**Signals from predators, injured conspecifics, and pesticide
modify the swimming behavior of the gregarious tadpole
Rhinella dorbignyi (Anura: Bufonidae)**

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2021-0075.R1
Manuscript Type:	Article
Date Submitted by the Author:	12-Aug-2021
Complete List of Authors:	<p>Curi, Lucila; Universidad Nacional del Nordeste Facultad de Ciencias Veterinarias, Instituto de Ictiología del Nordeste; Consejo Nacional de Investigaciones Científicas y Técnicas</p> <p>Cuzziol Boccioni, Ana; Consejo Nacional de Investigaciones Científicas y Técnicas ; Universidad Nacional del Litoral , Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (FBCB -UNL-CONICET)</p> <p>Peltzer, Paola; Consejo Nacional de Investigaciones Científicas y Técnicas ; Universidad Nacional del Litoral , Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas.</p> <p>Attademo, Andres ; Consejo Nacional de Investigaciones Científicas y Técnicas ; Universidad Nacional del Litoral , Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas</p> <p>Bassó, Agustín; Consejo Nacional de Investigaciones Científicas y Técnicas ; Universidad Nacional del Litoral , Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas</p> <p>León, Evelina; Consejo Nacional de Investigaciones Científicas y Técnicas ; Instituto Nacional de Limnología, Laboratorio de Biodiversidad y Conservación de tetrápodos</p> <p>Lajmanovich, Rafael; Consejo Nacional de Investigaciones Científicas y Técnicas; Universidad Nacional del Litoral , Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas</p>
Is your manuscript invited for consideration in a Special Issue?:	Not applicable (regular submission)
Keyword:	alarm cues, behavior, chemical signals, burrowing toad, PESTICIDES < Discipline, <i>Rhinella dorbignyi</i>

Signals from predators, injured conspecifics, and pesticide modify the swimming behavior of the gregarious tadpole *Rhinella dorbignyi* (Anura: Bufonidae)

L.M. Curi^{1,2}; A.P. Cuzziol Boccioni^{1,3}; P.M. Peltzer^{1,3}; A.M. Attademo^{1,3}; A. Basso³; E. J. Leon ^{1,3,4} and R.C. Lajmanovich ^{1,3}

¹ Consejo Nacional de Investigaciones Científicas Técnicas (CONICET). Buenos Aires, Argentina.

² Instituto de Ictiología del Nordeste (INICNE). Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste (FCV, UNNE), Sargento Cabral 2139, (CP: 3400) Corrientes Argentina.

³ Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (FBCB -UNL-CONICET), Ciudad Universitaria, Paraje “El Pozo”, RN N° 168, Km 472, (CP: 3000) Santa Fe, Argentina.

⁴ Instituto Nacional de Limnología, Laboratorio de Biodiversidad y Conservación de tetrápodos (INALI -UNL-CONICET), Ciudad Universitaria, Paraje “El Pozo”, RN n° 168, Km 472, (CP: 3000) Santa Fe, Argentina.

Corresponding author: Lucila M. Curi (email: lucilacuri@gmail.com)

Abstract: Tadpoles detect chemical signals released from predators and conspecifics, and those present in the environment, and adjust their behavioral responses. This study evaluated the swimming activity of *Rhinella dorbignyi* (Duméril and Bibron, 1941) tadpoles exposed to chemical signals, including cues from a predator fish *Synbranchus marmoratus* Bloch, 1975 and an injured conspecific; sublethal concentration of insecticide cypermethrin; and their combination. Swimming behavior (total distance moved, average speed, global activity, number of contacts between tadpoles) was evaluated in an individual (1) and groups of different size (3, 5, 7 and 10 tadpoles) using a video-tracking software tool. Predator exposure modified behavioral parameters, reducing encounters with predators and, therefore, mortality. Total distance moved and average speed increased in trials involving 1 tadpole and 3 interacting tadpoles exposed to injured conspecifics, whereas global activity increased in all group sizes, showing that gregarious tadpoles may be affected by alarm cues and their behavior may be disrupted. The insecticide treatments (alone and combined) increased parameters in all group sizes, causing hyperactivity due to its neurotoxic effect. The different responses observed after exposure to alarm cues and environmental signals in the different group sizes modified the normal behavior and the ecological dynamics of gregarious tadpoles.

Key words: Alarm cues, behavior, chemical signals, burrowing toad, pesticides, *Rhinella dorbignyi*

Introduction

In the last decades, studies on the behavior of amphibian tadpoles exposed to multiple stressors suggested not only behavioral shifts but also morphological and physiological modifications (Üveges et al. 2019; Saura-Mas and Benejam 2019). Ecotoxicological studies address several biological traits to determine the toxicity of single or complex chemical mixtures on amphibians (Robles-Mendoza et al. 2011; Cuzziol Boccioni et al. 2020). Moreover, behavioral endpoints proved to be effective biomarkers in contamination studies, since they are influenced by both endogenous and exogenous factors (Mann et al. 2009; Egea-Serrano et al. 2012). In amphibian tadpoles, impairment of swimming capacity has been linked to disturbances in neural, endocrine, and metabolic processes (Widder and Bidwell 2008) and has been used as an indicator of sublethal effects associated with exposure to several toxic chemicals (Denoël et al. 2012; Junges et al. 2017). The analysis of locomotor behavior in aquatic organisms (Juszczak et al. 2007; Henry et al. 2019) is useful to demonstrate the sublethal effect of some toxic compounds, as demonstrated in a few ecotoxicological studies (Peltzer et al. 2013; Egea-Serrano and Tejedo 2014; Da Costa Araujo and Malafaia 2020).

In aquatic environments, chemical signals are a major source of information for animals, which can assess the state of the environment and obtain information about the presence of predators (Mitchell et al. 2017). In addition, chemoreception may be one of the most effective sensory modalities for detecting environmental stressors (Hagman et al. 2009). In natural environments, tadpoles are exposed to a variety of natural and allochthonous chemical substances derived from anthropogenic activities (Hanlon and Relyea 2013). The effect of predation on a community is mainly driven by the ability of predators and prey to detect each other (Thetmeyer and Kils 1995). Chemical communication during predation events consists of signals (Wilson and Lefcort 1993) that can originate from the predators (named kairomones, Vazquez et al. 2017), or chemical cues released by conspecifics under predator attack, named alarm pheromones or alarm cues (Schoeppner and Relyea 2005, Mitchell et al. 2017). Amphibian tadpoles can recognize alarm cues, which alert them of the presence of predation risk; thus, alarm cues confer an adaptive and survival advantage (Kiesecker et al. 1996; Faulkner et al. 2017).

The changes in behavior and/or morphology of tadpoles in response to the presence of predators is known as predator-induced phenotypic plasticity (Benard 2004) and have been frequently studied using anuran tadpoles as a model organism (McCollum and Liemberger 1997; Mogali et al. 2011; Maher et al. 2013). Tadpoles can avoid areas with presence of predators by reducing their activity and changing several biological traits, such as time to reach metamorphosis (Gazzola et al. 2015; Mogali et al. 2016; Üveges et al. 2019), coloration, and morphology (Relyea 2001; Van Buskirk et al. 2003). Both types of signals (predator presence and tadpole injury due to predator attack) produce several ecological adjustments to reduce the risk of predation (Crossland and Shine 2012; Faulkner et al. 2017; Crossland et al. 2019). Moreover, some amphibians, like toads of the Bufonidae family, have epidermal secretory cells (giant cells) that release their secretion only when the skin is injured, eliciting a “scape reaction” in the tadpole school; therefore, these cells play an important role in chemical signaling defense mechanisms against predators (Regueira et al. 2016).

