Predatory Activity of Aquatic Insects (Odonata: Libellulidae and Coleoptera: Hydrophilidae) on tadpoles of *Rhinella* sp. and *Physalaemus* sp. under laboratory conditions

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Abstract

Predation regulates the functioning of communities and affects the population dynamics of organisms in the environment. The main aquatic predators are represented by invertebrates of the Insecta class, which feed on vertebrate and/or invertebrate organisms. Among vertebrates, tadpoles are the most consumed. This experiment studied the predatory activity of aquatic insects (Odonata: Libellulidae and Coleoptera: Hydrophilidae) on *Rhinella tadpoles* sp. and *Physalaemus* sp. in the city of Capitão Poço, Pará, Brazil. The experiment had six treatments performed in two phases (day and night). Significant differences were observed only between treatments 1 (control) and 2 (Libellulidae) (F= 7.21; p= 0.00). Insect predatory activity was performed in both phases, with no significant differences (F= 1.33; p= 0.26). The success of the Libellulidae family is related to the strategies and morphological aspects that their larvae use in the act of predation. In contrast, the Hydrophilidae family, represented by adults in this experiment, has characteristics that may have contributed to the low predatory activity observed, such as: base diet consisting of algae and organic matter.

Keywords: Amphibians, aquatic invertebrates, interspecific interaction.

Atividade predatória de insetos aquáticos (Odonata: Libellulidae e Coleoptera: Hydrophilidae) sobre girinos de *Rhinella* sp. e *Physalaemus* sp. em condições laboratoriais

Resumo

A predação regula o funcionamento das comunidades e afeta a dinâmica populacional dos organismos no ambiente. Os principais predadores aquáticos estão representados pelos invertebrados da classe Insecta, que se alimentam de organismos vertebrados e/ou invertebrados. Dentre os vertebrados, os girinos são os mais consumidos. Este experimento estudou a atividade predatória dos insetos aquáticos (Odonata: Libellulidae e Coleoptera: Hydrophilidae) sobre girinos de *Rhinella* sp. e *Physalaemus* sp. na cidade de Capitão Poço, Pará, Brasil. O experimento contou com seis tratamentos realizados em duas fases (dia e noite). Foram observadas diferenças significativas somente entre os tratamentos 1 (controle) e 2 (Libellulidae) (F= 7,21; p= 0,00). A atividade predatória dos insetos foi realizada em ambas as fases, não havendo diferenças significativas (F= 1,33; p= 0,26). O sucesso da família Libellulidae está relacionado as estratégias e aspectos morfológicos que suas larvas utilizam no ato de predação. Diferentemente, a família Hydrophilidae, representada por indivíduos adultos neste experimento possui características que podem ter colaborado na baixa atividade predatória observada, tais como: alimentação base composta por algas e matéria orgânica.

Palavras-chave: Anfíbios, invertebrados aquáticos, interação interespecífica.

Introduction

Communities of organisms interact in a variety of ways in the environment, both in abiotic and biological ways Begon *et al* 2006, Begon & Townsend 2020). These interspecific relationships control and/or maintain energy flow and nutrient cycling in the ecosystem (Ricklefs 2001, Laundré *et al* 2014).



Among these relationships, predation is considered one of the most important ecological events, since it influences both indirectly and directly the functioning of the present populations (Breviglieri *et al* 2013, Laundré *et al* 2014, Cinel *et al* 2020).

Brazil has the greatest richness and diversity of anuran

amphibians in the world, with approximately 1144 cataloged species (Segalla *et al* 2021). Due to their varying sizes and abundance in nature (Duellman & Trueb 1994, Pombal JR 2007, Wake & Koo 2018), they are consumed by various animals, from small invertebrates to vertebrates (Strussmann & Sazima 1993, Wells 2010). Tadpoles, in turn, are the most affected by predation pressure from aquatic insects, due to their size and abundance in temporary aquatic environments (Gunzburger & Travis 2004, Touchon & Vonesh 2016), and may suffer significant impacts on their populations thanks to this predatory pressure mainly in the breeding season (Jara 2008, Touchon & Vonesh 2016).

