Rol modulador de la estructura poblacional sobre los efectos de la contaminación en la dinámica poblacional de *Daphnia ambigua*

Tesis

Entregada a la

Universidad de Chile

en cumplimiento parcial de los requisitos

para optar al grado de

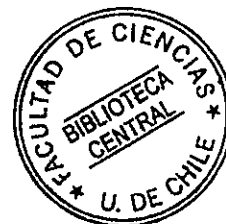### Magíster en Ciencias Biológicas

Facultad de Ciencias

por

Claudio Andrés Reyes Reyes

Diciembre de 2014

Director de Tesis: Rodrigo Ramos Jiliberto

Co-director de Tesis: Mauricio Lima Arce

FACULTAD DE CIENCIAS
UNIVERSIDAD DE CHILE

INFORME DE APROBACIÓN
TESIS DE MAGÍSTER

Se informa a la Escuela de Postgrado de la Facultad de Ciencias que la tesis de Magister presentada por el candidato

CLAUDIO ANDRES REYES REYES

Ha sido aprobada por la comisión de Evaluación de la tesis como requisito para optar al grado de Magíster en Ciencias Biológicas, en el examen de defensa privada de Tesis rendido el día 20 de noviembre de 2014.

Director de Tesis
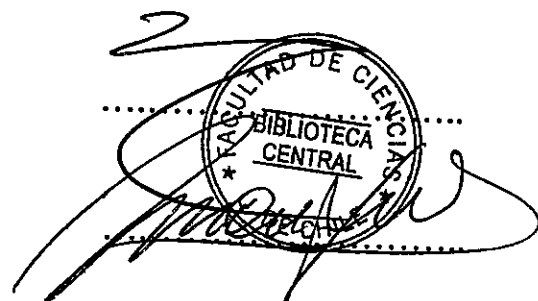Dr. Rodrigo Ramos Jiliberto
Co-director de Tesis
Dr. Mauricio Lima Arce

Comisión de Evaluación de Tesis

Dr. Pablo Sabat

Dr. Ramiro Bustamante

Dedicado a los cimientos de mi vida, mi madre y abuela.

Biografía

Claudio es un joven biólogo egresado el año 2011 de la facultad de ciencias de la Universidad de Chile. Luego de su pregrado continuó sus estudios de magíster en la misma casa de estudios impulsado por sus deseos de aprender y enfocar sus esfuerzos hacia la Ecología, en la cual se ha desempeñado los últimos años. En particular, sus intereses se han sesgado hacía la ecotoxicología en sistemas acuáticos, tema en el que desarrollo su última tesis.

Pero, más allá de sus intereses académicos y científicos, Claudio se caracteriza por ser un apasionado por las montañas a las cuales le dedica gran parte de su tiempo libre.

A pesar de todo sigo siendo la misma persona. Nada nuevo he sido todo menos frívolo. Dando gracias, así es como vivo yo por ser como soy, por querer seguir siéndolo....

Dedicado a los cimientos de mi vida, mi madre y abuela.

Agradecimientos

A mi familia, en especial a mi madre que aun me acompaña y mi abuela que me observa de arriba. Quienes dieron todo por mí y me enseñaron a caminar por la vida, incentivándome siempre a aprender y salir adelante. Sus esfuerzos por mí siempre serán el mejor regalo y estaré eternamente agradecido por ello.

A mi tutor Rodrigo Ramos Jiliberto por seguir confiando y darme la oportunidad de trabajar en su laboratorio. También agradezco su exigencia, la cual siempre fue por un buen objetivo.

Agradezco también a Ramiro Bustamante y Pablo Sabat por sus comentarios y voluntad.

Agradezco a mis amigos de toda la vida y a los amigos que han ido apareciendo en el camino. A los amigos de las montañas y todos aquellos que han vivido experiencias conmigo.

Agradezco a mis colegas y amigos del laboratorio de Biodiversidad, con quienes prácticamente he compartido todo mi crecimiento científico y he aprendido a encontrar grandes amigos en ellos. A Lidia Aliste quien me ayudó logísticamente en mis experimentos, su voluntad será siempre recordada. A Inger Heine por hacerme la vida imposible pero indispensable. A Javier González por ayudarme siempre cuando se lo pedí y por distraerme de mis labores cuando no se lo pedí. Gracias por confiar en mis capacidades y siempre darme consejos para seguir adelante.

A todos ustedes gracias por ayudarme a mantener el espíritu vivo.

Index

Índice

Figure list

Lista de figuras

Table list

Lista de tablas

Abstract

Natural zooplankton populations are continuously subjected to widely varying environmental conditions determining their distribution and abundance. Predation is an important natural stressor, which commonly exhibit a sharp selectivity of preys changing population size composition. In addition, increasingly pesticide exposure from released into freshwater systems exert detrimental effects on size structured zooplankton populations which may be different from the observed at individual level. The present study addressed experimentally the combined effects of anthropogenic stress (pesticide exposure) and natural stress (selective predation) and pointed out the significance of combined stressors in evaluating the impact of toxic chemicals on population dynamics. Simulated predation was performed to shift size structure of population towards smaller or larger body size by imitating fish or invertebrate predation on *Daphnia* populations. Our modeling analysis used theoretically based population dynamics models and Royama's theoretical framework for analyzing the dynamics of populations influenced by exogenous forces.

Our results showed that the effects of pesticide exposure led to different population sensitivity and their population dynamics was affected through different mechanism depending on the predation pressure. For exposed population under selective predation on newborns and selective predation on adults a nonlinear effect of pesticide exposure was observed. In populations under selective predation on newborns the population dynamics was affected by a decrease in the maximum reproductive rate. Conversely, for populations under selective predation on adults, changes in population dynamics were largely due to increased maximum reproductive rate and decreased competition intensity.

On the other hand, for populations under non-selective predation, the pesticide exposure exerted a weak lateral effect due to decreased competitive intensity. Findings from the present work could help to explain the seasonal variations of pollutant impact on population dynamics, mediated by the composition and activity of local predator assembly.

Resumen

Las poblaciones naturales del zooplancton están continuamente sometidas a una amplia variedad de condiciones ambientales que determinan su abundancia y distribución. La depredación es un factor natural que comúnmente exhibe una fuerte selectividad de presas, cambiando la distribución de tamaños dentro de las poblaciones. Adicionalmente, la creciente exposición a pesticidas liberados en los ecosistemas de agua superficiales ejerce efectos perjudiciales sobre las poblaciones tamaño-estructuradas que pueden ser diferentes a los efectos tamaño específicos observados a nivel individual. El presente estudio evaluó experimentalmente los efectos combinados de un factor antropogénico (pesticida) y un factor natural (depredación). Destacando la importancia de la depredación selectiva en la evaluación del impacto de compuestos químicos sobre la dinámica poblacional. Se efectuó depredación simulada con el objetivo de cambiar la estructura poblacional hacia tamaños corporales pequeños o grandes, imitando la depredación ejercida por peces o invertebrados sobre las poblaciones de *Daphnia*. Nuestro análisis teórico utilizó modelos de dinámica poblacional y el marco teórico desarrollado por Royama para analizar los efectos de fuerzas exógenas sobre la dinámica poblacional. Los resultados mostraron que los efectos del pesticida condujeron a sensibilidades y dinámica poblacionales diferentes dependiendo del tipo de depredación. Para las poblaciones expuestas al pesticida en condiciones de depredación selectiva sobre neonatos y depredación selectiva sobre adultos se observaron efectos no lineales. Sin embargo, los mecanismos por los cuales estas poblaciones modificaron su dinámica fueron diferentes. Para las poblaciones sometidas a depredación selectiva sobre neonatos la dinámica fue afectada mediante disminuciones de la tasa de reproducción máxima. Por el contrario, la dinámica de las poblaciones sometidas a una

depredación selectiva sobre adultos fue afectada por un incremento en la tasa de reproducción máxima y una disminución de la intensidad de competencia intraespecífica. Por otro lado, la exposición al pesticida en las poblaciones sometidas a depredación no selectiva, produjo un débil efecto lateral sobre su dinámica, disminuyendo solo la intensidad de competencia intraespecífica.

Los resultados de este trabajo podrían ayudar a explicar las variaciones estacionales de impacto de los pesticidas sobre la dinámica poblacional, mediadas por la composición y la actividad del ensamble de depredadores locales.

1. Introduction

Zooplankton from superficial waters is continuously subjected to a wide variation in environmental conditions determining distribution and abundance of populations. It is well known that species composition and abundance of freshwater zooplankton are strongly modulated by predation pressure (Hrbacek 1962; Hall et al. 1976; Stenson 1972; Nilsson & Peijler 1973; Dodson 1974) and predators in freshwater ecosystems commonly exhibit a sharp selectivity of preys (Brooks & Dodson 1965; Williamson 1986; Brandl 1998a,b). Fishes are visuals predators that choose prey of larger body size. Conversely, invertebrate predators such as larval stages of insects exert a higher predation pressure on smaller sized prey (Zaret 1980; Brett 1992; Gergs 2013). In prey species with relatively large adult sizes such as *Daphnia*, a size-selective predation means that fish predation exerts a preferential removal of adult individuals, while predation by invertebrates promotes preferential removal of newborns and early juveniles (Zaret 1980; Manca et al. 2008). Thus, functional diversity of predatory guilds, lead to changes in the age and size populations structure of their prey (Brooks & Dodson 1965).

Superficial fresh waters are widely affected by anthropogenic disturbances, such as byproducts of agriculture or the release of wastewater. These stressors could also exert profound changes in the dynamic and composition of freshwater populations (Agatz et al. 2012).The adverse effects of pollutants on non-target organisms such as zooplankton vary in magnitude according to the biological properties of species and populations and the physiological status of the exposed organisms. Thus, the effects of many pollutants depend on the age of exposed organisms (Medina et al. 2002; Willis & Ling 2004) and apparently, the most general pattern is that younger or smaller individuals have a lower tolerance

1

(Bodar et al. 1989; Forget et al. 1998). However, the age or size dependent effect of pollutants on the dynamics and abundance of a population with a given age structure cannot be understood solely on the basis of knowledge of the effect of pollution on individuals of different age or size. Predation may interact with pesticide effects via size-selectivity of both stressors leading to a non-linear (or non-additive) combination of effects at population level, as observed by Gergs and co-workers (2013). However, more studies are needed for understanding mechanisms behind interactive effects of long-term pesticide exposure and natural stressors on dynamics of populations structured by different patterns of predation pressure.

The present study aims to reveal how size-selective and non-selective predation, through regulating population size structure, determines the population level responses of *Daphnia* to stressor exposure. We analyze, by means of integrating laboratory experiments and modeling, the effects on *Daphnia ambigua* population dynamics exerted by sublethal long-term exposure to a neurotoxic pesticide (methamidophos) under contrasting patterns of simulated size-selective predation. In particular, we use the Royama classification of exogenous perturbations (vertical, lateral and non-linear) as an approach for evaluating the impacts on population dynamics (Royama 1992).

2. Methods

2.1. Zooplankton and phytoplankton cultures

All experiments were conducted using a clone of *Daphnia ambigua* isolated from natural populations from Peñuelas lake (33° 07' S y 71° 24' O), Valparaiso, Chile. Laboratory cultures of *Daphnia* were maintained in COMBO media (Kilham et al. 1998) at $20 \pm 1°C$ in light:dark cycle (16h:10h). The phytoplankton species *Pseudokirchneriella subcapitata* (ex *Selenastrum capricornutum*) was used as food for *Daphnia* during maintenance and trials. *P. subcapitata* was cultured using Bristol medium (James 1978) in 2L glass bottles constantly aerated through a 0.2 um filter. Prior to its use as food, the phytoplankton medium was removed through centrifugation at 3500 r.p.m. and the concentrate was kept at $5 \pm 1°C$.

