SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 34, No. 8, pp. 1751–1759, 2015
© 2015 SETAC
Printed in the USA

SPECIES INTERACTIONS AND CHEMICAL STRESS: COMBINED EFFECTS OF INTRASPECIFIC AND INTERSPECIFIC INTERACTIONS AND PYRENE ON *DAPHNIA MAGNA* POPULATION DYNAMICS

Karel P. J. Viaene,*† Frederik De Laender,‡ Andreu Rico,‖ Paul J. Van den Brink,§‖ Antonio Di Guardo,# Melissa Morselli,# and Colin R. Janssen†

†Laboratory of Environmental Toxicology and Aquatic Ecology, Environmental Toxicology Unit (GhEnToxLab), Ghent University, Ghent, Belgium
‡Research Unit in Environmental and Evolutionary Ecology, Namur University, Namur, Belgium
§Department of Aquatic Ecology and Water Quality Management, Wageningen University, Wageningen, The Netherlands
‖Alterra, Wageningen University and Research Centre, Wageningen, The Netherlands
#Department of Science and High Technology, University of Insubria, Como, Italy

(Submitted 22 December 2014; Returned for Revision 2 February 2015; Accepted 4 March 2015)

Abstract: Species interactions are often suggested as an important factor when assessing the effects of chemicals on higher levels of biological organization. Nevertheless, the contribution of intraspecific and interspecific interactions to chemical effects on populations is often overlooked. In the present study, *Daphnia magna* populations were initiated with different levels of intraspecific competition, interspecific competition, and predation and exposed to pyrene pulses. Generalized linear models were used to test which of these factors significantly explained population size and structure at different time points. Pyrene had a negative effect on total population densities, with effects being more pronounced on smaller *D. magna* individuals. Among all species interactions tested, predation had the largest negative effect on population densities. Predation and high initial intraspecific competition were shown to interact antagonistically with pyrene exposure. This was attributed to differences in population structure before pyrene exposure and pyrene-induced reductions in predation pressure by *Chaoborus* sp. larvae. The present study provides empirical evidence that species interactions within and between populations can alter the response of aquatic populations to chemical exposure. Therefore, such interactions are important factors to be considered in ecological risk assessments. *Environ Toxicol Chem* 2015;34:1751–1759. © 2015 SETAC

Keywords: Pyrene Species interactions Competition Predation *Daphnia magna*

INTRODUCTION

Current procedures for the ecological risk assessment of chemicals are generally based on the extrapolation of individual-level responses to the whole ecosystem and often fail to integrate a sufficient level of ecological realism [1,2]. In ecosystems, individuals exposed to a chemical are rarely isolated but interact with individuals of the same or of another species. Despite being one of the key characteristics of ecosystems, interactions within and between species are rarely included in current prospective ecological risk assessments, especially for nonpesticidal chemicals [3]. However, species interactions can alter the direct effects of a chemical on a sensitive species, such as increased mortality after pesticide addition because of decreased predator avoidance behavior [4]. Alternatively, by interacting with sensitive species, tolerant species also can be affected, leading to indirect effects of chemical stress, such as starvation of the consumer species when the prey species is affected [5,6] or reduced competition with the affected species [6]. The indirect effects of a chemical are often overlooked but can be as large as or even larger than the direct effects of the chemical [7]. Interactions with other species can either increase or decrease the susceptibility of populations and communities to a chemical [7,8]. For example, the no-observed-effect concentration of prometryn for ciliates was more than 2 orders of magnitude lower in microcosms compared with a single-species toxicity test because of the sensitivity of their food source to prometryn [9]. Also, elimination of grazers by the fungicide carbendazim allowed certain phytoplankton species to increase in abundance [10], and exposure to insecticides resulted in the development of antipredator structures in daphnids, potentially reducing the effect of predation [4]. Accurately assessing species interactions is thus essential to perform ecologically realistic chemical risk assessments [11].

