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Non-lethal effects of invertebrate predators on *Daphnia*: morphological and life-history consequences of water mite kairomone

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SUMMARY

1. Here, we report morphological and life-historical changes in the cladoceran *Daphnia ambigua* in response to chemical cues released by the predatory water mite *Piona chilensis*. Both species are common inhabitants of southern temperate lakes.
2. We found significant differences in adult body size at first, second and third reproduction. Also, individuals exposed to kairomones had longer tail spines at first reproduction, and the resultant offspring had smaller bodies and shorter tail spines.
3. Exposure to mite cues did not exert effects on brood size at first reproduction, but decreased offspring number in subsequent broods. Similarly, only the second and third reproduction events were delayed by kairomone exposure.
4. The intrinsic population growth rate of predator-induced animals was lower than that in controls, but simulations based on a parameterized matrix model showed that the fitness costs could be overcome if the reported phenotypic responses reduced predation rate moderately. The gain in protection from predators needed to cancel out the reduction in fitness associated with predator cues was directly related to juvenile survival and fertility, and inversely related to adult survival.
5. This is the first work reporting phenotypic plasticity in Cladocera in response to kairomones released by water mites, which are conspicuous predators in many austral fresh waters.

Keywords: Chile, costs, inducible defenses, *Piona chilensis*, trait-mediated indirect effects

Introduction

Induced defences are phenotypic adjustments, triggered by cues released by natural enemies, that suppress the negative effect of the interaction (Tollrian & Dodson, 1999). This particular type of phenotypic plasticity has been reported to occur in the form of shifts in life-historical, morphological and behavioural traits of many taxa, from protists to vertebrates (Miner *et al.*, 2005; Pigliucci, 2005). Cladocerans, for example, exhibit a variety of antipredator strategies (Larsson & Dodson, 1993; Tollrian & Dodson, 1999). Some of these operate through avoiding encounters with predators, often by means of spatial segregation (e.g. diel vertical migration; DVM) or the production of resting eggs. Other strategies act by decreasing the probability of prey detection by a predator (e.g. altering pigmentation, body size or swimming activity), or by decreasing predator capture efficiency (e.g. increased escape responses by prey). Furthermore, increased body size and the development of morphological structures in cladocerans (neck teeth, tail spines, helmets and crests) may hamper their handling or ingestion by predators and hence facilitate escape. A further

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category of defences consists of shifts in energy allocation and/or life-history traits that yield an optimal reproductive output under specific predation regimes (Taylor & Gabriel, 1992; Tollrian & Dodson, 1999). Given that predation risk is variable and defences are expected to have a cost in terms of some fitness component, inducible defences are considered to be cost-saving adaptations only displayed when necessary.

Planktonic crustaceans of the genus *Daphnia* have been extensively reported as prone to exhibit plastic responses associated with predation risk (Tollrian & Dodson, 1999). Although predators vary, most of them are size-selective. For example, planktivorous fish are visual predators that prefer larger *Daphnia*, whereas larvae of the phantom midge *Chaoborus* are tactile hunters and consume smaller *Daphnia* preferentially. As a consequence, predators impose different selective forces and generate predator-specific reactions in their prey (Brett, 1992; Stibor & Lüning, 1994; Long *et al*., 2007). In response to pelagic invertebrates, cladocerans display a variety of antipredator adaptations that include increased body size, development of neckteeth and elongation of the tail spine, as well as shifts in habitat use and life-history traits (see Tollrian & Harvell, 1999).

Ramos-Jiliberto *et al.* (2004) suggested that the inverse DVM observed in some cladoceran species from lake El Plateado could be a consequence of predation exerted by the water mite *Piona chilensis* Viets 1968 (Hidracarina). Water mites are tactile hunters, taking *Daphnia* and other cladocerans (Butler & Burns, 1991). The mite *P. chilensis* develops dense populations in several lakes in central Chile (R. Ramos-Jiliberto & L. R. Zuniga, unpubl. data), where the abundance of *Chaoborus* and other insect predators is negligible and there are no carnivorous copepods or cladocerans. Therefore, in contrast to *Chaoborus*-dominated pelagic communities from northern lakes, *Piona* is the only serious candidate in many lakes at these latitudes as a dominant zooplanktophagous invertebrate.