2.2. Size-specific individual tolerance to methamidophos

The effects of the pesticide methamidophos (O, S-dimethyl phosphoramidothioate) were evaluated at individual and population levels. This pesticide belongs to the organophosphorus family and has been used worldwide due to its high efficiency against insect pests in agroindustry (Malato et al. 1999). Methamidophos exerts its harmful effect on the central nervous system by inhibiting acetylcholinesterase activity (Hussain 1987). Its effects have been reported in mammals, birds and terrestrial insects. It is also highly toxic for aquatic organisms, registering LC_{50-96} values between 25-51 mg L^{-1} for fish (Tomin 1994).

To compare the magnitude of effects of the pesticide across different *Daphnia* body size, we performed acute toxicity tests on newborns (< 1 day old), juveniles (3 days old) and adults (7 days old) *D. ambigua*. The experiment was performed modifying the OECD (2004) procedure to determining the 24h-$LC_{50}$and 48h-LC_{50} for *Daphnia*. Nominal concentrations of methamidophos were 0, 50, 100,200, 400 y 800 µg L^{-1} (for newborns); 0, 5, 150, 300, 600, 1200 µg L^{-1} (for juveniles) and 0, 90, 180, 360, 720, 1440 µg L^{-1} (for adults). For each size class assay, five replicates were run per concentration, each one containing four individuals in 40 mL of media. The modification to the standard test consisted of feeding the individuals during the test with *P. subcapitata* at 10^6 cell mL^{-1}, in order to mimic conditions of the main experiments. The LC_{50}s values for methamidophos were calculated by probit analysis using the Spearman-Karber Method (Hamilton et al. 1977) with the statistical program Probit (U.S. EPA 1988).

2.3. Main experiments

2.3.1 Experimental concentration of methamidophos

A subset of concentrations below the 48h-LC_{50} value obtained on newborns (the most sensitive size class observed: see Results), were used in a chronic assay, in order to estimate the lowest observable effect concentration (LOEC) on reproduction and survival of *D. ambigua*. These results allowed to determining the sublethal concentration to be used in the main experiment (see appendix S2 for details).

2.3.2. Experimental design

To determine the harmful effects of methamidophos on size-structured populations of *D. ambigua*, experimental populations were subjected to simulated size-selective predation and exposure to pesticide. The experiment was started with parallel 24 experimental populations containing 10 individuals each (4 newborns, 3 juveniles and 3 adults), in 1L flasks with COMBO medium and fed with *P. subcapitata* at 10^6cel mL^{-1}. The size structure within each population during phase 2 and 3 was manipulated by three different kinds of simulated selective predation where a proportion of newborns (< 1.2 mm.), juveniles (1.2 – 1.5 mm.) and adults (> 1.5 mm.) were removed by manual pipetting, according to the corresponding simulated predation treatment: 30% of newborns in populations biased toward larger sizes, 30% of adult in populations biased toward smaller sizes and 10% of each size class in populations without bias. Selective predation and exposure treatments were performed considering three distinct experimental phases. **Phase 1 (transient):** According to the time determined in previous experiments (data not shown), the 24 experimental units were maintained without manipulation the first 12 days to ensure the stabilization of their abundances. **Phase 2 (selective predation):** after transient phase 1, treatments of size selective predation were initiated, subjecting 8 populations randomly selected to each of the three predation treatments described above. **Phase 3 (selective predation and pesticide exposure):** after 9 days in Phase 2, four populations from each of the predation treatments were subjected to chronic pesticide exposure. The concentration of pesticide used was 80 mg L^{-1}, chosen for being higher than the low observable effect concentration (LOEC = 60 mg L^{-1}) determined in the chronic assay, and just high enough to approach a reduction of 50% in reproduction (Appendix S2). During the whole phase 3, all populations were subjected to selective predation. The experiments were finished after

5

reaching a stable age structure, tested by the statistical tools described in the following section.

Each 3 days the densities of newborns, juveniles and adults were measured for each population (replicate) and the medium was renewed. The size classes described above were determined in an experiment showed in appendix S1. Total counts were performed at low densities. However, at high densities, three 40 mL aliquots were taken for each replicate.

2.4. Asymptotic analysis of population structure

Chi-square analysis was performed at each phase to determine the stable size structure within populations. Counts of newborns, juveniles and adults at a particular date were pairwise contrasted with the final count performed. This procedure allowed to creating a binary data set with 1 or 0 at each time for each replicate, indicating respectively significant or non-significant differences, where (i) ones values (p <0.05) corresponding to different size class structure and (ii) zeros values (p >0.05) corresponding to equal size class structure. A logistic regression was fitted to this binary data at each experimental phase for determining the time (t_f) at which each treatment reached their stable size structure, separating in this way the transient from the asymptotic dynamics in each experimental phase. In phase 2, one-way ANOVA was performed to determine differences in mean stable population densities between predation treatments. In phase 3, two-way ANOVA were performed to determine differences on mean stable population densities using pesticide exposure and predation treatments as factors. Post hoc Tukey-HSD test was used to make specific comparisons.

We also tested for changes in the asymptotic (i.e. in phase 3) size structure between exposed and unexposed populations under each predation treatment. This was done by means of a permutation test, randomly allocating (10,000 runs) the measured size structures between control and exposed treatments under each predation type. At each run, we measured the differences between size structures by mean of the χ^2 metrics. If the differences between observed control and exposed treatment (χ^2_{obs}) exceeded the 95% of the distribution of randomized χ^2 values (χ^2_{p95}), significant differences were accepted at the 5% level. In addition, generalized linear models (GLM) were performed, since parametric assumptions were not fulfilled, using pesticide exposure and size classes as factors.

2.5. Diagnosis and statistical models of population dynamics

To determine the endogenous structure of *D. ambigua* population dynamics, we first determined the order of the feedback structure of the obtained time series. Hence, we carried out an autoregressive analysis using the partial rate correlation function (PRCF), according to Berryman and Turchin (2001). In all cases the populations were dominated by first-order feedback (Figs. S4A-C).Therefore, we used a nonlinear version of the simple Ricker's (1954) equation as a starting point to model the R-function (Berryman 1999). R-functions represent the realized per capita population growth rates that represent the processes of individual survival and reproduction (Berryman 1999). This allowed us to model the basic influences of endogenous and exogenous forces on these dynamics.

7

2.5.1 Theoretical models of Daphnia population dynamics

Royama (1992) classified the R-functions by introducing three categories of exogenous perturbations: vertical, lateral and nonlinear perturbations. Vertical perturbations shift the maximum reproductive rate and the carrying capacity additively, changing the relative position of the whole R-function along y axis in an additive way. Lateral perturbations shift the carrying capacity without altering maximum reproductive rate, therefore the R-function shift along the X-axis in a non-additive manner. Nonlinear perturbations could affect together or independently the maximum reproductive rate and the carrying capacity shifting the curvature of the R-function. To understand how endogenous and exogenous forces determine population dynamics, we used a simple model of intra-specific competition, the exponential form of the discrete logistic model (Ricker 1954; Royama 1992):

$$N_t = N_{t-1} \llcorner r_m \llcorner \exp [\text{-c } \llcorner N_{t-1}^a] \tag{1}$$

where N_t represents the population abundance at time t, r_m is a positive constant representing the maximum finite reproductive rate, c is a constant representing competition and resource depletion, and a indicates the effect of interference on each individual as density increases (Royama 1992); $a>1$ indicates that interference intensifies with density and $a<1$ indicates habituation to interference. By defining Eq. 1 in terms of the R-function, $R_t = \log_e (N_t/N_{t-1})$, log-transforming Eq. 1, and defining the population density in logarithm $X_t = \log_e (N_t)$, we obtain:

$$R_t = R_m - \exp [a \llcorner X_{t-1} + C] \tag{2}$$

8

Where R_t is the realized per capita growth rate $R_t= \log_e(N_t/N_{t-1})$, $R_m = \log_e(r_m)$, a is the same parameter as in Eq. 1, $C = \log_e(c)$, and $X = \log_e(N)$. We fitted Eq. 2 by means of nonlinear regression analyses (Bates and Watts 1988) using the nls library in the program R (R Development Core Team 2014). For each replicate in phase 3 R-function parameters were estimated and compared among treatments by paired t-test.

3. Results

3.1. Size-specific individual tolerance to methamidophos

Acute toxicity test showed that tolerance to methamidophos, measured through both 24h and 48h LC_{50}, increased with age/size (Fig. S2A).

3.2 Asymptotic analysis of population structure

Population trajectories of *D. ambigua* under the two pesticide exposure and the three size-selective predation treatments are shown in Fig. 1. All *D. ambigua* populations exhibited an initial phase of exponential growth during the first 12 days. During this period, peak density of about 600-700 individuals L^{-1} were reached (Fig. 1). In phase 1, newborns were the most abundant size class, while juveniles and adults not differed in their densities (Fig. 4a). Figure 2 shows an example (under selective predation on newborns) of the measured trajectories of newborns, juveniles and adults. In appendix S3 we show the detailed trajectories for all treatments.

In phase 2 (from day 12), when size-selective predation started, population density decreased and then oscillated around 300 individuals L^{-1} (Fig. 1). One-way ANOVA

showed significant differences in asymptotic mean density among predation treatments ($F_{2,21}$ = 16.2; P < 0.001) and Tukey-HSD test showed that selective predation on adults led to higher densities, as compared to the others predation treatments (P < 0.001: Fig. 3b). In this phase, higher density of larger size class was observed in populations under selective predation on newborns. In contrast, populations under predation on adults were dominated by newborns. In populations under non-selective predation, slightly higher abundances of newborns were obtained (Tukey-HSD; Fig. 3a). The chi-square contingency analysis (Appendix S3) showed that size structure reached in populations at phase 1 differed significantly from the size structure reached in phase 2, for populations under selective predation on newborns (χ^2 = 42.56; P <0.0001), as well as for population subjected to non-selective predation (χ^2 = 8.039; P <0.05) and selective predation on adults (χ^2 = 6.73; P <0.05). Furthermore, significant differences were observed in phase 2 among size structures generated by each predation treatments 2 (χ^2 > 20; P <0.001 for all pairwise comparison). Therefore, the three patterns of simulated predation effectively generated different size structures in the experimental populations.

In phase 3, after the determination and addition of 80 μg L^{-1} of methamidophos (See Appendix S2) to the corresponding treatments, two-way ANOVA showed that predation regime ($F_{2,354}$ = 2238.5; P < 0.001), pesticide exposure ($F_{1,354}$ = 18076.8; P < 0.001) and their interaction ($F_{2,354}$ = 1889.1; P < 0.001) affected significantly the asymptotic population density (Fig. 4). Lower population densities were reached in populations exposed to the pesticide, under all predation treatments (Tukey-HSD test, P < 0.05; Fig. 4). During phase 3 all populations showed an initial decrease followed by an increase in densities (Fig. 1). However, populations under selective predation on adults were the most

