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Experimental assessment of interaction costs of inducible defenses in plankton

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The expression of phenotypically plastic traits in prey organisms, triggered by changes in the abundance of their predators, is customarily assumed to involve costs in some fitness components such as fecundity, growth or survival. However, these plastic responses may also have an interaction cost, which is assessed by the strength of interspecific interactions such as increased vulnerability to other predators or a reduction in competitive ability. This study assesses experimentally the effects of induced morphological defenses triggered by the carnivorous rotifer *Asplanchna brightwelli*, on the competitive ability of two herbivorous populations, and the reciprocal dominance between the rotifers *Brachionus calyciflorus* and *Brachionus havanaensis*. Our results support the existence of a trade-off between the expression of induced morphological defenses and competitive ability in aquatic animals. Specifically, we show that: (i) a consumer which reduces its vulnerability to predation via induced defenses increases its resource requirement to maintain zero population growth; and (ii) the expression of induced defenses promotes a qualitative shift in the relative competitive performance of the two herbivores over a single resource gradient. These results reinforce the general idea of functional community consequences of phenotypic plasticity, in particular the expression of inducible defenses of prey.

KEYWORDS: resource thresholds; exploitative competition; infochemicals kairomone; phenotypic plasticity

INTRODUCTION

Several animal and plant species, including planktonic organisms, respond to changes in the abundance of their predators by modifying the expression of some phenotypic traits. In rotifers, these responses involve the elongation of spines and appendages in the presence of infochemicals (kairomones) released by their predators. Such plastic morphological responses have been shown to diminish the probability of successful attacks by predators (van der Stap *et al.,* 2007; Aránguiz-Acuña *et al.,* 2010), and thus they are properly considered as induced anti-predator defenses.

The theory of phenotypic plasticity states that the survival benefit gained through the exhibition of an inducible defensive trait must be accompanied by costs on other fitness components; otherwise a fixed defense should evolve (Harvell, 1990; Tollrian and Harvell, 1999). Most often, costs of inducible defenses in plankton have been experimentally assessed at the individual level in free predation environments, by tracking alterations of vital rates such as survival and fertility in

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isolated populations (Gilbert, 1980; Epp and Lewis, 1984; Havel and Dodson, 1987; Stemberger, 1988; Zagarese and Marinone, 1992; Gilbert, 2009). In rotifers, the empirical evaluation of costs associated with induced phenotypes has led to contradictory results. In species of *Keratella*, a study by Stemberger (Stemberger, 1988) indicated costs in reproduction and sinking rates associated with predator-induced spine elongation. Nevertheless, a later study (Gilbert, 2009) found no evidence of such costs. Likewise, in *Brachionus calyciflorus*, one of the best-studied rotifers, earlier research found no costs of induced morphological defenses at all (Gilbert, 1980; Stemberger, 1990) in contrast to the results of Aránguiz-Acuña *et al.* (Aránguiz-Acuña *et al.*, 2010).

Controversial assessments of cost of inducible-defenses in rotifers may be due to methodological limitations, as discussed by Gilbert (Gilbert, 1999), but it is also possible that costs in isolated populations may be negligible in some species or under certain conditions, while other kinds of costs occurring in natural communities are being overlooked. It has been suggested that individuals may experience costs associated with induced defenses that emerge only when the focal species are inserted in an ecological community. These costs, called ecological costs (Strauss *et al.*, 2002) or community trade-offs (Garay-Narváez and Ramos-Jiliberto, 2009), are expressed as a fitness reduction as a consequence of modified strength of their interactions with other species. Such costs, hereafter called interaction costs, affect individual fitness by depleting the prey's ability to face natural enemies other than the inducer predator. In this regard, increased vulnerability to non-focal predators (Zagarese and Marinone, 1992; Tollrian, 1995; Tollrian and Dodson, 1999; Relyea, 2003; Benard, 2006; Hoverman and Relyea, 2009) as well as reduced interspecific competitive ability (Werner and Anholt, 1996; Pettersson and Brönmark, 1999; Peacor and Werner, 2000, 2001; Relyea, 2000) have been reported as examples of interaction costs of induced defenses. In particular, costs of defenses expressed in the competitive ability of prey are expected to influence species diversity within their trophic level.

Theoretical and empirical advances have shown that inducible defenses, by suppressing predator–prey interactions, represent a remarkable mechanism leading to trait-mediated indirect effects within food webs (Peacor and Werner, 1997; Abrams, 2007). Nevertheless, if there is an interaction cost, predators may act as modifying agents of the interaction strength between prey and other interacting species. This mechanism leads to trait-mediated indirect effects exerted by the inducer predator on the natural enemies of other prey involved in the interaction cost. This adds a new way in which phenotypic plasticity of prey may generate indirect effects in natural communities.

In addressing the effects of induced traits on the competitive ability of organisms, previous studies have shown that dominant and subordinate species can reverse their roles under the expression of predator-driven behavioral and morphological defenses in one of the actors (Werner, 1991; Peacor and Werner, 1997, 2000, 2001; Peacor, 2002; Relyea and Yurewicz, 2002; van der Stap *et al.*, 2008). Nevertheless, the mechanistic basis of observed changes in numerical dominance remains unclear, since competing causal hypotheses other than shifts in competitive ability cannot be discarded. For example, changes in numerical dominance driven by induced defenses might be a result of a trade-off between predation and starvation risks in behavioral decisions (Anholt and Werner, 1999), or between predation and sinking rates caused by morphological changes in aquatic species (Stemberger, 1988), rather than truly competitive costs. Under the framework of the mechanistic theory of resource competition (Tilman, 1982), a competitive cost associated with a biological activity should be expressed as an increase in the resource requirements for positive population growth, and it should be evaluated over a gradient of food availability. This approach has proven to generate reliable prediction of competitive outcomes in rotifer populations (Rothhaupt, 1988; Ciros-Pérez *et al.*, 2001).

