ARTICLE

Chemical compounds of a Neotropical plant constrain the anti-predator behaviour of sympatric tadpoles

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Abstract

The capacity to identify predator chemical cues is extremely advantageous as it allows prey to avoid the predation sequence from the beginning. However, for aquatic organisms, identification can be constrained by the presence of other substances, such as plant chemical compounds. Despite its ecological implications, there is a lack of knowledge on the potential chemical interference of sympatric plants to the surrounding aquatic fauna. In this context, our study aims to understand the consequences that chemical interference can entail in the anti-predator responses of tadpoles of the Cope's toad (*Rhinella diptycha*). We conducted an outdoor experiment, where we compared the anti-predator responses of *R. diptycha* tadpoles to a natural predator (giant water bug) with and without adding chemicals of a potentially toxic native plant (*Microlobius foetidus*) to the water. Tadpoles showed an increase in grouping behaviour and a reduction in activity in the predator treatment. Moreover, our results indicate that the chemical compounds of the sympatric plant modified tadpole behavioural responses, disrupting grouping behaviour while maintaining reduced activity. These findings help understand the complexity of chemical communication in aquatic habitats and the consequences on animal-plant interactions and conservation.

Keywords Animal-plant interaction · Behavioural response · Chemical interference · Garlic plant · Predator–prey interactions

Introduction

Predation plays a fundamental role in shaping ecology (Smith et al. 2003; Johnson 2010). This selective force occurs through two types of interactions: interactions that results in consumptions and interactions where no consumption occurs (Lima and Dill 1990; Barbosa and Castellanos 2005). When the predator affects prey density by directly consuming that prey, we have what we call consumptive effects, while non-consumptive effects are those where the simple presence of the predator can cause changes in prey's morphology, physiology, and behavior (Matassa and Trussell 2011). Although neglected for a long time, non-consumptive effects can imply major effects on prey, affecting prey behaviour, morphology, and physiology (Van Buskirk and Arioli 2002; Caro 2005).

To perform an anti-predator response, prey need first to detect the predator's presence. While visual stimuli can allow prey to locate predators (Hettyey et al. 2012) and finely assess the threat based on predator size (Chivers et al. 2001), chemical cues can convey other key information, such as predator diet or density (Schoeppner and Relyea 2005). Moreover, chemical cues are usually more effective in aquatic environments (Ferland-Raymond et al. 2010), particularly in turbid ones (Ferrari et al. 2010). However, the presence of other chemical compounds in the water can impair cue detection or risk assessment, which may

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consequently affect the prey response to avoid the predator (Lürling and Scheffer 2007; Ehrsam et al. 2016).

Previous studies have focused on understanding how chemical substances released by exotic plants may affect prey chemical communication and predator–prey interactions (Watling et al. 2011; Iglesias-Carrasco et al. 2017). For example, Burraco et al. (2018) investigated the effects of an exotic species of *Eucalyptus* on the antipredator responses of amphibian larvae and found that leachates of eucalypt leaf litter compromised amphibian antipredator responses and altered their metabolic rate. However, the effects of chemical compounds released by native plant species are scarcely explored. Based on this, we conducted an experiment with three sympatric species native to the Pantanal of Brazil: a prey, a predator, and a plant species. The plant has repellent properties and is allelopathic (i.e., that produces biochemicals influencing growth, survival, development or behaviour of other organisms; Cheng and Cheng 2015). Thus, we aimed to understand the consequences of the chemical interference between soluble compounds produced by a native plant and anti-predator responses of a coexisting toad species. We hypothesised that the presence of chemical compounds from the native plant may impair the detection of the predator by the prey, affecting the tadpoles' anti-predatory behaviour.

Methods

Study system

We conducted an outdoor experiment in temporary ponds of the Pantanal (Brazil, 19º55′10,77″S and 57º47′52,25″W), using *Rhinella diptycha* (Cope 1862) tadpoles as prey, the native giant water bug (Heteroptera: Belostomatidae) as predator, and a native allelopathic plant popularly known as garlic plant (*Microlobius foetidus*). The prey model species is broadly distributed, occurring in a wide variety of habitats, from tropical savannas to forests and even urban areas (Vasconcelos and Colli 2009). During the breeding season (from July to November) this species reproduces in shallow permanent or temporary water bodies (Vasconcelos and Colli 2009).