Here, we tested the ability of *Daphnia ambigua* Scourfield 1947 (Cladocera) to develop plastic phenotypic responses to the invertebrate predator *P. chilensis*. Particularly, we quantified morphological and life-historical responses to mite kairomones, and assessed the main fitness consequences of the observed changes.

Methods

Study organisms

The experiments were performed with a clone of *D. ambigua* isolated from the small eutrophic lake El Plateado (33°04′30″S and 71°39′12″W; altitude 340 m). The small *D. ambigua* exhibits DVM in the field (Ramos-Jiliberto & Zuniga, 2001), and it is a generalist consumer and one of the commonest *Daphnia* species in tropical and sub-tropical regions. Chemical and physical characterization of lake El Plateado can be found in Ramos-Jiliberto *et al.* (1997). The crustacean plankton was studied by Ramos-Jiliberto *et al.* (1998, 2004).

Predator kairomone was obtained from the pelagic water mite *P. chilensis* that cohabits with *D. ambigua* in lakes in central Chile. Water mites have complex life-cycles. Overwintering females lay their eggs during early spring. Hatching larvae eventually attach to some winged insect imago and leave the water. Late larvae drop back into the water and form a protonymph, from which emerges a nymph that feeds on microcrustaceans and gives rise to an imagochrysalis. The adult that emerge from this stage actively predates on crustacean plankton (Mitchell, 1964). Predatory water mites detect their prey by touch, and consequently have only a small radius of encounter with their prey (Riessen, 1982).

Experimental design and analyses

Cultures of cladocerans and water mites were grown in the laboratory using a mixture of 1/3 filtered lake water and 2/3 aged tap water. We followed standard procedures (see e.g. Dzialowski *et al*., 2003; Bernot *et al*., 2006) for obtaining kairomones (non-purified) from predator-conditioned medium. For simplicity, hereafter we call ‘kairomones’ the entire set of dissolved chemicals released in the presence of mites. The kairomone medium was obtained by placing 30 individuals of *P. chilensis*, with *Daphnia* as food, in 900 mL of water for 24 h. The experimental mite density was higher than reported previously from the field (Matveev, Martinez & Frutos, 1989; Balseiro, 1992) but was similar to local maxima we have observed in Chilean lakes. Every other day, the mites were put into fresh medium containing 20 daphniids. Based on previous tests, we know that this density of *D. ambigua* is far below the response threshold for observing intraspecific crowding effects in this clone. Both control and kairomone media were filtered through 0.45-μm mesh. During the experiments the medium and daphniids were replaced daily, using *Chlorella vulgaris* Beijerinck, 1890 at a saturating density of 10^6 cells mL^{-1} as food source for *Daphnia.* The pH, measured before and after each replacement of the medium, was constant at 7.4. Photoperiod (16 : 8 L : D) and temperature (20 ± 1.5 °C) were controlled inside an experimental chamber.

Animals for experiments were obtained as follows. Ten *D. ambigua* born from the third brood of a single mother were placed individually into 50 mL beakers containing 40 mL of medium. Just after they released their first brood, half of them were placed in control medium and the rest in kairomone medium. From their third brood we took a sample of 28 daughters as the experimental individuals, half of which were used as controls. In this way, the focal generation was exposed to the experimental medium throughout their ontogenesis, starting at the egg stage in the mother's brood chamber.

At birth, as well as after each individual reproductive event, we measured body length (from the top of head to the base of the tail spine), tail spine length and the angle of the tail spine respect to the main body axis (sketched form the centre of the eye to the base of the spine). These measurements were made on both the experimental animals and their daughters, from digital images using UTHSCSA IMAGE-TOOL for windows, version 3.0 (Wilcox *et al.*, 2002). At 24-h intervals, we inspected all animals and recorded their age at each reproduction, survival and offspring number. With the information obtained we constructed life-tables and estimated fitness as the intrinsic growth rate (r), by means of the discrete Euler-Lotka equation

$$\sum_{x=1}^{n} \mathrm{e}^{-rx} l_x m_x = 1$$

where x is age in days, n is age at third reproduction, and l_x and m_x are age-specific survival and fertility respectively.