The presence of stress factors can alter the recognition or response to the alarm cues (Burraco et al. 2013). Different studies investigated the interaction between presence of predators and exposure to agrochemicals in anuran tadpoles (Burraco et al. 2013; Moore et al. 2015; Mikó et al. 2017). However, few studies focused on the

synergistic effect of the presence of predators, signals of predation (injuries) from a conspecific, and pesticides in gregarious anuran tadpoles (Sontag et al. 2006; Wells 2007; Wei et al. 2014; Balestrieri et al. 2019). The deleterious effect of agrochemical contamination on anuran behavior was frequently investigated (see Relyea and Mills 2001; Zala and Penn 2004; Boone et al. 2007). The synergistic effect of different chemical stressors (natural and allochthonous) is environmentally relevant due to the massive amounts of pollutants reaching water bodies and the collapse of aquatic ecosystems worldwide (Bashir et al. 2020; Baseem 2020).

Therefore, this study aimed to evaluate the swimming activity in gregarious tadpoles of *Rhinella dorbignyi* (Duméril and Bibron, 1941) (Anura: Bufonidae) exposed to different signals: a) chemical cues of the predator *Synbranchus marmoratus* Bloch, 1975, b) alarm cues of an injured conspecific, c) sublethal concentration of a common pesticide that occurs in water bodies (cypermethrin), and d) a combination of the chemical stressors at the individual level and in groups of different sizes. We expected greater negative effects of the combination of chemical stimuli on the swimming behavior parameters of these gregarious anuran tadpoles than of each signal individually; we also expect a modification of normal ecological performance of tadpoles due to these alterations.

Material and Methods

Test species

Rhinella dorbignyi (Anura: Bufonidae) tadpoles were used as test organisms. This anuran species is widely distributed in the Neotropical region, with occurrence in Argentina, Brazil, Paraguay, and Uruguay (Ceï, 1980). Adults occur in forests, grasslands, and agricultural ecosystems such as rice plantations (Narvaes et al. 2004). The tadpoles are commonly found in temporary and semi-permanent ponds displaying schooling gregarious behavior during the reproductive period (November to March, Sanchez et al. 2009). In Argentina, this toad species is categorized as “not threatened” (Vaira et al. 2012).

Several fragments of gelatinous egg strings of *Rhinella dorbignyi* were randomly collected from temporary ponds in natural floodplains of the Paraná River (31° 39' 34.7" S, 60° 35' 31.1" W, Argentina) with collection permission of the Ministry of Environment of the Province of Santa Fe, Argentina (EXP. N° 02101-0018518-1). This site was previously found to be unpolluted (Attademo et al. 2014, 2015). The egg strings were immediately (1 h) transported in plastic flasks containing natural water and maintained in laboratory conditions until the eggs hatched (Gosner stage 19, Gosner 1960). Embryos and then tadpoles were maintained in glass aquaria with dechlorinated tap water until they reached Gosner stage 35 (Gosner 1960). Tadpoles were fed boiled lettuce *ad libitum* and water in the aquaria was changed every 48 h. At the start of the experiment, mean total length of tadpoles (9.66 ± 0.24 mm) was measured with a digital caliper (0.001 mm accuracy) and weight (0.09 ± 0.02 g) with digital balance (0.001 g accuracy).

A total of $N = 1040$ tadpoles at Gosner stage 36 were taken from the stock for the study. Tadpoles were treated according to ASIH (2004) criteria and with the approval from the Animal Ethics Committee of the Faculty of Biochemistry and Biological Sciences of the National University of the Littoral, FBCB number 388/06.

Experimental design

Chemical signals

Three chemical signals were selected and tested alone and in combination, as follows: a) cues from the predator fish *Synbranchus marmoratus* (PC), b) cues from injured conspecifics (IC), c) sublethal concentration of the pesticide cypermethrin ($10 \mu\text{g L}^{-1}$, CY), d) combination of signals, as illustrated in Figure 1. In total, eight treatments were performed, including individual and combined treatments, which were applied to different group sizes (see Figure 1).

a- Treatment involving chemical cues from predator

The eel *Synbranchus marmoratus* (Family: Synbranchidae) is widely distributed across Central and South America (from Mexico to northern Argentina, Kullander 2003). This eel species prefers shallow rivers with calm water, streams, lagoons, and estuaries, where it lives in caves (Maggese et al. 1993). Its conservation status is “not threatened” (Daniels and Maíz-Tome 2019). It was selected because it is a potential native predator of anuran tadpoles (Maffei et al. 2011; Junges et al. 2012). Fish juveniles used in predator chemical treatment experiments were collected from an unpolluted pond in the floodplain of Paraná River (Santa Fe province, Argentina; $31^{\circ}42'340''\text{S}$; $60^{\circ}34'60''\text{W}$). During one week, before the start of different trials, similar-sized eels (mean length $\pm$ SD = 24 ± 1.3 cm, mean weight $\pm$ SD = 12.8 ± 1.45 g) were acclimated to bioassay conditions and fed non-experimental tadpoles daily (between 2-3 tadpoles). One day before experimentation, one eel was randomly selected and housed in a flask containing 1 L of clean water to obtain water for each treatment involving the chemical cues from predator (PC treatment). Then, 200 mL of experimental water prepared for each PC treatment was randomly used for the different trials. The procedure was repeated for the four treatments containing PC and for each replicate ($n = 20$).

b-Treatment involving conspecific alarm cues

The alarm cues for each IC treatment were obtained by partially injuring the caudal fin of one tadpole (technique modified from Mitchel and McCormick 2013). This partial lesion was repeated for each treatment involving IC treatment at individual (1) and group sizes (3, 5, 7 and 10 tadpoles) and replicate. This procedure did not compromise tadpole survival. After being injured, the tadpoles were maintained in a petri dish containing 200 mL dechlorinated water for a maximum of one hour to ensure the persistence of the alarm cue in the water (Ferrari et al. 2007). Then, this water with chemical cues from injured tadpoles was used for the present treatment and in combination in the different group sizes. When experiments finished, injured tadpoles were maintained together until they completed metamorphosis.

c-Sublethal insecticide concentration treatment

Cypermethrin was selected due to its massive use in soybean and maize crops in Argentina and its deleterious effects on toad tadpoles at different biological levels (Izaguirre et al. 2001; Casco et al. 2006). Cypermethrin (CAS No. 52315-07-8; water solubility $4\text{--}10 \mu\text{g L}^{-1}$, Biga and Blaustein 2013) is a pyrethroid with a broad spectrum of insecticidal activity based on neurotoxicity. This insecticide has been detected at levels ranging from $3.5 \mu\text{g L}^{-1}$ to $194 \mu\text{g L}^{-1}$ in Argentine surface water (Marino and Ronco 2005). The concentration of $10 \mu\text{g L}^{-1}$ used for CY treatments was selected according to a previous study (Cabagna et al. 2006) and toxic values for other

aquatic organisms (WHO 1989). Cypermethrin was obtained from Argengric, Argentina. The commercial product consisted of 2.5% of active ingredient formulated in aqueous xylene.

