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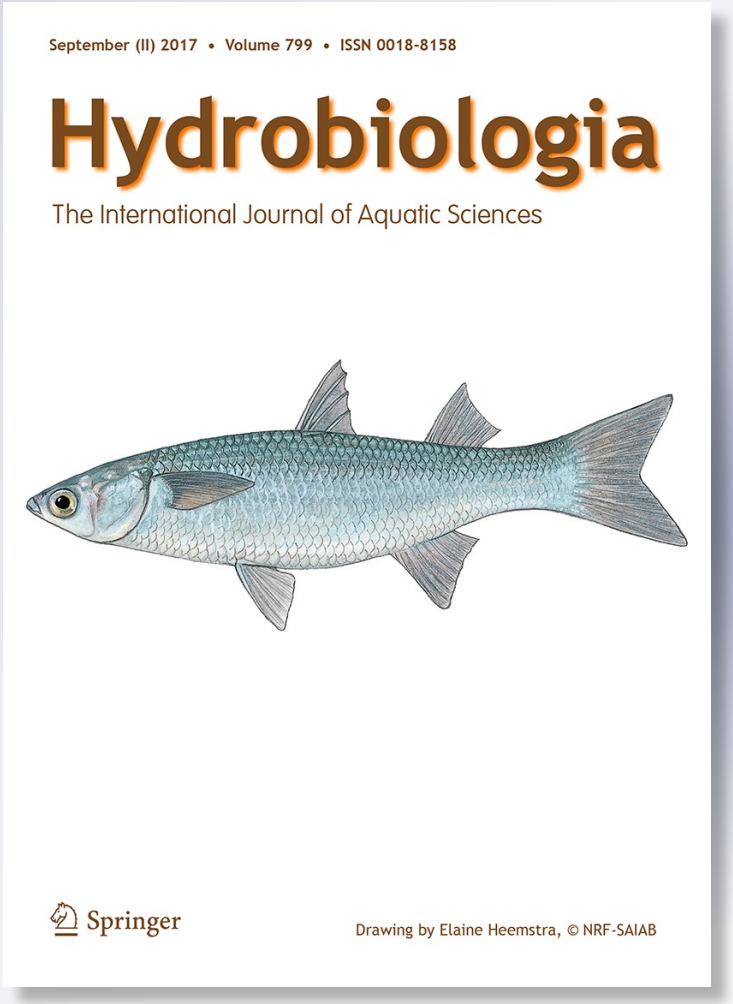

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PRIMARY RESEARCH PAPER

Pesticide increases transgenerational cost of inducible defenses in a freshwater rotifer

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Abstract In addition to natural stressors such as predation risk, aquatic organisms receive the simultaneous impact of anthropogenic stressors like pollution. In order to advance our understanding of multiple stressor effects, we evaluated the potential costs in the population growth rate derived from the sub-lethal effect of exposure to the pesticide methamidophos and from the expression of morphological defenses front to predation risk, in the rotifer *Brachionus calyciflorus*. Costs were evaluated both in the organisms that were exposed to the stressors and in their offspring. Our hypotheses were (1) plastic morphological defenses under exposure to pesticides have fitness costs, which may be transmitted from the parental to the filial generation, and (2) interactive effects between pesticides and predation are dependent of the mother's age. Our results indicate that pesticide exposure increased the costs, expressed as reduction in population growth rate, generated by the induction of defenses. Interestingly, these costs were detected only in the offspring of the treated organisms and were depended on the age of the mother. This indicates that the sub-lethal effects of predation were increased by pesticide pollution. This study provides experimental evidence of the transgenerational costs of phenotypic plasticity linked to interaction of natural and anthropic stressors.

Keywords Ecotoxicology · Infochemicals · Plankton · Predation · *Brachionus*

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Introduction

Organisms in aquatic ecosystems have developed strategies to face multiple environmental stressors, both natural and anthropogenic (Hanazato, 2001). Stressors often act simultaneously, producing either additive or interactive effects. In the case of interactive

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effects, i.e., when the magnitude or sign of the net effect of one stressor depends on the presence/dose of the other, predicting the ecological consequences of exposure to simultaneous stressors is especially difficult on the basis of individual effects (Christensen et al., 2006; Coors & De Meester, 2008; Altshuler et al., 2011, 2015). Predation is considered one of the strongest modulators of community structure in aquatic systems (Lampert, 1987). Predation exerts severe selective pressure on prey directly, but also on interacting species. One of the adaptive responses of prey to this pressure is the development of inducible antipredator defenses, where chemicals released by predators—kairomones—serve as signals that elicit prey response (see Tollrian & Harvell, 1999).

