

## Benefits, costs and reactivity of inducible defences: an experimental test with rotifers

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### SUMMARY

1. A key aspect of the ecology and evolution of adaptive prey responses to predator risk is the timing by which the former develop a defensive trait in response to inducing signals released by the latter. This property, called reactivity, has been shown to affect population stability and persistence.
2. Theoretically, the minimal predator density required by prey to exhibit induced defences is expected to increase with the effectiveness of the defence and decrease with its cost. Likewise, the time required for the prey population to exhibit an induced defence is expected to increase together with cost.
3. The freshwater rotifers *Brachionus calyciflorus* and *B. havanaensis* and their predator *Asplanchna brightwelli* were used to test the hypothesis that prey species exhibiting defences that offer a larger fitness benefit and lower fitness cost are more reactive to predator signals, in terms of requiring shorter exposure time and lower signal concentration to trigger a morphological defence reaction.
4. Our results showed that both prey species exhibited costly and effective defences after induction by predator infochemicals. Faster reactions were observed at higher levels of predator cues. Nevertheless, the observed relationship between reactivity and benefit/cost of defences did not agree with our expectations.
5. To our knowledge, this is the first study in which the timing of induction of morphological defences is experimentally assessed over a gradient of risk signals. We propose new research directions to disentangle the mechanisms and project the consequences of prey decisions at the morphological level.

**Keywords:** antipredator strategy, inducible defences, infochemicals, phenotypic plasticity, rotifers

### Introduction

In planktonic systems, antipredator inducible defences are an important type of prey response to chemical

signals released by their predators. In nature, many prey species respond adaptively to changes in the density of their predators by modifying behavioural, morphological, or life history traits, thus diminishing the probability of being killed (Tollrian & Harvell, 1999; Anholt, Werner & Skelly, 2000). From a functional point of view, induced prey defences have shown to exert important influences on the stability of the focal predator–prey system (Ramos-Jiliberto, 2003;

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Vos *et al.*, 2004; Kopp & Gabriel, 2006; Ramos-Jiliberto, Frodden & Aránguiz-Acuña, 2007), as well as that of small food web modules (Ramos-Jiliberto & Garay-Narváez, 2007; Ramos-Jiliberto, Duarte & Frodden, 2008a; Ramos-Jiliberto *et al.*, 2008b), and even the entire network of interacting species (Kondoh, 2007). Therefore, gaining knowledge about the mechanisms of induction and action of plastic defences is relevant for a better understanding of the functioning of ecological systems of any level of complexity.

A key aspect of adaptive prey responses relies on how fast organisms are able to react in the presence of an inducing environmental signal; a property hereafter termed 'reactivity'. Theoretically, the rate at which organisms can react phenotypically towards an induced optimal state enhances the stability of food webs and promotes species persistence (Kondoh, 2003, 2007). In aquatic systems, infochemicals released by predators (generically named kairomones) accumulate in their surrounding environment when predators are abundant, but they also break down and therefore decay over time when predators become scarce. Therefore, kairomones can be reliable environmental cues for prey to induce morphological defences in response to varying predation risk. From the last consideration, the reactivity of prey to predator cues may be analysed from two different perspectives: (i) how strong the environmental signal must be for the prey to perceive it and initiate the defensive response and (ii) how fast the defended phenotype is developed after the response is initiated. Although these functional traits are likely to be species-specific and constrained by the organisms' own physiology and life history, their optimal values should be affected by the set of costs and benefits associated with the available options.

It has been extensively reported that plastic defensive traits tend to be triggered where and when they confer a fitness benefit that overcomes their unavoidable costs (Stemberger, 1988; Harvell, 1990; Tollrian & Harvell, 1999). On this basis, it follows that the likelihood of organisms exhibiting induced defences should increase with the risk of being predated and decrease with the magnitude of costs associated with the expression of the defensive trait (Tollrian & Harvell, 1999).

An efficient defence, favoured by natural selection, would be exhibited whenever the defence cost is lower than the benefits of expressing it (i.e. cost of

harm). As the cost of harm increases with the density of predators there should be a threshold predator density at which the benefit equals the defence cost, above which the defence is triggered (Riessen, 1992; Van Buskirk & Arioli, 2002; Trimmer *et al.*, 2008). Therefore, this predator density threshold is expected to increase with the effectiveness of the defence and decrease with its cost (Nesse, 2005).

On the other hand, exhibiting a defensive plastic trait often requires a period of latency from the instant when the signal is perceived by the organism until the defence is displayed. According to the speed-accuracy hypothesis, this time delay would prevent the prey from responding to temporary false alarms (Tollrian & Harvell, 1999; Duquette, Altwegg & Anholt, 2005; Trimmer *et al.*, 2008; Chittka, Skorupski & Raine, 2009). A longer delay allows the prey to recover higher-quality information about the actual risk of predation and to develop a more accurate response. Nevertheless, to delay the response excessively may imply the inconvenient maintenance of a vulnerable state, increasing the risk of being killed. If the costs of making a wrong decision are low, a fast but inaccurate response would be promoted. Conversely, when accuracy is of vital importance to avoid costs, a slow but accurate response is expected (Chittka *et al.*, 2009).

