Animal Behaviour 180 (2021) 93–99

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Effects of a group-living experience on the antipredator responses of individual tadpoles

Bianca Guadin [a,*], Andrea Gazzola [a], Alessandro Balestrieri [a], Giovanni Scribano [a], José Martín [b], Daniele Pellitteri-Rosa [a]

[a] Dipartimento di Scienze Della Terra e Dell'Ambiente, Università di Pavia, Pavia, Italy
[b] Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

ARTICLE INFO

Article history:
Received 2 December 2020
Initial acceptance 6 April 2021
Final acceptance 21 June 2021

MS. number: 20-00867R

Keywords:
behavioural plasticity
conspecific density
dilution effect
predation risk
social experience

The tendency to aggregate during the larval stage is widespread and highly variable among anuran species. Several studies have highlighted the link between tadpole group density and their activity level, confirming that, usually, living in groups brings several antipredator benefits. However, nearly all studies have focused on the average behavioural responses of tadpoles tested in groups. In this study, we explored the effects of living in groups of three different sizes (1, 5 and 25 individuals per group) on the antipredator behaviour of individual green toad, *Bufotes balearicus*, tadpoles. We first assessed their basal activity and then examined changes in mobility rate and total distance after exposure to the chemical cues of predatory dragonfly, *Aeshna cyanea*, larvae. For both the pre- and poststimulus activity levels, we also tested the effects of the presence of conspecifics' chemical cues in the experimental tub. Our results showed that (1) a previous brief (8 days) experience of group living is sufficient to affect the basal level of activity of individual tadpoles, which increased with group size; (2) tadpoles that were reared alone did not lower their activity further when exposed to predators' odour; (3) the antipredator response of high-density-reared tadpoles decreased in the presence of conspecifics' cues, supporting the so-called dilution effect, which, anyway, may need a minimum group size to be apparent. We conclude that both previous group-rearing experience and current perception of the surrounding environment may affect antipredator behaviour in individual tadpoles.
© 2021 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Aggregation, considered in all its forms and variations, is widespread in many taxa, affecting individuals' behaviour on a large scale. Living in a group has been reported to bring several benefits, in terms of increased cooperation, foraging efficiency, reproduction and antipredator vigilance (Alcock, 1989; Krause & Ruxton, 2002). Regarding the latter, the mechanisms that can lead to benefits from grouping, such as the ‘dilution effect’ (Lehtonen & Jaatinen, 2016; Turner & Pitcher, 1986), ‘many eyes theory’ (Lima, 1995; Lima & Dill, 1990; Roberts, 1996) and ‘confusion effect’ (Landeau & Terborgh, 1986), have been well studied. These studies have focused on the effects of group living on predation rate in many taxa (e.g. *Oncorhynchus kisutch*, Grand & Dill, 1999; *Nasua narica*, Hass & Valenzuela-Galván, 2002; *Lampropholis delicata*, Downes & Höfer, 2004; protozoan, rotifer and crustacean species, Tollrian et al., 2015), suggesting that prey may use information about the density of both conspecifics and predators to determine their investment in inducible defences.

Prey can gather information on predation risk in multiple ways: direct cues include the visual, auditory or chemical detection of predators (kairomones), while indirect cues consist of any signal from injured or attacked conspecifics (or heterospecifics), as well as exudates produced by predators after consuming their prey (see Chivers & Smith, 1998; Kats & Dill, 1998). In aquatic environments, direct or indirect predation cues are mainly chemosensory (Hettyey et al., 2015; Van Buskirk & Arioli, 2002). Using anuran tadpoles as model organisms, it has been demonstrated that the detection of water-borne cues may elicit significant responses in prey species, in terms of changes in morphological characteristics, behaviour and life history traits (Marquis et al., 2004).

Behavioural responses, such as ‘freezing’ and reduction of activity rates, can be promptly elicited as predation risk is perceived, and have been recorded in many anuran larvae species (e.g. *Rana perezi*, Gómez-Mestre & Díaz-Paniagua, 2011; *Rana dalmatina*, Gazzola, Russo et al., 2018; *Rana latastei*, Scribano et al., 2020),

* Corresponding author.
E-mail address: bianca.guadin01@universitadipavia.it (B. Guadin).

https://doi.org/10.1016/j.anbehav.2021.08.009
0003-3472/© 2021 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

including toads (*Bufotes viridis*, Stav et al., 2007; *Bufo bufo*, Maag et al., 2012; *Bufo nebulifer*, Preston & Forstner, 2014).

Anuran tadpoles show different degrees of social aggregation, with bufonids usually forming large social groups (Blaustein & Waldman, 1992; Waldman, 1991), especially under predation risk (Stav et al., 2007). Consistently with the 'dilution effect', highly dense tadpole groups are subject to a higher number of attacks, but per capita predation rates are lower than in small groups (Spieler, 2005; Watt et al., 1997). Accordingly, lone tadpoles show lower activity levels and more cautious behaviours than tadpoles in pairs or groups (McClure et al., 2009; McCoy, 2007), even in the absence of predators' cues (Awan & Smith, 2007; Golden et al., 2001).

The relationship between conspecific density and predation risk assessment was investigated by Peacor (2003), who claimed that a single prey individual cannot accurately assess predation risk without gathering information on conspecific density. If this information is not included, the estimation of actual predation risk will be under- (at low prey density) or overestimated (at high prey density). Although, in tests of *Rana temporaria* tadpoles, the predictions of Peacor's risk assessment model have gained some support (Van Buskirk et al., 2011), the model has been poorly tested and little evidence of risk assessment based on conspecific density is available (Guariento et al., 2015). 'Thinning', i.e. density decline as an effect of predation, does not seem to decrease *R. dalmatina* tadpoles' activity levels, as the model would imply (Gazzola, Sacchi, et al., 2018). Notwithstanding, Peacor's model provides a useful starting point to investigate the effects of conspecific density on the assessment of predation risk.