Antipredator defenses are ubiquitous in nature and are developed by a variety of taxa including algae, plants, invertebrates, and vertebrates, which respond to changes in the abundance of their predators by modifying the expression of some phenotypic traits (Lampert et al., 1994, Agrawal et al., 1999; Van Buskirk, 2000). Although there are some contradictory reports, it is generally believed that predator-induced defenses generate some costs, and are limited to periods when the risk of predation is high and the benefit of the defense exceeds the cost (Tollrian & Harvell, 1999; Riessen, 2012; Gilbert, 2013). The costs include allocation costs, by use of energy and materials in generating and maintaining defenses, opportunity costs, associated with decreased population growth, and environmental costs, which emerge from the interaction of defenses and the environment (Harvell, 1990; Tollrian & Harvell, 1999). Depending on their nature, costs can be identified at the individual (Lima, 1998; Tollrian & Harvell, 1999; Relyea, 2002; Benard, 2004) as well as at population (Stemberger, 1988; Gilbert, 2009; Aránguiz-Acuña et al., 2011a; Riessen, 2012) and community levels (Werner & Peacor, 2003; Garay-Narváez & Ramos-Jiliberto, 2009; Aránguiz-Acuña et al., 2011b).

Freshwater systems are among the most stressed environments, affected by many kinds of pressures such as changes in water chemistry due to acidification, eutrophication, and input of contaminants (Altshuler et al., 2011). A large variety of pesticides enter aquatic systems in different ways as a by-product of agricultural activity, such as unintentional spills, air currents, and runoff, with diverse effects on resident non-target populations (see Schulz, 2004; Bendis & Relyea, 2016). Depending on the concentration, bioavailability, and toxicity of pesticides, their discharge into the environment may affect population density through lethal effects, which increase mortality, and through sub-lethal effects, which decrease reproductive rates by modifying behavioral, morphological or life history traits of individuals in exposed populations (Preston et al., 1999; Sakamoto et al., 2006; Relyea & Hoverman, 2006; Marcial & Hagiwara, 2007; DeMille et al., 2016). Methamidophos is an organophosphate pesticide soluble in water, used globally due to its high efficiency in eliminating insect pests in agriculture (Malate et al., 1999). It is known that this pesticide exerts harmful effects on the central nervous system of animals by reducing or inhibiting the activity of the enzyme acetylcholinesterase (Hussain, 1987). In aquatic systems, toxicological effects of metamidophos have been shown in organisms from different hierarchical levels (Tomlin, 1994; Lin et al., 2006; Tien & Chen, 2012).

Tolerance to different anthropogenic pressures, specifically toxicant exposure, is costly for organisms even at sub-lethal levels, since it involves the expression of a variety of defensive or compensatory mechanisms such as physiological detoxification and repair of injuries in the tissues of exposed organisms, with reduced resource allocation to other functions (Van Straalen & Hoffmann, 2000; Newman, 2001). Additionally, pollutants in the maternal environment of some aquatic invertebrates produce low-quality offspring as the result of energetically expensive mechanisms of reparation and detoxification which increase pollutant tolerance in the parental generation (Marshall, 2008; Fernández-González et al., 2011). There are reports of sub-lethal concentrations of pesticide producing transgenerational effects on the subsequent generation of insects (Forbes & Calow, 1999; Southwood & Henderson, 2000), mites (Ibrahim & Yee, 2000; Hamedi et al.,2011), parasitoids (Biondi et al., 2013), and rotifers (Guo et al., 2012b) shown by reduced development, reproductive and survival parameters.

Inducible defenses evolve in specific environmental conditions, and thus changes in the water chemistry, produced for example by addition of toxicants, may alter the effectiveness of the defenses or the ability to deal with predators, as has been shown in plankton species (Riessen et al., 2012; DeMille et al., 2016). Confronting predation risk and pollutant

exposure simultaneously, organisms face a physiological decision that may involve a trade-off between resource allocation that avoids being killed by predators and that avoids being damaged by toxicants. This potential trade-off could lead to an interactive effect of predation risk and toxicant exposure on the fitness of directly exposed organisms as well as on the performance of their offspring. Previous studies addressed the effect of predation and pesticide simultaneously (see Relyea & Hoverman, 2006), nevertheless our knowledge is still mainly limited to the lethal and immediate consequences of this interaction, with divergent results (Relyea & Mills, 2001; Relyea, 2005, 2012).