Brachionid rotifers constitute good biological models for testing reactivity to predator kairomones. They develop conspicuous and effective morphological defences in response to kairomones released by predators (Gilbert, 1966, 1999; Stemberger & Gilbert, 1987; Sarma, 1993; Iyer & Rao, 1996; Nandini, Pérez-Chávez & Sarma, 2003). The herbivores *Brachionus calyciflorus* Pallas and *B. havanaensis* Rousselet are common inhabitants of fresh water that are known to react, through the elongation of body spines, to kairomones released by the carnivore *Asplanchna* Gosse. In addition, it has been reported that these herbivores respond morphologically with different promptitude to variations in field density of *Asplanchna* (Gilbert, 2001; Garza-Mouriño *et al.*, 2005). In this study, we evaluate the costs, benefits and reactivity of morphological defence induction exhibited by *B. calyciflorus* and *B. havanaensis* in response to kairomones released by *Asplanchna*. Based on the theory summarised earlier, we test the following hypothesis: species with defences that confer a greater benefit (increase in protection) and that impose a lower cost (decrease in population growth

rate) will be more reactive to predator kairomones, requiring a shorter exposure and a lower concentration of infochemicals to trigger the defence.

## Methods

### *Cultures of the test species*

The predator *A. brightwelli* and the herbivores *B. calyciflorus* and *B. havanensis* were isolated from temperate lakes of central Chile: Tranque Pitama (33°27'S–70°49'W) and Rapel Reservoir (34°09'S–71°26'W). Clones were established from a single parthenogenic female and maintained under standard laboratory conditions (pH  $7.5 \pm 0.1$ , temperature  $25 \pm 0.5$  °C, photoperiod 12 : 12, 1 : d) for 2 months prior to their experimental use. For culture maintenance as well as for conducting the experiments, we used moderately hard water (U.S. EPA) enriched with vitamins: 100 mg L<sup>-1</sup> thiamine (B1), 0.5 mg L<sup>-1</sup> biotin and 0.5 mg L<sup>-1</sup> cyanocobalamine (B12). The stock cultures of herbivores were fed daily with the green alga *Chlorella vulgaris* at a density  $0.5 \cdot 10^6$  cell mL<sup>-1</sup>, which was cultured with Bold's basal medium (Borowitzka & Borowitzka, 1988). *A. brightwelli* was cultured in 500-mL vessels and daily fed *ad libitum* with a mixture of brachionid prey (*B. calyciflorus*, *B. caudatus*, *B. havanensis* and *B. patulus*). The culture medium was changed daily.

### *Population growth and cost of defences*

To assess the population growth rates of the herbivores exposed to predator infochemicals in the absence of predation, predator-conditioned EPA medium was prepared with *Asplanchna* at densities of 0.005, 0.01, 0.02, 0.1, 0.25 and 0.5 ind mL<sup>-1</sup> day<sup>-1</sup>. The control medium consisted of pre-conditioned fresh water with a mixture of the rotifers used as prey for the predator. Every 24 h *Asplanchna* individuals were filtered out, the conditioned medium was membrane-filtered (0.45 µm) and the pH adjusted to  $7.5 \pm 0.1$ .

For the experiments, we used 50-mL jars containing 20 mL of medium with food (*C. vulgaris*) at a density of  $10^6$  cell mL<sup>-1</sup>. For each brachionid species, the experiments were initiated with 20 randomly chosen rotifer individuals (i.e. 1 ind mL<sup>-1</sup>) obtained from the stock cultures in exponential growth phase. We used

56 experimental jars (two prey species × seven kairomone levels × four replicates). Brachionid density was assessed daily, either by an exhaustive count or by counting 1 mL samples from three to four aliquots. After density assessment, rotifers were transferred to new jars containing the appropriate test medium and food. Growth experiments were ended after 15 days, when most rotifer populations in the test jars began to decline.

The population density curves were compared using repeated measures ANOVA, after verifying parametric assumptions of normality (K-S test), homoscedasticity (Levene test) and sphericity (Mauchly test). The intrinsic growth rate  $r$  (per day) was calculated for each replicate as the slope of the curve  $\ln N_t$  versus time (Fernández-Araiza, Sarma & Nandini, 2005), from the exponential growth phase. These rates were compared with one-way ANOVA and a Dunnett test for multiple comparisons.

The estimates of  $r$  were used to identify the densities of *Asplanchna* that diminish the growth rate of *B. calyciflorus* and *B. havanensis*, relative to the control. We considered the cost of defence to be the percent decrease of  $r$  values calculated from kairomone treatments with respect to the values calculated from controls.