In this study, we explored the effects of group living on the defensive behaviour of Balearic green toad, *Bufotes balearicus*, tadpoles. As previous experiments mainly tested the collective behavioural responses of tadpoles in groups, here we specifically aimed at investigating the individual responses of tadpoles reared at three different densities (1, 5 or 25 individuals/container). Following Peacor (2003), we first hypothesized rearing conditions would affect tadpole antipredator behaviour (i.e. after exposure to a predatory cue), with tadpoles reared at the highest densities showing the lowest poststimulus reduction in activity levels. Second, since anuran larvae largely rely on chemosensory cues to assess the density of both conspecifics and predators, we expected responses to be more strongly elicited in tadpoles also exposed to conspecific chemical cues.

METHODS

Animal Collection

The Balearic green toad is native to Italy and Corsica and was introduced in the Balearic Islands in prehistoric times (Sindaco et al., 2009). It occurs in plain and hilly areas characterized by sandy and freshwater habitats, regularly used as sites for breeding and laying clusters ('strings') of eggs (Canestrelli et al., 2017; Fiacchini & Cavalieri, 2015; Gasparri et al., 2013; Lanza et al., 2007). During May 2020, six freshly laid green toad egg strings were collected from a network of canals flowing in an intensively cultivated area south of Milan (45°26′N, 9°20′E, Lombardy region, northern Italy). Egg clutches were immediately transported to the laboratory and prepared for the experiments. After hatching, tadpoles were transferred to 10 different containers (15 litres) and reared until the onset of the experiment, coinciding with Gosner's developmental stage 26–28, when they became appreciably active and social.

Twenty late-instar dragonfly, *Aeshna cyanea*, larvae, native predators of anuran tadpoles, were collected from an artificial pond located inside the protected natural area 'Bosco del Vignolo' (45°13′N, 8°56′E), using dip-nets. In the laboratory, they were kept individually in 0.8-litre plastic tubs.

Experimental Design

The experiment was planned as a $3 \times 2 \times 2$ full factorial design, which combined three different population density levels with two types of test environments (water with conspecifics' chemical cues versus aged tap water) and predation risk (predators' chemical cues versus water; Fig. 1). In the first, conditioning, phase of the experiment we prepared water containers (1.5 litres) with three levels of population density. On the first day, we prepared 35 containers, 25 at the lowest density (1 tadpole/container), five at the intermediate density (5 tadpoles/container) and five at the highest density (25 tadpoles/container). An equal number of containers for each density were prepared on each of the following 3 days, for a total of 140 rearing tubs. Each set was kept for 8 days before entering the testing phase. Throughout the conditioning period, tadpoles were fed rabbit food (dried grass pellets) ad libitum, to exclude the potential behavioural effects of food availability (Relyea, 2002), and water was changed every other day. Overall, 300 tadpoles were tested (25 individuals $\times$ 3 density treatments $\times$ 4 cues). On each of the 4 test days (the ninth since the start of the conditioning period of each set of tubs), we recorded the behavioural responses of 25 individual tadpoles from each density treatment. For the highest density treatment, five tadpoles from each container were tested; the remaining individuals were not included in the experiment. Tadpoles from each density treatment were selected, as far as practicable, to have a similar size and were randomly assigned to different stimuli (chemical cues).

Preparation of Odour Cues

To obtain the predators' cues, the day before the start of each test session, five dragonfly larvae were randomly selected and fed (at 2000 hours) green toad tadpoles. At the beginning of the experiment, we collected 50 ml aliquots of water from each predator's tub and mixed them in a separate container (Scribano et al., 2020). Aged tap water collected from a large tank (150 litres) was used as a control cue. Predators' cues have been observed to trigger strong behavioural responses after 36–48 h (even up to 72 h) of ageing in well water (Van Buskirk et al., 2014); thus, we were confident in their effectiveness.

Behavioural Trials

On each test day, tadpoles were randomly collected from the rearing containers and transferred individually in tubs (15×10.5 cm), filled with either 250 ml of aged tap water or 150 ml of aged water and 100 ml of water collected from the same container into which each individual tadpole had been conditioned, which, for the medium- and high-density treatments, was assumed to contain the chemical cues of conspecifics. Tadpoles were left to acclimatize for 20 min before the beginning of the test.

All tests were performed indoors and video-recorded with a digital camera (Canon Legria) hung 1.2 m above an arena, shielded with opaque panels and uniformly lit by spotlights. Overall, each trial lasted 20 min, 10 min before and 10 min after the injection of the stimulus, which consisted of 2 ml of either predator mixture or water (control) carefully injected with a 5 ml disposable syringe to minimize disturbance. The concentration of the predators' cues in the test tub (1:125) was consistent with previous studies (e.g. Gazzola et al., 2021; Gómez-Mestre & Díaz-Paniagua, 2011), and was expected to trigger a clear defensive response.