affected, relative to controls, reaching an evidently reduced asymptotic density as compared to each other predation treatment (Fig. 1). Furthermore, exposed populations differed significantly in asymptotic total population density among predation treatments (tukey-HSD, p < 0.001). Results from GLM analysis showed, for all predation treatments, an interactive effect between pesticide exposure and size classes suggesting significant differences in size structure between exposed and unexposed populations (Table S3A). We observed a lower F value for populations under selective predation on newborns and a higher F value for populations under selective predation on adults, which means a higher shift in size structure for the last populations (Table S3A). In contrast, the permutation analysis showed that pesticide exposure caused a shift in size structure of populations under selective predation on adults (χ^2_{obs} = 19.04, χ^2_{p95} = 13.10, P < 0.05), reversing the dominance of earlier stages (Fig. 4c). Also, the permutation test revealed a marginally significant difference in size structure for populations under unselective predation (χ^2 = 6.58, χ^2_{p95} = 4.86, P < 0.05; Fig. 4b). For selective predation on newborns no differences in population structure were detected by this test to pesticide exposure (χ^2 =1.18, χ^2_{p95} = 1.232, P > 0.05; Fig. 4a).
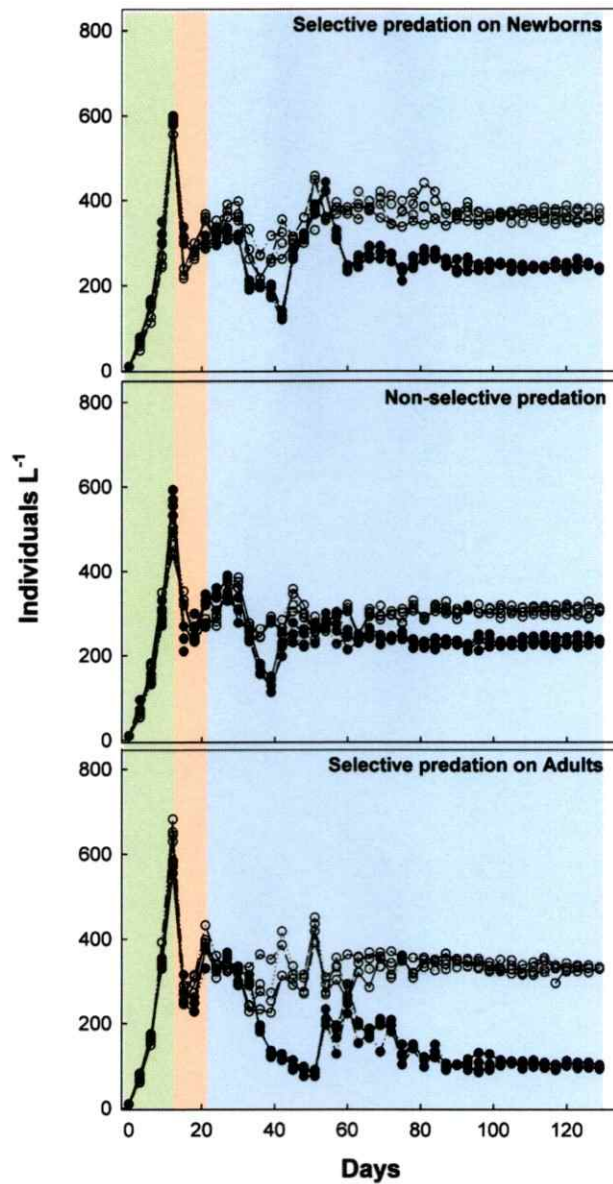
Figure 1. Time series of *D. ambigua* population density under selective predation on newborns (upper panel), non-selective predation (middle panel) and selective predation on adults (lower panel). Open circles and filled represent populations not exposed and exposed, respectively, to the pesticide methamidophos. Color areas represent phase 1 (green shading, growing populations), phase 2 (light orange shading, simulated predation) and phase 3 (light blue shading, simulated predation and pesticide exposure).

12
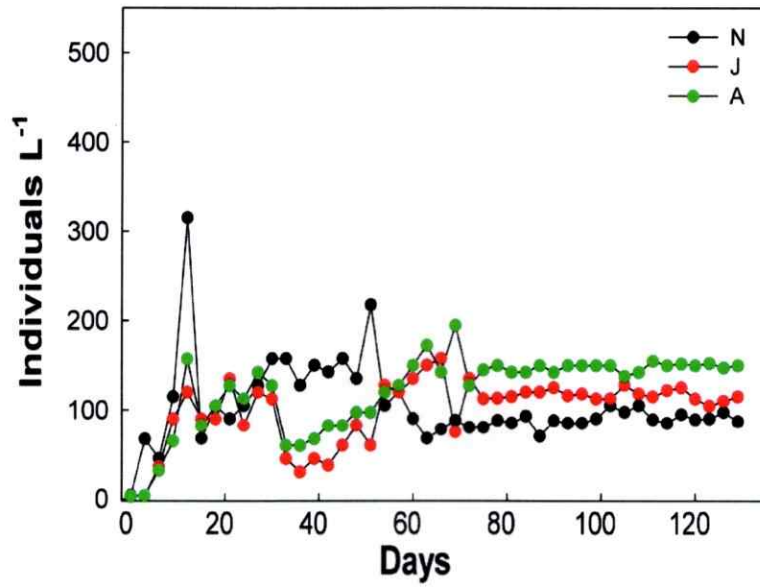
Figure 2. Time series of density for size classes newborns (black points), juvenils (red points) and adults (green points) for a replicate of populations under selective predation on newborns as an example.
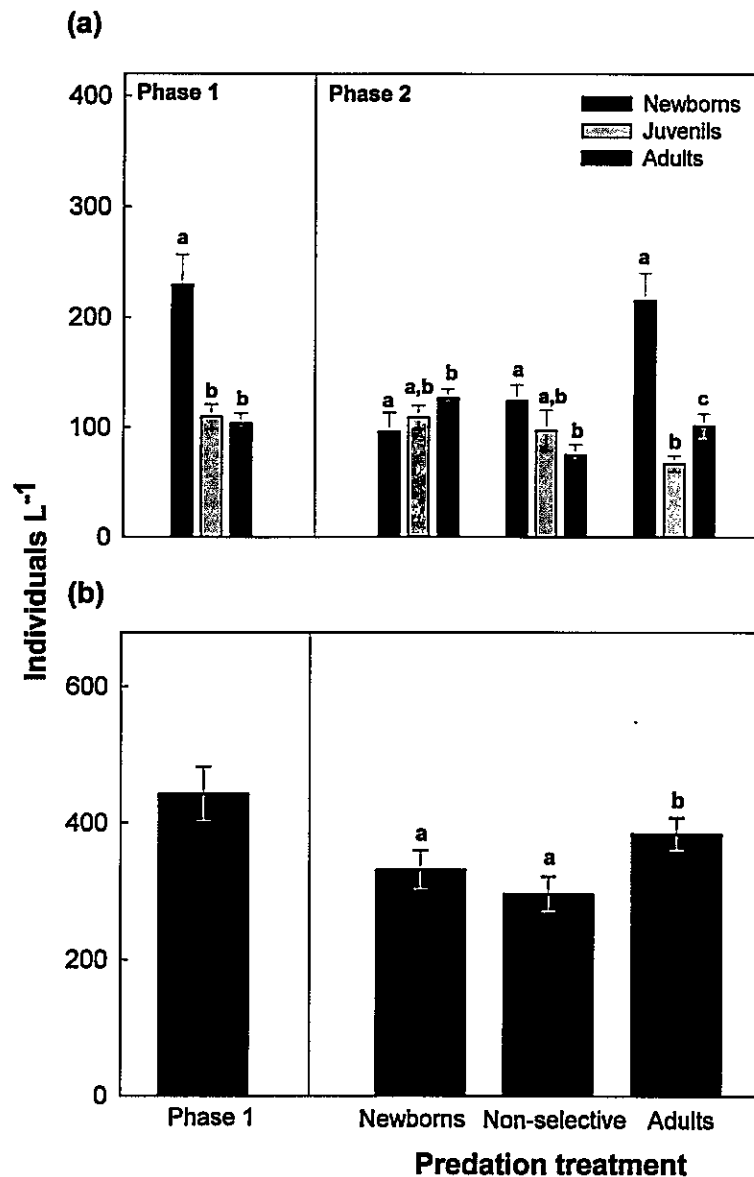
Figure 3. (a) Mean asymptotic density ± 95% C.I. of each size class during phase 1 (left panel) and for each predation treatment in phase 2 (right panel). Different letters above bars indicate significant differences among size class densities within each treatment predation treatments (Tukey-HSD test, P < 0.001). (b) Asymptotic total population density ± 95% C.I. for phase 1 (left panel) and each predation treatment in phase 2 (right panel). Different letters above bars indicate significant differences among treatments (Tukey-HSD test, P < 0.001).
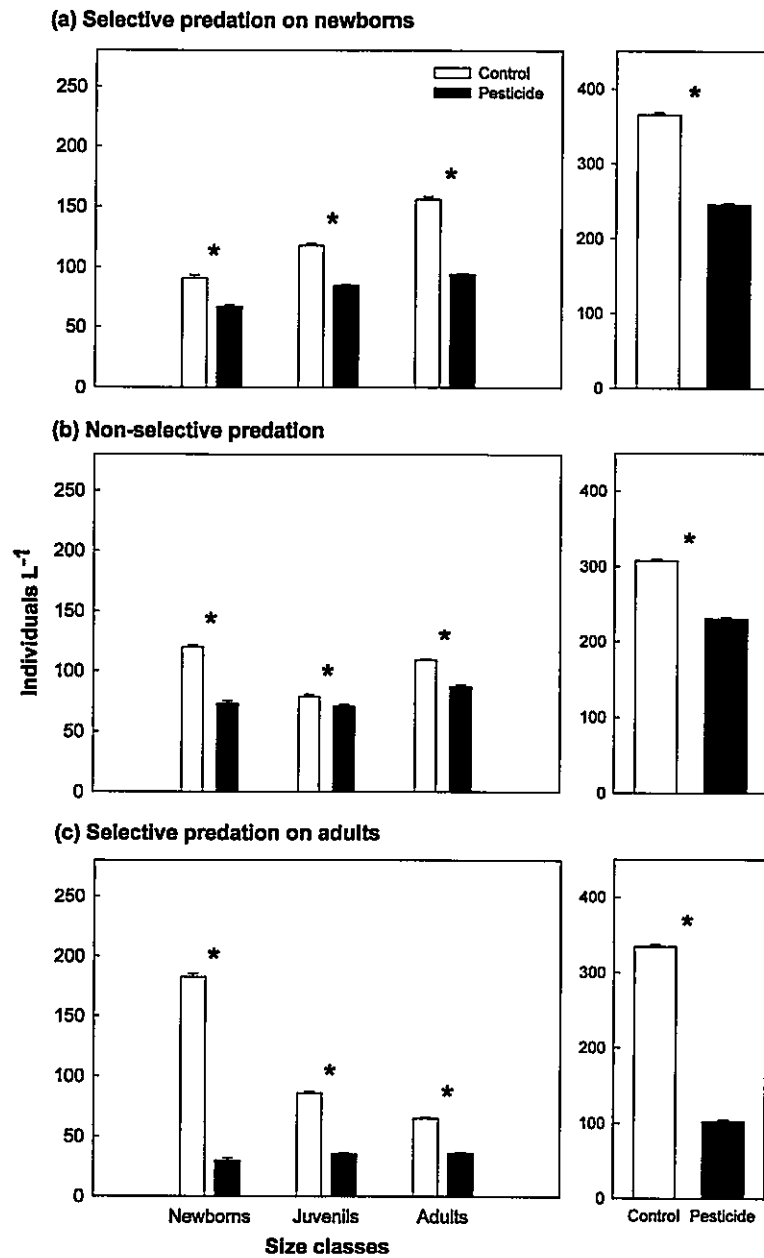
Figure 4. Mean asymptotic density ± 95% C.I. for size classes (left panels) and total population density (right panels) during phase 3 for unexposed populations (open bars) and exposed populations (filled bars) to pesticide, under the three experimental predation patterns: (a) selective predation on newborns, (b) non-selective predation and (c) selective predation on adults. Asterisks indicate significant differences among control an exposure treatments (Tukey-HSD test, $P < 0.001$).

15

3.3 Population dynamics

In phase 3, all *D. ambigua* populations showed a clear first-order dynamics caused by negative feedback of density at time *t*-1 (Berryman 1999). See Figure 5 as an example and Appendix S4 for a deeper analysis of partial rate correlation functions.

Accordingly, R-functions were constructed by fitting the realized per capita growth rate at *t* (R_t) to the logarithm of total abundance at *t*-1 (log N_{t-1}). All parameters in each fitted curves were significant (t < 5.5, p< 0.02) and the pesticide exposure exerted different effects in the shape of R-function depending on the predation treatment.

In terms of Royma's classification the pesticide exposure exerted nonlinear effects in both populations under selective predation on newborns and selective predation on adults (Fig. S4D). However, the mechanisms which led to changes in population dynamics were different for each treatment. For populations under selective predation on newborns, the changes in population dynamics exerted by pesticide exposure were driven by a significant decrease in the maximum reproductive rate R_m (t = 8.85, p<0.001; Fig. 5a), while maintaining without significant differences the competition intensity *C* (t = 1.22, p = 0.27; Fig. 5c) and the individual interference *a* (t = -1.18, p = 0.28; Fig. 5b). Conversely, for populations under selective predation on adult, the changes in dynamics exerted by pesticide exposure were driven by a significant increase in R_m parameter (t = -8.40, p<0.001; Fig. 5a) and a significant decrease in *C* parameter (t = -22.86, p<0.001; Fig. 5c), maintaining without significant differences the *a* parameter (t = 1.54, p = 0.17; Fig 5b).