### *Functional response and benefit of defences*

We quantified prey consumption by *Asplanchna* over a gradient of prey densities for two groups of prey: organisms previously grown under kairomone exposure (induced morphs) and organisms grown in control medium (non-induced morphs). The kairomone medium was obtained by preconditioning it with a predator density of 0.1 *Asplanchna* mL<sup>-1</sup> day<sup>-1</sup>. Previous tests showed that this predator density was effective to induce defended morphs. Seven different prey densities were used (0.25, 0.5, 1, 2, 4, 8 and 16 ind. mL<sup>-1</sup>) with five replicates each. Two individuals of *A. brightwelli*, previously starved for 2 h, were introduced into each experimental jar containing 20 mL of medium and the prey densities specified earlier. Predators were allowed to feed for 45 min, after which they were immediately fixed with diluted formalin (5%) and their stomach content analysed. Consumption rate was calculated as the mean number of prey in the stomach of each predator over 45 min.

We performed least squares nonlinear regression to fit the predator consumption data to a type II functional response,  $y = ax/(1 + aHx)$ , where  $a$  and  $H$  are the attack rate and handling time, respectively. We measured the benefits of the defence as the percent change of  $a$  and  $H$  values between kairomone and control treatments. The significance of differences in parameter values between induced and non-induced prey morphs was tested by means of a permutation test (3000 runs).

### Reactivity

A second series of experiments, following the same culture protocol as in the previous sections, was used to assess both the kairomone and time thresholds for induction of defensive structure in both herbivores. Kairomone concentrations were the same as before. Each culture of a brachionid species was initiated at a density of 1 ind mL<sup>-1</sup> in 150 mL. A total of 24 experimental jars were used (two rotifer species  $\times$  four kairomone levels  $\times$  three replicates). At days 0, 2, 4, 6 and 8, 50 egg-bearing females were sampled from each jar and fixed in 5% formalin. Digital images of the animals were taken under a dissecting microscope. We measured the length of the anterior and posterior spines on the images. Additionally, we measured the length and width of the body in each treatment. All measurements were made with the software UTHSCSA Image Tool for Windows v.3.0 (Department of Dental Diagnostic Science at The University of Texas Health Science Center, San Antonio, TX, U.S.A.).

For each replicate, each morphological measure of spine length made on individuals was compared against the 95th percentile of the morphological measure distribution obtained from control individuals (without kairomone). Thus, each measure was categorised as 1 (induced state) or 0 (not induced state) depending on whether the attribute was greater or less than the 95th percentile of the control.

Threshold kairomone concentration and threshold exposure times for defence induction were calculated by means of probit regression, as the kairomone concentration and exposure time to kairomones needed to induce 50% of the individuals in each replicate.

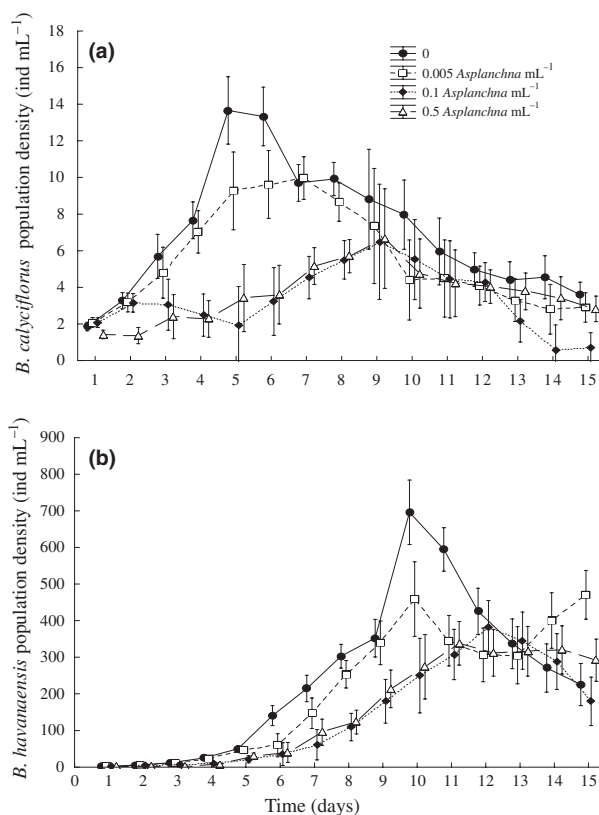
The relationship between body size measures and population growth rate was assessed by means of Pearson correlation analysis.