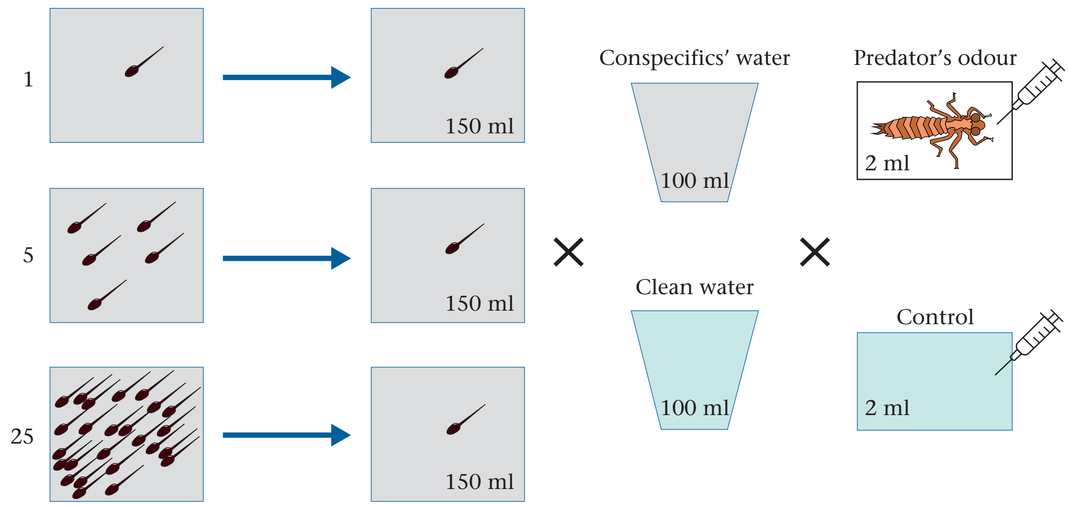

Figure 1. Experimental design. Tadpoles were placed in one of three density treatments (1, 5, 25 tadpoles) for 8 days. Individual tadpoles were then placed into the test tubs ($N = 100$ for each density treatment) in two types of test environment (presence or absence of conspecifics' cues × presence or absence of predators' cues).

Each tadpole was tested only once. On each test day, all trials were performed in random order between 1000 and 1500 hours. Videos were recorded at 25 frames/s and analysed using a source executable software for image-based tracking (ToxTrac; Rodriguez et al., 2018), which provides locomotor information by recording the *x* and *y* coordinates of the central point of each tadpole every 0.04 s.

Statistical Analysis

We tested two locomotor variables, namely 'mobility rate', i.e. the rate of instant speed above a threshold of 1 mm/s, and 'total distance', defined as the total distance (mm) covered by each tadpole in the test arena. Behavioural responses were recorded for both the pre- and poststimulus intervals. To test for variation in the tadpoles' level of activity with respect to the predators' cues, density treatment and presence of conspecifics' cues, we applied linear mixed models (LMMs), including, as response variable, the proportional change with respect to the prestimulus baseline in either mobility rate or total distance $\left[\frac{(\textit{locomotor variable after injection}-\textit{locomotor variable before injection})}{(\textit{locomotor variable before injection})}\right]$.

First, we explored the variation in the basal level of activity among treatments, i.e. prestimulus mobility rate and total distance, including density treatment (three levels), conspecific cues (two levels) and their interaction as fixed effects. The day of the experiment and behavioural trial within each day were inserted as random effects. To assess the proportional change in activity level after cue injection (for both mobility rate and total distance), we used a three-way interaction including predation risk (water or tadpole-fed dragonfly cue), test environment (presence or absence of chemical cues from conspecifics) and density treatment (1, 5, 25 tadpoles/container); the day of the experiment and behavioural trial within each day were included as random effects (all two-way interactions were also included in the model). LMMs were fitted through restricted maximum likelihood in the R package nlme (Pinheiro et al., 2019). Planned comparisons of interest were obtained using the R package emmeans (Length, 2018). We visually inspected the residuals to assess model fit and obtained confidence intervals for the estimates of fixed effects using the R package effects (Fox & Weisberg, 2018).

Ethical Note

Tadpoles were collected using sterilized dip nets to avoid the transmission of pathogens. All tadpoles were kept in indoor conditions at ambient temperature (20–23 °C) and natural light. Food was available ad libitum and water was changed periodically. During the experiments tadpoles were handled carefully to minimize physical stress. To obtain predators' cues, two tadpoles were supplied to predators by simulating, as much as possible, natural predation conditions. Once the experiments ended, all animals were released at the site of capture, following strictly the same sanitary procedures adopted in the collection phase. All procedures followed institutional guidelines and ethics, reviewed and approved by the Societas Herpetologica Italica (SHI), the national Institute for Environmental Protection and Research (ISPRA) and the Italian Ministry of Environment, Land and Sea (MATTM; permits code: 0006075–23/03/2018—PNM).

RESULTS

Prestimulus Activity

Prestimulus mobility rate was significantly affected by rearing conditions, increasing with density (density treatment: $\chi^2_2 = 43.98$, $P < 0.001$; $N = 50$ for each density level), but not by the presence of conspecifics' chemical cues ($\chi^2_1 = 0.06$, $P = 0.80$) or the density treatment*conspecifics' cues interaction ($\chi^2_2 = 0.67$, $P = 0.71$; Fig. 2). Pairwise comparisons showed a strong difference between the lowest and the highest density treatments, both in the presence ($t = -3.839$, $P = 0.002$) and in the absence ($t = -4.759$, $P < 0.001$) of conspecifics' cues. Similarly, the intermediate density treatment differed significantly from the highest one, both in the presence ($t = -2.91$, $P = 0.01$) and in the absence ($t = -3.18$, $P = 0.004$) of conspecifics' cues.