Competition and predation are regarded as the most important species interactions when considering indirect effects of chemicals [8]. Competition can occur between individuals of different species (interspecific competition) but also within 1 population of the same species (intraspecific competition). Although several studies exist on the combined effects of interspecific competition and chemicals [12,13], studies on how intraspecific competition affects the response of populations to chemical exposure are rather underrepresented in the ecotoxicological literature.

The objective of the present study was to investigate how initial differences in species interactions influence the response of aquatic invertebrate populations to chemical stress. To this end, *Daphnia magna* populations were initiated with different levels of intraspecific and interspecific competition and predation. After 7 d and 15 d, pyrene was added as a chemical stressor. Population size and structure of *D. magna* were evaluated using generalized linear models. Higher effects of pyrene were expected in populations that are experiencing

All Supplemental Data may be found in the online version of this article.
* Address correspondence to karel.viaene@ugent.be.
Published online 13 March 2015 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2973

increased competition or predation pressure compared with a control population.

MATERIALS AND METHODS

Experimental design

Daphnia magna populations were exposed to 6 levels of species interactions (i.e., species interaction control, low and high intraspecific competition, low and high interspecific competition, and predation) and to 5 different pyrene exposure profiles (i.e., control, solvent control, and low, medium, and high exposure; see Table 1). The experiment was performed in triplicate ($n = 3$). Two additional replicates were added for the species interaction control treatment without pyrene exposure ($n = 5$). The experiment was carried out in 94 glass vessels (1.5 L) filled with 0.5 L of freshwater RT medium [14]. The test vessels were randomly distributed within a water bath placed in a temperature-controlled room (20.8 ± 1 °C) and exposed to low artificial light conditions (1000–1500 lux). The experiment lasted for 29 d, with an adaptation period of 7 d (day 7 until day 0). Pyrene was added twice, on day 0 and day 8. After the second pyrene addition, population densities were monitored for another 14 d until day 22. The *D. magna* organisms used in the experiment were obtained from the laboratory culture of the department of Aquatic Ecology and Water Quality Management from Wageningen University (The Netherlands). *Scenedesmus obliquus* was used as a food source for the *D. magna* cultures before the experiment and throughout the course of the experiment. Test vessels were fed 6 times per week with *S. obliquus* (1 mg carbon L^{-1} d^{-1}). The rotifer *Brachionus calyciflorus*, which also feeds on *S. obliquus,* is expected to compete with *D. magna* for food and was used to simulate interspecific competition. *Brachionus calyciflorus* cysts were obtained from MicroBioTest (Mariakerke, Belgium), and a stock culture was set up in RT medium at 20 °C. *Chaoborus* sp. larvae, which were added to simulate predation, were collected from unpolluted mesocosms at Sinderhoeve research station (Renkum, The Netherlands).

Identical *D. magna* population structures were introduced in all test vessels. They were composed of 20% adults, 40% juveniles, and 40% neonates. The classification of *D. magna* organisms within these 3 groups was based on size and was performed by filtering the culture medium through sieves with different mesh sizes (i.e., adults > 800 μm; juveniles between 800 μm and 500 μm; and neonates < 500 μm) [15]. The neonate classification typically corresponded with individuals younger than 48 h. By using populations composed of different life stages, we wanted to simulate realistic population structures and study the sensitivity of different life stages and its implications for *D. magna* population dynamics. To study the effect of intraspecific competition on *D. magna* populations, initial densities of 10 (species interaction control), 20 (low intraspecific competition), and 40 (high intraspecific competition) *D. magna* individuals per test vessel were used. To study how interspecific competition affected the *D. magna* population, *B. calyciflorus* was added to the test vessels at the start of the experiment in densities of approximately 333 rotifers per $vessel^{-1}$ (low interspecific competition) and 999 rotifers per $vessel^{-1}$ (high interspecific competition). Predation was imposed by the addition of 1 *Chaoborus* sp. larva per test vessel. *Chaoborus* sp. larvae were added 3 d after the addition of daphnids to the test vessels to allow the daphnids to acclimatize. When a *Chaoborus* sp. larva died during the experiment, it was replaced to assure continuous predation pressure.