## Results

### Population growth and cost of defences

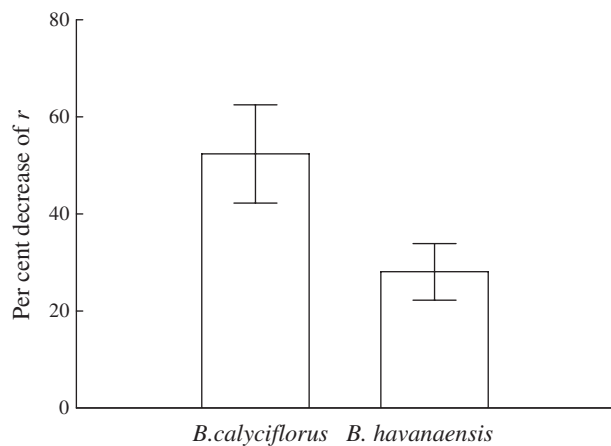
The initial phase of exponential growth of herbivores extended until days 5–9 for *B. calyciflorus* and days 10–12 for *B. havanaensis*, depending on the concentration of *Asplanchna* kairomone. The results of repeated measures ANOVA showed that mean population densities of both species were significantly affected ( $P < 0.001$ ) by the interaction between kairomone and time (Fig. 1).

Both *B. calyciflorus* and *B. havanaensis* populations showed greater values of  $r$  in both the control and the lowest kairomone concentration (0.005 *Asplanchna* mL<sup>-1</sup> day<sup>-1</sup>), compared to the higher densities of kairomone (Dunnett,  $P < 0.05$ ). There were no significant differences in  $r$  values among concentrations of kairomone equal to or greater than 0.01



**Fig. 1** Population density trajectories of (a) *Brachionus calyciflorus* and (b) *B. havanaensis* through time exposed to kairomones of *Asplanchna brightwellii* at the following levels: 0, 0.005, 0.1 and 0.5 *Asplanchna* mL<sup>-1</sup> day<sup>-1</sup>. Error bars indicate 95% confidence intervals.





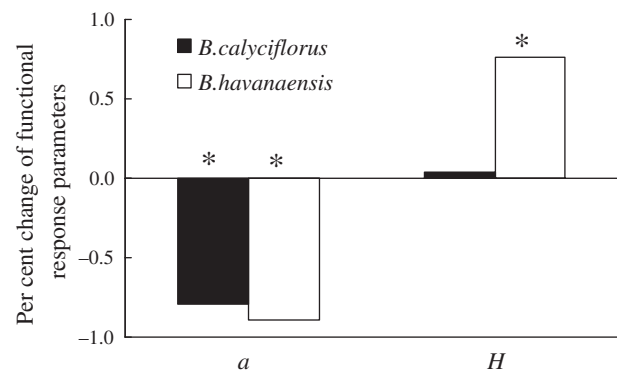
**Fig. 2** Cost of defences exhibited by *Brachionus calyciflorus* and *B. havanaensis*, calculated as percent decrease of intrinsic rates of population growth of rotifers exposed to kairomone treatments relative to controls. Error bars indicate 95% confidence intervals.

*Asplanchna*  $\text{mL}^{-1} \text{day}^{-1}$  (see detailed information in supplementary data Fig. S1). Therefore, our results revealed that *B. calyciflorus* and *B. havanaensis* showed costs of defences, measured as the reduction in  $r$ , but those costs were higher for *B. calyciflorus* than for *B. havanaensis* (Fig. 2).

Body size of both brachionid species increased with kairomone concentration, so that at lower concentrations (0 and  $0.005 \text{ mL Asplanchna mL}^{-1} \text{day}^{-1}$ ) both herbivores reached smaller body sizes than at high concentrations of kairomones ( $>0.1 \text{ mL Asplanchna mL}^{-1} \text{day}^{-1}$ ) ( $t$ -test,  $P < 0.05$ , supplementary data Fig. S2). The  $r$  values after exposure to kairomones were inversely correlated with the body size of both *B. calyciflorus*, ( $r_{\text{body width}} = -0.45$ ,  $r_{\text{body length}} = -0.58$ , Pearson,  $P < 0.05$ ) and *B. havanaensis* ( $r_{\text{body width}} = -0.43$ ,  $r_{\text{body length}} = -0.67$ , Pearson,  $P < 0.05$ ).