It has been shown in different invertebrate species that the value of certain fitness components of offspring varies with the age of the mother. Maternal age may have effects on offspring performance and survival, as has been reported in coleopterans (Opit & Throne, 2007) and in body size and nutrient content in a parasitic wasp (Giron & Casas, 2003), or egg, brood, and individual size in copepods (Jamieson & Santer, 2003). In rotifers, it has been observed that fecundity decreases after the age of first reproduction (Gilbert & Schröder, 2003). Maternal age also could have a birth-order effect to induce development of posterolateral spines on rotifers as a response to predation signals (Schröder & Gilbert, 2009). They reported a positive relationship between the age of the mother and the size of the spines, which may be caused by lesser allocation of resources to later eggs, because the costs of maintenance and development of spines appear to be low (Stemberger, 1990). Therefore, it is expected that investment in defenses may vary during the mother's life cycle with consequences on the offspring.

Monogonont rotifers include more than 1500 species that inhabit aquatic and humid habitats (Wallace et al., 2006; Wallace & Smith, 2009). Due to their high reproductive rates they frequently reach considerable population densities, thereby forming a substantial part of continental zooplankton and playing a critical role in aquatic food chains (Armengol et al., 2001; Wallace & Smith, 2009; Alvarado-Flores et al., 2015). Rotifers are a useful model system for aquatic ecology research due to their availability in natural conditions and easy cultivation, and have been used successfully in aquatic toxicology because of their small size, sensitivity to a great number of toxic substances, predominantly parthenogenetic reproduction (providing genetically and phenologically identical clones), standardized culture techniques, and rapid population growth (Snell & Joaquim-Justo, 2007; Dahms et al., 2011). Also, rotifers of several genera, especially *Brachionus*, respond to the presence of predators such as the carnivore *Asplanchna* rotifers. These responses involve the elongation of spines and appendages that persist throughout the lifetime of the individual in the presence of kairomones released by *Asplanchna* and accumulated in the medium, with consequent decreased probability of successful attacks by predators (van der Stap et al., 2007; Aránguiz-Acuña et al., 2010; Gilbert, 2013).

In this study, we evaluated the combined effects of pesticide exposure and morphological defenses induction in response to predation risk on the population growth rates exhibited by the exposed organisms as well as those exhibited by their unthreatened offspring, using rotifers as the study model. We tested the following hypotheses: (1) Induced morphological defenses have fitness costs, which may be increased under pesticide exposure and are expressed in the offspring of exposed rotifers, and (2) adverse effects of exposure to pesticides and predation risk are dependent on the mother's age. To test our hypotheses, we implemented an experimental model system composed of the herbivorous rotifer *Brachionus calyciflorus* Pallas, 1776, which develops morphological defenses in the presence of predator signals released by the carnivorous rotifer *Asplanchna girodi* de Guerne, 1888, using the microalga *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák as food resource for *B. calyciflorus* and the widely applied pesticide methamidophos.

Materials and methods

Cultures and isolation of the experimental species

Brachionus calyciflorus and *A. girodi* were collected from temperate lakes of central Chile: Tranque Lo Orozco (33°22′S–71°41′W) and Rapel Reservoir (34°09′S–71°26′W). One clone of each species was established from a single parthenogenic female and maintained under standard laboratory conditions: pH 7.5 ± 0.1, temperature 20 ± 1°C, photoperiod 16:8 h light: dark for 6 months before the experiments, to

avoid maternal environmental effects. Induction of male production was prevented by avoiding a high population density; that is, by removing the excess individuals above, the specified crowding cue threshold for *Brachionus* species (Aránguiz-Acuña & Ramos- Jiliberto, 2014). The culture medium for maintenance and experiments was MBL Woods Hole modified medium (Stemberger, 1981). The stock cultures of *B. calyciflorus* were fed daily with the green alga *Pseudokirchneriella subcapitata* at a density 5×10^5 cells ml^{-1}, which was cultured in Bold's basal medium (Borowitzka & Borowitzka, 1988). Algae were harvested in exponential growth phase, and densities were measured by direct counting. *A. girodi* was fed with *B. calyciflorus* at density 1 ind ml^{-1}. Culture medium with food of stocks was renewed every 48 h.