Within the structure of organization of temporary freshwater aquatic environments, the main organisms that act as predators are aquatic invertebrates, represented mainly by arthropods of the Insecta class (e.g. beetles, ants, water cockroaches and Odonata larvae) (Wellborn *et al* 1996, Bertoluci *et al* 2013, Petroni 2020). It is known that these organisms have a diversified diet that includes both other aquatic invertebrates and juveniles and larvae of vertebrates, such as tadpoles and fry (Ferreira-Júnior et al 2014).

Among aquatic insects, organisms of the orders Odonata and Coleoptera are present in various aquatic environments, such as rivers, lakes and temporary pools (Ferreira-Júnior *et al* 2014). The Order Odonata consists of approximately 5680 species distributed across all continents, except Antarctica (Kalkman *et al* 2007) of these, approximately 828 species are found in Brazil and are arranged in 14 families and 140 genera (Costa *et al* 2012). Their larvae are especially known for their high predatory capacity and for being one of the coexisting predators of amphibians (Júnior *et al* 2011).

In turn, the order Coleoptera constitutes the richest group in terms of species of the entire Kingdom Animalia, with more than 385,000 described species. They are grouped into four suborders: Adephaga , Myxophaga , Polyphaga and Archostemata and although most beetles are aerial, a small percentage use the aquatic environment as habitat (Slipinski *et al* 2011, Ferreira-Jr *et al* 2014). The Hydrophilidae family (suborder: Polyphaga), whose representatives are aquatic, has about 2,840 species and 169 genera (Short & Fikacek 2011). This family has eating habits involving decomposing material, however some species may also feed on other organisms, such as: tadpoles and aquatic invertebrates (Ferreira-Jr *et al* 2014).

In this study, two aquatic invertebrates were used, Odonata larvae and Coleoptera adults, seeking to clarify some information regarding the predatory activity involving invertebrates, given that it is very difficult to observe these activities in a natural environment (Pombal Jr 2007). Thus, the experiment aimed to study the predatory activity of these two families of aquatic insects (Odonata: Libellulidae and Coleoptera: Hydrophilidae) in relation to tadpole species of the genera *Rhinella* sp. and *Physalaemus* sp. under laboratory conditions.

Material and Methods

Study area

The experiment was carried out at the Laboratory of

Ecology and Conservation of the Amazon (Laboratório de Ecologia e Conservação da Amazônia: LABECA-CCP) at the Federal Rural University of the Amazon (UFRA-CCP) located in the municipality of Capitão Poço, northeast of Pará (-1.74833333333 and -47.061666667).

Sampling procedures

The samples were collected in February and March 2017, the local rainy season (Moraes *et al* 2005, Santos *et al* 2021a,b, Santos *et al* 2022), in temporary puddles in urban and rural regions and in the sewage treatment plant in the city of Capitão Poço. With the aid of small nets (mesh-2.0 mm) tadpoles of *Physalaemus* sp. and *Rhinella* sp. and specimens of aquatic insects belonging to the families Libellulidae (Odonata) and Hydrophilidae (Coleoptera). All organisms were packed separately in polyethylene containers with a capacity of 1L and transported to the laboratory, where the experiment was carried out.

Laboratory procedures

Aquatic insects and tadpoles were identified using specialized taxonomic keys (e.g. Ferreira-Jr *et al* (2014) and Ferreira-Júnior *et al* (2014) for aquatic insects; and Pezzuti (2011) and Hero (1980) for tadpoles. The scientific experiment was organized into three treatments (T1- Control without predator; T2- Libellulidae predator; T3-Hydrofilidae predator) with eight replicates each, being carried out in two phases (photoperiod): Phase 1 - Day (10 am to 18h) and Phase 2 - Night (00h to 08h) (Figure 1). To carry out the experiment, a polyethylene basket with a capacity of 7 liters was used. In the basket containing 1.5 liters of dechlorinated water, 20 tadpoles of a of the species.