Giant water bugs–belonging to the Belostomatidae family (Hemiptera)–are among the predators of *R. diptycha* tadpoles. These aquatic predators measure up to 30 mm in length and do not seem to be affected by the neurotoxins observed in the *Rhinella* genus (Cabrera-Guzmán et al. 2012). Giant water bugs are widely distributed and can be found in freshwater habitats such as ponds, lakes, and rivers (De Almeida et al. 2019). Besides, they feed on a wide variety of aquatic animals, ranging from invertebrates to adult and juvenile amphibians, including *Rhinella* tadpoles

(Toledo 2003; De Almeida et al. 2019). Many similar species of giant water bugs occur in the Pantanal, and identification of the exact species used for the study was not possible.

The native plant species displaying chemical defences is the garlic plant, *Microlobius foetidus* (LPWG 2017). This is a semi-deciduous tree that can reach up to 7 m, with a very ramified trunk (with branches almost reaching the ground) and a wide and low canopy. The chemical compounds of the garlic plant, possibly derived from its oils, exhibit high repellent action potential, inhibiting insect and plant growth (Silva et al. 2014). Furthermore, the species releases into the environment chemical substances with allelopathic properties, impairing or impeding the growth of other species in the area (Silva 2014). The basal stem and leaves of this semi-deciduous tree are commonly in contact with groundwater, particularly because this Pantanal area is subjected to seasonal flooding (from May to August; Galdino and Clarke 1995). Tadpoles used for the study were from puddles located less than 300 m from forest areas where this plant is abundant. Nonetheless, during the study period (that took place in the dry season) no tadpoles were observed in the forest puddles where the garlic plant is present.

Experimental design

Using metal stakes and plastic panels in the ponds, we created 30 field experimental enclosures (30×30 cm) isolated from external chemical signals (further details about the study system, experimental design and analysis are provided in the Online Resource 1). Water depth in the ponds ranged from 5 to 7 cm, so water volume of experimental enclosures ranged from 4.5 to 6.3 L, approximately, which allowed a clear view of the bottom of the pond and all individuals in the recordings. We performed trials for three treatments, each with ten replicates: (1) control, (2) predator, and (3) predator plus a garlic plant extract (predator/plant treatment). The control treatment consisted of a dummy predator –a plastic model of similar size, shape, and colour that an average adult water bug– that was carefully deposited with tweezers inside the enclosure to simulate the presence of an individual without a real predation risk. In the predator treatment, a giant water bug was placed inside the enclosure, where it could move freely. Lastly, in the predator/plant treatment, in addition to the presence of the giant water bug, we added 1 ml of garlic plant extract. The extract was prepared by crushing 25 fresh garlic plant leaves directly collected from the tree –in the same phenological state and of similar area (8–10 cm^2)– in 50 ml of water. This amount corresponds to near one leaf per trial, which is a realistic amount that can be found in nature (pers. obs.). We gently poured the plant extract into the enclosure immediately before releasing the predator.

In all treatments (control, predator, and predator plus garlic plant), we first checked for the absence of another possible predator, and then installed the enclosure and the video camera (1 m above the centre of the enclosure) and let tadpoles 10 min acclimation to the enclosure to minimise any disturbance due to the presence of the researcher (Melotto et al. 2021). Then, the plastic model, the predator, or the predator and the plant extract were added, depending on the treatment, and the immediate response of the tadpoles was filmed for 30 s. The whole experimental session was conducted within 1 h (all trials were performed between 8:30 and 9:30 am), and the duration of the trials was selected for two reasons: (1) to minimize daily environmental variation, and (2) because in pilot trials we noticed that behavioural shifts in response to predator presence occur almost instantaneously in this species.

The number of tadpoles inside the enclosure varied between 7 and 29 individuals (mean ± SE: 14.1 ± 0.83 tadpoles per trial), all ranging between Gosner's 26 and 30 developmental stages. To minimise the possibility of pseudoreplication within tadpole individuals, we conducted each trial at least 5 m from the previous one. To prevent contamination from either predator or plant chemicals in the water, we conducted the trials sequentially: first all the control trials ($N = 10$), then all predator trials ($N = 10$) and finally all the predator/plant trials ($N = 10$). No enclosure was allocated to a previously used location, even if for another treatment.

Behavioural variables

For each trial, we recorded two behavioural variables in 30 s videos: (1) grouping behaviour, and (2) tadpole activity. Tadpole grouping behaviour was recorded three times during each trial (at 1, 15 and 30 s), and assessed by means of a grouping index (GI; see Online Resource 1). We calculated activity based on the number of active (swimming, feeding, tail movement) and inactive (immobile, no tail movement) individuals (Austin et al. 2018) at seven timeframes during each trial (at 1, 5, 10, 15, 20, 25 and 30 s after the beginning of the trial). As the total number of tadpoles was different in each trial due to the natural conditions of our experiment, we calculated the proportion of active tadpoles (%). The proportion of active tadpoles was not correlated with the number of tadpoles per trial ($r = 0.083$; Online Resource 5).