Prestimulus total distance increased with density ($\chi^2_2 = 20.46$, $P < 0.001$, $N = 50$ for each density level) and was not affected by the presence of conspecifics' chemical cues ($\chi^2_2 = 0.76$, $P = 0.38$) or by the density treatment*conspecifics' cues interaction ($\chi^2_2 = 0.79$, $P = 0.67$; Fig. 2). Pairwise comparisons showed a strong difference between the lowest and highest density treatment, both in the presence ($t = -2.70$, $P = 0.02$) and in the absence ($t = -3.38$,

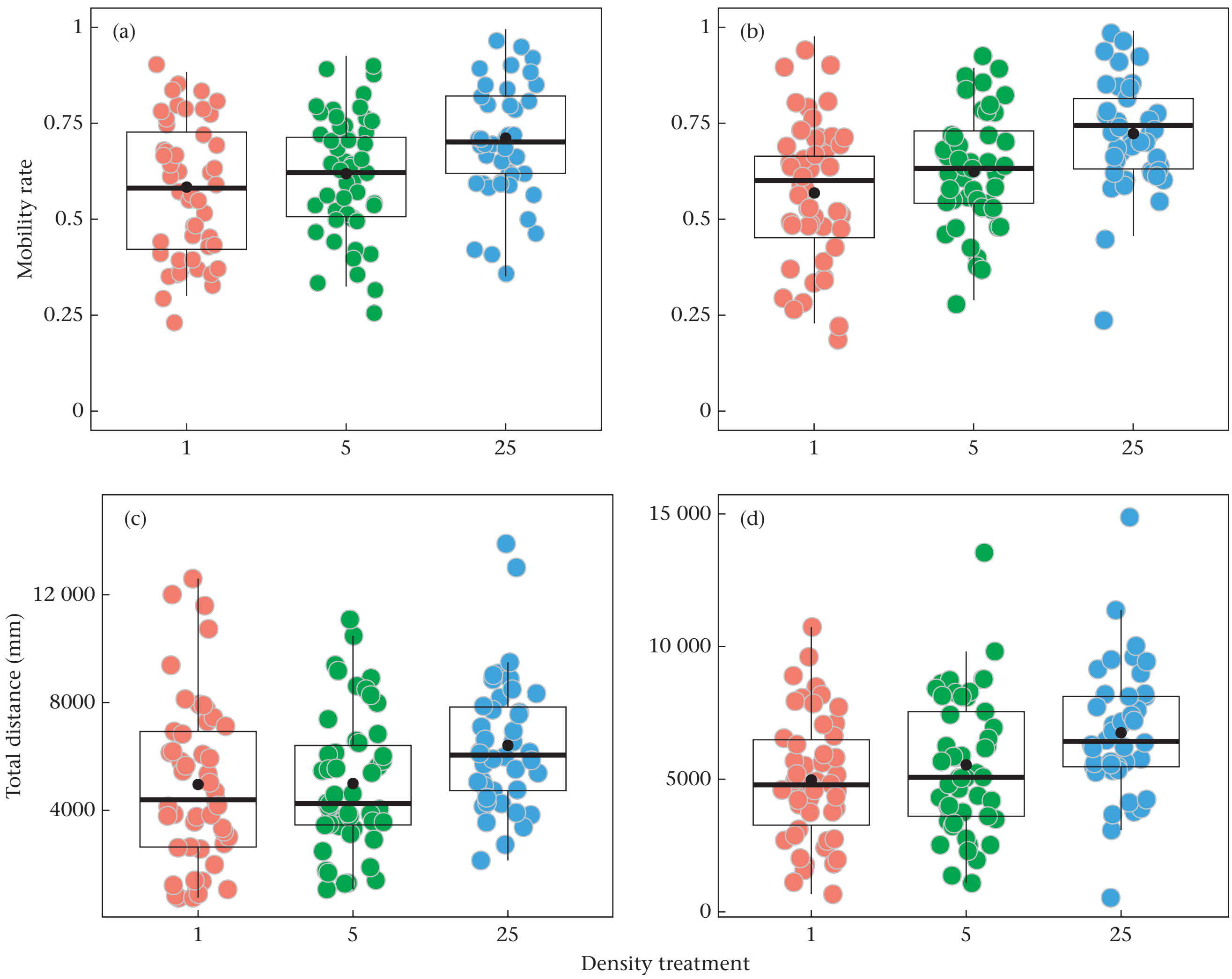

Figure 2. Prestimulus mobility rate and total distance moved for each of the three density treatments (1, 5, 25 tadpoles) (a, c) in the presence or (b, d) in the absence of conspecifics' cues. Coloured dots represent individuals' data; black dots represent means for each treatment ($N = 50$ for each combination of density and water-borne cues); box plots show medians (lines in the boxes), 25% and 75% quartiles (boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles).

$P = 0.002$) of conspecifics' cues. The intermediate density treatment differed significantly from the highest density treatment when in the presence of conspecifics' cues ($t = -2.64$, $P = 0.02$) but not in their absence ($t = -2.0$, $P = 0.09$). For both locomotor variables, no significant difference was detected between the lowest and intermediate density treatments ($P > 0.60$ for all).

Proportional Change in Activity

We found a significant interaction between predators' cues and density treatment ($\chi^2_2 = 8.67$, $P = 0.01$) in the proportional change in mobility rate, while none of the other two-way interactions was significant (density treatment*conspecifics' cues: $\chi^2_2 = 1.88$, $P = 0.38$; predators' cues*conspecifics' cues: $\chi^2_1 = 0.01$, $P = 0.91$; Fig. 3). The interaction among the three factors was marginally nonsignificant ($\chi^2_2 = 5.92$, $P = 0.051$). Lone-reared tadpoles did not respond differently from controls, either in the presence (estimated difference $= 0.07 \pm 0.07$, $t_{268} = 0.96$, $P = 0.33$) or in the absence (estimated difference $= -0.05 \pm 0.07$, $t_{268} = -0.73$, $P = 0.46$) of conspecifics' cues. Predators' cue-exposed tadpoles from the intermediate density group showed a significant reduction in mobility rate with respect to controls, in both environments (conspecifics' cues: estimated difference $= 0.25 \pm 0.07$, $t_{268} = 3.60$, $P < 0.001$; clean water: estimated difference $= 0.17 \pm 0.07$, $t_{268} = 2.45$, $P = 0.01$). Tadpoles from the highest density treatment showed a strong reduction in mobility rate when exposed to the predators' cues only in clean water (estimated difference $= 0.23 \pm 0.07$, $t_{268} = 3.00$, $P = 0.003$).