For comparison of trait values between treatments, as well as for comparing neonates from different broods, we used one-way randomization ANOVA and randomization *post hoc* multiple comparisons. The routines were run with the software RANDOM PROJECT version 2.0 lite (Jadwiszczak, 2003).

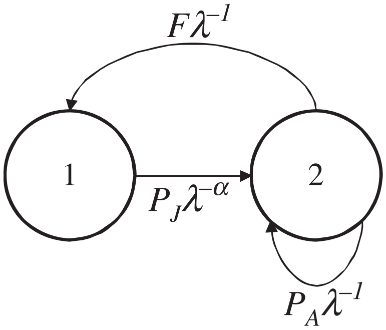

Fig. 1 Two-stage life cycle graph, with transition rates defined by time to maturity α, probability P_J of surviving to maturity, adult survival probability P_A and fertility F. Finite population growth rate is λ.

Finally, we used a modelling approach to estimate the amount of survival gain that induced phenotypes should exhibit for cancelling out the observed fitness reduction. For this purpose, from age-specific survival and fertility records we parameterized an age-structured matrix model for each of the two treatments (i.e. kairomone and control), according to Caswell's (2001) methods for birth-flow populations (see Ramos-Jilberto & Aránguiz-Acuña, 2007). The projection interval was equal to 1 day.

From each of the age-structured matrices we developed a simplified two-stage model with juveniles and adults as state-variables (Fig. 1). Here, juveniles survive with probability P_J to reach maturity, i.e. $\prod_{i=1}^{\alpha+1} P_i$, spending α time units in the process:

$$P_\mathrm{J} = \sigma_1^{\alpha}$$

For each treatment, age at maturity was considered to be the minimum age at which at least one individual in the treatment reproduced. On the other hand, adults survive with probability P_A during one projection interval:

$$P_\mathrm{A} = \sigma_2$$

Adult survivorship (σ_2) and fertility (F) were calculated according to Levin *et al.* (1996) as:

$$\sigma_2 = \frac{\sum_{i=\alpha+1}^{\infty} w_i P_i}{\sum_{i=\alpha+1}^{\infty} w_i}$$

$$F = \frac{\sum_{i=\alpha+1}^{\infty} w_i F_i}{\sum_{i=\alpha+1}^{\infty} w_i}$$

where w_i represents is the i-element of the stable age distribution vector, and P_i and F_i are respectively survival and fertility rates at age i, taken from the age-structured matrix.

The characteristic equation for the stage-structured model is:

$$\lambda^{\alpha+1} - P_A\lambda^{\alpha} - P_J F = 0$$

which was used for calculating population growth rate λ through finding its roots, as well as for calculating sensitivities of growth rate to changes in parameter values, i.e. $\frac{\partial\lambda}{\partial p}$, with $p = \{\sigma_1, \sigma_2, \alpha, F\}$, by means of implicit differentiation.

The contribution c_i of each parameter p_i to the observed change in λ as a product of the experimental treatment K (e.g. exposure to mite cues) relative to control C was evaluated through a retrospective analysis of the stage-structured model. The main equation is:

$$\lambda^{(K)} \approx \lambda^{(C)} + \sum_i c_i$$

with

$$c_i = \left(p_i^{(K)} - p_i^{(C)}\right) \frac{\partial\lambda}{\partial p_i}\bigg|_{P/2}$$

where $P/2$ indicates that sensitivities are evaluated at the mean of both parameter sets.

Results

The relationship between intrinsic growth rate and both body length and tail spine length of *Daphnia* at their age of first reproduction was different between controls and kairomone-exposed animals (Fig. 2). In terms of means, individuals exposed to kairomones had smaller bodies (*B*) and longer tail spines (*TS*), relative to controls (Table 1). From Fig. 2 it can be noted that (i) control organisms exhibited a positive correlation between standardized tail spine length (*TS/B*) and fitness r ($P < 0.05$), and (ii) individuals with similar values of the ratio *TS/B* had lower fitness when exposed to kairomones. Correlation analyses indicated that the observed association between *TS/B* and r in controls was mainly due to an inverse relationship between tail spine length and age at first reproduction (Spearman's rank correlation, $P < 0.05$).