Table 1. Overview of the different species interactions tested[a]

Treatment	No. of *Daphnia magna*	No. of *Brachionus calyciflorus*	No. of *Chaoborus* sp. larvae
Control	10	0	0
Intraspecific competition: low	20	0	0
Intraspecific competition: high	40	0	0
Interspecific competition: low	10	333	0
Interspecific competition: high	10	999	0
Predation	10	0	1

[a]The columns indicate how many individuals of each species were added to the test vessels for the different species interaction treatments. Each of these treatments was exposed to 5 different pyrene exposure profiles: no pyrene; solvent control; and low, medium, and high pyrene exposure.

Pyrene is a polycyclic aromatic hydrocarbon consisting of 4 benzene rings. Pyrene was chosen as model compound for this experiment because of its nonspecific, narcotic mode of action [16]. Phototoxicity of pyrene has been reported [17], and experiments were therefore performed under low light conditions (1000–1500 lux). Acetonitrile was used as solvent for pyrene and, therefore, a solvent control was included in the experimental design (38 μg L^{-1} and 75 μg L^{-1} added for the first and second additions, respectively). A stock solution of 0.75 g L^{-1} pyrene was prepared in acetonitrile and stirred intensively before addition to the test vessels. Pyrene was applied twice to the test vessels. The first dosing was applied 7 d after the start of the experiment (day 0) at a nominal concentration of 7.5 μg L^{-1}, 20 μg L^{-1}, and 55 μg L^{-1} for the low, medium, and high pyrene exposure profile, respectively. Pyrene concentrations were chosen between the 10% and 50% effective concentration (EC10 and EC50, respectively) values for immobilization. An $EC50_{immobilization}$ value of 68 μg L^{-1} (44–106 μg L^{-1}) was estimated based on a 48-h toxicity test with *D. magna* [18] (See Supplemental Data, Figure S1 for the concentration–response curve). Using a similar protocol, no mortality effects were observed for *B. calyciflorus* and the *Chaoborus* sp. larvae at pyrene concentrations up to 150 μg L^{-1}. Because the first pyrene addition had no observable effects on population densities, pyrene was added a second time at higher concentrations. The second application was performed 15 d after the start of the experiment (day 8) with a nominal pyrene concentration of 15 μg L^{-1}, 40 μg L^{-1}, and 110 μg L^{-1}, corresponding to the low, medium, and high pyrene exposure profiles, respectively.

Biological monitoring

Daphnia magna and *B. calyciflorus* abundances in the test vessels were monitored on day-4, day 0, day 2, day 4, day 7, day 10, day 15, and day 22 after the start of the experiment. *Daphnia magna* were counted and divided into the size classes adult, juvenile, and neonate by filtering the test medium over sieves with mesh sizes of 800 μm, 500 μm, and 200 μm, respectively. *Brachionus calyciflorus* abundances in the test medium of the interspecific treatments were monitored by taking two 6-mL subsamples per test vessel and counting swimming rotifers by using an inverted microscope (magnification 10×).

Chemical analyses

Samples for pyrene analysis were taken after the first pyrene application, before the second pyrene application and 2 d, 4 d, and 12 d after the second pyrene application. Pyrene samples were stored in the dark at –20 °C in glass tubes. The chemical

analysis was performed with gas chromatography–mass spectrometry (Trace GC 2000 series; Thermoquest, DSQ, Finnigan/Thermoquest). An apolar Zebron ZB 5-ms column (Phenomenex) was used for the analysis, and extraction and elution were performed by solid-phase extraction according to the manufacturer's instructions (Waters and Phenomenex). An internal standard (fluoranthene-d_{10}) at a concentration of 10 μg L^{-1} to 50 μg L^{-1} (depending on expected pyrene concentration) was used to control and correct for extraction losses. The method's recovery was always greater than 75%. Immediately before injection of the sample, a recovery standard was also applied to control for the injection itself.