With proportional change in total distance as a response variable, we detected a significant interaction between predators' cues and density treatment ($\chi^2_2 = 6.23$, $P = 0.04$); no other interaction was significant ($P > 0.25$ for all). Only the intermediate density treatment showed significant variation, total distance decreasing when both predators' and conspecifics' cues were present relative to the control treatment (estimated difference $= 0.37 \pm 0.18$, $t_{268} = 2.03$, $P = 0.04$; Fig. 3).

DISCUSSION

Many studies have explored the importance of group density for antipredator behaviour in a wide variety of species, demonstrating that group living is often intertwined with predation risk assessment, providing antipredator benefits while implying inevitable costs due to increased intraspecific competition and increased

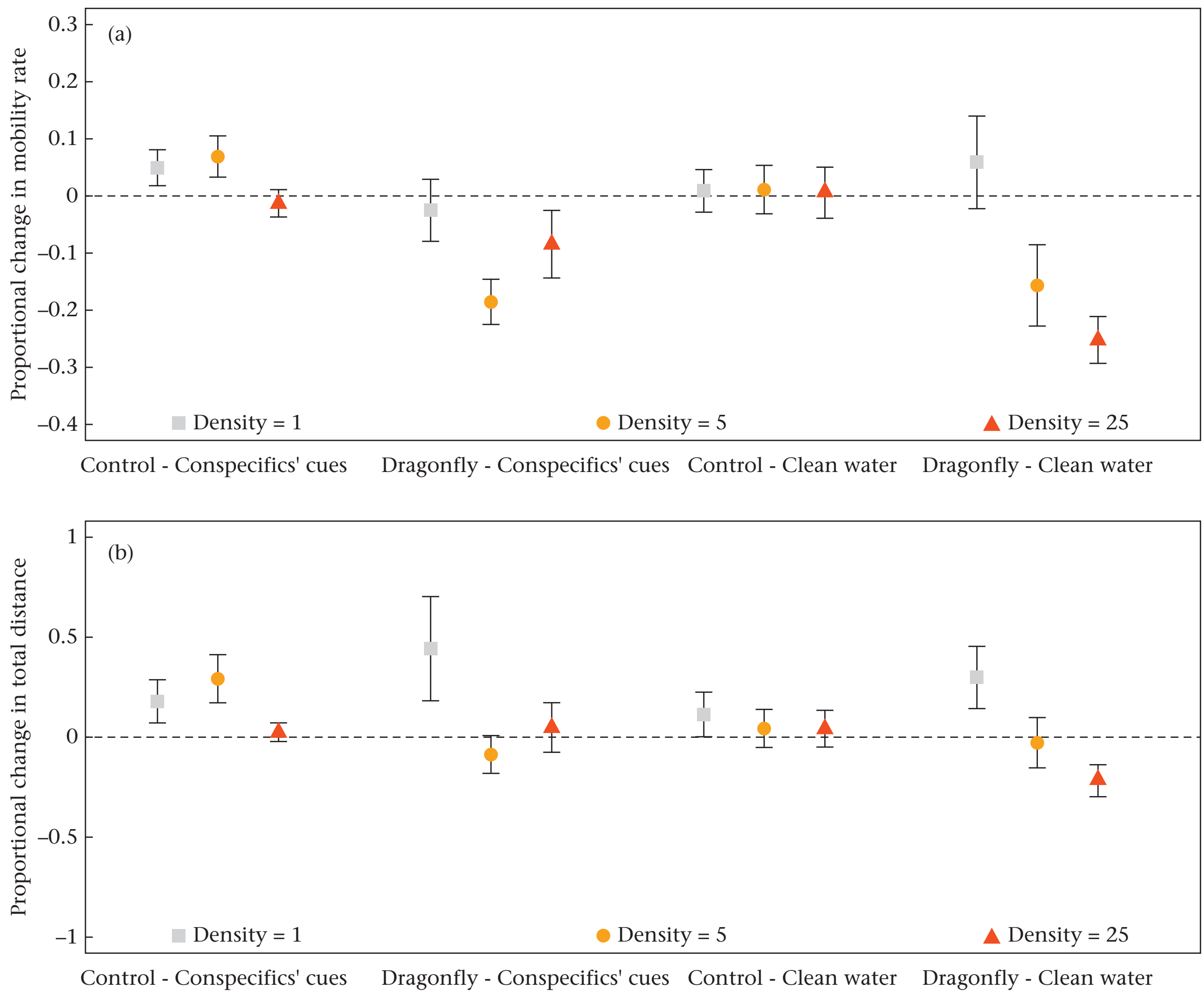

Figure 3. Mean (± SE) proportional change in (a) mobility rate and (b) total distance moved for tadpoles exposed to predatory cues (tadpole-fed dragonfly or control) either in the presence or in the absence of conspecifics' cues. Different colours and shapes indicate density treatments (1, 5 or 25 tadpoles; $N = 25$ for each combination).

attack rate on large groups (Krause & Ruxton, 2002). In this respect, anuran larvae have been poorly investigated, with studies using different approaches and experimental conditions, mainly with the aim of estimating the behavioural responses of tadpoles exposed to predation risk (e.g. Gazzola, Sacchi, et al., 2018; McCoy & Bolker, 2008; Nicieza, 1999; Spieler, 2003). Nearly all experiments have recorded the behavioural responses of tadpoles tested in groups of different size, hence acquiring data on activity levels by assessing the proportion of active individuals in each group. To investigate the effects of group-living experience on antipredator responses, we focused on individual *B. balearicus* tadpoles, showing that a brief conditioning period (8 days) was sufficient to later affect both their basal level of activity and defensive responses.