In order to deepen our understanding of the consequences of phenotypic plasticity for community functioning and species coexistence, in this study we assess the effects of induced morphological defenses on the competitive performance of two animal prey species. For this purpose, we experimentally evaluate the existence of a trade-off between inducible defenses triggered by the carnivorous rotifer *Asplanchna brightwelli* and competitive ability of two herbivore populations, and reciprocal dominance between them. As prey species, we used the rotifers *B. calyciflorus* and *Brachionus havanaensis*. These species differ in body size and use of food resources (Stemberger and Gilbert, 1985; Rothhaupt, 1988; Sarma *et al.*, 1996; Ciros-Pérez *et al.*, 2001), which we expected to be translated into different competitive abilities. Previous evidence (Sarma *et al.*, 1996) and our own experience with these organisms indicate that *B. calyciflorus* performs better than *B. havanaensis* under high resource density, while the latter exhibits a better performance at low resource levels. In addition, although both herbivores exhibit morphological defenses in response to *Asplanchna* kairomone, their defensive structures differ in anti-predator effectiveness (Gilbert, 2001; Garza-Mouriño *et al.*, 2005).

METHOD

Cultures of test species

The predator *A. brightwelli* and the herbivores *B. calyciflorus* and *B. havanensis* were isolated from temperate lakes of Central Chile: Pitama (33°27′S−70°49′W) and Rapel (34°09′S−71°26′W). Clones were established from a single parthenogenetic female and maintained under standard laboratory conditions (pH 7.5 ± 0.1, temperature 25 ± 0.5°C, diffuse light with a photoperiod of 12:12 L:D), during 2 months prior to their use in experiments. As culture medium, we used moderately hard water (US EPA, 2002) enriched with vitamins: 100 mg L^{-1} thiamine (B1), 0.5 mg L^{-1} biotin and 0.5 mg L^{-1} cyanocobalamin (B12). The herbivores in the stock cultures were fed daily with the green alga *Chlorella vulgaris* at a density of 0.5×10^6 cells mL^{-1}. The predator *A. brightwelli* was cultured in 500 mL vessels and fed daily *ad libitum* with a mixture of brachionid prey (*B. calyciflorus*, *Brachinonus caudatus*, *B. havanaensis* and *Brachinonus patulus*). The culture media were renewed every 24 h.

Defensive response to induction

Defensive morphological responses of prey were assessed under two kairomone concentrations: 0.005 and 0.1 *Asplanchna* mL^{-1} (low and high, respectively), based on previous laboratory measurements and ranges of reported field abundances (Nandini, 1999). The experimental animals, *B. calyciflorus* and *B. havanaensis* at a density of 1 ind. mL^{-1} in 150 mL, were exposed to low and high kairomone levels, in addition to a control without kairomone. Predator-conditioned medium was prepared daily with EPA water with the corresponding *Asplanchna* densities. *Asplanchna* was fed with a mixture of brachionids at a density of 4 ind. mL^{-1}. The control medium consisted of fresh water pre-conditioned with the mixture of rotifers was used as the prey for *Asplanchna*. The alga *C. vulgaris* served as food, at a density of 10^6 cells mL^{-1}. We used 18 experimental jars, consisting of 2 rotifer species × 3 kairomone levels × 3 replicates. Every 24 h, all rotifers were changed to fresh medium by filtering them through a 50 μm mesh. The medium obtained after 24 h of predator exposure was filtered through a 0.45 μm membrane and the pH was adjusted to 7.5 ± 0.1 before its use in the induction treatment. After 6 days, 50 egg-bearing individuals were sampled from each experimental jar and fixed in 5% formalin. Digital images of the animals were obtained under a dissecting microscope, from which the length of anterior and posterior spines, and width and length of the body were measured. Differences between morphological trait measures among treatments were evaluated for each species by means of one-way ANOVA and Dunnett's *post hoc* test after verification of parametric assumptions.

Assessment of competitive ability of single species

Single-species population growth experiments were conducted for each of the two prey, *B. calyciflorus* and *B. havanaensis*, under four different food densities: 0.25×10^6, 0.5×10^6, 1×10^6 and 2×10^6 cells mL^{-1}. These treatments were performed at two levels of the predation risk factor. The medium with predator kairomone was obtained daily through preconditioning EPA medium with 0.1 *Asplanchna* mL^{-1} for 24 h. Control and induction media were prepared following the same protocol explained in the previous section. We used 64 experimental vessels (2 rotifer species × 4 resource levels × 2 predation risk levels × 4 replicates). For each replicate, we used 50 mL jars containing 20 mL of medium and food at the corresponding density. The experiments were initiated with 20 individual rotifers per vessel, randomly chosen from the stock cultures in exponential growth phase. Population density of the herbivores was estimated daily by direct counting of each replicate, through either exhaustive censuses or by 3−4 aliquots. After counting, the individuals were transferred to fresh medium. These population growth experiments were ended after 15−29 days, when most experimental populations began to decline.

Temporal trajectories of population density were compared by repeated-measures ANOVA, after verification of parametric assumptions of normality (Kolmogorov−Smirnov's test), homoscedasticity (Levene's test) and sphericity (Mauchley's test). We used the square root transformation when needed.

Population growth rates were estimated for each daily interval as

$$r = \ln N_t - \ln N_{t-1} \qquad (1)$$

where N_{t-1} and N_t are rotifer densities on two consecutive days, excluding five initial days of transient phase. The exponential growth phase was identified for each time series by maximizing the explained variance of a linear regression of ln(N) versus time. We calculated a single growth rate r as the average of the r_t values obtained for the exponential phase identified.