Experimental cultures

For the experiments, a pre-conditioned medium was prepared with *A. girodi* at a constant density of 0.5 ind ml^{-1} plus *B. calyciflorus* at the same density, which served as its prey. This medium was called K medium, by the addition of *Asplanchna* kairomone. For the treatments without kairomone (control, called C medium), a medium was prepared without *Asplanchna*, with only the prey at the same density as in K medium (0.5 ind ml^{-1}). Every 24 h, both experimental media were prepared by removing the rotifers from the media using a 50 μm sieve. The K and C media were then filtered using a 0.2 μm membrane to remove particulate material, and the pH adjusted to 7.5 ± 0.1 to be used as experimental media. This was done to prevent pH changes caused by accumulation of metabolic products in the culture media from affecting the observed response. *B. calyciflorus* grown in the medium with kairomone should develop defenses, while those grown in the control medium should not.

The pesticide used in this study was O,S-dimethyl phosphoramidothioate ($C_2H_8NO_2PS$), commonly known as methamidophos. High toxicity of this pesticide has been observed in aquatic organisms, where values recorded for 96 h-LC50 were between 25 and 51 mg l^{-1} for fish and less than 0.22 mg l^{-1} for crustacean larvae (Tomlin, 1994); 24 h LC50 for *Daphnia magna* was 108.7 μg l^{-1} (Lin et al., 2006). This pesticide can affect algal community structures and would have great ecotoxicological impact on water quality and on higher trophic levels (Tien & Chen, 2012). The concentration used was selected based on preliminary toxicity tests on the basal resource *P. subcapitata* and on the rotifer *B. calyciflorus*, which found inhibition in the growth of the microalga at a concentration of 2 mg l^{-1} (LOEC) of the pesticide and a lethal concentration for 50% of the population (LC50) of 2.24 mg l^{-1} for the rotifer. Based on these results, a concentration of 0.2 mg l^{-1} of the pesticide, ten times lower than LOEC for the algae and the LC50 for the rotifer, was used for the experiments with *B. calyciflorus*, in order to quantify the subtle effects of pesticide interaction with the other stressor.

Experimental design

Neonates (<12 h age) were selected from the stock culture of *B. calicyflorus* and used to initiate two cultures at a constant density of 0.5 ind ml^{-1} in 500 ml of each type of medium: in control medium (C) without kairomone and in medium with kairomone (K), prepared as described above. These treatments were maintained with daily renewal of corresponding medium and fed with microalgae at a density of 5×10^5 cells ml^{-1} day^{-1}. After 5 days, 300 females carrying eggs were selected from each culture, from which 150 neonates of age less than 6 h were obtained for the P generation from each medium, our experimental first generation. (Online Resource 1).

Thirty egg-bearing females from each treatment, C and K, were reserved to be photographed and measured to corroborate the presence of defenses in the individuals exposed to the induction signal. This was done by contrasting the length of the posterior spines recorded for each individual with the 95th percentile of the distribution of measures obtained in individuals without induction (C). Each individual was scored as 1 or 0 (induced or not induced) according to whether its value was greater or less than this 95th percentile. The result was expressed as the percentage of individuals showing induction.

The remaining 120 individuals from each treatment were used as the *P* generation in the following experiment. Each experimental unit consisted of five individuals in a test tube with 10 ml of medium, fed with a density of 5×10^5 cells ml^{-1} day^{-1}; the medium was renewed daily. Neonates whose mothers

were cultured in C medium (mothers without defenses) were submitted to the following treatments: (a) control, without kairomone or pesticide (C medium) and (b) with methamidophos pesticide at a concentration of 0.2 mg l^{-1} (P medium); and neonates whose mothers were cultured in K medium (mothers with induced defenses) were submitted to the following treatments: (c) with only kairomone (K medium); and (d) kairomone and pesticide (KP medium), with 0.2 mg l^{-1} of methamidophos. The experiment was performed at 1000 lux, photoperiod 16:8 light: dark and at 17°C. This temperature allowed adequate recording of the life cycle of the organisms, differentiating each mother from its offspring. Thus, the design was a 2 × 2 full factorial with two levels of kairomone (present and absent) and two levels of pesticide (0 and 0.2 mg l^{-1}) with 6 replicates each, with a total of 24 experimental tubes. The survival and reproduction of the P generation were recorded daily. Based on this information, we obtained complete life tables, which we used to construct and estimate an age-structured demographic matrix for each treatment. The finite population growth rate was calculated as the dominant eigenvalue (λ) of the demographic matrix (Caswell, 2001).