Increased basal activity in tadpoles raised at growing densities may be explained by each individual's perception of conspecifics as a potential threat for future (as food was not limiting during the conditioning period) resource availability (i.e. the risk of future food shortage; Tollrian et al., 2015). Larger rearing containers might have lowered perceived competition, but at the risk of reducing proximity among tadpoles and erasing the effects of density conditioning and thus undermining the aim of our experiment.

The behaviour of green toad tadpoles after cue injection differed between density treatments. Although single tadpoles usually respond to predators' cues (*Hyla femoralis*, McCoy, 2007; *Hyla squirella*, McCoy & Bolker, 2008; *Rana sylvatica*, McClure et al., 2009; *R. perezi*, Gómez-Mestre & Díaz-Paniagua, 2011; *R. latastei*, Scribano et al., 2020), green toad tadpoles reared alone in our experiment showed the lowest activity level and did not significantly reduce their activity level when exposed to predators' cues.

Toad tadpoles rarely grow alone, and usually tend to aggregate with up to thousands of siblings and congenerics as a predator avoidance behaviour (Watt et al., 1997). In the absence of conspecifics, competition is no longer crucial and, whenever food is not a limiting factor, activity levels can be sharply reduced (Gazzola, Balestrieri et al., 2018; Van Buskirk & McCollum, 2000). In the lowest density treatment, activity levels were probably too low to decline further in the presence of predation risk (Nunes et al., 2014). Accordingly, Peacor (2003) stated that prey response to predation risk is a function of the per capita amount of predators' cues. This implies that individuals cannot accurately assess predation risk without gathering information on conspecific density. Since predation risk may exist even in the absence of cues (Lima & Dill, 1990), low conspecific density systems (in which cues propagate more slowly and irregularly) should hypothetically always induce ‘higher-risk phenotypes’, that is, more cautious and risk-avoiding behaviours.

On the other hand, individuals living in high-density groups should be better able to evaluate predation risk, and thus invest less energy and resources in inducible defences to the benefit of a more efficient competitive behaviour. Consistently, tadpoles reared within the largest groups strongly decreased their mobility rate when exposed to predators' cues in clean water, but their response was substantially weakened when conspecifics' cues were present; the latter probably provided information on group size, mimicking the dilution effect. The behaviour of tadpoles from the intermediate density treatment, which responded to predation risk in both test environments, may suggest that there needs to be a minimum group size, or concentration of conspecifics' cues, for individuals to experience the antipredator benefits of group living. Indeed, we may argue that water from a five times higher group density might have conveyed more reliable information on the number of conspecifics and, consequently, might also have induced a different perception of the per capita amount of cues present in the environment.

Total distance moved gave less straightforward results when tadpoles were exposed to predators' cues. Recently Gazzola et al. (2021) have shown that under predation threat tadpoles incorporate protean elements into their movement, increasing path complexity. Such changes in movement patterns may explain why the total distance covered by high-density-reared tadpoles did not vary compared to controls, despite the decrease in their mobility rate.

By applying video-tracking techniques to individual tadpoles, we have provided evidence of the effects of group living on the defensive responses of green toad tadpoles. Peacor's model predictions were only partially confirmed, and our results suggested that the contextual occurrence of conspecifics' cues plays a bigger role than previously experienced group size in shaping tadpole responses to predators' cues.

Our results cannot rule out the possibility that the short-term (30 min) aggregation experienced by tadpoles before the trials may have overrode the previous conditioning period (8 days) altering their activity levels. Further research would be needed to evaluate this possibility and to underline the differences that may arise with different density treatments' exposure times.

Anuran larvae are considered to rely mainly on chemical stimuli to assess both predation risk and conspecifics' density. To assess both predation risk and conspecifics' density, anuran larvae are considered to rely on chemical rather than visual stimuli. As neither dragonfly larvae nor conspecifics were physically present in the test tubs, visual information was not anyway available to the potential prey. The reinforcement of tadpole behavioural responses by visual stimuli, however, is worth exploring further in future studies.

Author Contributions

The study was conceived by B.G., A.G. and D.P.-R.; B.G., A.G. and G.S. collected data; A.G. analysed the data; B.G. wrote the first draft and all the authors contributed to the final version.

Declaration of Interest

None.

Acknowledgments

We thank Fausto Pistoja for giving us access to the area of Bosco del Vignolo, for the collection of the predators needed in this experiment, and Mara Battaiola, for her help in the collection of the green toad egg clusters.

References

Alcock, J. (1989). *Animal behavior: An evolutionary approach*. Sunderland, MA: Sinauer Associates.

Awan, A. R., & Smith, G. R. (2007). The effect of group size on the activity of leopard frog (*Rana pipiens*) tadpoles. *Journal of Freshwater Ecology, 22*, 355–357. https://doi.org/10.1080/02705060.2007.9665059

Blaustein, A. R., & Waldman, B. (1992). Kin recognition in anuran amphibians. *Animal Behaviour, 44*, 207–221.