A Monod curve was fitted by means of least squares non-linear regression to the relationship between r and resource density R, based on the following equation

$$r = \frac{r_{\max}(R - R^*)}{R - R^* + K_c} \tag{2}$$

Fitting parameters of the model were: $r_{\max}$, maximum population growth rate, R^*, resource density for zero population growth, and K_c, Monod constant. Based on the mechanistic theory of resource competition (Tilman, 1982), the threshold R^* was our measure of competitive ability (Rothhaupt, 1993; Ciros-Pérez *et al.*, 2001). In environments with a single and constant resource density, species with lower R^* are predicted to exclude competitively all other species that exploit the same resource, thus a lower R^* value represents a higher consumer competitive ability. An F test was conducted to assess significance of estimated R^* thresholds and a t-test to examine differences in the parameters R^* and $r_{\max}$ between induced and non-induced states.

Competition experiments

We used 32 experimental vessels (4 resource levels × 2 predation risk levels × 4 replicates). Procedures for each treatment were the same as in the previous section, but with both brachionid species growing together in the same vessel. Rotifers from each vessel were counted daily, using 3–4 10 mL aliquots, and then transferred to fresh medium. The experiment was terminated when exclusion was observed in one of the two species or when densities of both populations stabilized. Population performance was measured through the mean and the coefficient of variation of population density over time, excluding a 5-day transient phase. These attributes were analyzed by two-way ANOVAs after verifying parametric assumptions, with resource level and kairomone as factors. The Tukey *post hoc* test for multiple comparisons was performed.

From time series of population densities in the competition experiments, rates of competitive exclusion η were calculated (Grover, 1988, 1991). The response variable $Y(t) = \ln[N_1(t)/N_2(t)]$, where N_1 and N_2 are population densities at time t of *B. calyciflorus* and *B. havanaensis*, respectively, was regressed against time. η was calculated as $\eta = [\hat{Y}(t_2) - \hat{Y}(t_1)]/(t_2 - t_1)$ where $\hat{y}$ is the predicted value from the fitted linear or quadratic regression at references times t_1 and t_2. Between t_1 and t_2, both competitor species coexist. Exclusion rates were compared between treatments by means of two-way ANOVA with kairomone and resource as factors. The Holm–Sidak *post hoc* test for multiple comparisons was performed when differences were found.

Defense effectiveness

An assessment of the level of effectiveness of the defensive traits exhibited by the two experimental herbivores was carried out through a comparison between induced and non-induced morphs of the fitted parameters governing the type II functional responses of predator. Induced morphs were obtained by exposing the herbivores during 7 days to predator kairomone produced by 0.1 *Asplanchna* mL^{-1}. Seven prey densities were used (0.25, 0.5, 1, 2, 4, 8 and 16 ind. mL^{-1}), with 5 replicates each. Two previously starved adults of *A. brightwelli* were introduced into each experimental jar containing 20 mL of medium and the specified prey densities. Predators were allowed to feed for 45 min, after which they were fixed in diluted formalin (5%) and their stomach content was analyzed. Predator consumption was expressed as the mean number of prey per predator observed in the stomachs. For each herbivore species and each morph, the consumption data were fitted, by means of least squares non-linear regression, to a type II functional response of the form, $y = ax/1 + aHx$, where a and H represent the attack rate and handling time, respectively.

RESULTS

Morphological changes were observed in both prey species exposed to a high concentration (0.1 *Asplanchna* mL^{-1}) of predator kairomone (Supplementary data, Fig. S1). Elongation of anterior and posterior spines and increase in body size were effectively induced in both prey species by the high kairomone treatment (Table I). On the other hand, morphological structures exhibited in organisms exposed to the low kairomone level (0.005 *Asplanchna* mL^{-1}) did not differ from the control, except for a slight decrease in body length of *B. havanaensis* (Table I). Based on these results, the low and high kairomone levels are considered not to induce and to induce, respectively, morphological defenses in the test organisms.

The relationship between rotifer population growth rate and food density was well characterized by an asymptotically increasing curve (Fig. 1). The food level R^* at which population growth rate is zero is an indicator of competitive ability, whereas the asymptote $r_{\max}$ represents the potential growth rate when food is abundant. Figure 2 shows that both species increased their food thresholds when they were induced, when compared with their non-induced states (t-test, $P < 0.05$). On the other hand, defended morphs of *B. havanaensis* had a lower $r_{\max}$ relative to the undefended morphs.

Table I: Measures (mean ± 95% CI) of anterior spines, posterior spines, body width and body length of the prey B. calyciflorus *and* B. havanaensis

	B. calyciflorus			*B. havanaensis*		
	Control	Low kairomone	High kairomone	Control	Low kairomone	High kairomone
Anterior spine	32.9 ± 0.8	32.3 ± 0.8	46.3 ± 0.9	38.7 ± 1.3	36.9 ± 0.8	56.3 ± 1.7
Posterior spine	39.6 ± 0.8	44.9 ± 1.6	76.6 ± 3.9	96.3 ± 4.5	101.4 ± 1.9	164.3 ± 4.3
Body width	179.9 ± 10.6	190.1 ± 4.4	225.4 ± 4.6	112.3 ± 3.3	108.2 ± 2.4	143.0 ± 4.8
Body length	225.7 ± 8.5	232.9 ± 9.9	299.4 ± 3.3	155.4 ± 3.6	132.2 ± 1.3	166.4 ± 4.6

Rotifers were exposed to medium control, with low kairomone concentration and with high kairomone concentration.