On the other hand, under non-selective predation, populations showed a weak, although statistically significant lateral effect of pesticide exposure (Fig. S4D). In which, the *C* parameter was decreased significantly (t = -9.75, p<0.001; Fig. 5c), while R_m parameter (t = 0.69, p = 0.53; Fig 4a) and *a* parameter (t = 1.50, p = 0.11; Fig 4b) remained without

16

significant differences. However, the observed shift in the R-function of populations under non-selective predation suggests a vertical effect of pesticide exposure, since both R_m and C were decreased and the whole curve was displaced (Fig. S4D).

Fig. 5 Partial rate correlation function for a time serie in an unexposed population of *D. ambigua* subjected to selective predation on newborns (as a graphical example). See appendix S4 for detailed plots in each predation treatment.
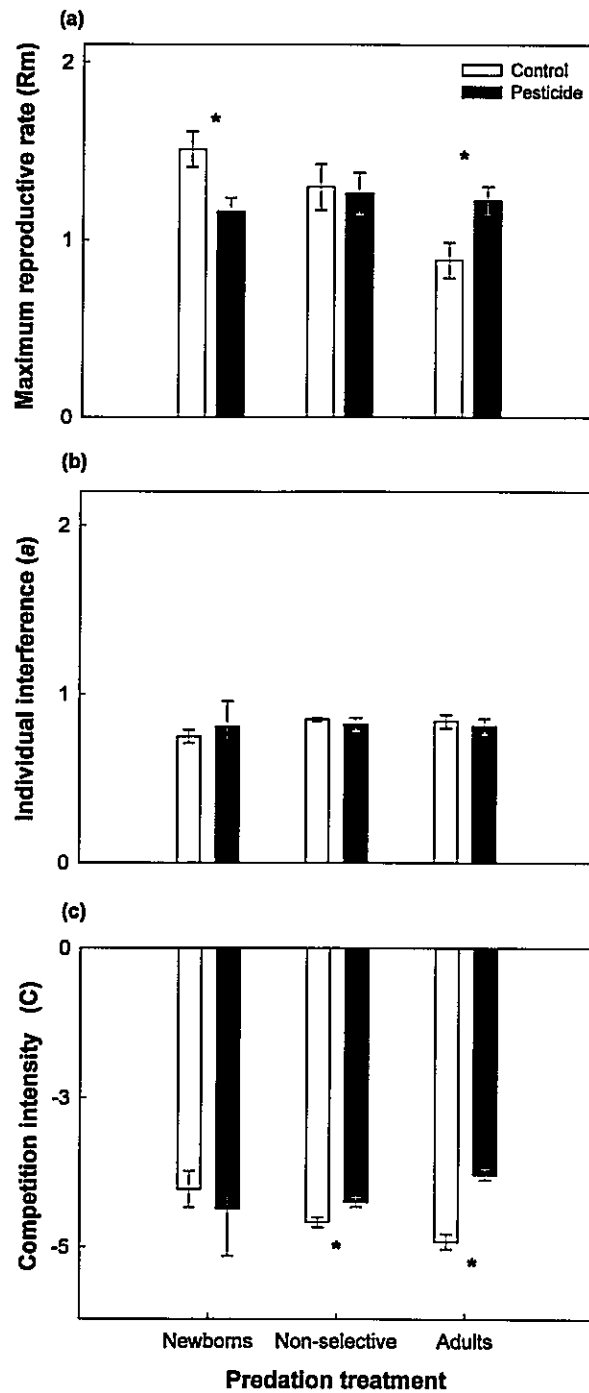
Fig. 6. Estimated parameters for reproductive function (R-function) in phase 3 for unexposed (White bars) and exposed (black bars) populations of *D. ambigua* subjected to different predation treatments. Asterisks indicate significant differences among control an exposure treatments (t-Test, P < 0.001).
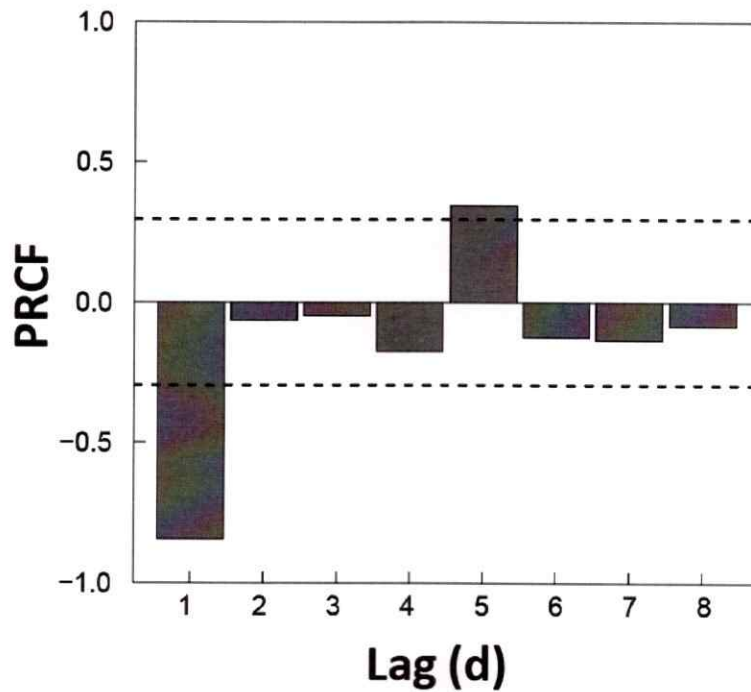
4. Discussion

In this study, our aim was to determine how population size-structure resulting from size-selective predation modulates the effects of pesticide exposure on population dynamics of *D. ambigua*. Simulated size-selective predation was effective in driving significant changes in the size structure of the experimental *Daphnia* populations. Pesticide exposure decreased population density in each predation treatment, exerting a higher density decrease in populations under selective predation on adults. Regarding the frequency of the different size classes, all exposed populations were dominated by larger individuals, while control populations showed different patterns of size structures depending on the predation treatment. Pesticide exposure exerted changes in population structure only in populations under selective predation on adults.

The modeling analysis showed that pesticide exposure promoted different population dynamics depending on the predation selectivity exerted on the experimental populations. In our analyses, pesticide effects on population dynamics was evaluated through its impact on each of three parameters that governs the shape of the R-function: maximum reproductive rate R_{max}, competition intensity parameter C and individual interference parameter a. A significant and stronger decrease on the maximum reproductive rate was observed in exposed populations under selective predation on newborns. Conversely, exposed populations under selective predation on adults showed a significant increase in their maximum reproductive rate. Whereas, in exposed populations under non selective predation the maximum reproductive rate did not show significant changes. Pesticide exposure exerted a decrease in the competition intensity parameter in populations under selective predation on adults as well as in populations under non selective predation,

19

although the effect was strongest in the former. Conversely, this parameter was not shifted by pesticide exposure in population under selective predation on newborns. Finally, the individual interference parameter remained without significant differences among control and exposed populations.

During the initial phase all populations showed a dominance of smaller individuals, which is typical for growing populations under high food availability and weak intraspecific interactions (Hanazato & Hirokawa 2004; Takahashi & Hanazato 2007). After, in phase 2 (only predation) population structure in each treatment differed respect to the initial phase and the internal organization of *D. ambigua* populations was shifted in similar way to the natural changes exerted either by fish (selective predation on adults) or invertebrates (selective predation on newborns) in natural freshwater populations, leading to an accumulation of smaller or larger individuals, respectively (Zaret 1980; Brett 1992; Gliwicz el al. 2010; Greg 2013). The greater effects of predation pressure were observed in populations under selective predation on adults, in which density of newborns predominates likely due to lower intraspecific competition for food resources between remained adults (Glazier 1992; Preuss et al 2009).

During the experimental pesticide exposure, greater negative effects on population density were observed in populations under selective predation on adults, which were composed predominantly by smaller individuals. This reduction reflects the observed results of our individual level test, where smaller individuals were less tolerant to methamidophos and confirms the greater sensitivity of smaller sizes or early stages to pesticide exposure observed early for cladocerans (Klein 2000; Takahashi & Hanazato 2007). Furthermore, a recent study conducted on populations of *Daphnia magna* subjected to selective predation

20

pressure of invertebrates and short-term pesticide exposure revealed a higher decrease in abundance driven by pesticides in populations composed largely by smaller individuals (Gergs 2013).

However, our populations under selective predation on newborns did not follow the same pattern found above. Pesticide exposure removed a greater proportion of individuals of larger size classes, which could be attributed to lower demographic transitions from smaller to larger individuals (Liess et al. 2006). In environments under higher crowding and stronger intraspecific interactions as observed in our populations under selective predation on newborns and non-selective predation, the pesticide exposure increased the negative effects on all size classes present in the population (Hanazato & Hirokawa 2004; Takahashi & Hanazato 2007).

Our modeling analysis showed different mechanisms by which the pesticide exposure drove population level effects, in dependence on the predation treatment to which were subjected. The parameter analysis showed that exposed populations under selective predation on newborns not changed their competition intensity parameter but decreased their maximum reproductive rate, as compared to control. Conversely, exposed populations under selective predation on adults decreased the competition intensity parameter and increased their maximum reproductive rate. Selective predation on newborns could have decreased the food condition and increasing the individual stress as observed previously (Knops et al. 2001; Smolders 2005), affecting consequently the further reproduction in remained individuals (Burns 1995; Barata et al. 2002; Liess 2002; Beketov & Liess 2005; Foit et al. 2011).We observed that the selective predation, either by smaller or large size classes drove to stronger and qualitative changes in the population dynamics. Exposed

populations under those treatments showed nonlinear effects but differed in the mechanisms by which the population dynamics was shifted. In contrast, for populations under non-selective predation weaker lateral effects of pesticide exposure were observed on population dynamics. However a vertical effects also could be suggested, which could have emerged in conditions of stronger predation pressure or pesticide exposure for these populations.

The ways in which endogenous process (population feedbacks) and exogenous factors (environmental perturbations) may interact have implications for the resulting of population dynamics and for the sensitivity of a particular species to environmental conditions (Johst & Drechsler 2003; Owen-Smith 2011). Changes in population dynamics had considered predominantly environmental variation as food fluctuations (Lima & Berryman 2006; Previtali et al. 2009; Jhost et al. 2012), weather changes (Lima et al. 2008; Jhost et al. 2012) and predation (Lima et al. 2002; Previtali et al. 2009). Specially, the joint effects of climate variability, seasonality and changes in the predator guild are likely to result in a large nonlinear and seasonal impact on demography and population dynamics. As observed for semiarid rodent species, which during the breeding season, population dynamics appear to be limited by food and regulated by intraspecific competition. By contrast, during the non-breeding season, predation and to a lesser degree food availability appear to determine population reproductive rates (Lima et al. 2002). This finding, in line with our results shows how the environmental variability shifts the qualities of population dynamics through diverse mechanisms. We observed how both pollution effects and selective predation modulate the qualitative properties of population dynamics and could help to extend the vision of exogenous perturbation by incorporating in the same and simple

22

framework the effects of anthropogenic stressors on population dynamics of freshwater ecosystems.

We concluded that aquatic populations in natural environments could respond in different ways to pesticide exposure depending on size structure and diversity of the dominant predators. In presence of fishes, which are characterized by a selective predation towards larger size classes, *Daphnia* population dynamics could be affected by strongly reducing population densities and changing size structure, through changes in both the interaction between individuals and the population reproductive rate. On the other hand, the selective predation on smaller prey sizes as the exerted by invertebrates or non selective predation which could be present at low fish densities and presence of invertebrates, promote changes in population dynamics by exerting lower decrease in population densities, through changes in the reproductive rate of populations and in the interaction between individuals, respectively. Of further consideration for research is the consequences of the seasonal variation in the composition and activity of the predator assembly in interaction with the seasonal variation in exposure to agrochemicals, and the potential shifts expected for these seasonal trends as a product of climate change. Only through a deeper understanding of the interactive effects of natural and anthropogenic stressors we could advance our ability to manage natural systems.