#### Functional response and benefit of defences

Functional response curves of *Asplanchna* feeding on both types of prey were significantly fitted ( $P < 0.001$ ) to a type II function (see data and best fit in supplementary data Fig. S3). This held for both the induced (*B. calyciflorus*,  $R^2 = 0.50$ , *B. havanaensis*,  $R^2 = 0.70$ ) and non-induced prey morphs (*B. calyciflorus*,  $R^2 = 0.52$ , *B. havanaensis*,  $R^2 = 0.62$ ). The attack rate of *Asplanchna* on both prey species was reduced as a result of the induced defences (*B. calyciflorus*,  $a_{\text{non-induced}} = 0.02 \pm 0.02$ ,  $a_{\text{induced}} = 0.004 \pm 0.005$ ; *B. havanaensis*,  $a_{\text{non-induced}} = 0.076 \pm 0.08$ ,  $a_{\text{induced}} = 0.01 \pm$



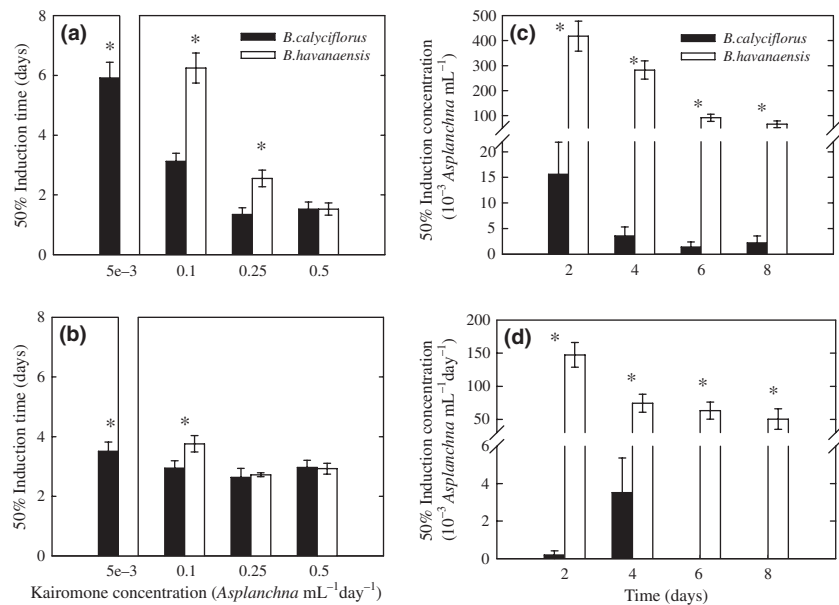
**Fig. 3** Benefit of defences exhibited by *Brachionus calyciflorus* and *B. havanaensis* in response to kairomones of *Asplanchna brightwelli*. The benefits were evaluated as the percent change of attack rate  $a$  and handling time  $H$  of predators feeding on induced prey, relative to values from predators feeding on control prey. Asterisks indicate values significantly different from zero (3000 permutations).

0.01). The ratio of predator attack rate on induced prey versus the attack rate on non-induced prey indicated that both prey species acquired a significant benefit from the defence, of similar magnitude for this parameter (Fig. 3). In addition, induced *B. havanaensis* was also favoured by increasing the handling time ( $H$ ) required to be consumed by the predator (*B. calyciflorus*,  $H_{\text{non-induced}} = 0.66 \pm 0.59$ ,  $H_{\text{induced}} = 0.69 \pm 0.84$ ; *B. havanaensis*,  $H_{\text{non-induced}} = 0.38 \pm 0.31$ ,  $H_{\text{induced}} = 0.68 \pm 0.55$ ). The benefit of defences from increasing  $H$  was significant only for *B. havanaensis* (Fig. 3). The abovementioned results suggest that induced defences provide a greater benefit to *B. havanaensis* than to *B. calyciflorus*.

#### Reactivity

In general, the time of kairomone exposure required to induce spine elongation in 50% of the population decreased with increasing kairomone concentration, and the kairomone concentration required to induce defences decreased with exposure time (Fig. 4). *B. calyciflorus* required a shorter exposure time and a lower kairomone concentration to induce spine elongation compared to *B. havanaensis*. There was an exception to the abovementioned rule at the highest kairomone concentration for the anterior spine and at the two highest concentrations for the posterior spine, where no significant differences between species were found. These results indicate that *B. calyciflorus*

**Fig. 4** Reactivity of the inducible defence of prey *Brachionus calyciflorus* and *B. havanaensis* in response to *A. brightwellii* kairomone. Left: exposure time required to induce morphological changes in 50% of the population (a: anterior spines and b: posterior spines). Right: kairomone concentration required to induce morphological changes in 50% of the population (c: anterior spines and d: posterior spines). Error bars indicate 95% confidence intervals and asterisks show significant differences ( $P < 0.05$ , Tukey HSD test) between species.



required lower exposure times and concentrations of kairomone to express morphological defences compared to *B. havanaensis*. In other words, *B. calyciflorus* was more reactive than *B. havanaensis* to the kairomone dose.

Integrating our results, the initial hypothesis is rejected since the species gaining the highest benefit and suffering the lower cost from expressing the induced defences, *B. havanaensis*, was less reactive than the species exhibiting higher costs and lower benefits, *B. calyciflorus*.