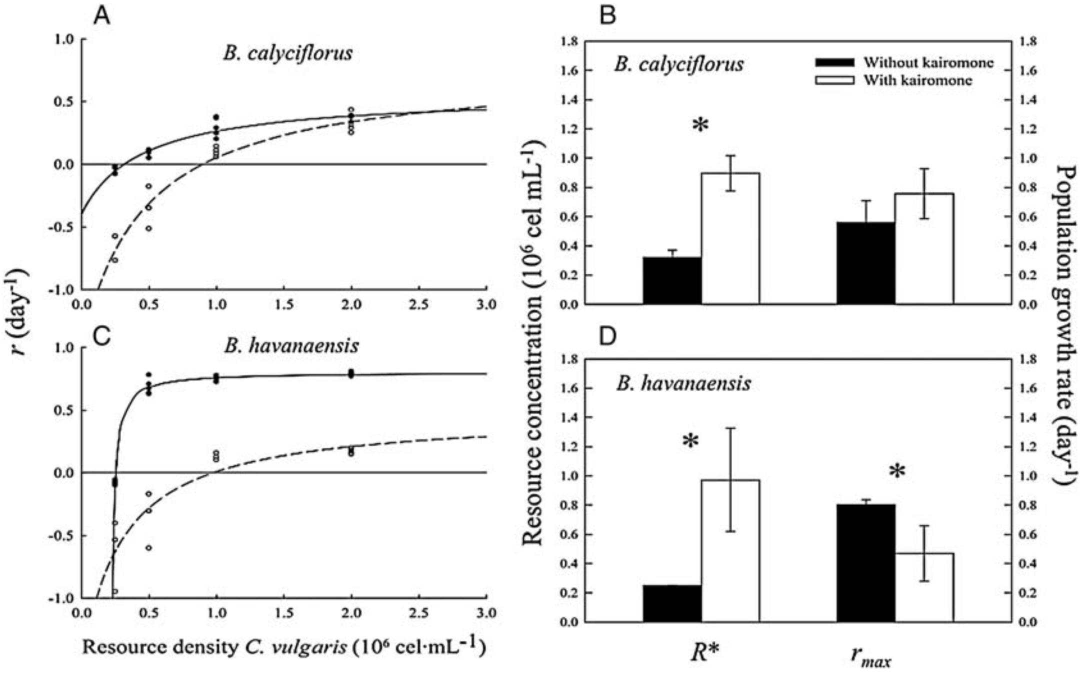

Fig. 1. Relationship between resource (*C. vulgaris*) density and growth rate of (**A**) *B. calyciflorus* and (**C**) *B. havanaensis.* Continuous line and filled circles: herbivores not induced; dashed line and empty circles: herbivores induced with predator kairomone. Data were fitted to a Monod type equation (*F* test, $P < 0.01$). From these curves, resource thresholds for zero growth (R^*) and maximum growth rate ($r_{\max}$) for *B. calyciflorus* (**B**) and *B. havanaensis* (**D**) were calculated, with and without kairomone of the predator *A. brightwelli*. Error bars correspond to 95% CI. *Significant differences between treatments (*t*-test, $P < 0.05$).

These results indicate that both herbivores decreased their competitive ability when defenses were expressed. In other words, at low food densities ($<R^*$), both species should exhibit decreased growth rate and an increased likelihood of local extinction when defenses are expressed. Conversely, at high food level, the defense would reduce the population growth of *B. havanaensis* but would not alter the performance of *B. calyciflorus*.

The two-species experiment was designed to assess the effect of induced defenses on the relative performance of the two herbivores sharing a single food resource (Fig. 2). This experiment showed that in fresh medium, *B. havanaensis* reached higher mean densities and less variable trajectories at low food densities (0.25 and 0.5×10^6 cells mL^{-1}) compared with *B. calyciflorus*. At higher food densities (1 and 2×10^6 cells mL^{-1}), *B. calyciflorus* reached higher mean values, while the densities of both species were equally stable (Fig. 3). These results agree with calculated rates of competitive exclusion, which show that the exclusion rate of *B. havanaensis* by *B. calyciflorus* increased with resource availability (Table II). No exclusion was predicted at 1×10^6 cells mL^{-1} food, which matched our empirical results. In the treatments with predator kairomone, at all resource levels $\geq 0.5 \times 10^6$ cells mL^{-1}, mean density of *B. calyciflorus* was higher and its trajectories were less variable than those of *B. havanaensis*. At the lowest food