References

Agatz, A., Hammers-Wirtz, M., Gabsi, F., Ratte, H. T., Brown, C. D. & Preuss, T. G. (2012) Promoting effects on reproduction increase population vulnerability of Daphnia magna. Environ. Toxicol. Chem. 31, 1604–10.

Barata C, Baird D.J. (2000). Determining the ecotoxicological mode of action of chemicals from measurements made on individuals: Results from instar-based tests with Daphnia magna Straus. Aquat Toxicol, 48, 195–209.

Bates, D., Watts, D. (1988). Nonlinear regression analysis and its applications. Wiley New York, 341 pp.

Berryman, A. A. (1999). Principles of population dynamics and their application. Stanley Thornes Publ.

Berryman, A.A, Turchin, P. (2001). Identifying the density-dependent structure underlying ecological time series. Oikos, 92, 265–270.

Beketov, M.A., Liess, M. (2005). Acute contamination with esfenvalerate and food limitation: chronic effects on the mayfly. Cloeon dipterum. Environ Toxicol Chem, 24(5), 1281–1286.

Bodar, C.W.M., Van Leeuwen, C.J., Voogt, P.A., Zandee, D.I. (1988). Effect of cadmium on reproduction strategy of *Daphnia magna*. Aquat Toxicol, 12, 301–309.

Brandl, Z. (1998a). Feeding strategies of planktonic cyclopoids in lacustrine ecosystems. J Mar Syst, 15, 87–95.

Brandl, Z. (1998b). Life strategy and feeding relations of Cyclops vicinus in two reservoirs.Int Rev Hydrobiol, 83, 381–388.

Brett, M.T. (1992). Chaoborus and fish-mediated influences on Daphnia longispina population structure, dynamics and life history strategies. Oecologia, 89, 69–77.

Brook, J., Dodson, S.I., (1965). Predation, body size and composition of the plankton. Science, 150, 28–35.

Dodson, S.I., (1974). Zooplankton competition and predation: an experimental test of the size-efficiency hypothesis. Ecology, 55, 605–613.

Foit, K., Kaske, O., Liess, M. (2011). Competition increases toxicant sensitivity and delays the recovery of two interacting populations. Aquat Toxicol 106–107:25–31.

Forget, J., Pavillon, J.F., Menasria, M.R., Bocquené, G., (1998). Mortality and LC50 values for several stages of the marine copepod Tigriopus brevicornis (Muller) exposed to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos and malathion. Ecotox Environ Safe, 40, 239–244.

Gergs, A., Zenker, A., Grimm, V., Preuss, T.G. (2013). Chemical and natural stressors combined: from cryptic effects to population extinction. Scientific reports (Nature publishing group) 3, 2036.

Glazier, D.S. (1992). Effects of food, genotype, and maternal size and age on offspring investment in Daphnia magna. Ecology, 73, 910–926.

Gliwicz, Z.M., Szymanska, E., Wrzosek, D. (2010). Body size distribution in Daphnia populations as an effect of prey selectivity by planktivorous fish. Hydrobiologia, 643, 5–19.

Hall, D.J., Cooper, W.E., Werner, E.E. (1970). An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol Oceanograph, 15, 839–929.

Hamilton, M.A., Russo, R.C., Thurston, R.V. (1977) Trimmed Spearman–Karber method for estimating median lethal concentrations. Environ Sci Tech, 11, 714–719.

Hanazato, T., Hirokawa, H. (2004). Change in vulnerability of Daphnia to an insecticide application depending on the population phase. Freshw Biol, 49, 402–409.

Hrbacek, J. (1962). Species composition and the amount of zooplankton in relation to fish stock.Rozpravy CSAV, Rada MPV 72(10), 1–117.

Hussain, M.A. (1987). Anticholinesterase properties of methamidophos and acephate in insects and mammals. Bull Environ Contam Toxicol, 38, 131–8.

James, D.E. (1978). Culturing algae. Carolina Biological Supply Company, Burlington, NC, USA.

Johst, K., Drechsler, M. (2003) Are spatially correlated or uncorrelated disturbance regimes better for the survival of species? Oikos, 103, 449–456.

Johst, K., Lima M., Berryman, A.A. (2012). Scaling up: how do exogenous fluctuations in individualbased resource competition models re-emerge in aggregated stochastic population models? Popul Ecol, 55(1), 173–182.

Kilham, S.S., Keeger, D.A., Lynn, S.G., Goulden, C.E, Herrera, DL. (1998). COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia, 377, 147–159.

Klein, B. (2000). Age as a factor influencing results in the acute daphnid test with Daphnia magna Straus. Water Research, 34, 1419–1424.

Knops, M., Altenburger, R., Segner, H. (2001). Alterations of physiological energetics, growth and reproduction of Daphnia magna under toxicant stress. Aquat Toxicol, 53, 79–90.

Liess, M. (2002). Population response to toxicants is altered by intraspecific interaction. Environ Toxicol Chem, 21, 138–142.

Liess, M., Pieters, B., Duquesne, S. (2006). Long-term signal of population disturbance after pulse exposure to an insecticide: rapid recovery of abundance, persistent alteration of structure. Environmental Toxicology and Chemistry, 24, 1326–1331.

Lima, M., Stenseth, N.C., Jaksic, F.M. (2002). Population dynamics of a South American small rodent: seasonal structure interacting with climate, density-dependence and predator effects. Proc R Soc Lond B Biol Sci, 269, 2579–2586.

Lima, M., Berryman, A.A. (2006). Predicting nonlinear and non-additive effects of climate: the Alpine ibex revisited. Clim Res, 32, 129–135.

Lima, M., Harrington, R., Saldana, S., Estay, S. (2008). Nonlinear feedback processes and a latitudinal gradient in the climatic effects determine green spruce aphid outbreaks in the UK. Oikos, 117, 951–959.

Manca, M., Vijverberg, J. L., Polishchuk, V., Voronov, D.A. (2008). Daphnia body size and population dynamics under predation by invertebrate and fish predators in Lago Maggiore: an approach based on contribution analysis. J Limnol, 67(1), 15–21.

Medina, M., Barata, C., Telfer, T., Baird, D. (2002). Age-and sex related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod Acartia tonsa Dana. Arch Environ Contam Toxicol, 42, 17–22.

Malato, S., Blanco, J., Richter, C., Milow, B., Maldonado, M.I. (1999). Solar photocatalytic mineralization of commercial pesticides: methamidophos. Chemosphere, 38(5), 1145–56.

Nilsson, N.A., Pejler, B. (1973). On the relation between fish fauna and zooplankton composition in north Swedish lakes.Report, Institute of Freshwater Research, Drottningholm, 53, 51–77.

OECD (2004). OECD guidelines for testing of chemicals. Guideline 202: Daphnia sp., Acute immobilisation test, adopted April 2004.

Owen-Smith, N. (2011). Accommodating environmental variation in population models: metaphysiological biomass loss accounting. J Anim Ecol, 80,731–741

Preuss, T.G., Hammers-Wirtz, M., Hommen, U., Rubach, M.N., Ratte, H. T. (2009). Development and validation of an individual based Daphnia magna population model: The influence of crowding on population dynamics. Ecol Model, 220, 310–329.

Previtali, M.A., Lima, M., Meserve, P.L., Kelt, D.A., Gutierrez, J.R. (2009). Population dynamics of two sympatric rodents in a variable environment: rainfall, resource availability, and predation. Ecology, 90, 1996–2006

Ricker, W. (1954). Stock and recruitment. J. Fish. Res. Board Can. 11: 559–623.

Royama, T. (1992). Analytical population dynamics. London, UK: Chapman and Hall.

Smolders, R., Baillieul, M., Blust, R. (2005). Relationship between the energy status of Daphnia magna and its sensitivity to environmental stress. Aquatic Toxicology, 73, 155–170.

Stenson, J., 1972. Fish predation effects on the species composition of the zooplankton commnity in eight small forest lakes. Report, Institute of Freshwater Research, Drottningholm, 52, 132-148.

Takahashi, H. & Hanazato, T. (2007). Synergistic effects of food shortage and an insecticide on a Daphnia population: rapid decline of food density at the peak of population density reduces tolerance to the chemical and induces a large population crash. Limnology, 8, 45–51.

Tomin, C. (1994). The pesticide manual, a world compendium, 10th ed. Croydon, UK: British Crop Protection Council.

U.S. EPA (1989). Pesticide Environmental Fate One Line Summary: Methamidophos. U.S. EPA Environmental Fate and Effects Division. Washington, DC.

Williamson, C.E. (1986). The swimming and feeding behavior of Mesocyclops. Hydrobiologia, 134, 11-19.

Willis, K., Ling, N. (2004). Toxicity of the aquaculture pesticide cypermethrin to planktonic marine copepods. Aquacult res, 35(3), 263-270.

Zaret, T.M. (1980). Predations and freshwater Communities. New Haven and London Yale University Press: 187 pp.

APPENDIX S1

*Body growth and population size classes*

Aiming to determine the body size of individuals and set population size classes, ten individuals of *D. ambigua* arranged individually in 50 mL flasks with 40 mL of COMBO media were used. Daily and during 20 days, body length measures (from the top of the head to the base of the tail spine) were obtained with a digital camera attached to a microscope, keeping individuals at the same focal point and subsequently analyzed by Biopix IQ 2.0 software (BioPix. AB, Gothenburg, Sweden). The precision of the length measurements was 10-4 mm. In addition, successive molts were recorded, which indicate the way to a higher stage of development (instar) to maturity. For each replicate, data of body length versus instar were fitted to the following sigmoid model by nonlinear regression analyses (Bates and Watts 1988) using the nls library in the program R (R Development Core Team 2011):

$$L(t) = \left(\frac{L_{max} - L_{min}}{1 + (t/t_{50})^a}\right) + L_{min}$$

where $L(t)$ = body length at time t, L_{max} = asymptotic body length (i.e. projected body length at infinite age), L_{min} = initial body length, t_h = age at which the middle value between L_{min} and L_{max} is reached, and a= abruptness of the curve.

Table S1A. Summary of parameters fitted by means of nonlinear regression analyses for body growth of *D. magna*.

| Parameter	value	Std. Error	t	p-value	R^2
L_{min}	0.631	0.035	18.3	<0.0001	0.965
L_{max}	2.776	0.090	30.8	<0.0001	
t_h	4.348	0.222	19.5	<0.0001	
a	1.979	0.169	11.7	<0.0001	
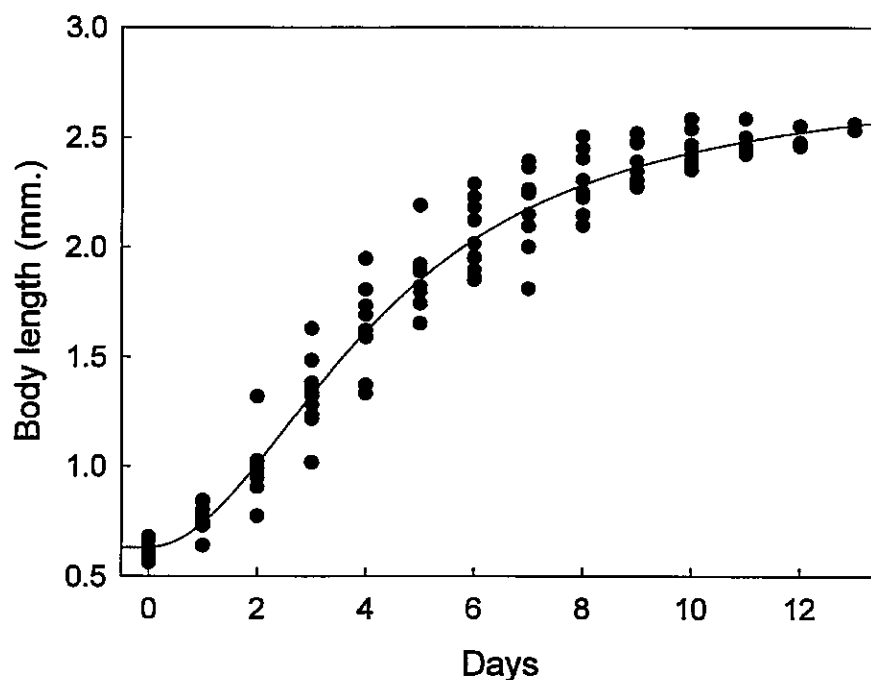
Fig.S1A. Measurement of body length versus days in *D. ambigua*. Points show measurements obtained for each replicate. Sigmoidal regression is presented in solid line.