The offspring of the *P* generation, the F_1 generation, were separated daily and cultured in kairomone-free and pesticide-free medium at a density of 1 ind ml^{-1}, recording the age of their mothers. We defined reproductive age as the time (in days) after the first reproductive event of each treatment. Given that the original stock cultures consisted of one single clone, the offspring was grouped in an experimental unit according to the mother's reproductive age and the treatment to which they were exposed. We considered each of these groups as a cohort. Considering that the time between successive broods in *B. calyciflorus* could be less than 24 h, we were especially careful in spacing sampling events evenly. Furthermore, given the temperature dependence of reproductive rates in this species (Ma et al., 2010) we chose a relatively low temperature of 17°C so that the rate of clutch production would be slow. The survival and reproduction of the F_1 cohorts were recorded daily until the fifth day after their first reproduction, in order to construct partial life tables per cohort. This period of growth was considered sufficient to satisfy an ecotoxicological criterion of chronic toxicity in rotifers (Snell & Moffat, 1992; APHA et al., 2012).

Data analysis

For the P generation, the differences among values of λ were evaluated using 2-way ANOVA with pesticide and kairomone as factors, and a post hoc Tukey test for multiple comparisons.

Partial cohort life tables of the of F_1 generation were obtained daily without replication. In this way, we obtained five partial life tables per treatment. To make comparison among treatments, we lumped the five life tables per treatment into a single population life table. For this, each single cohort life table built from i_a individuals born from a mother of reproductive age a was decomposed into i_a identical individual life tables. We composed a quasi-empirical population life table by adding the $m = \sum_a i_a$ individual life tables. Then from the m individual life tables we randomly sampled with replacement m individual life histories from which we built a bootstrapped population life table. This procedure was repeated 5000 times, thus obtaining 5000 bootstrapped population life tables per treatment. Rates of population growth λ were estimated as the dominant eigenvalue of each matrix. Ninety five percent confidence intervals of λ were calculated from the bootstrapped λ values by the percentile method (Manly, 2006). Finally, differences in λ between treatments were estimated using standard permutation tests (Manly, 2006). This test consists, for a given pair of population life tables $\{A, B\}$, of randomly constructing a new pair $\{A', B'\}$ of life tables from the set of individual life histories of A ∪ B, calculating the difference $D_{A'B'} = \lambda_{A'} - \lambda_{B'}$, and repeating the process many times to obtain a distribution of $D_{A'B'}\lambda$ from which to calculate 95% CI.

In addition, we evaluated if the effects of maternal exposure to kairomone and pesticide on the λ of the F_1 depended on maternal age. For this, we used a generalized lineal model (GLM) with a gamma distribution, and an inverse-link function. The gamma distribution is preferred for continuous dependent variables that are rates (Van Allen & Rudolf, 2013). Nonetheless, we also performed the same analysis using a Gaussian distribution and an identity-link function, obtaining the same results.

The effect size (in absolute value) of kairomone exposure on λ is indicative of the costs of expressing defenses. We estimated the effect size of kairomone exposure in the P generation on the λ of the F_1 as $(\lambda_K - \lambda_C)/\lambda_C$, where subscripts K and C represent

treatments with and without kairomone exposure of the P generation. The same ratio was calculated for treatments with pesticide as $(\lambda_{KP} - \lambda_P)/\lambda_P$. Calculating the error propagation allowed estimating the confidence intervals of the effect size from confidence intervals of λ, as follows. $D_{kc} = \lambda_k - \lambda_c$ is the difference between λ values obtained for treatments K and C; $\delta_{kc} = \sqrt{(\delta_k)^2 + (\delta_c)^2}$ is the propagated error estimated for the difference between λ values, δ_k and δ_c are the errors associated with k and c, respectively; and $\frac{D_{kc}}{\lambda_c}$ is the relative difference between λ values. Finally, the error associated with the relative difference δ_{RD} was estimated as: $\delta_{RD} = \frac{D_{kc}}{\lambda_c}\sqrt{\left(\frac{\delta_{kc}}{D_{kc}}\right)^2 + \left(\frac{\delta_c}{\lambda_c}\right)^2}$ (Lo, 2005).

A similar procedure was used to estimate the effect size as a function of the reproductive age of the mothers, but without the associated error due to lack of replicates.

Results

Exposure of *B. calyciflorus* to *A. girodi* kairomone caused an average increase of 145% in the length of the posterior spines of the offspring, whose length was 0.067 ± 0.012 µm (mean ± SD), relative to non-exposed organisms, which had length = 0.028 ± 0.013 µm (mean ± SD). This morphological response was observed in 96.6% of the treated organisms, which confirms the inductive effect of the kairomones used.

