, W 95 039°51´55.5”; S 15º05´13.2”, W 038º59’55.1”, and S 15º4’23.75”, W 38º59’55.51”). These places are altered Restinga areas with anthropic influence where bromeliads were planted forming clusters.

3.2.2 *Sampling procedures*

We randomly set as many as necessary plots (10x1m), divided in 10 quadrants (1x1m) (Figure 1). Following, we randomly selected five quadrants. In each quadrant, we enumerated all bromeliads with permanent marker and randomly selected only one bromeliad. We sampled five bromeliads in each plot. We replicated these sample method in nine parcels, three in each area, totalizing 90 bromeliads sampled.

Figure 1. Study area scheme, representing the sampling plot with the smaller quadrats.



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We collected the water with biological material from the central tank and from one axil. We only considered the samples with presence of *P. luteolus* tadpoles and/or mosquito larvae. Samples without any of the focus species were reconsidered and resampled after a new raffle. To collect the material, we sucked with a plastic hose with 1 cm of diameter. This collection method was performed always by the same person. The collected material was immediately placed in individual plastic jars of 125 ml duly identified (Tn: transect number; Bn: bromeliad number; CT: central tank; An: axil number), and preserved with formalin 10%. The jars with collected material were taken to the laboratory at the Universidade Estadual de Santa Cruz (Ilhéus, Brazil). At the laboratory we quantified the number of mosquito larvae, presence or absence of *P. luteolus* tadpoles from each sample and measured all the tadpoles collected in each sample.

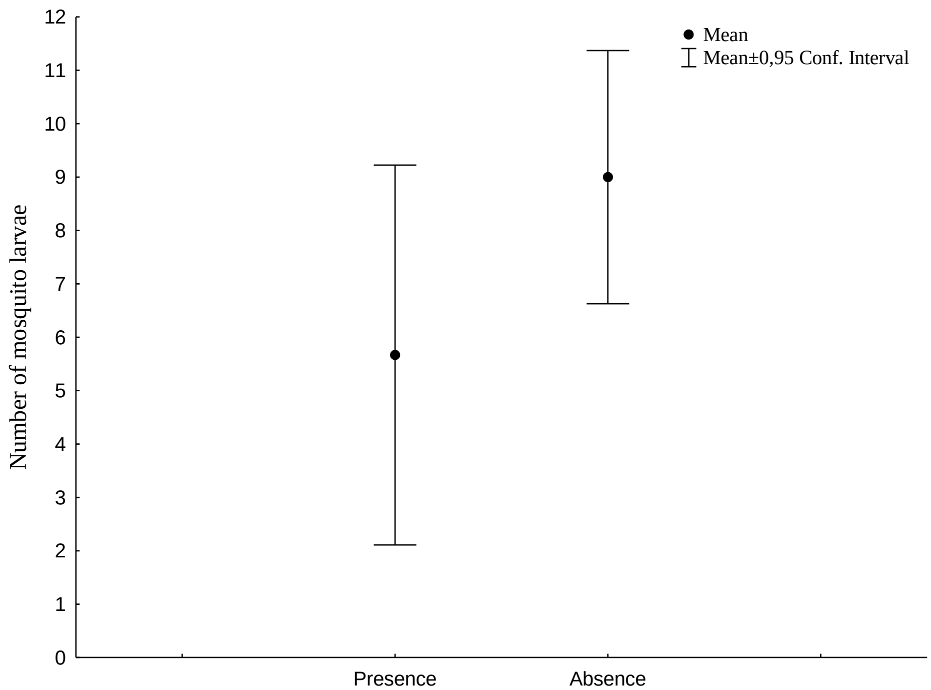
3.3.3 *Statistical analyses*

We performed a *t-test* to compare the abundance of mosquito larvae in sites with presence or absence of *Phyllodytes luteolus* tadpoles. Using only data from sites where tadpoles were present, we made a simple linear regression to evaluate if the mosquito larvae abundance was influenced by tadpole abundance. We measured all tadpoles collected and with this size data (for sites with more than one individual we calculated sizes' mean) performed a simple linear regression between tadpoles size and mosquito larvae abundance.