Body growth closely matched a sigmoid curve of body length versus instar (Fig. 1A). The nonlinear regression was significant ($R^2 = 0.97$; $P < 0.0001$). Table 3A summarizes regression analysis, from which size class division was made considering L_{min} value and age at maturity in our clone of *D. ambigua*. Adult size class was considered to be comprised by individuals larger that L_A= 1.5 mm, size attained at day 4, when they started laying their eggs into the brood chamber. Neonate and juvenile size classes were defined as comprised by individuals with body size smaller and larger than $(L_A - L_{min})/2 = 1.2$ mm, respectively. Therefore the size classes in our experiments were neonates (<1.2 mm), juveniles (1.2-1.5 mm) and adults (>1.5 mm).

APPENDIX S2

1. Size-specific individual tolerance to methamidophos
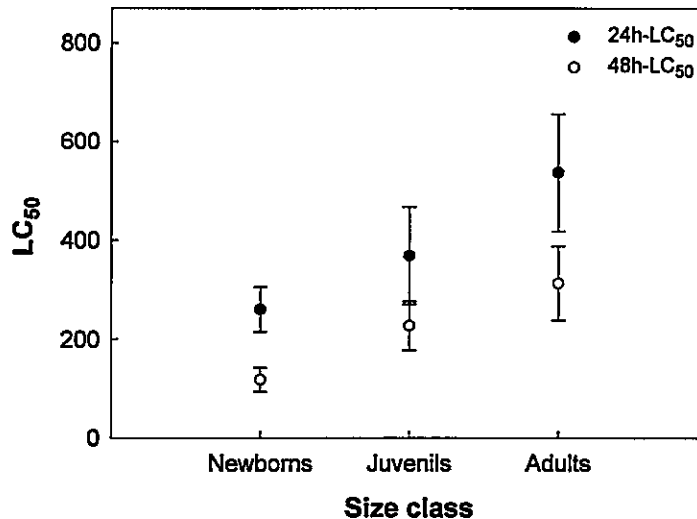
Fig.S2A. Acute toxicity 24h-LC_{50} (black circles) and 48h- LC_{50} (white circles) values (µg L^{-1}) ± C.I. 95% for *D. ambigua* of different sizes exposed to methamidophos.

2. Experimental concentration of methamidophos

Individuals were exposed since birth to reproductive maturity completing a period of 7 days of exposure to sublethal concentrations of 40, 60, 80, 100 µg L^{-1}. For each concentration three groups of 4 individuals were disposed in vessels of 50 mL with 40 mL of medium COMBO and the pesticide. A control group was included to compare results of exposure. Individuals were kept and fed according to the conditions described for acute toxicity test. The aqueous medium was renewed daily to maintain food and pesticide levels. Each replicate was reviewed every day to determine mortality, age at first reproduction and clutch size. Comparisons between exposure concentrations were made by one-way ANOVA. In addition, a post hoc Tukey's HSD test was used to make specific comparisons.

Table S2A. Summary of one-way ANOVA results for testing the chronic effects of exposure to different methamidophos concentrations on life history traits of *Daphnia ambigua.*

Trait	Source	d.f	F	P
Survival	Concentration	4	>103.98	< 0.0001
	residual	15		
Reproduction	Concentration	4	103.98	< 0.001
	residual	15		
Age at first reproduction	Concentration	3	7	0.006
	residual	12		
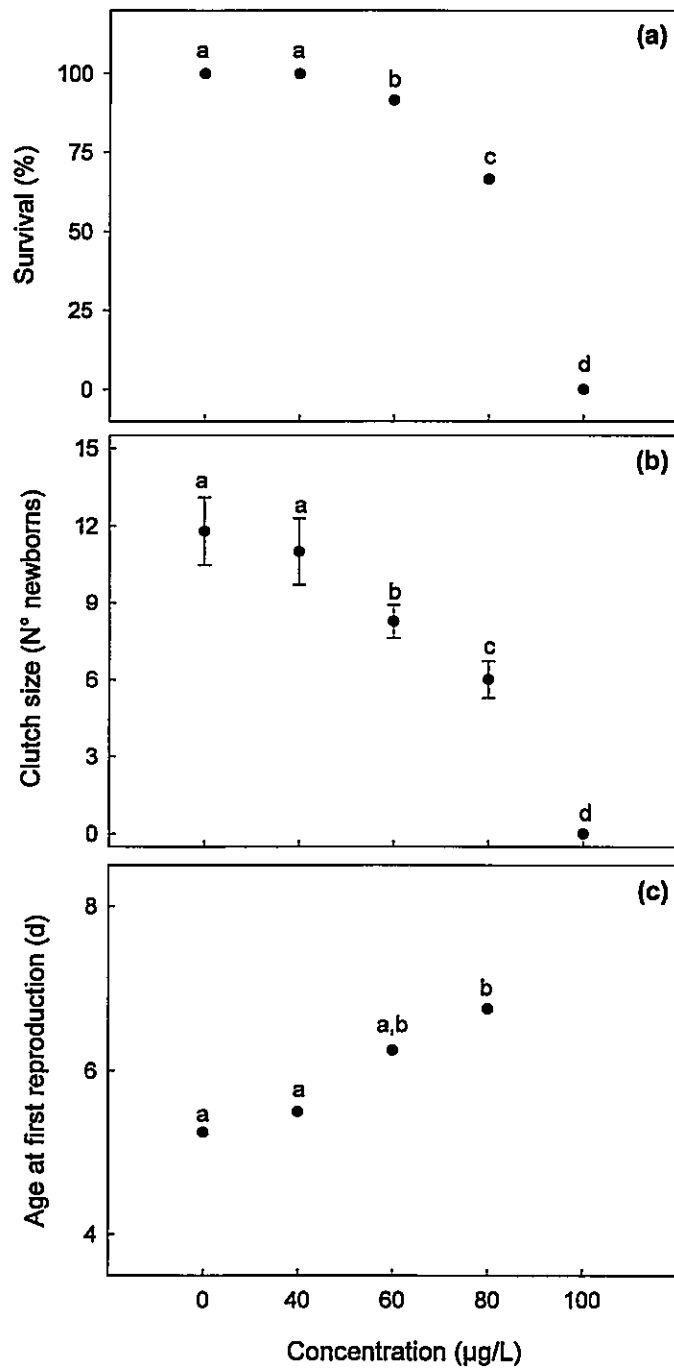
Fig.S2B. Effects of methamidophos exposure at different concentrations on (a) survival, (b) clutch size and (c) age at first reproduction of *D. ambigua*. Each symbol represents mean values. Different letters above symbols indicate significant differences among treatments (one-way ANOVA followed by Tukey-HSD test, $P < 0.001$).