There was no significant interaction between exposure to pesticide and kairomones on the values of λ Induction of defenses did not show a demographic cost in growth rate in the P generation, independent of pesticide exposure, since there were not significant differences between treatments C and K or between treatments P and KP. Significant differences were observed for pesticide as main factor (Two way ANOVA, d.f. = 1, $F = 12.57$, $P = 0.002$). Exposure to pesticide decreased the values of λ both with and without kairomones, but the exposure to kairomones did not exert any effect on λ in the P generation (Fig. 1).

Conversely, in the F_1 generation there were significant differences in λ (permutation test, $P < 0.05$) between all treatments (Fig. 2), indicating that both defense induction and pesticide exposure exerted effects on the offspring growth rate. The largest λ decrease was observed in the offspring of mothers exposed to the KP medium (33% decrease with respect to controls). The cost of expressing defenses, measured as the effect size of the induction of defenses of mothers on the λ of daughters, was significantly higher in media with pesticide compared to media without pesticide (Fig. 3). We did not find sexual females or males in any of our treatments.

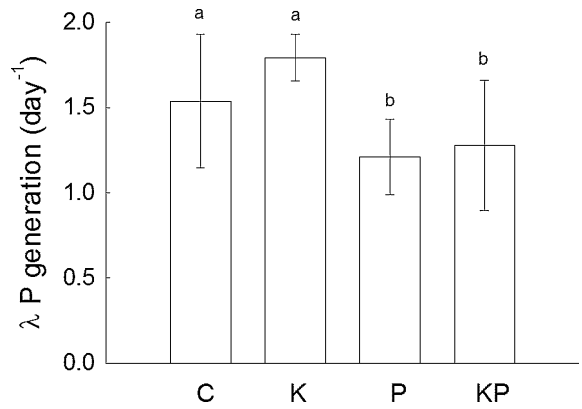

Fig. 1 Finite rate of population increase (λ) (mean values ± 95% CI) of *B. calyciflorus* of the first (P) generation exposed to different treatments. Control medium (C), kairomone medium (K), pesticide medium (P), and medium with kairomone and pesticide (KP). *Different letters* indicate significant differences (Tukey test $P < 0.05$)

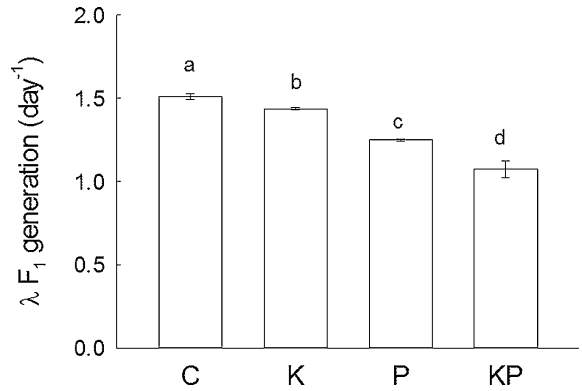

Fig. 2 Finite rate of population increase (λ) (mean values ± 95% CI) of offspring (F_1) of *B. calyciflorus* whose parental generation P was exposed to control medium (C), with predator kairomone (K), with metamidophos pesticide (P), and with kairomone and pesticide (KP). *Different letters* indicate significant differences (permutation test, $P < 0.05$)

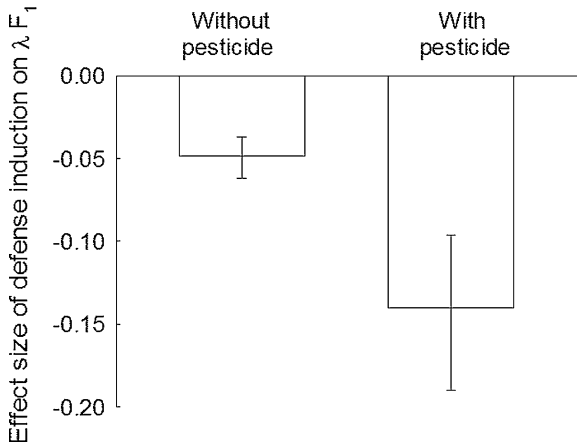

Fig. 3 Effect size of induction of defenses on the rate of population growth (λ) of the F_1 offspring in treatments with and without pesticides. Shown are mean values ± 95% CI

Population growth rates of the F_1 generation were dependent on the interaction of the reproductive age of their mothers and the treatment to which the mothers were exposed (gamma-GLM, d.f. = 11, $F = 5.83$, $P = 0.01$). Increasing growth rates ($\lambda > 1$) were reached by all offspring, except by those cohorts whose mothers had reproductive age of more than 4 days, in both treatments with pesticide (treatments KP and P) (Fig. 4). Without pesticides (C and K treatment), the offspring of the youngest mothers (reproductive age = 1) achieved lower growth rates than those reached by the offspring of older mothers. Nevertheless, in the control condition, with increasing maternal age the growth rate of the offspring reached the highest values observed (compared to the remaining treatments) with a maximum at intermediate ages; the λ values for K treatment did not show wide variation due to the age of mothers compared to the other treatments. In pesticide treatment, λ values were independent on maternal age up to age 4, after which a steep decrease occurred. Under treatment KP, λ values decreased monotonically with increasing maternal age.