Experimental conditions and behavioral endpoints

Individual (one tadpole) and group (3, 5, 7, and 10 tadpoles) behavior responses were recorded for 5 min in a petri dish (15 cm diameter and 2 cm height) containing 200 mL in each chemical treatment. Eight treatments (with five replicates each) were performed in the laboratory, as shown in Figure 1. Dechlorinated water was used as control treatments. The laboratory conditions consisted of $24 \pm 2^\circ\text{C}$ and the digital records were performed between 1400 and 1800 h. Different individuals were used in each trial, treatment, and replicate to ensure a mean of response that can vary at intra-specific and inter-individual levels.

Total distance moved (cm), average speed (cm s^{-1}), and global activity (cm^2) were evaluated individually ($n = 1$) and in interactions of an increasing number of tadpoles ($n = 3, 5, 7$ and 10). Likewise, the number of interactions (contacts between pairs) was also analyzed. These parameters were quantified and analyzed using the Smart video-tracking software (3.0.02 PANLAB HARVARD APPARATUS®).

Statistical analysis

MANOVA (Wilks' lambda multivariate test statistic, λ) was used to determine overall significant differences among treatments involving chemical cues in the response vectors (four behavior parameters: total distance moved, global activity, average speed, and number of contacts). If MANOVA was significant, univariate analysis of variance (ANOVA, F) followed by Tukey or Dunnett post-hoc tests were used for treatment significance. The Shapiro-Wilk test and the Levene median test were used to assess normality and homogeneity of variance of the data, respectively (Zar 1999). Statistical analyses were performed using the IBM software version 18 ($p < 0.05$).

Results

The four variables measuring tadpole swimming behavior were significantly altered after exposure to the different chemical signals in all group sizes: 1 ($\lambda = 0.49$, $F = 2.26$, $p < 0.006$); 3 ($\lambda = 0.51$, $F = 5.86$, $p < 0.0001$), 5 ($\lambda = 0.037$, $F = 12.2$, $p < 0.0001$), 7 ($\lambda = 0.004$, $F = 38.8$, $p < 0.0001$), and 10 tadpoles ($\lambda = 0.016$, $F = 58.3$, $p < 0.0001$). The individual analysis of each MANOVA and the ANOVA for each behavior variable are shown in Table 1S¹.

Total distance moved and average speed

Total distance moved and average speed were the most affected parameters in all the treatments and group sizes (Figure 2, Table 1). Both parameters were higher than control in all treatments; this difference was significantly higher in the CY and CY + PC treatments with 3 and 5 interacting tadpoles than in control. Total distance moved and average speed were also significantly lower than CO in PC and IC + CY + PC treatments with 7 interacting tadpoles (Dunnett post-hoc test $p < 0.05$; Figure 2, Table 1). In the group with 10 interacting tadpoles, these parameters were significantly higher in IC and CY than in control (Dunnett post-hoc test $p < 0.05$; Figure 2, Table 1).

¹ Supplementary material are available with the article through the journal Web site

Global activity

The global activity was higher in groups with 3, 5 and 7 interacting tadpoles exposed to IC, CY, IC + CY, IC + PC, CY + PC, and IC + CY + PC than in control (Dunnet post-hoc test $p < 0.05$, Figures 2 and 3, Table 1). Moreover, the global activity was lower in the group with 10 interacting tadpoles exposed to PC than in control (Dunnet post-hoc test $p < 0.05$, Figure 3, Table 1).

Intraspecific interaction

The number of contacts was higher in the groups with 3 and 5 interacting tadpoles exposed to CY and CY + PC, respectively (Dunnet post-hoc test $p < 0.05$, Figure 2, table 1) than in control. In contrast, in the group with 5 interacting tadpoles, the number of contacts was lower in IC + CY + PC than in control. In the group with 7 interacting tadpoles, the number of contacts was lower than in control in all chemical signal treatments. The number of contacts increased in the group with 10 interacting tadpoles exposed to CY, but it declined in PC with respect to control (Dunnet post-hoc test $p < 0.05$, Figure 2, Table 1).

Discussion

Predator chemical cues

Avoidance behavior is a more common response to alarm signals in Bufonidae than in other anuran families, and is useful to prevent predation (Rödin Mörch et al. 2011). Our results showed a decrease in total distance moved, average speed, global activity, and number of contacts in tadpoles exposed to fish predator chemical cues, principally with a high number of interacting tadpoles. Reduced activity decreases the probability of a random encounter with predators and thus increases survivorship of tadpoles (Chuang et al. 2019; Ramamonjisoa et al. 2019). Under perceived predation risk, tadpoles usually react by changing the level of activity according to the ratio between the amount of predatory cue and conspecific density (Van Buskirk et al. 2011; Gazzola et al. 2018). Our results revealed higher activity in treatments involving 1, 3 and 5 interacting tadpoles, as indicated by the low total distance moved, average speed and global activity with respect to control. In groups with a higher number of interacting tadpoles (7 and 10), the antipredatory strategy observed in this gregarious tadpole consists of reducing their activity instead of escaping. In several other studies involving other species, different species-specific behaviors were described, such as freezing, escape behavior, shelter seeking, avoidance of the predator's vicinity, and colony defense (Wisenden 2000, Mogali et al. 2019, 2020). Moreover, different defense responses to specific alarm signals were described for different predators (Schoeppner and Relyea 2009). Some authors recorded that the presence of predators not only reduced the activity of tadpoles, but also decreased their growth rate (Van Buskirk 2002; McCoy 2007), modifying their development and phenotypes (Benard 2004; Vonesh and Warkentin 2006), and altering their morphology (McCollum and Liemberger 1997; Ferrari et al. 2010; Gomez-Mestre and Diaz-Paniagua 2011).

Conspecific alarm cues

The ability to recognize conspecific alarm cues confers an adaptive advantage by alerting individuals about a risk (such as predator presence) and then eliciting a behavioral response (Faulkner et al. 2017). Alarm cues can provide temporal and spatial information about predation risk, allowing prey to regulate the expression of

inducible defense in several aquatic species (Chivers and Smith 1998). In our results, alarm cues from injured conspecific tadpoles increased total distance moved and average speed in all the groups of interacting tadpoles, being reinforced with the increase of global activity. As recorded by Ferrari et al. (2010), intrinsic and extrinsic factors affect the response to conspecific alarm cues. Similarly, an increased swimming activity was observed when tadpoles were exposed to both alarm cues of predator presence and injured conspecific. Specifically, as mentioned by Schoeppner and Relyea (2005), the additional information provided by the simultaneous exposure to both kairomones (predator presence) and pheromones (from attacked prey) provides complete and effective antipredator defenses in gregarious species. In addition, Rödin Mörch et al. (2011) concluded that in solitary species, the behavior of tadpoles (such as *Rana cascadae* Slater, 1939; *Rana aurora* Baird and Girard, 1852; *Bombina variegata* (Linnaeus, 1758)) is typically unaffected by alarm cues, but in gregarious taxa, avoidance is the most frequent response to alarm cues.