Chronic toxicity, which aimed to determine methamidophos concentration to be used in main experiments showed significant differences on survival, clutch size and age at first reproduction between sublethal concentration assessed ($p < 0.001$; Table S2A). A heavy reduction in survival was observed at 80 and 100 µg L-1 (Tukey-HSD; Fig. S2A). Clutch size was diminished 25 and 50% at 60 and 80 µg L^{-1}, respectively. Furthermore, no reproduction was observed at 100 µg L-1 (Tukey-HSD; Fig. S2A). Finally, age at first reproduction was delayed in individuals exposed to 60 and 80 µg L-1 (Tukey-HSD; Fig. S2A). Given these results, a sublethal concentration of 80 µg L-1 was chosen to be used in main experiment.
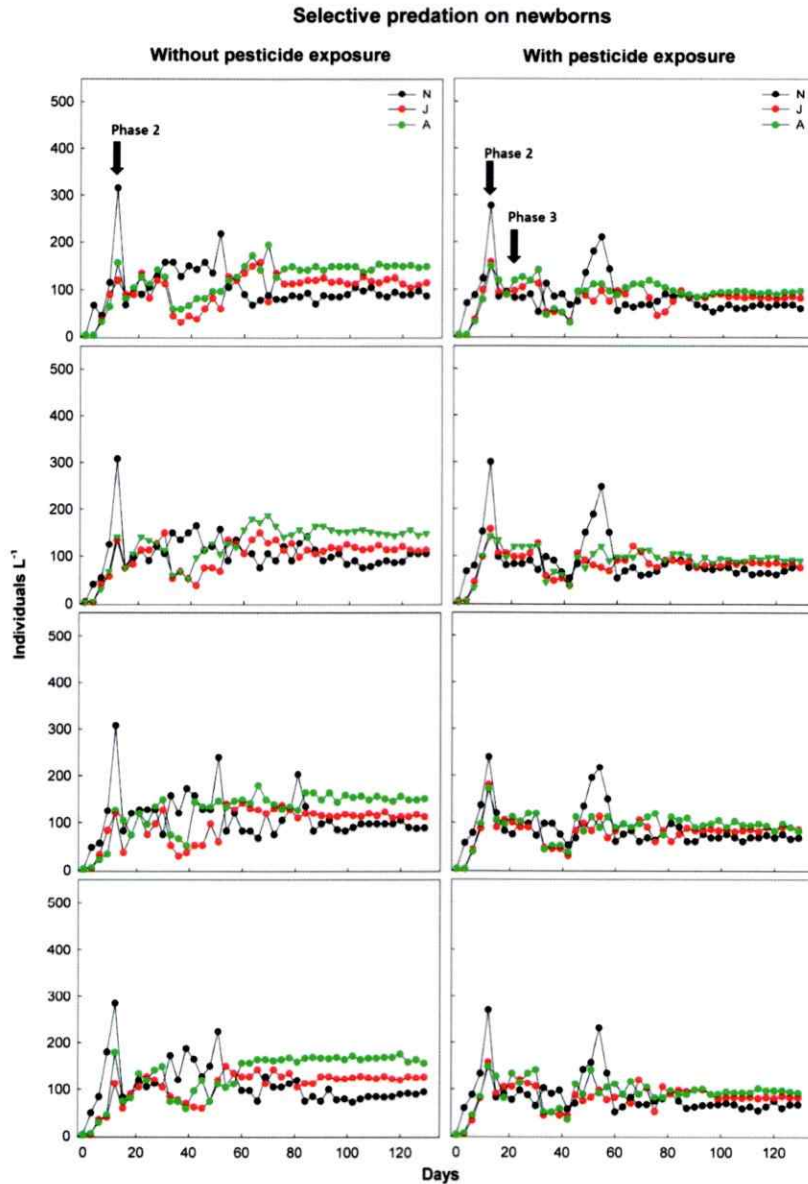
Fig. S3A. Time series of density for size classes newborns (black points), juveniles (red points) and adults (green points) for populations under selective predation on **newborns**. Left panel shows populations subjected to phases 1-2 and right panel shows populations subjected to phases 1-2-3. Each plot represents one replicate for treatments described. Arrows shows start of predation (phase 2) and chronic pesticide exposure (phase 3).
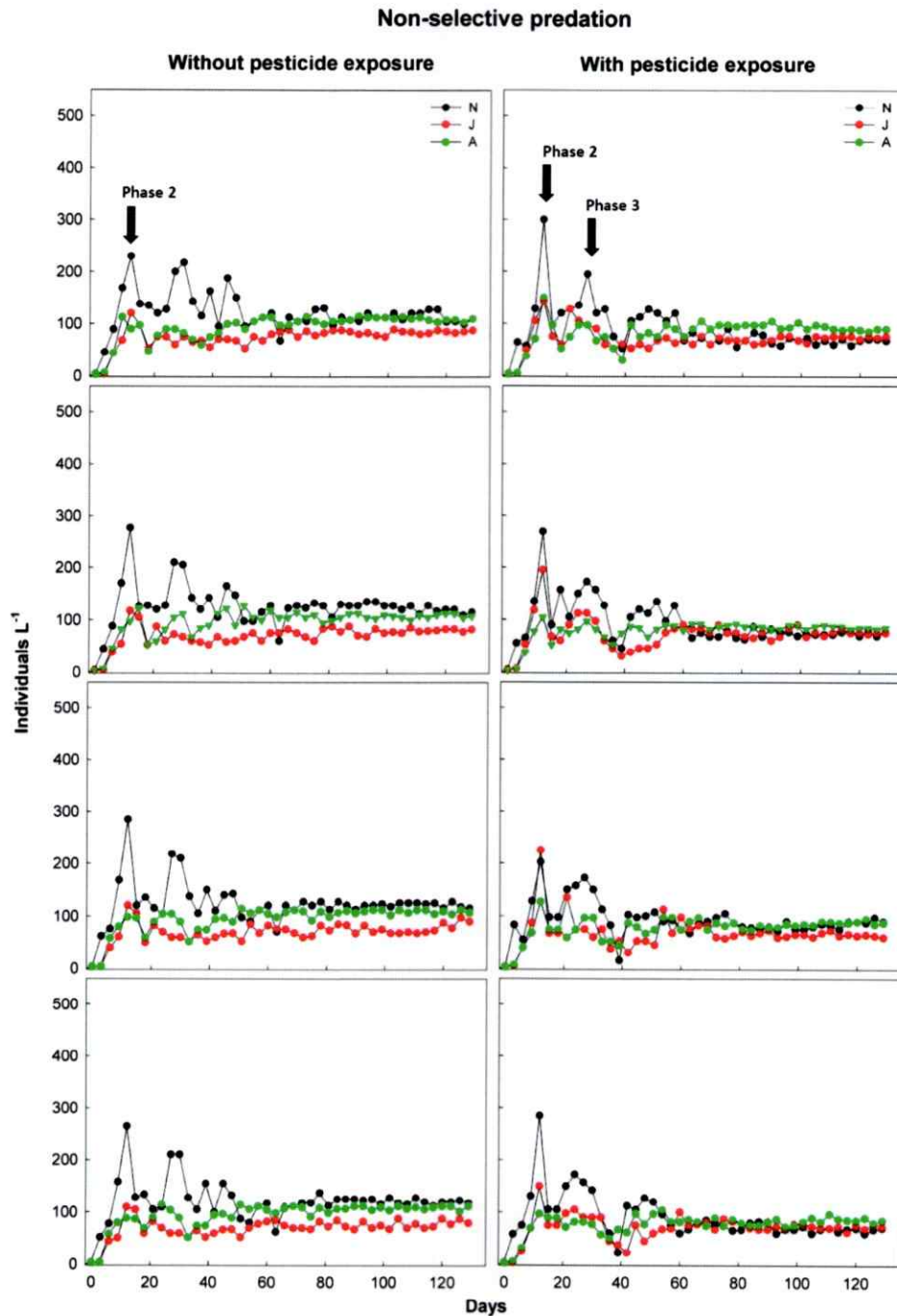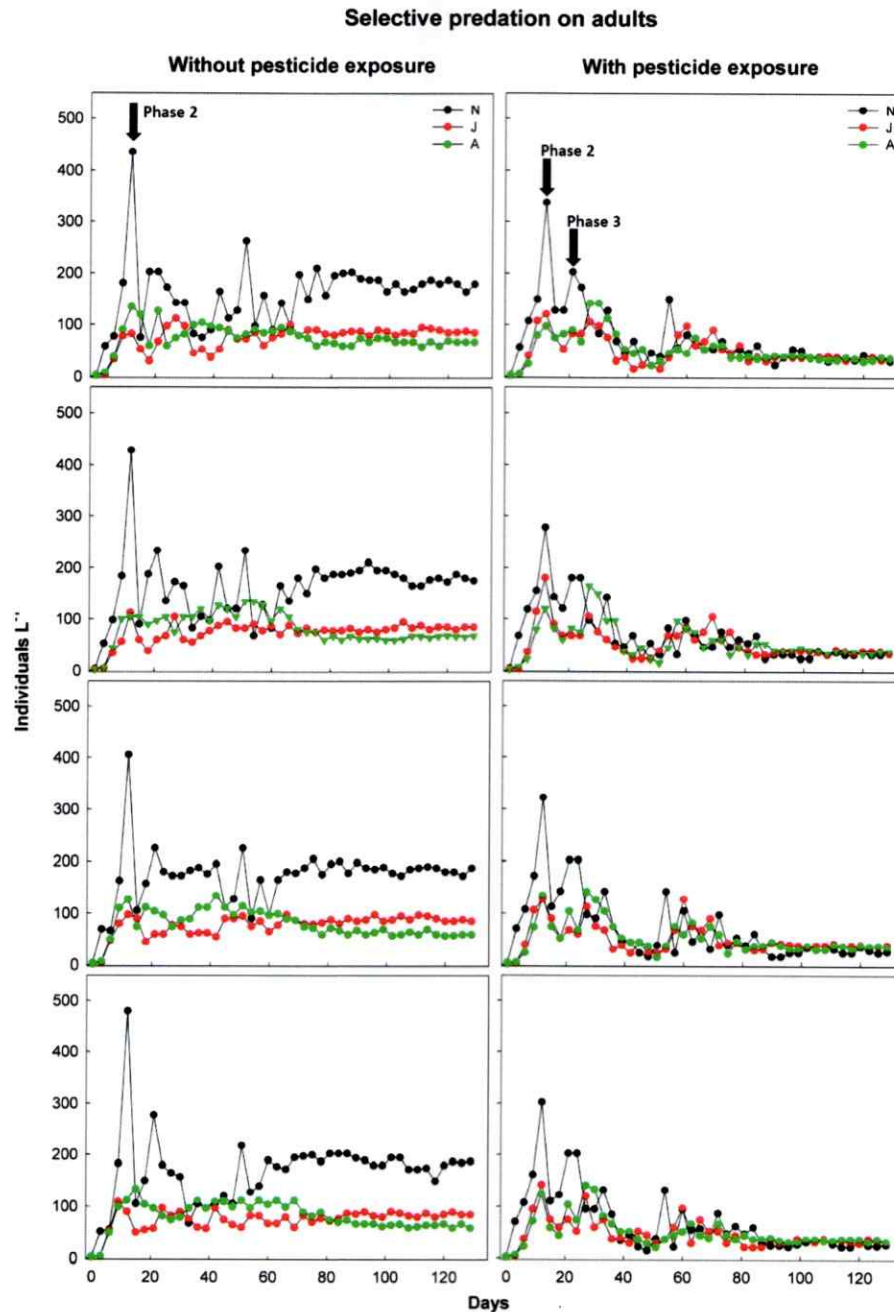
Fig. S3B. Time series of density for size classes newborns (black points), juveniles (red points) and adults (green points) for populations under **non-selective predation**. Left panel shows populations subjected to phases 1-2 and right panel shows populations subjected to phases 1-2-3. Each plot represents one replicate for treatments described. Arrows shows start of predation (phase 2) and chronic pesticide exposure (phase 3).

Fig. S3C. Time series of density for size classes newborns (black points), juveniles (red points) and adults (green points) for populations under **selective predation on adults**. Left panel shows populations subjected to phases 1-2 and right panel shows populations subjected to phases 1-2-3. Each plot represents one replicate for treatments described. Arrows shows start of predation (phase 2) and chronic pesticide exposure (phase 3).

Table S3A. Summary of GLM results for testing differences of size class distribution on *Daphnia ambigua* populations exposed and unexposed to methamidophos under different predation treatment.

Predation treatment	Source	d.f.	F	p
Predation on newborns	Pesticide (P)	1	1276.21	<0.001
	Size class (Sc)	2	441.74	<0.001
	P x Sc	2	246.29	<0.001
	Error	359		
Non selective	Pesticide (P)	1	3009.36	<0.001
	Size class (Sc)	2	1354.29	<0.001
	P x Sc	2	250.08	<0.001
	Error	359		
Predation on adults	Pesticide (P)	1	13167.56	<0.001
	Size class (Sc)	2	2621.27	<0.001
	P x Sc	2	3220.79	<0.001
	Error	359		

Table S3B. Observed mean stable abundance for each size class at phase 1, phase 2 (predation) and phase 3 (predation and pesticide exposure). Parenthesis represents abbreviations for predation treatments, pesticide exposure and their combinations.

	Treatment	Newborns	Juveniles	Adults
Phase 1		230	110	103
Phase 2	Predation on newborns (N)	96	109	127
	Predation on all classes (NJA)	124	97	75
	Predation on adults (A)	216	67	101
Phase 3	N-Control (NC)	91	117	149
	N-Pesticide (NP)	65	84	93
	NJA-Control (NJAC)	113	83	110
	NJA-Pesticide (NJAP)	67	72	93
	A-Control (AC)	181	87	68
	A-Pesticide (AP)	37	36	36

Table S3C. Observed Chi-square results (χ^2_{obs}) for paired comparisons in *D. ambigua* population size structures between (a) phase 1 and predation treatments, between predation treatments and (b) control and exposure under each predation treatments. See abbreviations and data in Table 1C. Significant differences are shown in bold.

|	Pairs	χ^2	P
(a) *Phase 2*	Phase 1 - N	42,56	**< 0,0001**
	Phase 1 - NJA	8,03	**0,02**
	Phase 1 - A	6,73	**0,03**
	N-NJA	15,64	**< 0,0001**
	N-A	55,66	**< 0,0001**
	NJA-A	23,22	**< 0,0001**
(b) *Phase 3*	NC-NP	1,057	0,59
	NJAC-NJAP	6,583	**0,04**
	AC-AP	19,035	**< 0,0001**