The observed kairomone-induced shifts of morphological traits depended strongly on the developmental state of *D. ambigua*. For neonates, while neither differences in *B* (marginally) nor in *TS* were significant between groups, the *TS/*B ratio was significantly smaller for kairomone-exposed individuals (Table 1). When animals reached maturity we found significant effects of kairomone medium on *B*, *TS* and *TS/*B, although the direction of the effect on *TS/B* was opposite to that in neonates (Table 1). While individuals exposed to kairomones had smaller bodies, longer tail spines and consequently a larger *TS/*B ratio at first reproduction, only body length was still significantly smaller in the kairomone treatments after *Daphnia* had hatched their second and third broods (Table 1). There were no significant differences in the tail spine angle (*SA*) between groups at any developmental state (Table 1).

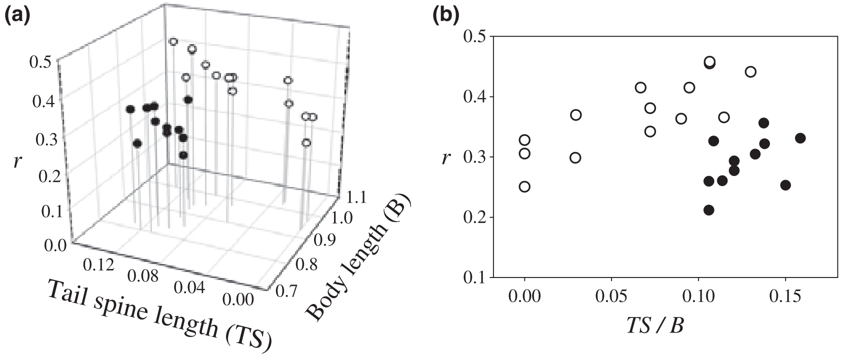

Fig. 2 Main morphological responses of *Daphnia* at their age of first reproduction, in relation with their calculated population growth rate (r). Empty and filled circles show control and kairomone treatments respectively: (a) body length and tail spine length versus r, (b) ratio of tail spine length to body length versus r. Correlation is significant (Spearman's rank test, $P < 0.05$) only for controls.

Table 1 Morphological and life-history traits of experimental *Daphnia* under kairomone and control treatments

Trait	Control mean ± SD	Kairomone mean ± SD	*F*-value	*P*-value
Neonates				
$nC = 14$, $nK = 14$			$F_{(1,26)}$	
B (mm)	0.497 ± 0.052	0.531 ± 0.039	3.384	0.07
TS (mm)	0.151 ± 0.017	0.143 ± 0.011	2.009	0.15
*TS/*B	0.307 ± 0.053	0.271 ± 0.031	4.605	<0.05
SA (degrees)	170.5 ± 3.652	169.1 ± 3.473	1.001	0.31
First reproduction				
$nC = 14$, $nK = 11$			$F_{(1,23)}$	
B (mm)	0.965 ± 0.036	0.843 ± 0.035	69.92	<0.01
TS (mm)	0.062 ± 0.043	0.106 ± 0.012	10.02	<0.01
*TS/*B	0.065 ± 0.045	0.126 ± 0.018	17.66	<0.01
SA (degrees)	184.3 ± 7.209	178.5 ± 7.756	3.701	0.06
AR (days)	5.143 ± 1.231	5.000 ± 0.679	1.082	0.84
F (ind)	3.785 ± 1.121	3.545 ± 1.035	0.110	0.87
Second reproduction				
$nC = 13$, $nK = 11$			$F_{(1,21)}$	
B (mm)	1.005 ± 0.028	0.905 ± 0.049	38.50	<0.01
TS (mm)	0.067 ± 0.043	0.076 ± 0.026	0.334	0.57
*TS/*B	0.067 ± 0.042	0,085 ± 0.032	1.349	0.24
SA (degrees)	178.4 ± 7.517	185.6 ± 11.70	3.289	0.24
AR (days)	7.000 ± 1.290	10.00 ± 3.098	10.17	<0.01
F (ind)	3.615 ± 0.767	2.000 ± 0.632	30.88	<0.01
Third reproduction				
$nC = 12$, $nK = 11$			$F_{(1,18)}$	
B (mm)	1.035 ± 0.035	0.930 ± 0.047	34.10	<0.01
TS (mm)	0.062 ± 0.035	0.055 ± 0.020	0.276	0.60
*TS/*B	0.060 ± 0.033	0.060 ± 0.023	0.00003	0.99
SA (degrees)	186.6 ± 13.90	189.9 ± 8.045	0.399	0.53
AR (days)	8.750 ± 0.965	12.66 ± 2.061	33.87	<0.01
F (ind)	5.000 ± 1.206	2.111 ± 0.781	39.03	<0.01
$nC = 14$, $nK = 11$			$F_{(1,23)}$	
r (day^{-1})	0.370 ± 0.062	0.299 ± 0.044	13.28	<0.01