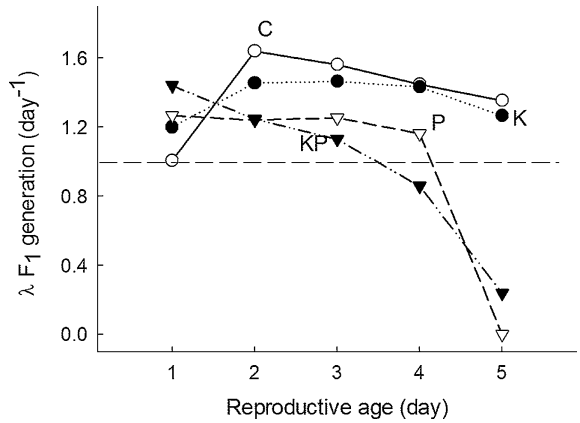

Fig. 4 Population growth rates λ of offspring (F_1) according to the reproductive age of their mothers (F_1) exposed to different treatments of kairomone and pesticide. *Open circles* with *continuous lines* correspond to mothers cultured in control medium (C); *black circles* with *dashed lines* are mothers cultured in medium with kairomone (K); *open triangles* with *discontinuous lines* are mothers cultured in medium with pesticide (P); *filled triangles* with *dashed lines* correspond to mothers cultured in medium with kairomone and pesticide (KP). Reference value of population growth ($\lambda = 1$) is shown with *dashed line*

The change in the transgenerational cost of defense induction due to the reproductive age is summarized in Fig. 5. There were opposite trends between effect sizes on offspring from mothers of 1-day reproductive age (positive) and offspring from older mothers (negative), either with or without pesticide exposure. Pesticide exposure exerted a negative effect on λ, proportionally with the reproductive age of mothers, whereas the negative effect of the addition of kairomones was independent of the age of mothers not exposed to pesticide. It was not possible to determine the effect size for the F_1 of mothers exposed to pesticide with reproductive age of 5 days, because there were no surviving offspring.

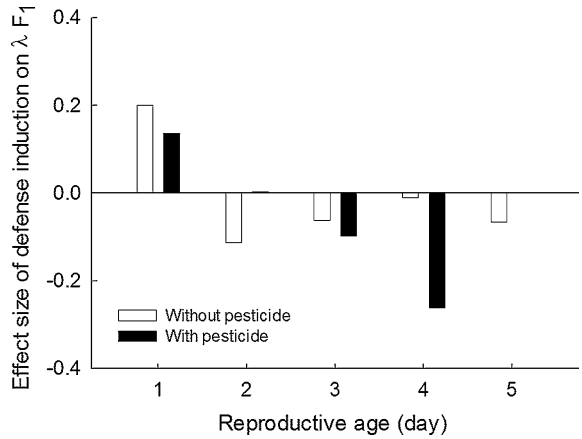

Fig. 5 Effect size of kairomone addition on the population growth rate (λ) of the offspring F_1 at different reproductive age of mothers, in treatments with (*black bars*) and without (*white bars*) pesticide. Was not possible to determine the effect size for F_1 from age of mother with 5 days

Discussion

Exposure of rotifers to the pesticide methamidophos did not reveal costs derived from the expression of defenses on population growth in the parental generation, where only the direct toxicity of pesticide caused a decrease in fitness. Nevertheless, our results showed costs in the offspring of the induced mothers. These costs were greater with pesticide, and depended upon the age of the mother. These results are in agreement with previous studies which reported that the development of spines in *B. calyciflorus* induced by *Asplanchna* kairomone occurs without reproductive costs for the induced generations (Stemberger, 1990; Gilbert, 2012), and that parental exposure of *B. calyciflorus* to organophosphate pesticide may result in decreased fitness in the offspring (Guo et al., 2012b). The higher cost observed for F_1 in medium with pesticides may be in line with results showing that offspring from toxicant-exposed mothers were more resistant to predation than offspring from mothers from toxicant-free medium (Moran et al., 2010). Although our experimental design was not focused on testing the defense effectiveness on the offspring, higher defense cost in the absence of predators could be related to higher benefits when an effective predator is present (Tollrian & Harvell, 1999).