Furthermore, global activity was higher in *R. dorbignyi* tadpoles exposed to injured conspecific cue in all the group sizes evaluated. In the presence of the injured conspecific alarm cues, tadpoles displayed escape and disaggregation behavior; this behavior was indicated by a lower number of contacts and an increased swimming activity, which probably allows tadpoles to reach a safer location, as may occur in natural environments. However, in *Rhinella arenarum* (Hensel, 1867) tadpoles exposed to injured conspecifics, a decrease in individual activity was observed (Jungblut 2012). These authors also found that tadpoles perceive different stimulus intensity or predation risk levels.

Synergisms between agrochemicals and natural signals

The swimming activity of *R. dorbignyi* was also affected by exposure to cypermethrin alone or combined with other chemical signals. This insecticide is lipophilic, which facilitates its fast access to various tissues, and has a high affinity to the central nervous system due to its neurotoxic effect (Anadon et al. 1996), as demonstrated for *R. arenarum* tadpoles (Izaguirre et al. 2000, 2001; Casco et al. 2006). Cypermethrin effects incremented the behavioral parameters (total distance moved, average speed and global activity) in the different group sizes. This effect can be associated with cypermethrin neurotoxicity, with short-term of this pesticide causing excitation. Likewise, several toxic compounds can inhibit cholinesterase activity on non-target organisms, such as fish or amphibians, and produce excitatory locomotion (Peltzer et al. 2013; Sandrini et al. 2013). Cypermethrin prolongs the normally transient increase in sodium permeability of the nerve membrane during excitation, leading to long-lasting trains of repetitive impulses in sense organs and a frequency-dependent depression of the nerve impulse in nerve fibers (WHO 1989). Here, cypermethrin caused hyperactivity, even when predator cues were also present, indicating that individuals were not able to carry out their normal anti-predatory activity. In biological terms, changes in tadpole locomotion are a threat to their survival, since even small changes can limit animals' movements in their habitats and, consequently, reduce their ability to escape from predators (Araújo and Malafaia 2020). Likewise, Relyea and Mills (2001) demonstrated that carbaryl became even more lethal for *Dryophytes versicolor* (LeConte, 1825) tadpoles when combined with predatory stress. The tadpoles of wood frogs exposed to Glyphosate Roundup® sublethal concentrations failed to exhibit antipredator behavior (Moore et al. 2015). That work also provides considerable evidence that injured conspecific cues are deactivated by Roundup, and the tadpoles failed to reduce activity when we mixed the alarm cue solution with this herbicide. The responses can

vary according to the differential sensitivity to the compounds. However, other authors did not find a synergistic effect between predator and chemical contamination (Reeves et al. 2011; Burraco et al. 2013).

When tadpoles were exposed to the combination of the three chemical signals, an increase of global activity in all the group sizes and, therefore, a decrease in the number of contacts between tadpoles, were observed. In this context, it can be assumed that the tadpoles were overstimulated by synergisms of natural and environmental signals. The increase in global activity and decrease in other swimming parameters could be due to signs of cypermethrin toxicity, which acts synergistically with predator signals. Salibian (1992) described spontaneous arrhythmic flutter movements in *R. arenarum* early response to deltamethrin. Similarly, in our trials, such behavior could mean hyperactivity but non-displacement, limited by the predator signals. The overexcitation and continuous spasms of individuals are due to cypermethrin exposure and could limit the normal “escape and disaggregation” and “anti-predatory” behavior caused by conspecific and predator signals, respectively. The alteration of swimming behavior and social interaction represents an indirect impact of the pollutant on population dynamics.

These results contribute to the understanding of how environmental contamination influences the ecological dynamics of tadpoles, principally on the normal antipredatory and gregarious behavior of tadpoles. Due to the increased use of pesticides in agricultural ecosystems, not only the effects of a particular chemical substance but also its synergistic effects with other natural or anthropogenic factors are of ecological concern.

This study demonstrated that swimming behavior parameters of amphibian tadpoles are suitable biomarkers to evaluate the influence of different chemical signals in aquatic environments. The swimming activity as a measurable response of the interaction between exposure to toxicants and to chemical signals provides ecological insight into how contaminants modify intra- and interspecific interactions that ultimately affect the organisms’ performance and survival in the environment. These signals also altered the gregariousness; this effect might have severe consequences on survival of tadpoles in natural environments (Araújo and Malafaia 2020). Further studies are needed to elucidate the interaction mechanism of chemical signals from different sources and how those signals modify the behavioral responses of tadpoles. Overall, the study of those behavior endpoints may contribute to a better understanding of susceptibility features throughout development and natural history of gregarious species of anurans in response to the allochthonous chemicals.

Author Statements

Acknowledgements

To Junges C. for lab assistance and Brasca J. for English editing and comments. This study was supported in part by National Agency for Promotion of Science and Technology, Argentine (ANPCyT FONCyT PICT, N° 1069), and Course of Action for Research and Science Promotion, Argentine (CAI D-UNL, PIC N° 100004 LI).

Competing interest statements

The authors declare that there are no competing or financial interests

Contributors’ statements

RCL and PMP designed the experiments. LMC, AMA performed the experiment. APC, PMP, LMC analyzed the data. LMC, PMP, APC wrote the manuscript. EJJ, AB, AMA revised and wrote the manuscript.

318

319 **Funding statements**

320 This study was supported in part by National Agency for Promotion of Science and Technology, Argentina
321 (ANPCyT FONCyT PICT, N° 1069), and Course of Action for Research and Science Promotion, Argentina (CAI
322 D-UNL, PIC N° 100004LI).

323 **Availability of data and material**

324 The datasets used and/or analyzed during the current study are available from the corresponding author on
325 reasonable request.

326 **References**

327 Anadón, A., Martínez-Larrañaga, M.R., Fernández-Cruz, M.L., Díaz, M.J., Fernandez, M.C., and Martinez, M.A.
328 1996. Toxicokinetics of deltamethrin and its 4'-HO-metabolite in the rat. *Toxicol. App. Pharmacol.* **141**: 8–16.
329 [https://doi.org/10.1016/S0041-008X\(96\)80003-2](https://doi.org/10.1016/S0041-008X(96)80003-2).

330

331 Araújo, A.P.C., and Malafaia, G. 2020. Can short exposure to polyethylene microplastics change tadpoles'
332 behavior? a study conducted with neotropical tadpole species belonging to order anura (*Physalaemus cuvieri*). *J.*
333 *Hazard. Mat.* **391**: 122214. <https://doi.org/10.1016/j.jhazmat.2020.122214>.

334

335 ASIH: American Society of Ichthyologists and Herpetologists. 2004. Guidelines for use of live amphibians and
336 reptiles in field and laboratory research, 2nd edn. Washington, D.C.: Herpetological Animal Care and Use
337 Committee (HACC) of the American Society of Ichthyologists and Herpetologists.