B, body length; *TS*, tail spine length; *SA*, angle of tail spine relative to body axis; *AR*, age at each reproductive event; *F*, brood size; *r*, intrinsic population growth rate.
P-values after randomization ANOVA. Sample size for controls and kairomone treatment are given by *nC* and *nK* respectively.

We also found differences in both offspring number and offspring quality in predator-induced animals relative to controls. First-brood neonates had smaller bodies, shorter *TS* and larger *SA* when exposed to kairomones (Table 2), although the *TS/*B ratio did not differ between treatments. Second-brood neonates showed no significant differences in *B*, but both *TS* and *TS/*B were significantly smaller in the kairomone treatment (Table 2). Similarly to their older sisters, the *SA* of second-brood neonates increased with exposure to kairomones. Finally, only the *SA* remained significantly different between third-brood neonates (Table 2). The analyses also revealed that neonates born from mothers exposed to kairomones exhibited significant differences among broods in *B*, *TS/*B and *SA*. In contrast, neonates born from control mothers did not exhibit any difference among broods. Multiple comparisons tests revealed that first-brood neonates differed in *B* ($P < 0.05$) compared with their second- and third-brood sisters, whereas *TS/*B was different between neonates from first and second broods. Finally, *SA* measured in first-brood neonates significantly differed from that of third-brood individuals.

Reproductive life-history traits also responded to mite kairomones, leading to a decreased intrinsic growth rate (Table 1). No differences were recorded in age at first reproduction, but the production of second and third broods was delayed when exposed to kairomones. Similarly, brood size did not differ between treatments on first reproduction, but was

Table 2 Morphological and life-history traits of *Daphnia* offspring under kairomone and control treatments

Trait	Control mean ± SD	Kairomone mean ± SD	*F*-value	*P*-value
First brood neonates				
$nC = 45$, $nK = 49$			$F_{(1,93)}$	
B (mm)	0.445 ± 0.028	0.415 ± 0.024	30.21	<0.01
TS (mm)	0.159 ± 0.012	0.147 ± 0.012	27.98	<0.01
TS/B	0.360 ± 0.033	0.354 ± 0.029	0.790	0.37
SA (degrees)	169.5 ± 7.216	172.1 ± 4.032	4.448	<0.05
Second brood neonates				
$nC = 44$, $nK = 19$			$F_{(1,61)}$	
B (mm)	0.436 ± 0.022	0.451 ± 0.056	2.445	0.12
TS (mm)	0.155 ± 0.021	0.143 ± 0.017	4.411	<0.05
TS/B	0.354 ± 0.042	0.319 ± 0.037	9.922	<0.01
SA (degrees)	170.3 ± 8.654	175.6 ± 9.555	4.690	<0.05
Third brood neonates				
$nC = 45$, $nK = 19$			$F_{(2,62)}$	
B (mm)	0.442 ± 0.028	0.437 ± 0.028	0.3096	0.58
TS (mm)	0.153 ± 0.013	0.147 ± 0.013	2.554	0.11
TS/B	0.348 ± 0.033	0.338 ± 0.031	1.364	0.24
SA (degrees)	171.8 ± 5.274	175.7 ± 4.867	7.413	<0.01

B, body length; *TS*, tail spine length; *SA*, angle of tail spine relative to body axis. *P*-values after randomization ANOVA. Sample size for controls and kairomone treatment are given by *nC* and *nK* respectively.

significantly lower in kairomone treatments in subsequent broods. These effects had the consequence that predator-exposed *Daphnia* exhibited lower population growth rate.