Canestrelli, D., Bisconti, R., Chiocchio, A., Maiorano, L., Zampiglia, M., & Nascetti, G. (2017). Climate change promotes hybridisation between deeply divergent species. *PeerJ, 5*, Article e3072. https://doi.org/10.7717/peerj.3072

Chivers, D. P., & Smith, R. J. F. (1998). Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. *Écoscience, 5*, 338–352. https://doi.org/10.1080/11956860.1998.11682471

Downes, S., & Hoefer, A. (2004). Antipredatory behaviour in lizards: Interactions between group size and predation risk. *Animal Behaviour, 67*, 485–492. https://doi.org/10.1016/j.anbehav.2003.05.010

Fiacchini, D., & Cavalieri, C. (2015). Il rospo smeraldino *Bufo balearicus - B. viridis* complex (Anura: Bufonidae) nelle marche: Aspetti corologici, ecologici e conservazionistici. *Biologi Italiani, XLV*, 55–60.

Fox, J., & Weisberg, S. (2018). Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. *Journal of Statistical Software, 87*, 1–27. https://doi.org/10.18637/jss.v087.i09. URL: https://doi.org/10.18637/jss.v087.i09).

Gasparri, R., Casavecchia, S., Galiè, M., & Biondi, E. (2013). The restoration of the wetlands with standing waters constituting the habitat of the Italian green toad (*Bufo balearicus* Boettger, 1880). *Plant Sociology, 50*, 109–119. https://doi.org/10.7338/pls2013501/08

Gazzola, A., Balestrieri, A., Martín, J., & Pellitteri-Rosa, D. (2018). Is it worth the risk? Food deprivation effects on tadpole anti-predatory responses. *Evolutionary Biology, 45*, 47–74. https://doi.org/10.1007/s11692-017-9430-y

Gazzola, A., Russo, G., & Balestrieri, A. (2018). Embryonic and larval defensive responses of agile frog (*Rana dalmatina*) to alien crayfish. *Ethology, 124*, 347–356. https://doi.org/10.1111/eth.12737

Gazzola, A., Sacchi, R., Ghitti, M., & Balestrieri, A. (2018). The effect of thinning and cue: Density ratio on risk perception by *Rana dalmatina* tadpoles. *Hydrobiologia, 813*, 75–83. https://doi.org/10.1007/s10750-018-3510-6

Gazzola, A., Balestrieri, A., Scribano, G., Fontana, A., & Pellitteri-Rosa, D. (2021). Contextual behavioural plasticity in Italian agile frog (*Rana latastei*) tadpoles exposed to native and alien predator cues. *Journal of Experimental Biology*, jeb240465. https://doi.org/10.1242/jeb.240465

Golden, D. R., Smith, G. R., & Rettig, J. E. (2001). Effects of age and group size on habitat selection and activity level in *Rana pipiens* tadpoles. *Herpetological Journal, 11*, 69–73.

Gómez-Mestre, I., & Díaz-Paniagua, C. (2011). Invasive predatory crayfish do not trigger inducible defences in tadpoles. *Proceedings of the Royal Society B: Biological Sciences, 278*, 3364–3370. https://doi.org/10.1098/rspb.2010.2762

Grand, T. C., & Dill, L. M. (1999). The effect of group size on the foraging behaviour of juvenile coho salmon: Reduction of predation risk or increased competition? *Animal Behaviour, 58*, 443–451. https://doi.org/10.1006/anbe.1999.1174

Guariento, R. D., Carneiro, L. S., Esteves, F. A., Jorge, J. S., & Caliman, A. (2015). Conspecific density affects predator induced prey phenotypic plasticity. *Ecosphere, 6*, Article art106.

Hass, C., & Valenzuela-Galván, D. (2002). Anti-predator benefits of group living in white-nosed coatis (*Nasua narica*). *Behavioral Ecology and Sociobiology, 51*, 570–578. https://doi.org/10.1007/s00265-002-0463-5

Hettyey, A., Tóth, Z., Thonhauser, K. E., Frommen, J. G., Penn, D. J., & Van Buskirk, J. (2015). The relative importance of prey-borne and predator-borne chemical cues for inducible antipredator responses in tadpoles. *Oecologia, 179*, 699–710. https://doi.org/10.1007/s00442-015-3382-7

Kats, L. B., & Dill, L. M. (1998). The scent of death: Chemosensory assessment of predation risk by prey animals. *Écoscience, 5*, 361–394. https://doi.org/10.1080/11956860.1998.11682468

Krause, J., & Ruxton, G. D. (2002). *Living in groups*. Oxford, U.K.: Oxford University Press.

Landeau, L., & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour, 34*, 1372–1380. https://doi.org/10.1016/S0003-3472(86)80208-1

Lanza, B., Andreone, F., Bologna, M. A., Corti, C., & Razzetti, E. (2007). *Fauna d'Italia, Amphibia, XLII*. Bologna, Italy: Calderini.

Lehtonen, J., & Jaatinen, K. (2016). Safety in numbers: The dilution effect and other drivers of group life in the face of danger. *Behavioral Ecology and Sociobiology, 70*, 449–458. https://doi.org/10.1007/s00265-016-2075-5

Lenth, R. (2018). *Emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package Version 1.3.0 https://CRAN.R-project.org/package=emmeans.