## Discussion

Both *B. calyciflorus* and *B. havanaensis* exhibited costly and effective inducible defences triggered by *Asplanchna* kairomones. In addition, our results showed that the decrease in population growth (cost) was greater for *B. calyciflorus* than for *B. havanaensis*. Nevertheless, the observed decrease in population growth under kairomone treatments could be a by-product of the increase in body size associated with the defensive response (see supplementary data, Fig. S2). Our results also indicated that the expression of morphological defences gave a greater benefit to *B. havanaensis* than to *B. calyciflorus*, since predator functional response in the former decreased to a greater degree after the induction of defences. Contrary to our expectations, defence reactivity to predator cues exhibited by *B. calyciflorus* was greater

compared to *B. havanaensis*, especially with regard to the kairomone concentration required to induce the defence. Consequently, our results do not support the hypothesis that species presenting more beneficial and less expensive defences should be more reactive to predator signals.

Reactivity to hazard signals has been linked to optimal behavioural responses exhibited by organisms of many species (Van Buskirk & Arioli, 2002; Chittka *et al.*, 2003; Dukas & Morse, 2003; Franks *et al.*, 2003; Ings & Chittka, 2008). Conversely, to our knowledge, this is the first study in which the timing of induction of morphological defences is experimentally assessed over a gradient of risk signals. Our results are in agreement with current theory (Franks *et al.*, 2003) in that faster decisions are made under riskier conditions, as imposed by increasing levels of predator cues. Thus, rules of behavioural decisions also seem to apply for morphological responses.

Phenotypic plasticity involves potential variation in behaviour, morphology, physiology, development or life history of organisms as a consequence of environmental heterogeneity (Agrawal, 2001). When to move, when to forage or when to trigger the development of a defensive structure are all decisions with fitness consequences and are all prone to error in dependence on the amount and quality of the information gathered from the environment (Chittka *et al.*, 2009). Planktonic prey often evaluates predation risk through the perception of kairomones. Therefore,

the more time a prey is exposed to a cue or the more intense the signal is, the more certain the organism will be about the actual risk of being predated. Conversely, a high reactivity implies more risk of exhibiting an unnecessary and costly defended state. From the abovementioned arguments, it follows that rules governing adaptive behavioural decisions should also hold, at least partially, for developmental decisions leading to morphological variation.

Unlike behavioural defences, the induction of defended morphs in rotifers occurs during the formation of eggs produced by mothers exposed to *Asplanchna* kairomone. Once spine induction is triggered in developing eggs, the expression of defences is irreversible even if the stimulus is removed. Hence, there is a one-generation delay in the expression of morphological defences (Gilbert, 1966), which imposes a lower limit to reaction time. This implies that an instantaneous induction of morphological responses is not achievable, contrary to the case of behavioural responses where this limit to reactivity can be negligible. In time units, the response delay will be a function of developmental rate, which in turn is affected by several factors such as temperature, feeding and physiological condition of organisms (Atkinson, 1994; Kirk, 1997; Stelzer, 2001, 2002).

In the same vein, induced populations exhibiting faster growth will express defences earlier, all other things being equal. In our experiments, *B. calyciflorus* showed a lower  $r$  compared to *B. havanaensis*. Therefore, if the time of kairomone exposure required by the populations to express defences is scaled by  $r$ , the observed interspecific differences in reactivity to predator cues will be even higher.

Although the observed relationship between reactivity and benefit/cost of defences did not agree with our *a priori* expectations, our results open new directions for future research. First, the magnitude of the benefit of the defensive response could be a function of the relative abundances of alternative prey (Van Buskirk & Arioli, 2002; Nandini *et al.*, 2003; Sarma & Nandini, 2007). In this case, a next step would be to provide the predator with an offer of multiple prey species in a gradient of relative densities, to measure the defence benefits allowing for shifts in consumer preferences. On the other hand, the net cost of defence expression in a natural context could result from reductions in prey growth rate derived from changes in the strength of interactions with other species and

therefore may be not accurately evaluated in isolated populations. Trade-offs have been reported between induction of defences and competitive ability of organisms (Werner & Anholt, 1996; Relyea, 2000, 2001; Werner & Peacor, 2003) and between defences and vulnerability to other predators (Taylor & Gabriel, 1992; Tollrian & Dodson, 1999). Thus, the reduction of  $r$  in our isolated system may give an underestimate of the cost of defences in the scenario where organisms evolve. In addition, the assessment of energetic costs associated with defensive responses to kairomones may require an experimental setup with more limited food availability (Sarma & Rao, 1987; Sarma, 1989; Gilbert, 1999; Van Buskirk, 2000). It follows that further experiments are needed to assess the cost of defences in a more realistic community context. Our understanding of the functioning of natural systems will increase with new empirical and theoretical research focused on disentangling the mechanisms and projecting the consequences of prey decisions at the morphological level.