From the mean values of life-history data obtained in our experiments (Table 1), we parameterized a simple stage-structured matrix model (Table 3) to assess: (i) the contribution of each demographic parameter to the observed fitness change (Fig. 3) and (ii) the minimal survival advantage of induced phenotypes required to balance their associated reproductive handicap. Note that (ii) presumes that the longer tail spine in *Daphnia* exposed to mite kairomones at the age at first reproduction confers some degree of protection against invertebrate predators (Havel & Dodson, 1984). The calculations were made for various values of juvenile survival, fertility and adult survival (Fig. 3).

Table 3 Model parameter values estimated from our experiments

	Control	Kairomone
Juvenile survival (σ_1)	1	0.98
Adult survival (σ_2)	0.94	0.94
Time to first reproduction (α)	3	3
Fertility (*F*)	1.97	0.95

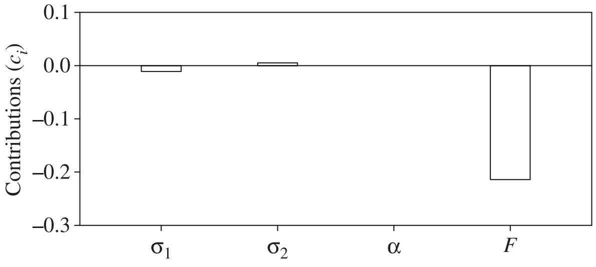

Fig. 3 Contributions of life-history parameters to observed decrease in growth rate as a consequence of exposure to mite cues. Symbols σ_1 and σ_2 indicate juvenile and adult survival rate respectively, α is time to first reproduction and *F* is mean fertility of adults.

Retrospective analyses indicated that the fitness reduction associated with predator cues was mostly due to a decrease in mean fertility (Fig. 4). Although population growth rate was more sensitive to changes in the probability of survival, the substantial reduction in the fertility of *Daphnia* exposed to kairomones, together with a moderate sensitivity of λ to this parameter, resulted in the largest contribution of *F* to the observed changes in λ.

On the other hand, our analyses revealed that, assuming a 75% adult survival (i.e. $\sigma_2 = 0.75$), and values of fertility and juvenile survival as measured in our laboratory experiments, induced individuals must

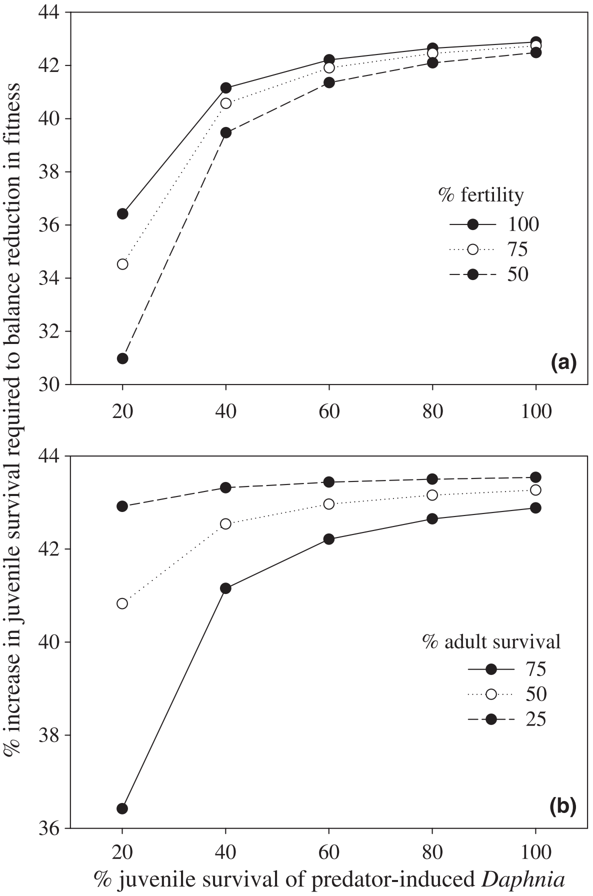

Fig. 4 Results of matrix model projections with parameter values estimated in our experiments. The curves show the amount (%) of juvenile survival that kairomone-induced individuals must gain to balance the fitness reduction associated with the response to predator cues. The plots show the dependence of this value on basal juvenile survival (*x*-axis in a and b), average fertility (a), and adult survival (b).