Treatment 1 (control) was carried out to verify the mortality conditions of the tadpoles in the absence of the predator and the possible effects with the water used (dechlorinated tap water). In treatment 2, in each replica, 1 predator of the Libellulidae family was used together with 20 tadpoles of one of the species in each basket; and in treatment 3 in each replica, 1 predator of the Hydrophilidae family was used for the 20 tadpoles. At the end of each photoperiod, the water was changed and the remaining tadpoles that were alive were counted, noted down and reused in the replicas.

Data analysis

After the end of the experiment, the data obtained were digitized and submitted to tests of normality and homoscedasticity of variances, using the *Shapiro-Wilk test* and *Cochran's C test*, respectively, for an adequate choice of subsequent tests (whether parametric or non-parametric). *One-way* and *factorial* ANOVA analysis of variance (in this case, treatment x period) was used to study the effect of treatments, where significant differences between factors were recorded whenever (p=0.05). *Tukey* 's test was used as a method for comparing means a posteriori whenever significant differences were observed.

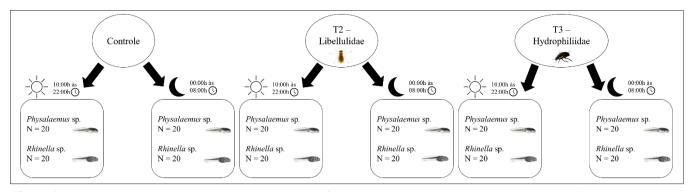


Figure 1. Scheme used to carry out the experiment. From left to right: treatments 1 (control), treatment 2 (Libellulidae predator) and treatment 3 (Hydrophilidae predator), respectively); number of *Physalaemus tadpoles* sp. and *Rhinella* sp. available in each treatment according to the photoperiod (morning and evening).

Results and Discussion

We provided 640 tadpoles for each family of aquatic insects, 320 of which belong to the genus *Physalaemus* and 320 from *Rhinella*. The Libellulidae family preyed on 107 *Physalaemus* tadpoles and 67 of *Rhinella*, consuming an average of 6.68 and 4.18 tadpoles, respectively, while the family Hydrophilidae preyed on 105 and 7, consuming an average of 6.56 and 0.43 tadpoles per day.

Significant differences (F= 7.21; p= 0.00) were observed only between treatments 1 (control – no predator) and 2 (predator – Libellulidae). This difference did not occur with treatment 3 (predator - Hydrophilidae) (Figure 2). Despite this, the family Libellulidae preyed on both species of tadpoles.

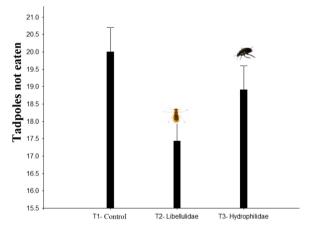


Figure 2. Analysis of variance ANOVA (one-way) for the Treatments (T1-control; T2-Libellulidae and T3-Hydrophilidae) according to the total number of uneaten tadpoles.

In contrast, the Hydrophilidae family consumed more tadpoles of the species *Physalaemus* sp. Thus, differences were observed according to the species consumed (F= 4.73; p= 0.03), where tadpoles of *Physalaemus* sp. were the most preyed (Figure 3A). Both families of aquatic insects showed predatory activity in both periods, thus, there was no statistically significant difference (F= 1.33; p= 0.26), although the Libellulidae family preyed slightly more at night compared to Hydrophilidae which foraged equally in both periods (Figure 3A).

3B).