338

339 Attademo, A.M., Peltzer, P.M., Lajmanovich, R.C., Cabagna-Zenklusen, M.C., Junges, C.M., and Basso, A. 2014.
340 Biological endpoints, enzyme activities, and blood cell parameters in two anuran tadpole species in rice
341 agroecosystems of mid- eastern Argentina. *Environ. Monit. Assess.* **186**: 635–649. [https://doi.org/10.1007/s10661-](https://doi.org/10.1007/s10661-013-3404-z)
342 [013-3404-z](https://doi.org/10.1007/s10661-013-3404-z).

343

344 Attademo, M.A., Peltzer, P.M., Lajmanovich, R.C., Cabagna-Zenklusen, M., Junges, C.M., Lorenzatti, E., Aró, C.,
345 and Grenón, P. 2015. Biochemical changes in certain enzymes of *Lysapsus limellum* (Anura: Hylidae) exposed to
346 chlorpyrifos. *Ecotoxicol. Environ. Saf.* **113**: 287–294. <https://doi.org/10.1016/j.ecoenv.2014.12.021>.

347

348 Balestrieri, A., Gazzola, A., Pellitteri-Rosa, D., and Vallortigara, G. 2019. Discrimination of group numerosness
349 under predation risk in anuran tadpoles. *Anim. Cogn.* **22**: 223–230. <https://doi.org/10.1007/s10071-019-01238-5>.

350

351 Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A., and Dar, S.A. 2020. Concerns and Threats of
352 Contamination on Aquatic Ecosystems. *Bioremediation and Biotechnology: Sustainable Approaches to Pollution*
353 *Degradation*. 1–26.

354

- 355 Bassem, S.M. 2020. Water pollution and aquatic biodiversity. *Biodivers. Int. J.* **4**(1): 10–16.
356 <https://doi.org/10.15406/bij.2020.04.00159>.
- 357
- 358 Benard, M.F. 2004. Predator-induced phenotypic plasticity in organisms with life histories. *Annu. Rev. Ecol. Evol.*
359 *Syst.* **35**: 651–673. <https://doi.org/10.1146/annurev.ecolsys.35.021004.112426>.
- 360
- 361 Biga, L.M., and Blaustein, A.R. 2013. Variations in lethal and sublethal effects of cypermethrin among aquatic
362 stages and species of anuran amphibians. *Environ. Toxicol. Chem.* **32**(12): 2855–2860.
363 <https://doi.org/10.1002/etc.2379>.
- 364
- 365 Boone, M.D., Semlitsch, R.D., Little, E.E., and Doyle, M.C. 2007. Multiple stressors in amphibian communities:
366 effects of chemical contamination, bullfrogs, and fish. *Ecol. Appl.* **17**(1): 297–301. [https://doi.org/10.1890/1051-](https://doi.org/10.1890/1051-0761(2007)017[0291:MSIACE]2.0.CO;2)
367 [0761\(2007\)017\[0291:MSIACE\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2007)017[0291:MSIACE]2.0.CO;2).
- 368
- 369 Burraco, P., Duarte, L.J., and Gomez-Mestre, I. 2013. Predator-induced physiological responses in tadpoles
370 challenged with herbicide pollution. *Curr. Zool.* **59**(4): 475–484. <https://doi.org/10.1093/czoolo/59.4.475>.
- 371
- 372 Cabagna, M.C., Lajmanovich, R.C., Peltzer, P.M., Attademo, A.M., and Ale, E. 2006. Induction of micronuclei in
373 tadpoles of *Odontophrynus americanus* (Amphibia: Leptodactylidae) by the pyrethroid insecticide cypermethrin.
374 *Toxicol. Environ. Chem.* **88**(4): 729–737. <https://doi.org/10.1080/02772240600903805>.
- 375
- 376 Casco, V.H., Izaguirre, M.F., Marín, L., Vergara, M.N., Lajmanovich, R.C., Peltzer, P.M., and Soler, A.P. 2006.
377 Apoptotic cell death in the central nervous system of *Bufo arenarum* tadpoles induced by cypermethrin. *Cell Biol.*
378 *Toxicol.* **22**: 199–211. <https://doi.org/10.1007/s10565-006-0174-1>.
- 379
- 380 Cei, J.M. 1980. Amphibians of Argentina. *Monit. Zool. Ital.* **2**: 1–609.
- 381
- 382 Chivers, D.P., and Smith, R.J.F. 1998. Chemical alarm signalling in aquatic predator-prey systems: a review and
383 prospectus. *Ecoscience*, **5**: 338–352. <https://doi.org/10.1080/11956860.1998.11682471>.
- 384
- 385 Chuang, M.F., Choe, M., Kang, H., Borze'e, A., Kim, A., Kwon, S., Sung, M., and Jang, Y. 2019. Microhabitat
386 preference in American bullfrog tadpoles (*Lithobates catesbeianus*) in relation to predation pressure. *Aquat.*
387 *Invasions*, **14**(3): 444–457. <https://doi.org/10.3391/ai.2019.14.3.04>.
- 388
- 389 Crossland, M.R., and Shine, R. 2012. Embryonic exposure to conspecific chemicals suppresses cane toad growth
390 and survival. *Biol. Lett.* **8**: 226–229. <https://doi.org/10.1098/rsbl.2011.0794>.
- 391
- 392 Crossland, M.R., Salim, A.A., Capon, R.J., and Shine, R. 2019. The effects of conspecific alarm cues on larval
393 cane toads (*Rhinella marina*). *J. Chem. Ecol.* **45**(10): 838–848. <https://doi.org/10.1007/s10886-019-01111-2>.
- 394