Selective predation on newborns
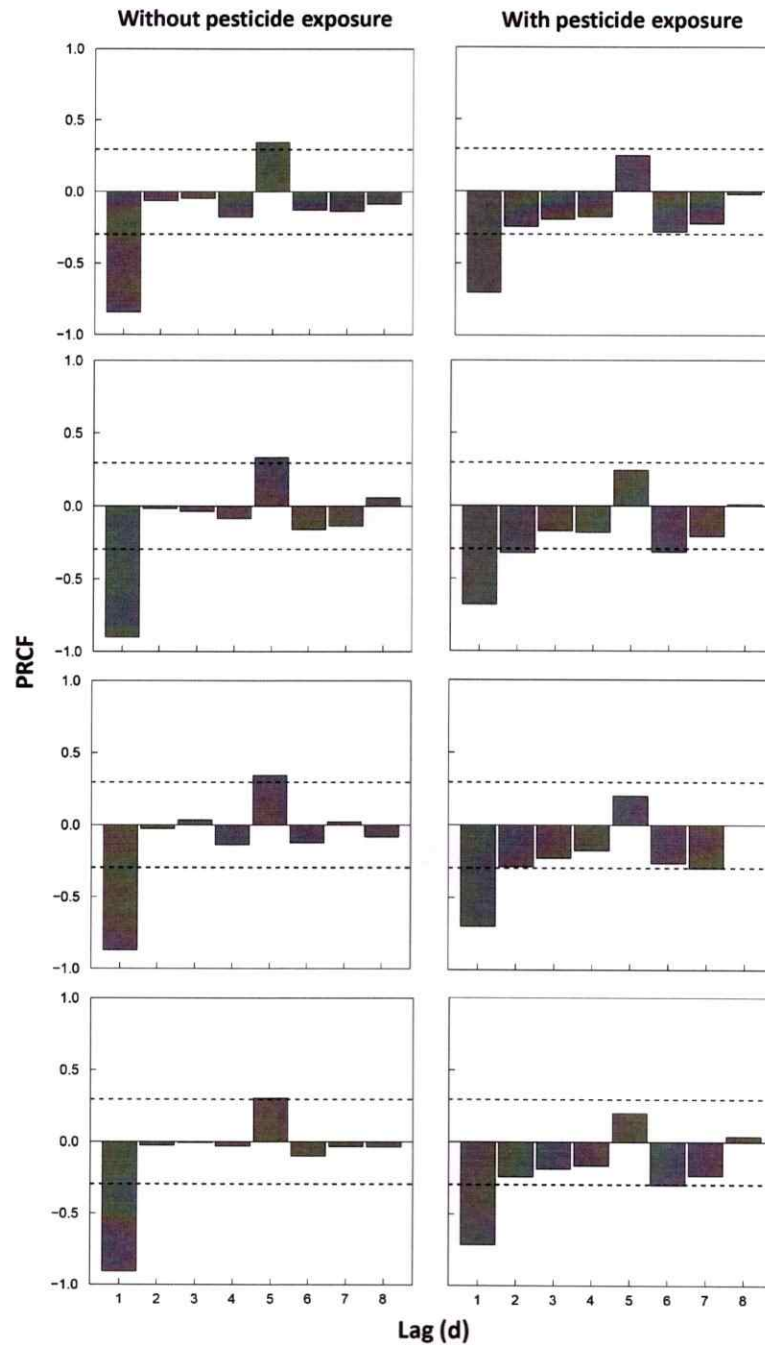
Fig. S4A. Partial rate correlation functions for time series in unexposed populations (left panel) and exposed populations (Right panel) of *D. ambigua* subjected to **selective predation on newborns**. Each plot represents one replicate within treatments.
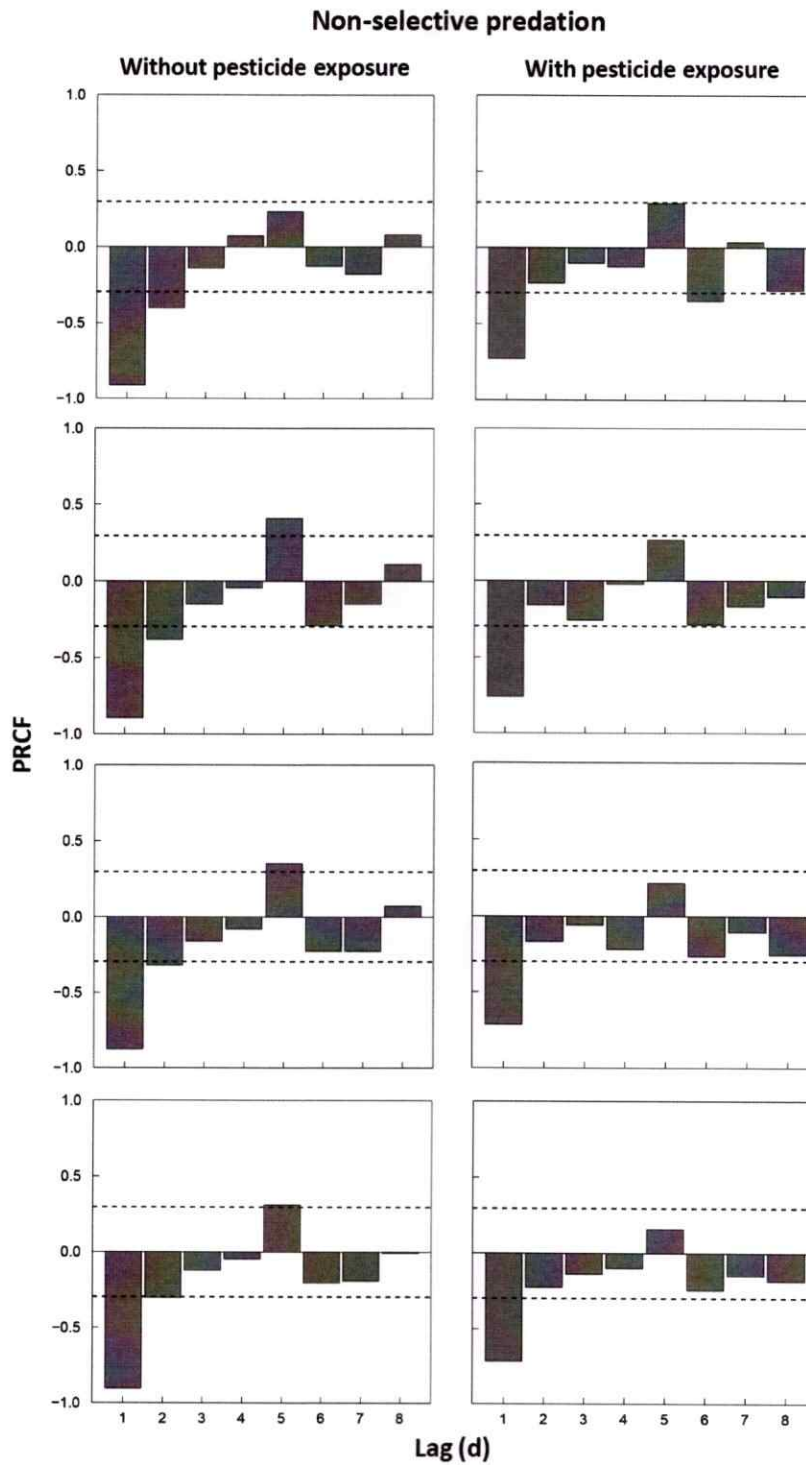
Fig. S4B. Partial rate correlation functions for time series in unexposed populations (left panel) and exposed populations (Right panel) of *D. ambigua* subjected to **non-selective predation**. Each plot represents one replicate within treatments.
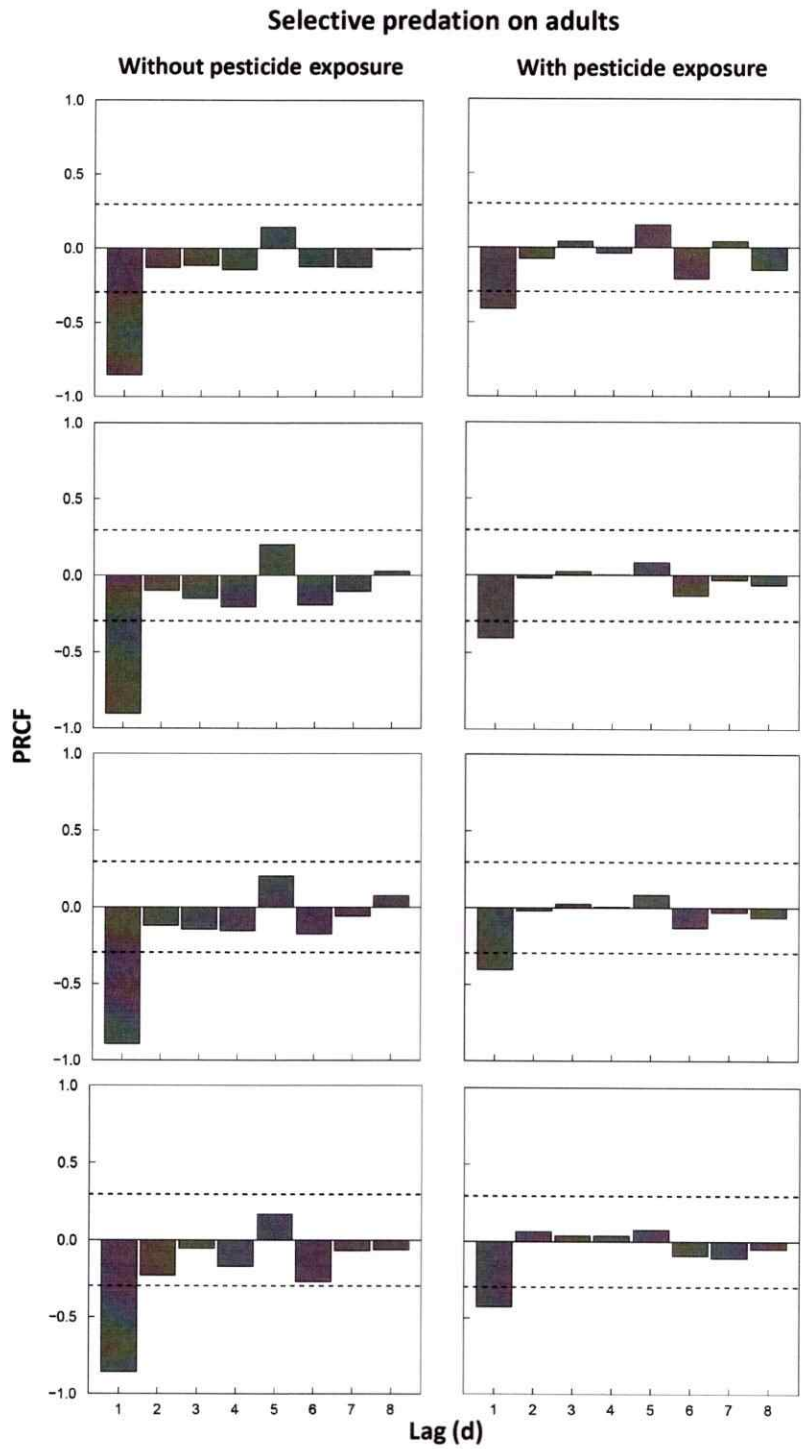
Fig. S4C. Partial rate correlation functions for time series in unexposed populations (left panel) and exposed populations (Right panel) of *D. ambigua* subjected to **selective predation on adults**. Each plot represents one replicate within treatments.
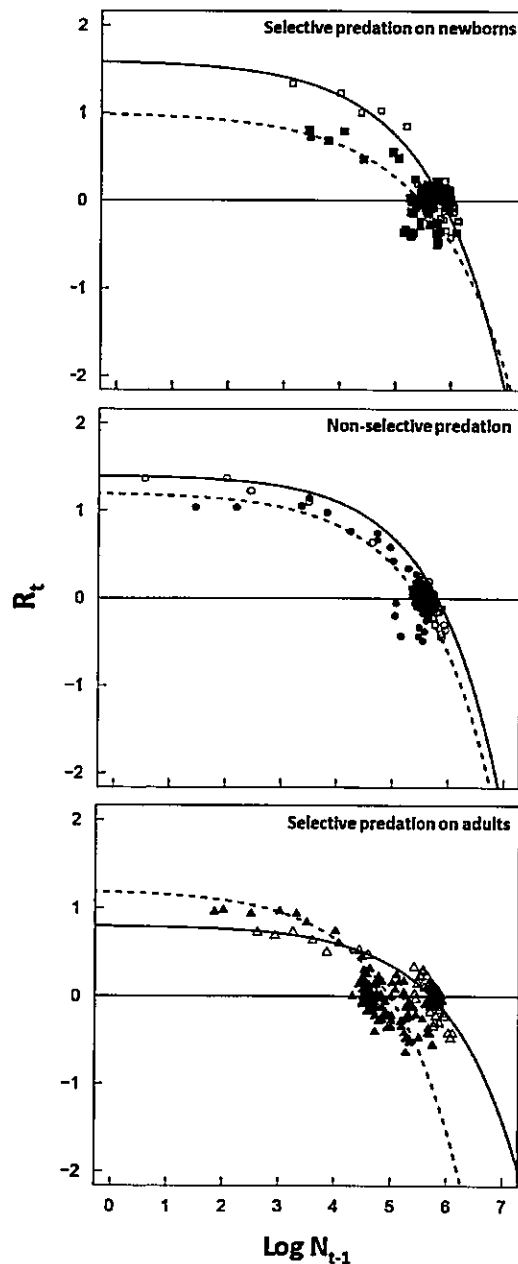
Fig.S4D. Estimated reproductive function (R-function) of *D. ambigua* in phase 3 subjected selective predation on newborns (upper panel), non-selective predation (middle panel) and selective predation on adults (lower panel). Unexposed (white symbols and solid fitted lines) and exposed (black symbols and dashed fitted lines) populations for each predation treatment are shown. The X-axis represents the logarithm of abundance (N) at time t-1 and the Y-axis the realized per capita growth rate at time t.