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### References

- Agrawal A.A. (2001) Phenotypic plasticity in the interactions and evolution of species. *Science*, **294**, 321–326.
- Anholt B.R., Werner E. & Skelly D.K. (2000) Effect of food and predators on the activity of four larval ranid frogs. *Ecology*, **81**, 3509–3521.
- Atkinson D. (1994) Temperature and organism size—a biological law for ectotherms? *Advances in Ecological Research*, **25**, 1–58.
- Borowitzka M.A. & Borowitzka L.J. (1988) *Micro-Algal Biotechnology*. Cambridge University Press, UK.
- Chittka L., Dyer A.G., Bock F. & Dornhaus A. (2003) Bees trade off foraging speed for accuracy. *Nature*, **424**, 388.
- Chittka L., Skorupski P. & Raine N.E. (2009) Speed–accuracy tradeoffs in animal decision making. *Trends in Ecology and Evolution*, **24**, 400–407.

- Dukas R. & Morse D.H. (2003) Crab spiders affect flower visitation by bees. *Oikos*, **101**, 157–163.
- Duquette S.L., Altwegg R. & Anholt B.R. (2005) Factors affecting the expression of inducible defences in *Euplotes*: genotype, predator density and experience. *Functional Ecology*, **19**, 648–655.
- Fernández-Araiza M.A., Sarma S.S.S. & Nandini S. (2005) Combined effects of food concentration and temperature on competition among four species of *Brachionus* (Rotifera). *Hydrobiologia*, **546**, 519–534.
- Franks N.R., Dornhaus A., Fitzsimmons J.P. & Stevens M. (2003) Speed versus accuracy in collective decision making. *Proceedings of the Royal Society B Biological Sciences*, **270**, 2457–2463.
- Garza-Mouriño G., Silva-Briano M., Nandini S., Sarma S.S.S. & Castellanos-Páez M.E. (2005) Morphological and morphometrical variations of selected rotifer species in response to predation: a seasonal study of selected brachionid species from Lake Xochimilco (Mexico). *Hydrobiologia*, **546**, 169–179.
- Gilbert J.J. (1966) Rotifer Ecology and Embryological Induction. *Science*, **151**, 1234–1237.
- Gilbert J.J. (1999) Kairomone-Induced Morphological Defences in Rotifers. In: *The Ecology and Evolution of Inducible Defences* (Eds R. Tollrian & C.D. Harvell), pp. 127–141. Princeton University Press, Princeton, NJ.
- Gilbert J.J. (2001) Spine development in *Brachionus quadridentatus* from an Australian billabong: genetic variation and induction by *Asplanchna*. *Hidrobiologia*, **446/447**, 19–28.
- Harvell C.D. (1990) The ecology and evolution of inducible defences. *Quarterly Review of Biology*, **65**, 323–340.
- Ings T.C. & Chittka L. (2008) Speed-accuracy tradeoffs and false alarms in bee responses to cryptic predators. *Current Biology*, **18**, 1520–1524.
- Iyer N. & Rao R.T. (1996) Responses of predatory rotifer *Asplanchna intermedia* to prey species differing in vulnerability: laboratory and field studies. *Freshwater Biology*, **36**, 521–533.
- Kirk K.L. (1997) Egg size, offspring quality and food level in planktonic rotifers. *Freshwater Biology*, **37**, 515–521.
- Kondoh M. (2003) High reproductive rates result in high predation risks - a mechanism promoting the coexistence of competing prey in spatially structured populations. *The American Naturalist*, **161**, 299–309.
- Kondoh M. (2007) Anti-predator defence and the complexity-stability relationship of food webs. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 1617–1624.
- Kopp M. & Gabriel W. (2006) The dynamic effects of an inducible defence in the Nicholson-Bailey model. *Theoretical Population Biology*, **70**, 43–55.
- Nandini S., Pérez-Chávez R. & Sarma S.S.S. (2003) The effect of prey morphology on the feeding behaviour and population growth of the predatory rotifer *Asplanchna sieboldi*: a case study using five species of *Brachionus* (Rotifera). *Freshwater Biology*, **48**, 2131–2140.
- Nesse R.M. (2005) Natural selection and the regulation of defences. A signal detection analysis of the smoke detector principle. *Evolution and Human Behavior*, **26**, 88–105.
- Ramos-Jiliberto R. (2003) Population dynamics of prey exhibiting inducible defences: the role of associated costs and density-dependence. *Theoretical Population Biology*, **64**, 221–231.
- Ramos-Jiliberto R. & Garay-Narváez L. (2007) Qualitative effects of inducible defences in trophic chains. *Ecological Complexity*, **4**, 58–70.
- Ramos-Jiliberto R., Frodden E. & Aránguiz-Acuña A. (2007) Pre-encounter versus post-encounter inducible defences in predator-prey model systems. *Ecological Modelling*, **200**, 99–108.
- Ramos-Jiliberto R., Duarte H. & Frodden E. (2008a) Dynamic effects of inducible defences in a one-prey two-predators system. *Ecological Modelling*, **214**, 242–250.
- Ramos-Jiliberto R., Mena-Lorca J., Flores J.D. & Morales-Álvarez W. (2008b) Role of inducible defences in the stability of a tritrophic system. *Ecological Complexity*, **5**, 183–192.
- Relyea R.A. (2000) Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology*, **81**, 2278–2289.
- Relyea R.A. (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, **82**, 523–540.
- Riessen H.P. (1992) Cost-benefit model for the induction of an antipredator defence. *American Naturalist*, **140**, 349–362.
- Sarma S.S.S. (1989) Effect of *Chlorella* density and temperature on somatic growth and age at maturity of the rotifer *Brachionus patulus* (Muller) (Rotifera). *Current Science*, **58**, 788–791.
- Sarma S.S.S. (1993) Feeding responses of *Asplanchna brightwelli* (Rotifera): laboratory and field studies. *Hydrobiologia*, **255/256**, 275–282.
- Sarma S.S.S. & Nandini S. (2007) Small prey size offers immunity to predation: a case study on two species of *Asplanchna* and three brachionid prey (Rotifera). *Hydrobiologia*, **593**, 67–76.
- Sarma S.S.S. & Rao T.R. (1987) Effect of food level on body size and egg size in a growing population of the rotifer *Brachionus patulus* Muller. *Archiv fur Hydrobiologie*, **111**, 245–253.