- 395 Cuzziol Boccioni, A.P., Peltzer, P.M., Martinuzzi, C.S., Attademo, M.A., Leon, E.J., and Lajmanovich, R.C. 2020.
396 Morphological and histological abnormalities of the neotropical toad, *Rhinella arenarum* (Anura: Bufonidae)
397 larvae exposed to dexamethasone. J. Environ. Sci. Health., Part B.
398 <https://doi.org/10.1080/03601234.2020.1832410>.
399
- 400 Da Costa Araujo, A.P., and Malafaia, G. 2020. Can short exposure to polyethylene microplastics change tadpoles'
401 behavior? a study conducted with neotropical tadpole species belonging to order anura (*Physalaemus cuvieri*). J.
402 Haz. Mat. **391**: 122214. <https://doi.org/10.1016/j.jhazmat.2020.122214>.
403
- 404 Daniels, A., and Maiz-Tome, L. 2019. *Synbranchus marmoratus*. The IUCN Red List of Threatened Species 2019:
405 e.T186540A1814657. <https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T186540A1814657.en>
406
- 407 Denoël, M., D'Hooghe, B., Ficetola, G.F., Brasseur, C., De Pauw, E., Thomé, J.P., and Kestemont, P. 2012. Using
408 sets of behavioral biomarkers to assess short-term effects of pesticide: a study case with endosulfan on frog
409 tadpoles. Ecotoxicol. **21**: 1240–1250. <https://doi.org/10.1007/s10646-012-0878-3>.
410
- 411 Egea-Serrano, A., and Tejedo, M. 2014. Contrasting effects of nitrogenous pollution on fitness and swimming
412 performance of Iberian waterfrog, *Pelophylax perezi* (Seoane, 1885), larvae in mesocosms and field enclosures.
413 Aquat. Toxicol. **146**: 144–153. <https://doi.org/10.1016/j.aquatox.2013.11.003>.
414
- 415 Egea-Serrano, A., Relyea, R., Tejedo, M., and Torralva, M. 2012. Understanding of impact of chemicals on
416 amphibians: a meta-analytic review. Ecol. Evol. **2**: 1382–1397. <https://doi.org/10.1002/ece3.249>.
417
- 418 Faulkner, A.E., Holstrom, I.E., Molitor, S.A., Hanson, M.E., Shegrud, W.R., Gillen, J.C., Willard, S.J., and
419 Wisenden, B.D. 2017. Field verification of chondroitin sulfate as a putative component of chemical alarm cue in
420 wild populations of fathead minnows (*Pimephales promelas*). Chemoecology, **27**: 233–238.
421 <https://doi.org/10.1007/s00049-017-0247-z>.
422
- 423 Ferrari, M.C.O., Wisenden, B.D., and Chivers, D.P. 2010. Chemical ecology of predator prey interactions in
424 aquatic ecosystems: a review and prospectus. Can. J. Zool. **88**: 698–724. <https://doi.org/10.1139/Z10-029>.
425
- 426 Gazzola, A., Brandalise, F., Rubolini, D., Rossi, P., and Galeotti, P. 2015. Fear is the mother of invention: anuran
427 embryos exposed to predator cues alter life history traits, post-hatching behaviour and neuronal activity patterns. J.
428 Exp. Biol. **218**: 3919–3930. <https://doi.org/10.1242/jeb.126334>.
429
- 430 Gazzola, A., Sacchi, R., Ghitti, M., and Balestrieri, A. 2018. The effect of thinning and cue: density on risk
431 perception by *Rana dalmatina* tadpoles. Hydrobiologia, **813**: 75–83. <https://doi.org/10.1007/s10750-018-3510-6>.
432
- 433 Gomez-Mestre, I., and Díaz-Paniagua, C. 2011. Invasive predatory crayfish do not trigger inducible defenses in
434 tadpoles. Proc. R. Soc. Part B. **278**: 3364–3370. <https://doi.org/10.1098/rspb.2010.2762>.

435

436 Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification.
437 Herpetologica, **16**: 183–190. <https://doi.org/10.2307/3890061>.

438

439 Hagman, M., Hayes, R.A., Capon, R.J., and Shine, R. 2009. Alarm cues experienced by cane toad tadpoles affect
440 post-metamorphic morphology and chemical defenses. Func. Ecol. **23**: 126–132. <https://doi.org/10.1111/j.1365-2435.2008.01470.x>.

442

443 Hanlon, S.M., and Relyea, R. 2013. Sublethal Effects of Pesticides on Predator-Prey interaction in Amphibians.
444 Copeia, **2013**(4): 691–698. <https://doi.org/10.1643/CE-13-019>.

445

446 Henry, J., Rodriguez, A., and Wlodkowic, D. 2019. Impact of digital video analytics on accuracy of
447 chemobehavioural phenotyping in aquatic toxicology. Peer J. **7**: e7367. <https://doi.org/10.7717/peerj.7367>.

448

449 Izaguirre, M.F., Lajmanovich, R.C., Peltzer, P.M., Peralta Soler, A., and Casco, V.H. 2000. Cypermethrin-induced
450 apoptosis in the telencephalon of *Physalaemus biligonigerus* tadpoles (Anura: Leptodactylidae). Bull. Environ.
451 Contam. Toxicol. **65**: 501–507. <http://dx.doi.org/10.1007/s001280000152>.

452

453 Izaguirre, M.F., Lajmanovich, R.C., Peltzer, P.M., Peralta Soler, A., and Casco, V.H. 2001. Induction of cell death
454 by the synthetic pyrethroid insecticide cypermethrin in the developing brain of *Physalaemus biligonigerus* tadpoles
455 from Argentina. FrogLog, **43**: 3–4.

456

457 Jungblut, L.D. 2012. El sistema olfatorio y vomeronasal en larvas de anfibios anuros y su participación en la
458 detección de estímulos químicos en el ambiente. Http://DigitalBIFcenUbaAr 151.

459

460 Junges, C.M., Peltzer, P.M., Lajmanovich, R.C., and Attademo, M.A. 2012. Toxicity of the fungicide
461 trifloxystrobin on tadpoles and its effect on fish-tadpole interaction. FrogLog, **20**(3): 43–44.

462

463 Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., and Attademo, M.A. 2017. Acute toxicity and
464 etho-toxicity of three insecticides used for mosquito control on Amphibians tadpoles. Water Air Soil Pollut. **228**:
465 143. <https://doi.org/10.1007/s11270-017-3324-6>.

466

467 Juszczak, G.R., Sliwa, A., Wolak, P., Tymosiak-Zielinska, A., Lisowski, P., and Swiergiel, A. 2007. The usage of
468 video analysis system for detection of immobility in the tail suspension test in mice. Pharmacol. Biochem. Behav.
469 **85**(2): 332–338. <https://doi.org/10.1016/j.pbb.2006.08.016>.

470

471 Kiesecker, J. M., Chivers, D.P., and Blaustein A.R. 1996. The use of chemical cues in predator recognition by
472 Western toad tadpoles. Anim. Behav. **52**: 1237–1245. <https://doi.org/10.1006/anbe.1996.0271>.

473

- Kullander, S.O. 2003. Family Synbranchidae (Swamp-eels). p. 594-595. In R.E. Reis, S.O. Kullander and C.J. Ferraris, Jr. (eds.) Checklist of the Freshwater Fishes of South and Central America. Porto Alegre: EDIPUCRS, Brazil.
- Maffei, F., Ubaid, F.K., and Jim, J. 2011. Anurans in an open cerrado area in the municipality of Borebi, São Paulo state, Southeastern Brazil: habitat use, abundance and seasonal variation. *Biota Neotrop.* **11**(2): 221-233. <http://dx.doi.org/10.1590/S1676-06032011000200023>.
- Maggese, M.A., Ravaglia, M., and Guerrero, G. 1993. Cultivo de *Synbranchus marmoratus*, un desafío productivo para América Latina. En: From discovery to commercialization (Eds. Carrill, M.; Dahle, L.; Morales, J.; Sogeloos, P.; Svennevig, N. y Wyban, J.). Spec. Publ. Eur. Aquacult. Soc. (19), European Aquaculture Society, Oostende, Belgium, p: 238.
- Maher, J.M., Werner, E.E., and Denver, R.J. 2013. Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proc. Biol. Sci.* **280**: 20123075. <https://dx.doi.org/10.1098%2Frspsb.2012.3075>.
- Mann, R.M., Hyne, R.V., Choung, C.B., and Wilson, S.P. 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environ. Pollut.* **157**: 2903–2927. <https://doi.org/10.1016/j.envpol.2009.05.015>.
- Marino, D., and Ronco, A. 2005. Cypermethrin and chlorpyrifos concentration levels in surface water bodies of the Pampa Ondulada, Argentina. *Bull. Environ. Contam. Toxicol.* **75**(4): 820–826. <https://doi.org/10.1007/s00128-005-0824-7>.
- McCollum, S.A., and Liemberger, J.D. 1997. Predator-induced morphological changes in an Amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia*, **109**: 615–621. <https://doi.org/10.1007/s004420050124>.
- McCoy, M.W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. *Oecologia*, **153**: 871–878. <https://doi.org/10.1007/s00442-007-0795-y>.
- Mikó, Z., Ujszegi, J., Gál, Z., and Hettyey, A. 2017. Effects of a glyphosate-based herbicide and predation threat on the behaviour of agile frog tadpoles. *Ecotoxicol. Environ. Saf.* **140**: 96–102. <https://doi.org/10.1016/j.ecoenv.2017.02.032>.
- Mitchel, M.D., and McCormick, M.I. 2013. Ontogenetic differences in chemical alarm cue production determine antipredator responses and learned predator recognition. *Behav. Ecol. Sociobiol.* <https://doi.org/10.1007/s00265-013-1537-2>.
- Mitchell, M.D., Bairos-Novak, K.R., and Ferrari, M.C.O. 2017. Mechanisms underlying the control of responses to predator odours in aquatic prey. *J. Exp. Biol.* **220**: 1937–1946. <https://doi.org/10.1242/jeb.135137>.