Statistical analyses

Due to the hierarchical structure of the data (two behavioural variables measured several times for each trial, and 10 trials per treatment), we analysed the effect of the treatment on tadpole grouping behaviour and activity fitting Linear Mixed Models (LMMs) and Generalised Linear Mixed Models (GLMMs). Time of observation within the video (1 s, 15 s, 30 s) and the ID of the trial were included as random factors, using random slope for observation time and random intercept for the trial ID. Fixed factors comprised the treatment (control, predator, and predator/plant), the time of observation (again), and the interaction between treatment and time.

Given the nature of the data, which followed a normal distribution, we modelled the GI using LMMs. For activity, we fitted a logistic GLMM, due to the nature of the response variable (proportion of active tadpoles; Douma and Weedon 2019). Logistic GLMM are more convenient to fit proportion data than Gaussian models (Douma and Weedon 2019), and the output of the mixed-effects logistic model allows for interpretations of the coefficients in terms of the odds ratio (see Online Resource 1 for more information). Analyses were conducted using the R software (R Core Team 2018). Models were fitted with the 'lmer' and the 'glmer' functions of the lme4 package (Bates et al. 2007).

Results

Rhinella diptycha tadpoles responded to the presence of their potential predator, the giant water bug, by grouping themselves (Online Resource 2) and reducing their activity (Online Resource 3 and 4). Plant extract addition impaired tadpole grouping response while playing no effect on activity (Fig. 1). There was a subtle, but significant interaction ($\beta = -0.025$, $p = 0.041$) between the treatment and the time of the video recording affecting the activity of tadpoles. Thus, the tadpoles tended to decrease their activity with time under the predator treatment, while they mainly tended to increase activity with time under the control treatment (Online Resource 4; Fig. 1).

Discussion

As suggested by studies on non-consumptive effects, predation risk changed the behaviour of *R. diptycha* tadpoles, resulting in a reduction of activity and an increase of grouping behaviour. Furthermore, chemical compounds from the native plant caused changes in the tadpoles' anti-predatory responses. Previous studies have shown that the presence of chemical compounds dissolved in water can alter not only the ability to respond to the presence of predators (Lürling and Scheffer 2007) but also the chemical communication between conspecifics. For example, Burraco et al. (2018) showed that the presence of leachates of eucalypt leaf compromised tadpole anti-predatory responses, affecting activity patterns and decreasing the hiding time of individuals. Iglesias-Carrasco et al. (2017) also described how the exposure to eucalypt extract altered the chemosensory ability of male palmate newts to detect ponds containing female or conspecific alarm cues and to find food. Both studies analysed the effect of long-term exposure (days) to toxic
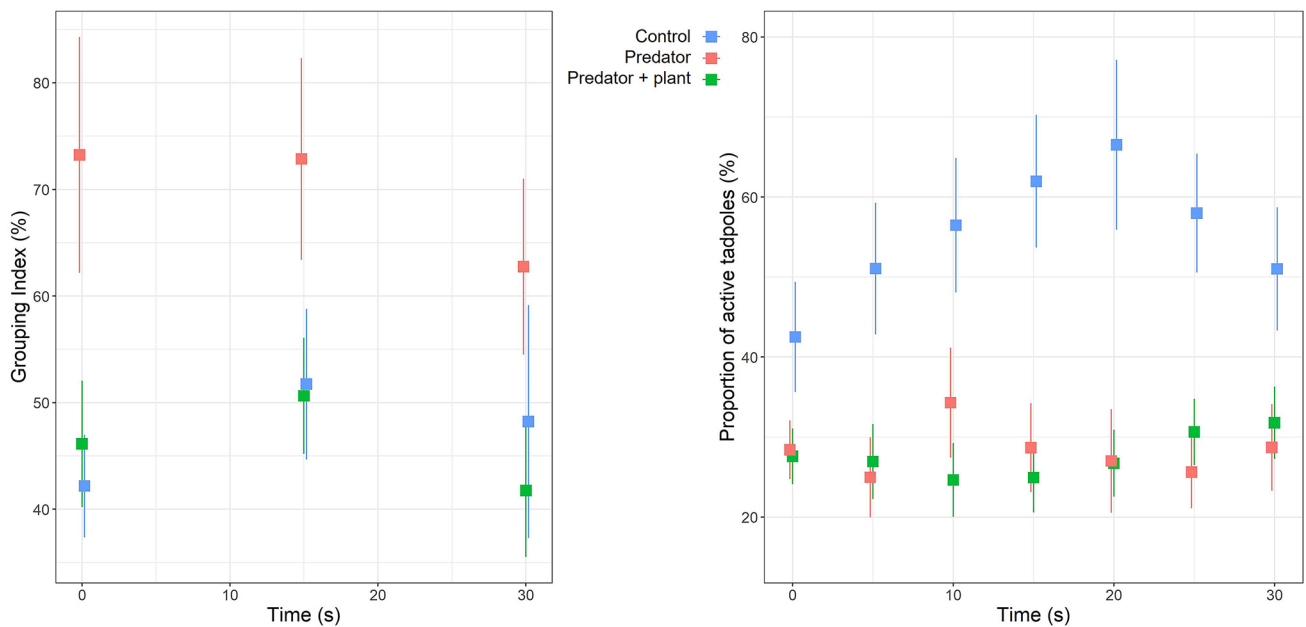
Fig. 1 Results of mixed models showing mean and error bars (CI 95%) of tadpole grouping behaviour (calculated by the grouping index, see Online Resource 1 for details; left plot; LMM) and the proportion of active tadpoles (right plot; GLMMs) in response to the three experimental treatments: (1) control, tadpole exposure to a dummy predator (plastic model of similar size and shape than a giant water bug; blue); (2) predator treatment, tadpole exposure to predator presence (adult giant water bug; red), and (3) predator/plant treatment, tadpole exposure to predator presence and a garlic plant extract (*M. foetidus*; green)