With respect to vertical transmission, described as responses to environmental change transmitted across successive generations (Ramos-Jiliberto & Aránguiz-Acuña, 2013), on one hand, the results indicate that exposure to methamidophos increased the costs of induced antipredator defenses in the unexposed descendants. On the other hand, the results also show that, due to exposure to the pesticide, the kairomone or to both, the organisms reacted by modifying their reproductive effort in the first brood, producing individuals with better performance, reflected in a higher rate of population growth (λ). Although some studies have shown interactive effects of the toxicity of certain pesticides and the presence of predation signals on the demographic response (Barry, 1998; Janssens & Stoks, 2013; Trekels et al., 2013), or even an increase of antipredator defense costs in zooplankton in the presence of pesticides (Pestana et al., 2010), to the best of our knowledge, this is the first study to report an increased transmission over a generation in the costs of the defenses derived from the exposure to pesticides. In agreement with previous research (Martínez-Jerónimo & Muñoz-Mejía, 2007), we found that maternal age influences the sensitivity of their offspring to toxicants. In particular, our results show that the reduction in offspring's λ driven by pesticide exposure was stronger in organisms of older mothers.

In synthesis, greater allocation of resources to increasing the quality of the early descendants appears to be the strategy used by *B. calyciflorus* as a response to the effect of the pesticide in females exposed to the predation signal. Treatments with pesticide generated in the first brood offspring with greater quality, and the quality of the descendants decreased with the age of the mother. These results may be considered as first indications for further studies on this topic. It is useful to track the fitness changes across several generations and to reveal how persistent the adverse effects of stressors are. We found it interesting to clarify the age dependence of stressor effects and how this age dependency is modified across generations. It is possible that the relationship between offspring quality and maternal age influences the long-term population dynamics, for example via an increase in the extinction probability of the isogenic lines of older mothers due to a progressive decrease in their fecundity (see Schröder & Gilbert, 2009). Also, other studies have reported an inverse relationship between the proportion of females with sexual reproduction and the age of their mothers (Gilbert & Schröder, 2007), which may be a determinant in the balance between population growth via parthenogenesis and long-term survival via production of resting eggs by sexual reproduction (Snell, 1987; Serra & King, 1999; Serra et al., 2004). It is important to note that the propensity to sexual reproduction is dependent on the strain (Gilbert & Diéguez, 2010). Although in our case, we did not detect induction to sexual females, other strains could be prone to sexual reproduction, so a multi-clone study would be welcome.

Several organophosphate pesticides, including methamidophos, exert their action by an inhibitory effect on the enzyme acetylcholinesterase (AChE). AChE inhibition can cause an increase in acetylcholine in the synapses of cholinergic neurons, resulting in neurotransmission of abnormal duration and intensity (Pope et al., 2005). As result, this could modify the swimming activity or feeding behavior, which may explain observed changes in the growth and reproduction as a consequence of increasing

metabolic costs due to extra activity under long-term exposure (Pestana, 2010; Guo et al., 2012a). This mechanism could be invoked to explain, at least partially, the decreased fitness of offspring (F_1) as a consequence of pesticide exposure of their mothers, an effect that could be intensified in older mothers. Also, acetylcholinesterase activity is localized in the cell membrane and cytoplasm of developing oocytes as well as in the cytoplasmic bridge that connects the oocyte to the vitellarium. Through this structure, the maternal factors synthesized in the vitellarium are transported to the forming oocytes (see Rainieri, 1984). This may explain the effects on unexposed descendants, in which maternal exposure to chemical stressors generated a fitness reduction in their offspring and not in the exposed P generation, whose oocytes formed in toxicant-free medium.

In conclusion, our results showed that the exposure of the organisms to two environmental pressures, the signal of predation risk and a pesticide, triggered complex responses at the individual and population levels, revealing an interactive effect of the two factors. The costs of reaction to the presence of predators were compromised by the exposure to the pesticide, and this response depended on maternal age, in agreement with our hypotheses. Moreover, diverse environmental pressures transmit their effects to the offspring of the organisms exposed, where these responses have the potential to generate important impacts on population and community dynamics (Beckerman et al., 2002; Ramos-Jiliberto & Aránguiz-Acuña, 2013), shaping the functioning of ecosystems. This study makes a novel contribution to advance our understanding of the mechanisms and ecological consequences of the interaction between ubiquitous natural and anthropogenic factors, and highlights the relevance of vertical transmission of ecological effects of disturbance.

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