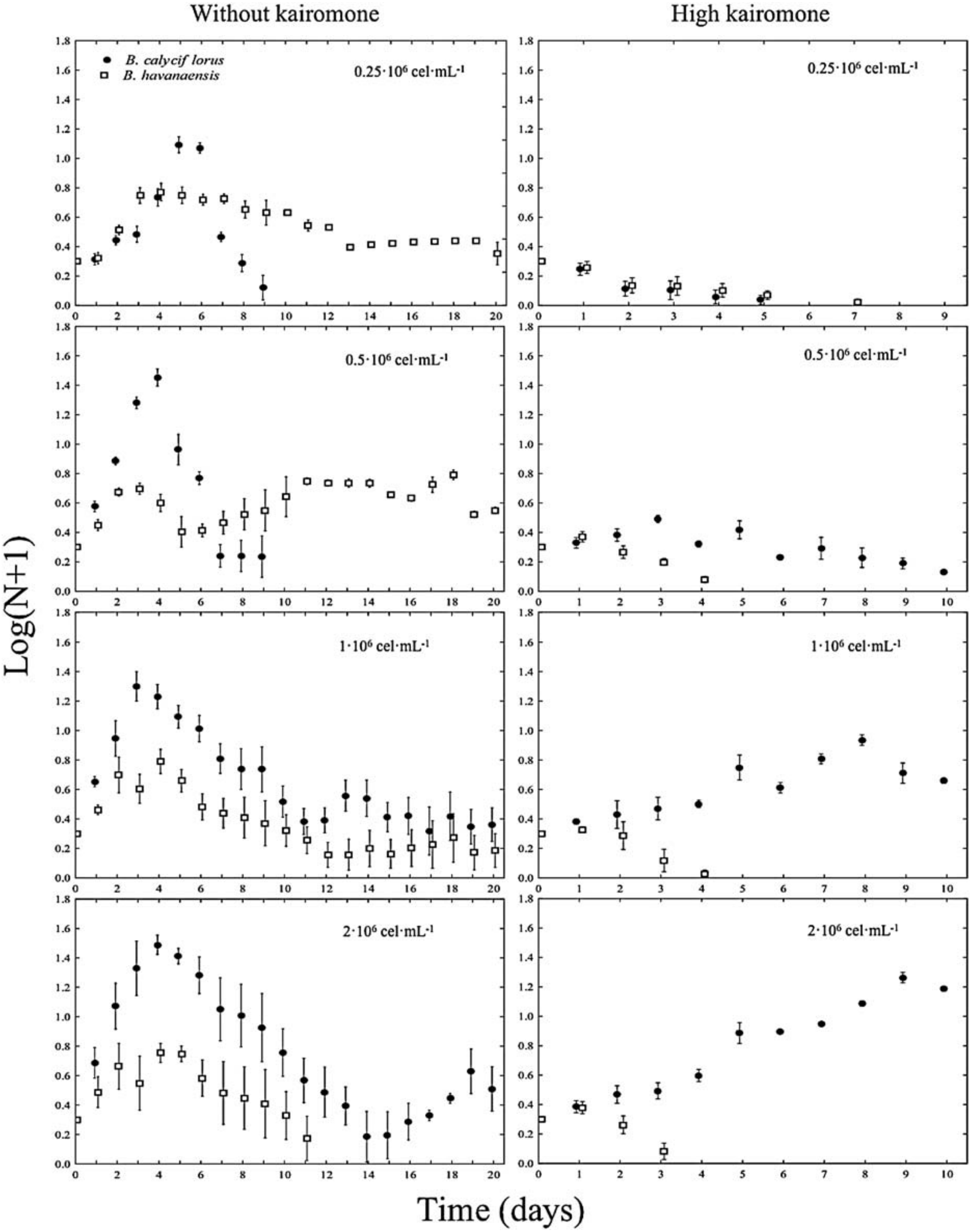

Fig. 2. Population density trajectories of *B. calyciflorus* and *B. havanaensis*, in competition experiments: without kairomone (left column), and with high kairomone concentration (right column). Experiments were run under a gradient of the resource *C. vulgaris*: Error bars correspond to 95% confidence intervals.

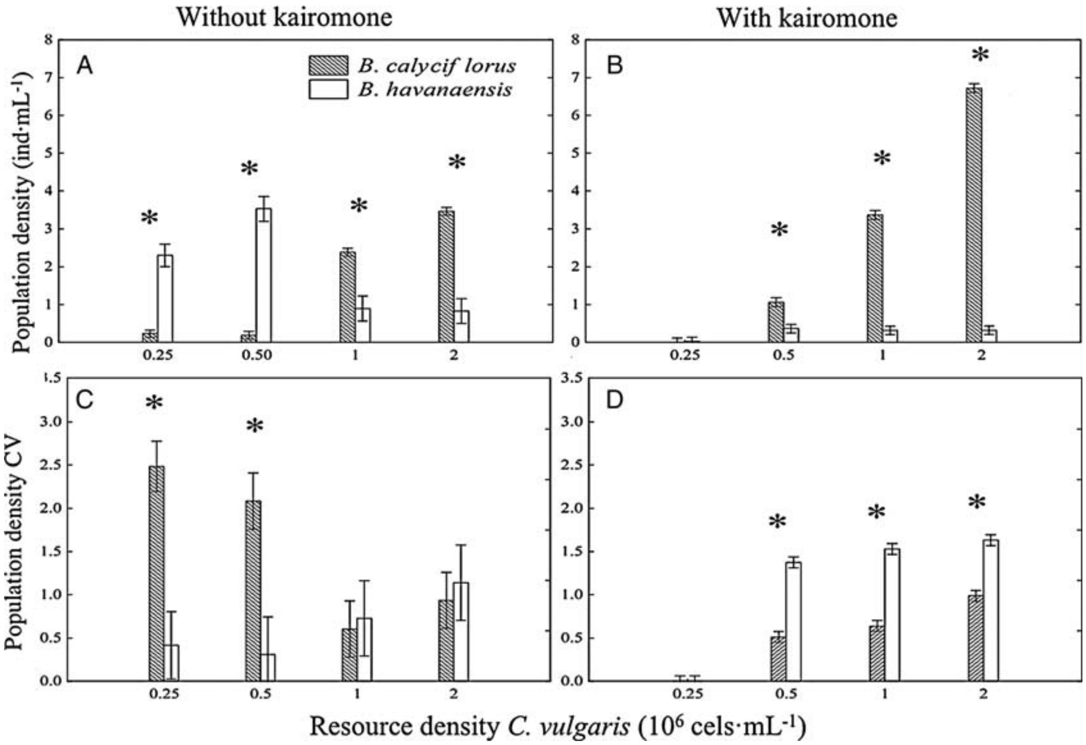

Fig. 3. Mean population density (**A** and **B**) and coefficient of variation of population density (**C** and **D**) over time in the competition experiment between *B. calyciflorus* and *B. havanaensis* carried out in control medium (without kairomone), and with predator kairomone. Error bars correspond to 95% CI. *Significant differences between species (Tukey, $P < 0.05$).