chemicals, while this study is assessing the immediate (seconds) effects of chemical compounds in the water. Besides toxics affecting communication, they found an effect of toxics on prey body condition that ultimately affected anti-predatory responses. In both studies, eucalypt was allochthonous (originated in a place other than where it is found), while in this study we investigated the effects of chemicals released by a native plant.

Although quite speculative, some hypotheses can be formulated to explain how chemical compounds from the native plant can affect tadpole response. For instance, plant chemical compounds could somehow interfere with chemical signals released from startled conspecifics, impairing their ability to aggregate. The activity pattern, which remained the same in both the predator and the predator/plant treatment, is an individual behaviour, while the grouping, which was altered in the predator/plant treatment, is a behaviour that requires communication between other individuals within the group. Thus, an interference in communication would lead to changes in behaviour that require chemical communication, but not in individual behaviours. For example, plant extract could have possibly induced a change in pH, which is known to alter chemical communication (Brown et al. 2002; Turner and Chislock 2010). Another hypothesis could be that the non-aggregation behaviour shown in the presence of chemical plant extracts may confer some adaptive advantage to the prey by tuning their anti-predatory

responses depending on the chemical context. For example, plant extract can signal the presence of leaves where to take shelter, rather than promoting grouping. This could be a plausible explanation since both species share a common evolutionary history, and therefore tadpoles might have developed differential conditional strategies depending on the presence of the plant litter (Phillips and Shine 2006). To better understand how these behaviours change according to different situations, a baseline pre-exposure is necessary, investigating how the group of individuals behaves initially (without any interference) and how the behaviour is changed given a certain stimulus.

As far as we know, this is the first study focused on the effect of chemical components of a native plant species on the anti-predatory behaviour of tadpoles, and although innovative, the study has some caveats that are recognized and should be corrected in future studies. The lack of a fourth treatment –with the plant extract alone– makes some conclusions impossible. For example, it is not possible to disentangle the effect of plant chemical compounds on tadpole behaviour from the effect of predator presence. Furthermore, how tadpoles may alter their behavioural response when only garlic exudates are present remains to be ascertained. Clearly, future experiments should consider the effects of the plant compounds alone on tadpole activity.

An important point of our study is that *M. foetidus* is a native semideciduous plant, and although it is not common

to see ponds with tadpoles occurring directly under *M. foetidus* trees (pers. obs.), in times of flood, the plant branches can touch the ground and old leaves that fall to the ground can be transported, putting the tadpoles in direct contact with the chemical components. Previous studies addressing the interference of plant chemicals on the behaviour of amphibians have assessed exotic plant species, and knowledge on the effects of native plants is scarce (e.g., Watling et al. 2011). Besides being naturally present, *M. foetidus* has been increasingly cultivated for wood, restoration, agroecology, as it contains symbiotic nitrogen-fixing bacteria, and to obtain repellent products. Moreover, our study provides evidence on interference between native and sympatric species, raising interest about possible consequences of an expanding native species for the local ecosystem. We suggest in-depth studies seeking to understand how the relationships between plants and anti-predator behaviours may influence mortality rates, population dynamics and even the occurrence of amphibian species.

Data availability Data will be available by request.

Code availability Code will be available by request.

Declarations

Conflict of interest The author declares that there are no conflicts of interest.

Ethical approval
Ethics authorization: ICMBio/SISBIO #63653.

Consent to participate Not applicable.

Consent for publication Not applicable.

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