514

515 Mogali, S.M., Saidapur, S.K., and Shanbhag, B.A. 2011. Levels of predation modulate antipredator defense
516 behavior and metamorphic traits in the toad *Bufo melanostictus*. J. Herpetol. **45**: 428–431.
517 <https://doi.org/10.2307/41415311>.

518

519 Mogali, S., Saidapur, S., and Shanbhag, B. 2016. Influence of desiccation, predatory cues, and density on
520 metamorphic traits of the bronze frog *Hylarana temporalis*. Amphibia-Reptilia, **37**: 199–205.
521 <https://doi.org/10.1163/15685381-00003044>.

522

523 Mogali, S., Shanbhag, B., and Saidapur, S. 2019. Experience of predacious cues and accessibility to refuge
524 minimize mortality of *Hylarana temporalis* tadpoles. Acta Herpetol. **14**: 15–19.
525 https://doi.org/10.13128/Acta_Herpetol-22855.

526

527 Mogali, S.M., Shanbhag, B.A., and Saidapur, S.K. 2020. Behavioral responses of tadpoles of *Duttaphrynus*
528 *melanostictus* (Anura: Bufonidae) to cues of starved and fed dragonfly larvae. Phyllomedusa, **19**: 93–98.
529 <http://dx.doi.org/10.11606/issn.2316-9079.v19i1p93-98>.

530

531 Moore, H., Chivers, D.P., and Ferrari, M.C.O. 2015. Sub-lethal effects of Roundup™ on tadpole anti-predator
532 responses. Ecotoxicol. Environ. Saf. **111**: 281–285. <https://doi.org/10.1016/j.ecoenv.2014.10.014>.

533

534 Narvaes, P., Kwet, A., Silvano, D., Lavilla, E., and Langone, J. 2004. *Rhinella fernandezae*. The IUCN Red List
535 of Threatened Species 2004: e.T54637A11179477.
536 <https://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T54637A11179477.en>.

537

538 Peltzer, P.M., Junges, C.M., Attademo, A.M., Bassó, A., Grenón, P., and Lajmanovich, R.C. 2013. Cholinesterase
539 activities and behavioral changes in *Hypsiboas pulchellus* (Anura: Hylidae) tadpoles exposed to glufosinate
540 ammonium herbicide. Ecotoxicology, **22**: 1165–1173. <https://doi.org/10.1007/s10646-013-1103-8>.

541

542 Ramamonjisoa, N., Nakanishi, K., and Natuhara, Y. 2019. The efficacy of a generalized antipredator defense
543 against a novel predator depends on the source of induction in prey. Hydrobiologia, **836**(1): 197–205.
544 <https://doi.org/10.1007/s10750-019-3951-6>.

545

546 Reeves, M.K., Perdue, M., Blakemore, G.D., Rinella, D.J., and Holyoak, M. 2011. Twice as easy to catch? A
547 toxicant and a predator cue cause additive reductions in larval amphibian activity. Ecosphere, **2**(6): 1–20.
548 <https://doi.org/10.1890/ES11-00046.1>.

549

550 Regueira, E., Dávila, C., and Hermida, G.N. 2016. Morphological changes in skin glands during development in
551 *Rhinella arenarum* (Anura: Bufonidae). Anat. Rec. **299**: 141–156. <https://doi.org/10.1002/ar.23284>.

552

- Relyea, R.A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, **82**: 523–540. <https://doi.org/10.2307/2679877>.
- Relyea, R.A., and Mills, N. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to grey treefrog tadpoles (*Hyla versicolor*). *Proc. Natl. Acad. Sci. U. S. A.* **98**: 2491–2496.
- Robles-Mendoza, C., Zúñiga-Lagunes, S.R., Ponce de León-Hill, C.A., Hernández-Soto, J., and Vanegas-Pérez, C. 2011. Esterases activity in the axolotl *Ambystoma mexicanum* exposed to chlorpyrifos and its implication to motor activity. *Aquat. Toxicol.* **105**: 728–734. <https://doi.org/10.1016/j.aquatox.2011.09.001>.
- Rödin Mörch, P., Forsman, A., and Hagman, M. 2011. Taxonomic patterns of tadpole behavioural responses to alarm cues. (pp. 123–140). In: James L. Murray (ed.). *Frogs: Biology, Ecology and Uses*. Nova Science Publishers, Inc. New York, USA.
- Salibián, A. 1992. Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ. Contam. Toxicol.* **48**: 616–621. <https://doi.org/10.1007/bf00199082>.
- Sánchez, L.C., Peltzer, P.A., and Lajmanovich, R.C. 2009. Structure of wetland-breeding anuran assemblages from the southern section of the Paraná river, Argentina. *Herpetol. J.* **19**: 173–184.
- Sandrini, J.Z., Rola, R.C., Lopes, F.M., Buffon, H.F., Freitas, M.M., Martins, C.M., and da Rosa, C.E. 2013. Effects of glyphosate on cholinesterase activity of the mussel *Perna perna* and the fish *Danio rerio* and *Jenynsia multidentata*: in vitro studies. *Aquat. Toxicol.* **130–131**: 171–173. <https://doi.org/10.1016/j.aquatox.2013.01.006>.
- Saura-Mas, S., and Benejam, L. 2019. Effects of the invasive crayfish *Procambarus clarkii* on growth and development of *Pelophylax perezi* tadpoles in field conditions. *Anim. Biodivers. Conserv.* **42**(2): 245–252, <https://doi.org/10.32800/abc.2019.42.0247>.
- Schoeppner, N.M., and Relyea, R.A. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecol. Lett.* **8**: 505–512. <https://doi.org/10.1111/j.1461-0248.2005.00744.x>.
- Schoeppner, N.M., and Relyea, R.A. 2009. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Funct. Ecol.* **23**: 1114–1121. <https://doi.org/10.1111/j.1365-2435.2009.01578.x>.
- Sontag, C., Sloan Wilson, D., and Wilcox, S. 2006. Social foraging in *Bufo americanus* tadpoles. *Anim. Behav.* **72**: 1451–1456. <https://doi.org/10.1016/j.anbehav.2006.05.006>.