Table II: Rates of competitive exclusion η *(estimate* ± *SE) in the competition experiment between* B. calyciflorus *and* B. havanaensis *at four resource levels*

Resource level (10^6 cells mL^{-1})	Without kairomone	With kairomone
0.25	−0.22 ± 0.05	−0.01 ± 0.018*
0.5	−0.14 ± 0.07	0.60 ± 0.09*
1	0.036 ± 0.06	0.73 ± 0.05*
2	0.14 ± 0.09	0.80 ± 0.22*

η values >0 represent competitive dominance of *B. calyciflorus*, and η values <0 represent dominance of *B. havanaensis*. *Significant differences between kairomone levels (Holm–Sidak, $P < 0.001$).

availability (0.25×10^6 cells mL^{-1}), both species tended to extinction as predicted by the calculated food thresholds (Figs. 2 and 3). Correspondingly, exclusion rates of *B. havanaensis* by *B. calyciflorus* increased with resource availability, but with higher values compared with the no-kairomone level. These results predict exclusion of *B. havanaensis* at all but the lowest food levels in the presence of kairomones (Table II).

Table III summarizes the evaluation of the effectiveness of the defensive traits. Defended morphs of both species benefited from a decreased predator attack rate (*a*). In addition, *B. havanaensis* also benefited from an increased handling time (*H*). These values indicate that the defense of *B. havanaensis* can be up to 18 times more effective in avoiding predation losses compared with that of *B. calyciflorus*.

DISCUSSION

The results of this study support the existence of an interaction cost of inducible defenses in rotifers as model organisms, expressed as a trade-off between the expression of morphological defenses and competitive ability. The two experimental species used here showed a significant increase in resource thresholds for zero population growth when defenses were induced. That is, defended morphs required a higher resource density to maintain positive rates of population growth relative to non-induced morphs. This is consistent with previous knowledge (Barry, 1994; Tollrian and Dodson, 1999; van Buskirk, 2000), which associated morphological defenses with metabolic costs derived from energy allocation to defensive structures. Studies focused on evaluating the cost of the kairomone-induced phenotype in single rotifer populations have produced variable results (Gilbert, 1980; Stemberger, 1988), and even results

Table III: Fitted parameters (± SE) of the functional responses of A. brightwelli *to* B. calyciflorus *and* B. havanaensis *abundances, exhibiting basic and induced morphs*

	Basic morph			Induced morph		
	a	*H*	R^2	*a*	*H*	R^2
B. calyciflorus	0.02 ± 0.02	0.66 ± 0.59	0.52	0.004 ± 0.005	0.69 ± 0.84	0.50
B. havanaensis	0.076 ± 0.08	0.38 ± 0.31	0.62	0.01 ± 0.01	0.68 ± 0.55	0.70

Type II curves, $y = ax/(1 + aHx)$, were fitted by non-linear least squares ($P < 0.001$).

opposite to ours, with defended morphs associated with reduced threshold food concentration (Stemberger, 1990). Nevertheless, it must be noted that the plastic response exhibited by both tested clones was characterized not only by a notable spine elongation (40−100% increase) but also by an increase in body size (27−36%). Positive allometric relationships have been recorded between body size of several rotifer species and R^* (Stemberger and Gilbert, 1985). On this basis, it is expected that large-bodied defensive phenotypes would have higher R^* relative to smaller undefended morphs within the same species, which is consistent with our results.

Experimental evaluations of fitness costs of defended phenotypes in rotifer populations have shown variable results, explained by interspecific differences in body sizes and sinking rates, and effects of food concentration (Gilbert, 1999). The observed effects of defense induction on competitive ability assessed in single-species experiments predicted the outcome of the coexistence experiments. First, both species drastically reduced their ability to maintain viable populations and species coexistence at very low food levels when defenses were induced, as was predicted by the increase in R^*. Second, the induction of defenses promoted a shift in the relative competitive performance of the herbivores. The species that without predator cues dominated at moderately low resource levels (*B. havanaensis*) decreased its mean population density after kairomone induction, stepping down as the dominant competitor (Fig. 2). Conversely, the dominant species under high resource availability (*B. calyciflorus*) reinforced its dominant role after induction of defenses. In addition, rates of competitive exclusion increased with the addition of kairomones. Furthermore, calculated rates predicted the exclusion of *B. calyciflorus* at low food levels and of *B. havanaensis* at high levels without kairomones. Adding predator cues promoted competitive exclusion of *B. havanaensis* over the resource gradient, except at the lowest food level where both species went extinct. This suggests that the ability of *B. havanaensis* to exploit scarce resources is overwhelmed by high metabolic or allocation costs of defense; at a high resource level, its growth was limited by a lower reproductive potential ($r_{\max}$) compared with its non-induced state. *Brachionus calyciflorus* was likely favored by the release of resources at intermediate food levels, but adding kairomones in non-limiting food conditions promoted the equalization of trajectories in the initial phase of population growth, maintaining higher densities during the last phase (Fig. 2). Thus, the addition of kairomones mainly affected the dominant competitor over the resource gradient. These shifts in patterns of coexistence are in agreement with the kairomone-driven shifts in the relationship between growth rate and resource availability shown above. The increase in R^* after kairomone induction affected both herbivores, but only *B. havanaensis* diminished its maximum growth rate $r_{\max}$. In other words, exposure to predator cues decreased the performance of both species at limiting resource levels, but as food increased, the performance of *B. havanaensis* became increasingly poorer. Our results are in agreement with earlier expectations of resource-dependent costs driven by morphological defenses (Gilbert, 1980; Stemberger, 1990). Nevertheless, those expectations had not been experimentally contrasted with competitive outcomes.