- Stelzer C.P. (2001) Resource limitation and reproductive effort in a planktonic rotifer. *Ecology*, **82**, 2521–2533.
- Stelzer C.P. (2002) Phenotypic plasticity of body size at different temperatures in a planktonic rotifer: mechanisms and adaptive significance. *Functional Ecology*, **16**, 835–841.
- Stemberger R.S. (1988) Reproductive costs and hydrodynamic benefits of chemically induced defences in *Keratella testudo*. *Limnology and Oceanography*, **33**, 593–606.
- Stemberger R.S. & Gilbert J.J. (1987) Multiple species induction of morphological defences in the rotifer *Keratella testudo*. *Ecology*, **68**, 370–378.
- Taylor B.E. & Gabriel W. (1992) To grow or not to grow: optimal resource allocation for *Daphnia*. *American Naturalist*, **139**, 248–266.
- Tollrian R. & Dodson S.I. (1999) Inducible defences in Cladocera: constraints, costs, and multipredator environments. In: *The Ecology and Evolution of Inducible Defences* (Eds R. Tollrian & C.D. Harvell), pp. 177–202. Princeton University Press, Princeton, NJ.
- Tollrian R. & Harvell C.D. (1999) The Evolution of Inducible Defences: Current Ideas. In: *The Ecology and Evolution of Inducible Defences* (Eds R. Tollrian & C.D. Harvell), pp. 306–321. Princeton University Press, Princeton, NJ.
- Trimmer P.C., Houston A.I., Marshall J.A.R., Bogacz R., Paul E.S., Mendl M.T. & McNamara J.M. (2008) Mammalian choices: combining fast-but-inaccurate and slow-but-accurate decision-making systems. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2353–2361.
- Van Buskirk J. (2000) The costs of an inducible defence in anuran larvae. *Ecology*, **81**, 2813–2821.
- Van Buskirk J. & Arioli M. (2002) Dosage response of an induced defence: how sensitive are tadpoles to predation risk? *Ecology*, **83**, 1580–1585.
- Vos M., Verschoor A.M., Kooi B.W., Wäckers F.L., DeAngelis D.L. & Mooij W.M. (2004) Inducible defences and trophic structure. *Ecology*, **85**, 2783–2794.
- Werner E.E. & Anholt B.R. (1996) Predator induced behavioural indirect effects: consequences to competitive interactions in anuran larvae. *Ecology*, **77**, 157–169.
- Werner E.E. & Peacor S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1** Population growth rate of (a) *Brachionus calyciflorus* and (b) *B. havanaensis*, exposed to a gradient of kairomone concentration. Bars indicate mean values and errors are 95% confidence intervals. Different symbols indicate significant differences among treatments ( $P < 0.05$ , Dunnett test).

**Fig. S2** Body length (a) and body width (b) of *Brachionus calyciflorus* and *B. havanaensis* over a gradient of kairomone concentration. Low kairomone level grouped treatments of 0 and 0.005 mL *Asplanchna* mL<sup>-1</sup> day<sup>-1</sup>. High kairomone level includes treatments >0.1 mL *Asplanchna* mL<sup>-1</sup> day<sup>-1</sup>. Mean values and 95% confidence intervals are shown.

**Fig. S3** Functional responses of *Asplanchna brightwelli* feeding on non-induced (a, c) and induced (b, d) states of *Brachionus calyciflorus* (a, b) and *B. havanaensis* (c, d). Continuous lines show least squares best fits.

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