- Thetmeyer, H., and Kils, U. 1995. To see and not be seen: the visibility of predator and prey with respect to feeding behavior. *Mar. Ecol. Prog. Ser.* **126**: 1–8. <https://doi.org/10.3354/meps126001>.
- Üveges, B., Szederkenyi, M., Mahr, K., Moricz, A.M., Krüzselyi, D., Bokony, V., Hoy, H., and Heltyey, A. 2019. Chemical defense of toad tadpoles under risk by four predator species. *Ecol. Evol.* **9**: 6287–6299. <https://doi.org/10.1002/ece3.5202>.
- Vaira, M., Akmentins, M., Attademo, A., Baldo, D., Barrasso, D., et al. 2012. Categorización del estado de conservación de los Anfibios de la República Argentina. *Cuad. Herpetol.* **26**: 131–159.
- Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *Am. Nat.* **160**: 87–102. <https://doi.org/10.1086/340599>.
- Van Buskirk, J., Anderwald, P., Lüpold, S., Reinhardt, L., and Schuler, H. 2003. The lure effect, tadpole tail shape, and the target of dragonfly strikes. *J. Herpetol.* **37**: 420–424. <http://www.jstor.org/stable/1566166>.
- Van Buskirk, J., Ferrari, M., Kieng, D., Näpflin, K., and Ritter, N. 2011. Prey risk assessment depends on conspecific density. *Oikos*, **120**: 1235–1239. <https://doi.org/10.1111/j.1600-0706.2010.19311.x>.
- Vázquez, L.A., Rendón, M.Á., Díaz-Paniagua, C., and Gómez-Mestre, I. 2017. Among-species variation in the morphological responses of larval amphibians to native and invasive species. *Ecosistemas*, **26**(3): 32–38. <http://dx.doi.org/10.7818/ECOS.2017.26-3.05>.
- Vonesh, J.R., and Warkentin, K.M. 2006. Opposite shifts in size at metamorphosis in response to larval and metamorph predators. *Ecology*, **87**: 556–562. <https://doi.org/10.1890/05-0930>.
- Wei, L., Shao, W., Ding, G., Fan, X., and Lin, Z. 2014. Density but not Kinship regulates the growth and developmental traits of Chinese Tiger Frog (*Hoplobatrachus chinensis*) tadpoles. *Asian Herpetol. Res.* **2**(2): 113–118. <https://doi.org/10.3724/SP.J.1245.2014.00113>.
- Wells, K.D. 2007. The ecology and behavior of Amphibians. University of Chicago Press, Chicago, USA.
- WHO. World Health Organization. 1989. Environmental Health Criteria 82. Cypermethrin.
- Widder, P.D., and Bidwell, J.R. 2008. Tadpole size, cholinesterase activity, and swim speed in four frog species after exposure to sub-lethal concentrations of chlorpyrifos. *Aquat. Toxicol.* **88**: 9–18. <https://doi.org/10.1016/j.aquatox.2008.02.008>.
- Wilson, D.J., and Lefcort, H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Anim. Behav.* **46**: 1017–1019. <https://doi.org/10.1006/anbe.1993.1285>.
- Wisenden, B.D. 2000. Olfactory assessment of predation risk in the aquatic environment. *Philos. Trans. R. Soc. Lond., B Biol. Sci.* **355**: 1205–1208. <https://dx.doi.org/10.1098%2Frsb.2000.0668>.

Zala, S.M., and Penn, D.J. 2004. Abnormal behavior induced by chemical pollution: a review of the evidence and new challenges. *Anim. Behav.* **68**: 649–664. <https://doi.org/10.1016/j.anbehav.2004.01.005>.

Zar, J.H. 1999. *Biostatistical analysis* (4th. Ed.) Upper Saddle River. Prentice Hall, USA.

Figure Captions

Figure 1. Experimental conditions used to evaluate *R. dorbignyi* tadpoles, showing the compounds and the number of individuals assayed in each group.

Figure 2. Swimming behavior of *R. dorbignyi* exposed to treatments with different chemical cues (control; IC: cue from injured conspecifics; CY: sublethal concentration of cypermethrin; PC: cue from predator fish), based on total distance moved and average speed (A), global activity (B) and number of contacts (C).

Figure 3. Global activity of *R. dorbignyi* tadpoles exposed to treatments with different chemical cues (CO: control; IC: cue from injured conspecifics; CY: sublethal concentration of cypermethrin; PC: cue from predator fish). For each treatment, in and individual (1) and group of different sizes (3, 5, 7 and 10 individuals, in order of occurrence in the figure from bottom to top) are represented by their respective video track.

Table 1. Summary of treatments, showing their effects (increase ↑, or decrease ↓ with respect to controls) on different endpoints of the swimming behavior of *Rhinella dorbignyi* tadpoles. IC: treatment involving cues from injured conspecifics, CY: cypermethrin (pesticide) treatment, PC: treatment involving cues from predator fish; combinations of signals are named with the acronyms of each involved treatment. Arrows in bold indicate changes (increase or decrease) significantly different from the control.

Treatments	Tadpole groups	Distance moved	Average speed	Global activity	No. of contacts
IC	1	↑	↑	↑	-
	3	↑	↑	↑	↓
	5	↓	↓	↑	↓
	7	↓	↓	↑	↓
	10	↑	↑	↑	↑
CY	1	↑	↑	↑	-
	3	↑	↑	↑	↑
	5	↑	↑	↑	↑
	7	↑	↑	↑	↓
	10	↑	↑	↑	↑
PC	1	↑	↑	↑	-
	3	↑	↑	↑	↑
	5	↓	↓	↑	↓
	7	↓	↓	↓	↓
	10	↓	↓	↑	↓
IC + CY	1	↑	↑	↑	-
	3	↑	↑	↑	↓
	5	↑	↑	↑	=
	7	↑	↑	↑	↓
	10	↑	↑	↑	↑
IC + PC	1	↑	↑	↑	-
	3	↑	↑	↑	=
	5	↑	↑	↑	↓
	7	↑	↑	↑	↓
	10	↑	↑	↑	↑
CY + PC	1	↑	↑	↑	-
	3	↑	↑	↑	↑
	5	↑	↑	↑	↑
	7	↑	↑	↑	↓
	10	↑	↑	↑	↓
IC + CY + PC	1	↑	↑	↑	-
	3	↑	↑	↑	↑
	5	↓	↓	↑	↓
	7	↓	↓	↑	↓
	10	↑	↑	↑	↓

Figure 1. Experimental conditions used to evaluate *R. dorbignyi* tadpoles, showing the compounds and the number of individuals assayed in each group.

500x500mm (300 x 300 DPI)

Figure 2
© The Author(s) or their Institution(s)

Figure 3