Calculated R^*'s were not different between species with predator cues, and were marginally different without predator cues. These similarities suggest that the outcome of competition will depend on the states of the competing organisms when resources become limiting. Nevertheless, given the dependence exhibited by the calculated R^* values with respect to the level of induced defenses, the competitive ability of species cannot be assumed to be a fixed trait, but is rather a plastic attribute associated with predation risk and presumably with other factors affecting the development of defenses. Kairomone concentration, in addition to affecting population densities (Jeschke and Tollrian, 2000) and bioavailability of several pollutants (Lürling and Scheffer, 2007), is known to determine the development of defenses in planktonic organisms and thereby they should modulate their competitive ability. This highlights the link between phenotypic plasticity and competition-mediated coexistence in variable environments.

In addition, an assessment of the effectiveness of the induced defenses, measured as changes in the functional response of predators facing both undefended and defended prey, indicated that *B. havanaensis* benefited more than *B. calyciflorus* from predation by *Asplanchna*. Therefore, the species exhibiting the more effective defense also paid the stronger interaction cost (i.e. diminished competitive performance). This preliminary result highlights the importance of phenotypic plasticity of organisms in the generation of optimal responses to simultaneous threats posed by natural enemies such as predators and competitors. This result, although requiring a broader validation across species, highlights the relevance of individual prey responses to fluctuating environments while undergoing multiple selective pressures.

The observed decrease in competitive performance of both defended species at limiting resource levels suggests that interaction costs generated by inducible defenses increase the strength of competition among populations, which is expected to promote exclusion of species and community instability. Most theoretical (Vos *et al.*, 2004; Miner *et al.*, 2005; Kondoh, 2007; Ramos-Jiliberto *et al.*, 2008) and experimental studies (Verschoor *et al.*, 2004; van der Stap *et al.*, 2007; Aránguiz-Acuña *et al.*, 2010) have shown that the incorporation of inducible defenses stabilizes the dynamics of bi- and tri-trophic systems, enhancing species persistence. Nevertheless, stabilization effects of inducible defenses could be compromised with potential destabilization effects of an interaction cost and other feedback mechanisms such as response delays (Garay-Narváez and Ramos-Jiliberto, 2009). This issue deserves further theoretical and empirical research in order to understand the relevance of interaction costs and its community consequences.

Our results complement those of Werner and Anholt (Werner and Anholt, 1996), Relyea (Relyea, 2000) and van der Stap *et al.* (van der Stap *et al.*, 2008), who verified that behavioral responses as well as morphological responses of prey to cues released by their predators impose individual costs that are only measurable in a multispecific scenario. Additionally, we offer novel evidence on the link between a mechanistic theory of resource competition, based on individual attributes, and community functioning mediated by inducible plastic responses of competitors. Dynamic consequences of adaptive plasticity appear, therefore, to be sensitive to the actual community structure in which the responsive organisms are inserted.

The results presented in this study experimentally confirmed the existence of functional community consequences derived from the expression of inducible defenses. This highlights the intricacy of mechanisms leading to both quantitative and qualitative adjustments of the interspecific effects taking place in natural communities, and draw attention to the interplay between individual-level responses and community functioning for a better understanding of complex ecologies in the wild.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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REFERENCES

Abrams, P. (2007) Defining and measuring the impact of dynamic traits on interspecific interactions. *Ecology*, **88**, 2555–2562.

Anholt, B. R. and Werner, E. E. (1999) Density-dependent consequences of induced behavior. In Tollrian, R. and Harvell, C. D. (eds), *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, New Jersey, pp. 218–230.

Aránguiz-Acuña, A., Ramos-Jiliberto, R., Nandini, S. *et al.* (2010) Benefits, costs and reactivity of inducible defences: an experimental test with rotifers. *Freshwater Biol.*, **55**, 2114–2122.

Barry, M. J. (1994) The costs of crest induction for *Daphnia carinata*. *Oecologia*, **97**, 278–288.

Benard, M. F. (2006) Survival trade-off between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). *Ecology*, **87**, 340–346.

Ciros-Pérez, J., Carmona, M. J. and Serra, M. (2001) Resource competition between sympatric sibling rotifer species. *Limnol. Oceanogr.*, **46**, 1511–1523.

Epp, R. W. and Lewis, W. M. (1984) Cost and speed of locomotion for rotifers. *Oecologia*, **61**, 289–292.

Garay-Narváez, L. and Ramos-Jiliberto, R. (2009) Induced defenses within food webs: the role of community trade-offs, delayed responses, and defense specificity. *Ecol. Complex.*, **6**, 383–391.

Garza-Mouriño, G., Silva-Briano, M., Nandini, S. *et al.* (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. (1980) Further observations on developmental polymorphism and its evolution in the rotifer *Brachionus calyciflorus*. *Freshwater Biol.*, **10**, 281–294.

Gilbert, J. J. (1999) Kairomone-induced morphological defenses in rotifers. In Tollrian, R. and Harvell, C. D. (eds), *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, New Jersey, pp. 127–141.

Gilbert, J. J. (2001) Spine development in *Brachionus quadridentatus* from an Australian billabong: genetic variation and induction by *Asplanchna*. *Hydrobiologia*, **446/447**, 19–28.

Gilbert, J. J. (2009) Predator-specific inducible defenses in the rotifer *Keratella tropica*. *Freshwater Biol.*, **54**, 1933–1946.

Grover, J. P. (1988) Dynamics of competition in a variable environment: experiments with two diatom species. *Ecology*, **69**, 408–417.

Grover, J. P. (1991) Dynamics of competition among microalgae in variable environments: experimental tests of alternative models. *Oikos*, **62**, 231–243.

Harvell, C. D. (1990) The ecology and evolution of inducible defenses. *Q. Rev. Biol.*, **65**, 323–340.

Havel, J. E. and Dodson, S. I. (1987) Reproductive costs of *Chaoborus*-induced polymorphism in *Daphnia pulex*. *Hydrobiologia*, **150**, 273–281.