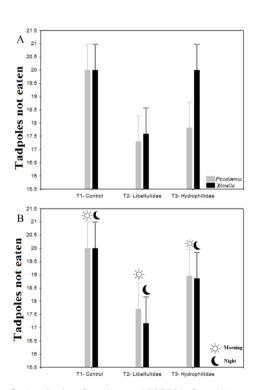


Figure 3. Analysis of variance ANOVA for: (A) species of tadpoles (*Physalaemus* sp. and *Rhinella* sp) and treatments (T1-control; T2-Libellulidae and T3-Hydrophilidae) in relation to the total number of uneaten tadpoles and (B) species of tadpoles (*Physalaemus* sp. and *Rhinella* sp.) between periods (morning and evening) in relation to the total number of uneaten tadpoles

The high predation rates observed for Libellulidae are justified due to the high predatory potential of these organisms, both in the larval and adult stages (Júnior *et al* 2011). Dragonflies have a highly modified buccal structure (lip) that facilitates the act of predation (Rodrigues *et al* 2016). They are considered generalists, presenting voracious behavior, which can directly influence the populations of their prey, feeding on a wide variety of organisms, contributing to 95% of mortality rates in some groups in the natural environment (Neiss & Hamada 2014, Silva-Son 2021). Dragonflies also have the strategy of remaining inert during predation, achieving more success in capturing their prey (Azevedo-Ramos & Magnusson 1999). Because they have this predation capacity, Odonata larvae play a fundamental role in the environment, being used as biological controllers of various organisms (Costa *et al* 2012).

The predatory activity of the Hydrophilidae family was lower compared to that of the Libellulidae family. This can be explained by the fact that the predatory habit is more related to their larvae (Oliveira *et al* 2004). In this study, adults of the family were used, as we did not find a sufficient number of larvae to carry out the experiment. Adults have a more generalist eating habit, involving algae and detritus of organic material (Ferreira-Jr *et al* 2014). Another factor that may have influenced the timing of family predation Hydrophilidae is the fact that beetles move in circles in the water column (Gambale *et al* 2014). This movement is observed by prey that immediately seek ways of defense that prevent them from being preyed on, among these, the escape behavior becomes the most used (Teplitsky *et al* 2005).

Although consumption of hydrophilids was lower than that of the Libellulidae family, the family preyed on more Physalaemus tadpoles sp., possibly due to the fact that tadpoles of Rhinella sp. produce toxic substances (bufotoxin), which makes them unpalatable (Cavalheri 2010). This toxin can act by inhibiting the sodium and potassium pump and also affect the cardiovascular and digestive systems of some organisms, which can even lead to death (Gadelha et al 2015). In addition, tadpoles of this genus also tend to form aggregates, which was also observed in this study, so that predatory activity is lower, facilitating the survival of most individuals (Cavalheri 2010). Aggregation can act in two ways: the confusion effect - where tadpoles disperse in groups, leaving predators confused - and the dilution effect, decreasing the chance of individuals being preved on. The larger the aggregate, the smaller the chance of predation (Uetz et al 2002).

Libellulidae family preyed on both Physalaemus sp. as well as Rhinella sp., showing no preference for any of the genera, proving that their larvae, as well as adults, are natural predators (Neiss & Hamada 2014). Furthermore, the unpalatability of Rhinella tadpoles does not always function as a defense for some predators that have specialized behavioral and physiological strategies, which may have been one of the reasons why Odonata larvae preyed on them more easily (Zanelato et al 2010). In this study, families of aquatic insects were foraging in both study periods. However, the Libellulidae family was more active for foraging at night, even though this difference was not significant. Unlike this study, Mandal et al (2008) observed Odonata nymphs consuming more in the phase they called photophase (day) compared to the scotophase (night). However, Libellulidae nymphs tend to forage especially at night, as they have characteristics that help them capture prey, such as large compound eyes (Neiss & Hamada 2014).

Conclusion

The success of the Libellulidae family may be related to the strategies that their larvae have to help in predatory activity, such as: Modified lip (jagged), large compound eyes and inert behavior. The Hydrophilidae family, represented here by the

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