exhibit *c.* 42% higher survival, compared to controls, to counterbalance the reduction in fitness associated with predator cues. As juvenile survival decreases, the defence needs to be progressively less effective to remain adaptive (Fig. 4).

In Fig. 4a,b we estimated the sensitivity of the model predictions to variations in fertility and adult survival. When fertility is decreased (e.g. because food is limited), less extra protection is needed to cancel out the reduction in fitness associated with predator cues, although this effect was more pronounced under lower juvenile survival (Fig. 4a). On the other hand, lower adult survival increased the defense-effectiveness threshold for the induced phenotype (Fig 4b). Overall, our simulations indicated that induced phenotypes would be favoured if they confer, up to first reproduction, a moderate level of protection (30–44%) against predation by mites. This level will be lower at lower juvenile survival, higher adult survival and lower fertility.

Discussion

Previously, behavioural plasticity in *D. ambigua* in lake El Plateado was reported in the form of DVM (Ramos-Jiliberto & Zuniga, 2001), the behaviour being attributed to the predation risk from fish (De Meester *et al.*, 1999). However, the guild of pelagic predators in El Plateado consists of planktivorous fish (such as *Cheirodon* sp., *Odontesthes bonariensis* Valenciennes 1835 and *Basilichtys* sp.) together with the water mite *P. chilensis*. Hence, the morphological and life-history responses reported in this work, in addition to prior evidence, reveal that *D. ambigua* exhibits a wide spectrum of responses to cues released by several predators (Tollrian & Dodson, 1999; Lass & Spaak, 2003).

The phantom midge *Chaoborus* is a well-known pelagic invertebrate predator in the northern hemisphere, exerting a major role in structuring the herbivore assemblage of lakes (Hanazato & Yasuno, 1989; Yan *et al.*, 1991). Nevertheless, it has been suggested that other predators, such as *Leptodora (*Herzig & Auer, 1990), *Notonecta* (Lüning, 1992), copepods (Caramujo, Crispim & Boavida, 1997; Caramujo & Boavida, 2000) and water mites (Matveev & Martinez, 1990; Balseiro, 1992), could exert a similar role in particular waterbodies.

Our results showed that kairomone-induced organisms were significantly smaller at their age of first reproduction (Table 1). Remarkably, smaller body size was not associated with a smaller brood at first reproduction nor delayed maturation, but the quality of first-brood offspring could be compromised by their smaller body size. The second brood of kairomone-induced individuals hatched at a later age and were smaller in size, but the body size of their offspring did not differ from controls. The same occurred at the third reproduction. Thus, the fitness reduction in induced phenotypes was due to a diminished number of offspring, as well as to delays in subsequent reproductive events. An additional source of variation in fitness could be apparent through transgenerational effects, which were not explicitly tested here. Our results suggest that the offspring of kairomone-induced *Daphnia* are of lower quality (smaller body and spine length) as a side-effect of the maternal response. Therefore, it is possible that our experiments underestimated the total reduction in long-term fitness of *Daphnia* exposed to mites.

The information available shows a consistent pattern of smaller size and age at first reproduction when *Daphnia* is exposed to fish kairomones (Macháček, 1991; Weber & Declerck, 1997; Stibor & Lampert, 2000; Mikulski, 2001; Doksaeter & Vijverger, 2001; Sakwinska, 2002; Bernot *et al*., 2006). This evidence supports the hypothesis of a size refuge, in which zooplankters avoid detection by fish through a decreased body size at maturity (Taylor & Gabriel, 1992). Nevertheless, size at first reproduction has been shown to increase (Dodson, 1984; Lüning, 1992; Walls & Ventelä, 1998; Stibor & Lampert, Sell, 2000) or decrease (Havel & Dodson, 1987; Lüning, 1992; Caramujo & Boavida, 2000) when *Daphnia* is exposed to tactile invertebrate predators. Similarly, invertebrate predators have been shown to induce both a delayed (Ketola & Vuorinen, 1989; Caramujo & Boavida, 2000) and an accelerated (Weber & Declerck, 1997) first reproduction. This may suggest that adult body size is a critical trait for *Daphnia* faced with visual predators, while the evidence is contradictory in relation to invertebrate predators.