Hoverman, J. T. and Relyea, R. A. (2009) Survival trade-offs associated with inducible defences in snails: the roles of multiple predators and developmental plasticity. *Funct. Ecol.*, **23**, 1179–1188.

Jeschke, J. M. and Tollrian, R. (2000) Density-dependent effects of prey defences. *Oecologia*, **123**, 391–396.

Kondoh, M. (2007) Anti-predator defence and the complexity–stability relationship of food webs. *P. Roy. Soc. B: Biol. Sci.*, **274**, 1617–1624.

Lürling, M. and Scheffer, M. (2007) Info-disruption: pollution and the transfer of chemical information between organisms. *Trends Ecol. Evol.*, **22**, 374–379.

Miner, B. G., Sultan, S. E., Morgan, S. G. *et al.* (2005) Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.*, **20**, 685–692.

Nandini, S. (1999) Variations in physicochemical parameters and plankton community structure in a series of sewage stabilization ponds. *Rev. Biol. Trop.*, **47**, 149–156.

Peacor, S. D. (2002) Positive effect of predators on prey growth rate through induced modifications of prey behavior. *Ecol. Lett.*, **5**, 77–85.

Peacor, S. D. and Werner, E. E. (1997) Trait-mediated indirect interactions in a simple aquatic food web. *Ecology*, **78**, 1146–1156.

Peacor, S. D. and Werner, E. E. (2000) Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. *Ecology*, **81**, 1998–2010.

Peacor, S. D. and Werner, E. E. (2001) The contribution of trait-mediated indirect effects to the net effects of a predator. *Proc. Natl Acad. Sci. USA*, **98**, 3904–3908.

Pettersson, L. B. and Brönmark, C. (1999) Energetic consequences of an inducible morphological defence in crucian carp. *Oecologia*, **121**, 12–18.

Ramos-Jiliberto, R., Mena-Lorca, J., Flores, J. D. *et al.* (2008) Role of inducible defenses in the stability of a tritrophic system. *Ecol. Complex.*, **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. (2003) How prey respond to combined predators: a review and an empirical test. *Ecology*, **84**, 1827–1839.

Relyea, R. A. and Yurewicz, K. L. (2002) Predicting community outcomes from pairwise interactions: integrating density- and trait-mediated effects. *Oecologia*, **131**, 569–579.

Rothhaupt, K. O. (1988) Mechanistic resource competition theory applied to laboratory experiments with zooplankton. *Nature*, **333**, 660–662.

Rothhaupt, K. O. (1993) Steady-state growth and carbon metabolism of *Brachionus rubens* and *Brachionus calyciflorus*. In: Walz, N. (ed.), *Plankton Regulation Dynamics: Experiments and Models in Rotifer Continuous Cultures*. Springer, Berlin, pp. 123–132.

Sarma, S. S. S., Nandini, I. and Dumont, H. J. (1996) Competitive interactions between herbivorous rotifers: importance of food concentration and initial population density. *Hydrobiologia*, **331**, 1–7.

Stemberger, R. S. (1988) Reproductive costs and hydrodynamic benefits of chemically induced defenses in *Keratella testudo*. *Limnol. Oceanogr.*, **33**, 593–606.

Stemberger, R. S. (1990) Food limitation, spination, and reproduction in *Brachionus calyciflorus*. *Limnol. Oceanogr.*, **35**, 33–44.

Stemberger, R. S. and Gilbert, J. J. (1985) Body size, food concentration, and population growth in planktonic rotifers. *Ecology*, **66**, 1151–1159.

Strauss, S. Y., Rudgers, J. A., Lau, J. A. *et al.* (2002) Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.*, **17**, 278–285.

Tilman, D. (1982) *Resource Competition and Community Structure*. Princeton University Press, New Jersey.

Tollrian, R. (1995) Predator-induced morphological defenses: costs, life-history shifts, and maternal effects in *Daphnia pulex*. *Ecology*, **76**, 1691–1705.

Tollrian, R. and Dodson, S. I. (1999) Inducible defenses in Cladocera: constraints, costs, and multipredator environments. In Tollrian, R. and Harvell, C. D. (eds), *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, New Jersey, pp. 177–202.

Tollrian, R. and Harvell, C. D. (1999) The evolution of inducible defenses: current ideas. In Tollrian, R. and Harvell, C. D. (eds), *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, New Jersey, pp. 306–321.

US EPA. (2002) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edn. EPA-821-R-02-012.

van Buskirk, J. (2000) The costs of an inducible defense in anuran larvae. *Ecology*, **81**, 2813–2821.

van der Stap, I., Vos, M., Tollrian, R. *et al.* (2008) Inducible defenses, competition and shared predation in planktonic food chains. *Oecologia*, **157**, 697–705.

van der Stap, I., Vos, M., Verschoor, A. M. *et al.* (2007) Induced defenses in herbivores and plants differentially modulate a trophic cascade. *Ecology*, **88**, 2474–2481.

Verschoor, A. M., Vos, M. and van der Stap, I. (2004) Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. *Ecol. Lett.*, **7**, 1143–1148.

Vos, M., Kooi, B. W., DeAngelis, D. L. *et al.* (2004) Inducible defenses and the paradox of enrichment. *Oikos*, **105**, 471–480.

Werner, E. E. (1991) Nonlethal effects of a predator on competitive interactions between two anuran larvae. *Ecology*, **72**, 1709–1720.

Werner, E. E. and Anholt, B. R. (1996) Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology*, **77**, 157–169.

Zagarese, H. E. and Marinone, M. C. (1992) Induction and inhibition of spine development in the rotifer *Keratella tropica*: evidence from field observations and laboratory experiments. *Freshwater Biol.*, **28**, 289–300.