Lima, S. (1995). Back to the basics of anti-predatory vigilance: The group-size effect. *Animal Behaviour, 49*, 11–20. https://doi.org/10.1016/0003-3472(95)80149-9

Lima, S., & Dill, L. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology, 68*, 619–640. https://doi.org/10.1139/z90-092

Maag, N., Gehrer, L., & Woodhams, D. C. (2012). Sink or swim: A test of tadpole behavioral responses to predator cues and potential alarm pheromones from skin secretions. *Journal of Comparative Physiology A, 198*, 841–846. https://doi.org/10.1007/s00359-012-0750-1

Marquis, O., Saglio, P., & Neveu, A. (2004). Effects of predators and conspecific chemical cues on the swimming activity of *Rana temporaria* and *Bufo bufo* tadpoles. *Archiv für Hydrobiologie, 160*, 153–170. https://doi.org/10.1127/0003-9136/2004/0160-0153

McClure, K., Mora, J., & Smith, G. R. (2009). Effects of light and group size on the activity of wood frog tadpoles (*Rana sylvatica*) and their response to a shadow

stimulus. *Acta Herpetologica, 4*, 103–107. https://doi.org/10.13128/Acta_Herpetol-2959
McCoy, M. (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
McCoy, M., & Bolker, B. (2008). Trait-mediated interactions: Influence of prey size, density and experience. *Journal of Animal Ecology, 77*, 478–486.
Nicieza, A. (1999). Context-dependent aggregation in common frog *Rana temporaria* tadpoles: Influence of developmental stage, predation risk and social environment. *Functional Ecology, 13*, 852–858. https://doi.org/10.1046/j.1365-2435.1999.00375.x
Nunes, A. L., Orizaola, G., Laurila, A., & Rebelo, R. (2014). Rapid evolution of constitutive and inducible defences against an invasive predator. *Ecology, 95*, 1520–1530. https://doi.org/10.1890/13-1380.1
Peacor, S. (2003). Phenotypic modifications to conspecific density arising from predation risk assessment. *Oikos, 100*, 409–415. https://doi.org/10.1034/j.1600-0706.2003.12043.x
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). *nlme: Linear and Nonlinear Mixed Effects Models* (pp. 1–139). R Package Version 3 https://CRAN.R-project.org/package=nlme. >.
Preston, D. B., & Forstner, M. R. J. (2014). Aggregation status and cue type modify tadpole response to chemical cues. *Journal of Fish and Wildlife Management, 6*, 199–207. https://doi.org/10.3996/042014-JFWM-028
Relyea, R. (2002). Competitor-induced plasticity in tadpoles: Consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs, 72*, 523–540. https://doi.org/10.2307/3100055
Roberts, G. (1996). Why individual vigilance declines as group size increases. *Animal Behaviour, 51*, 1077–1086. https://doi.org/10.1006/anbe.1996.0109
Rodríguez, A., Zhang, H., Klaminder, J., Brodin, T., Andersson, P. L., & Andersson, M. (2018). ToxTrac: A fast and robust software for tracking organisms. *Methods in Ecology and Evolution, 9*, 460–464. https://doi.org/10.1111/2041-210X.12874
Scribano, G., Balestrieri, A., Gazzola, A., & Pellitteri-Rosa, D. (2020). Strong behavioural defensive responses of endemic *Rana latastei* tadpoles induced by a native predator's odour. *Ethology, 126*, 922–930. https://doi.org/10.1111/eth.13072
Sindaco, R., Romano, A., & Martínez-Solano, I. (2009). *Bufotes balearicus*. The IUCN Red List of Threatened Species, Article e.T153567A4517245. https://doi.org/10.2305/-IUCN.UK.2009.RLTS.T153567A4517245.en, 2009.
Spieler, M. (2003). Risk of predation affects aggregation size: A study with tadpoles of *Phrynomantis microps* (Anura: Microhylidae). *Animal Behaviour, 65*, 179–184. https://doi.org/10.1006/anbe.2002.2030
Spieler, M. (2005). Can aggregation behaviour of *Phrynomantis microps* tadpoles reduce predation risk? *The Herpetological Journal, 15*, 153–157.
Stav, G., Kotler, B. P., & Blaustein, L. (2007). Direct and indirect effects of dragonfly (*Anax imperator*) nymphs on green toad (*Bufo viridis*) tadpoles. *Hydrobiologia, 579*, 85–93.
Tollrian, R., Duggen, S., Weiss, L., Laforsch, C., & Kopp, M. (2015). Density-dependent adjustment of inducible defences. *Scientific Reports, 5*, 12736. https://doi.org/10.1038/srep12736
Turner, G., & Pitcher, T. (1986). Attack abatement: A model for group protection by combined avoidance and dilution. *American Naturalist, 128*, 228–240. https://doi.org/10.1086/284556
Van Buskirk, J., & Arioli, M. (2002). Dosage response of an induced defense: How sensitive are tadpoles to predation risk? *Ecology, 83*, 1580–1585. https://doi.org/10.2307/3071977
Van Buskirk, J., Ferrari, M., Kueng, D., Näpflin, K., & Ritter, N. (2011). Prey risk assessment depends on conspecific density. *Oikos, 120*, 1235–1239.
Van Buskirk, J., Krügel, A., Kunz, J., Miss, F., & Stamm, A. (2014). The rate of degradation of chemical cues indicating predation risk: An experiment and review. *Ethology, 120*, 942–949. https://doi.org/10.1111/eth.12266
Van Buskirk, J., & McCollum, S. A. (2000). Functional mechanisms of an inducible defence in tadpoles: Morphology and behaviour influence mortality risk from predation. *Journal of Evolutionary Biology, 13*, 336–347. https://doi.org/10.1046/j.1420-9101.2000.00173.x
Waldman, B. (1991). Kin recognition in amphibians. In P. G. Hepper (Ed.), *Kin recognition* (pp. 162–219). Cambridge, U.K.: Cambridge University Press.
Watt, P. J., Nottingham, S. F., & Young, S. A. (1997). Toad tadpole aggregation behaviour: Evidence for a predator avoidance function. *Animal Behaviour, 54*, 865–872. https://doi.org/10.1006/anbe.1996.0512