The exposure of *D. ambigua* to mite kairomone also led to an increase in mean tail spine length during the pre-reproductive stages, which has previously been reported as a response to cues from various invertebrate predators including copepods (Dodson, 1984; Caramujo & Boavida, 2000; Laforsch & Tollrian, 2004), the predatory cladoceran *Leptodora* (Laforsch & Tollrian, 2004) and *Chaoborus* (Sell, 2000; Dzialowski *et al*., 2003; Weber, Vesela & Repka, 2003; Laforsch & Tollrian, 2004). Since a long tail spine could confer protection on *Daphnia* against predation by invertebrates (Lüning, 1995; Sell; Caramujo & Boavida, 2000), our results suggest that *D. ambigua* exhibits an elongated tail spine around the time females release the first brood, maintaining age at maturation and offspring number at the cost of reduced offspring quality in the first brood, together with a decreased number of young in subsequent reproductive events, which were also delayed. These changes resulted in the induced phenotypes bring about a fitness reduction as a consequence of decreased fertility. In summary, when exposed to mite cues, *Daphnia* maximizes the probability of reproducing at least once.

Finally, assuming that the probability of adult survival after first reproduction is reduced for *Daphnia* in nature (e.g. Lampert, 1993), our model-based analyses predict that a moderate level of protection against mite predators (as a consequence of a modification of the spine) would be enough to gain a fitness advantage over non-induced animals. The protection 'needed' to balance the fitness reduction associated with predator cues is directly related to juvenile survival and fertility, and inversely related to adult survival.

Our work has some limitations that should be considered. Since experimental exposure to kairomones started when the animals were still inside the mother's brood chamber, our procedures did not control for possible maternal effects. However, the embryonic phase of *Daphnia* is particularly sensitive to chemical signals (Mikulski, Lipowska & Pijanowska, 2004) and, therefore, avoiding maternal effects through eliminating embryonic exposure could generate more serious errors. Moreover, since embryos and mothers are physiologically independent, maternal effects should mainly act via behavioral mechanisms, which should be unimportant in our batch cultures. Neither were we able to isolate the effects of the 'pure' kairomone, from those possibly exerted by the entire set of substances released by the predator, which would have included excretory products that might act as inhibitors rather than as true infochemicals. Our protocol was oriented towards minimizing this source of error; nevertheless this does limit this kind of study until we have a good method for purifying kairomones.

There is a large body of literature reporting morphological defences in *Daphnia* induced by invertebrate predators such as *Chaoborus*, *Leptodora*, *Notonecta* and some copepods in the north hemisphere (see Tollrian & Harvell, 1999). Our results expand the list of predators able to induce non-lethal responses in zooplankton, to include the water mite *P. chilensis*. In the southern Andes, water mites appear to be one of the most abundant invertebrate pelagic predators (Matveev & Martinez, 1990; Balseiro, 1992). Water mites are known to be very unpalatable to fish (Kerfoot, 1982; own unpubl. data). Therefore, their populations are probably to be regulated either by resources, physical constraints or predation on larval stages. We anticipate that the next steps for research should include an experimental evaluation of the hunting and handling effectiveness of water mites preying on induced versus non-induced prey, as well as a closer examination of the factors regulating different stages of the life cycle of predatory mites.

Despite the potentially interesting differences between pelagic systems dominated by *Chaoborus* and those dominated by water mites, little work has been devoted to this topic (Proctor & Pritchard, 1989). To our knowledge, this is the first work showing non-lethal effects of water mites on their cladoceran prey, and we stress that much understanding about the structure and function of southern fresh waters can be gained through studying trophic relationships of mite predators in the light of current knowledge about predation by invertebrates, mainly developed in northern latitudes.

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