3.3 RESULTS

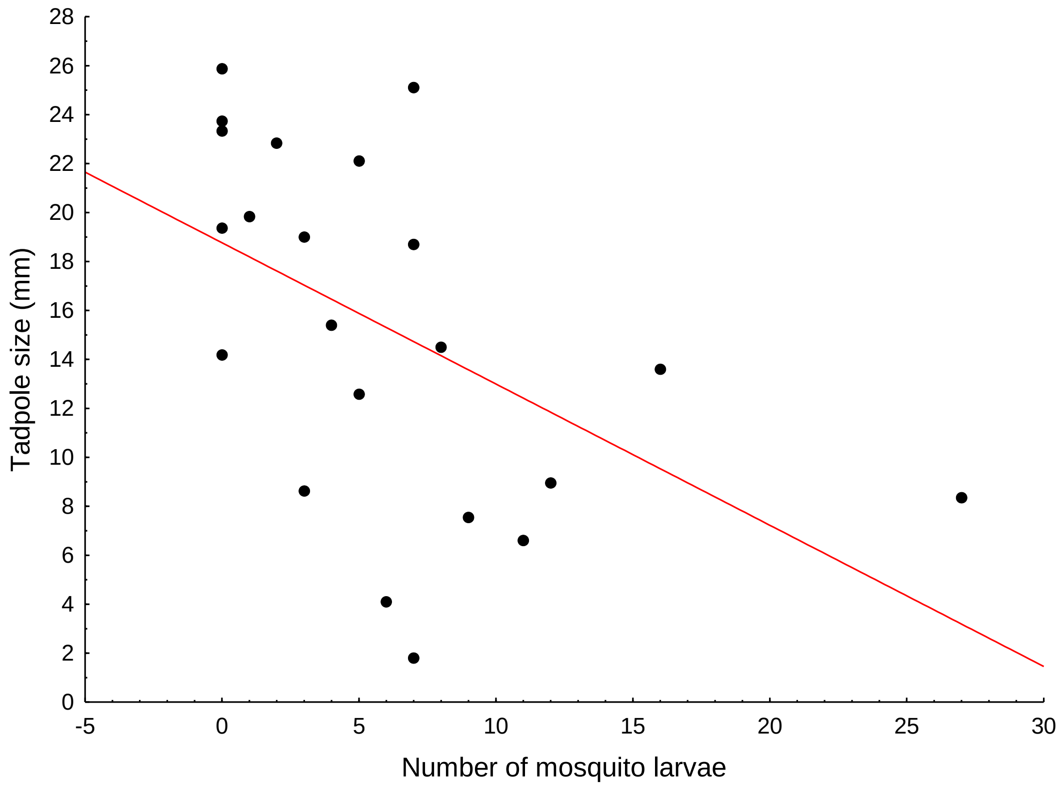
The *t-test* showed no difference between sites where tadpoles were present or absent (t= -1.552, d.f. = 76, p= 0.124) (Figure 3).

Figure 3- Relationship between the number of mosquito larvae and presence/absence of tadpoles.



The simple linear regression to compare abundances of mosquito larva and tadpoles did not show a relation between the increase in the number of tadpoles and the abundance of mosquito larvae.. The relation between tadpole size and mosquito larvae abundance had a significant relation, so, sites with larger tadpoles had lower abundance of mosquito larvae, although there was not a huge relation (0.261). Figure 4 shows a trend that larger predators have greater potential to control prey populations (p= 0.014).

Figure 4- Relation between tadpoles size and mosquito larvae abundance.



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In the collected samples we quantified only the Culicidae larvae as potentially transmitters of viral diseases.

3.4 DISCUSSION

Our results suggest that tadpole body size influenced the mosquito larvae density in bromeliads. We did not found density dependent effects on Culicidae larvae, there was no difference in mosquito larvae abundance between sites where tadpoles were present or absent, neither relation between increasing tadpole density and decreasing mosquito larvae density.

Possibly, the great abundance of mosquito larvae in bromeliads with the presence of small tadpoles may have occurred due to the order of colonization of the environment, that is, the eggs of Culicidae may have been deposited before the spawning of *P. luteolus*, resulting in an interspecific competition for resources. As many species of tadpoles are herbivores, detritivores or suspensory feeders, this may explain the competition between smaller tadpoles and mosquito larvae (HAGMAN; SHINE, 2007). It is important to consider the temporal overlap in the reproductive season of frogs and mosquitoes (HAGMAN; SHINE, 2007), to better understand the dynamics of predation and competition between tadpoles of *P. luteolus* and mosquito larvae.

Previous studies have shown that competition effects between tadpoles and mosquito larvae can affect pupations (for mosquito larvae), and body size for tadpoles (MOKANI; SHINE, 2002). With higher mosquito larvae density tadpoles can exhibit body size reduction due to competition for resources, which prevents tadpoles from growing and being physically able to prey on mosquito larvae (MORIN et al., 2012)

Even for *Phyllodytes luteolus* being a species with prolonged reproductive season, rain plays an important role in its reproductive habits. Therefore, the dry season may have affected our samplings, since many of the bromeliads sampled did not contain water in their axils, nor in their tanks. This lack of water in bromeliads may influence the low amount of *P. luteolus* tadpoles collected. There was not any difference in mosquito larvae density between bromeliads with presence or absence of tadpoles. However, we found a tendency in mosquito larvae density decrease where tadpoles were larger. Studies with other animal groups suggest that body size influences in the predator effectiveness. As showed by Marian (1983) in a study with *Hoplobatrachus tigerinus* tadpoles as body size increases the number of prey consumed also increases.

Hagman and Shine (2007) demonstrated that mosquito larvae are negatively influenced by tadpole presence. In small waterbodies the tadpoles of cane toad can suppress growth and development of mosquito larvae. Under experimental conditions, mosquito larvae are negatively affected by the presence of tadpoles (HAGMAN; SHINE, 2007). Tadpoles can reduce mosquito populations through predation of the larvae, however the removal of predators as a side effect of pesticides may increase the outbreak of viral diseases (SARWAR, 2015). This previous findings show that more field experiments should be performed to assess the relation between tadpoles and mosquito larvae.

3.5 ACKNOWLEDGEMENTS

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3.6 APPENDIX – A

Links to predations movies:

https://vimeo.com/177309039

https://vimeo.com/177316114