Fate model analysis

The recently developed dynamic water–sediment organism model EcoDyna [19] was used to predict the temporal fate of pyrene during the experiments. The model was calibrated using the nominal water volume (500 mL) of the experiment, and the water–sediment interaction was minimized to simulate negligible exchange, given the lack of a sediment phase in the vessels used. To calculate potential algal uptake, a daily contribution of 1 mg carbon L^{-1} was assumed, and organism biomass was calculated by using length–weight relationships [20,21]. Physical–chemical properties for pyrene were obtained from literature [22].

Statistical analyses

All analyses were performed using the statistical software package R (Ver 3.1.1 [23]). For each sampling time, generalized linear models were constructed. Total, adult, juvenile, and neonate *D. magna* abundances were considered as response variables, allowing for the examination of population structure. The effect of intraspecific competition (control, low, high), interspecific competition (control, low, high), and predation (nonpredation and predation) was assessed by constructing a generalized linear model with the respective species interaction, pyrene exposure (control, low, medium, high), and their interaction as predictor variables.

The generalized linear models were initially constructed assuming a Poisson distribution [24], but this led to unsatisfactory model validation. We therefore opted to perform generalized linear model analyses with a normal distribution on the log10-transformed *D. magna* abundance data. The solvent control treatment was not included in the generalized linear model analysis, because preliminary tests showed no significant differences between the control and the solvent control treatments. Backwards model selection was used, dropping predictor variables based on the Akaike's Information Criterion, hypothesis testing, and model validation analysis [24]. As model validation analysis, we investigated whether patterns in the data were present using predicted versus observed plots, investigated whether patterns in the residuals were present using predictor versus residuals plots, and tested the normality of the residuals using quantile-quantile plots [24].

RESULTS

Pyrene concentrations

Measured pyrene concentrations in water were lower than expected from the nominal values (Table 2). Nevertheless, a clear difference was seen between the 3 pyrene exposure profiles at any given point in time. The EcoDyna model was used to simulate pyrene concentration variations in water. The model was run to fit actual water concentrations and the importance of the main fluxes dominating the change in concentration with time after the spikes. As a result of the fitting procedure, a chemical half-life in water of 30 h was necessary to reproduce the observed concentrations (no distinction could be made between biotic and abiotic processes), whereas volatilization accounted for approximately 20% of losses. Simulations confirmed that pyrene uptake in algae and animal biomass was negligible.

Statistical analyses

We included only the results for the total *D. magna* abundance in the present study; results for the size classes are included in the Supplemental Data. Independent of the explanatory variables, a clear trend in the model intercept value could be observed. An increase in the intercept was seen until day 7; afterwards, the intercept slowly decreased (Tables 3–5). The intercept is the value estimated by the generalized linear models without any effect of the predictor variables (pyrene and species interactions) and thus reflects the population dynamics of *D. magna* without stress. The initial increase and then the decline of the intercept indicated that the population was growing until the carrying capacity was reached (Figure 1).

Effects of pyrene

The estimated direct effects of pyrene were almost identical between the different treatments of species interactions (Tables 3–5). We therefore only refer to Table 3 in this section of the present study. The first pyrene addition did not significantly affect *D. magna* population densities (Figure 1 and Table 3). However, the highest pyrene exposure did reduce total population densities 7 d after the second pyrene addition (day 15). The description and discussion of the experiment results therefore focus on the observed effects after the second pyrene addition. Effects of the medium and low pyrene exposure profiles on total *D. magna* abundance were absent or negligible (<0.13; Table 3). The variance of the total population densities explained by pyrene exposure at day 15 was greater than 45% (Supplemental Data Tables 1–3). Fourteen days after the second pyrene addition, *D. magna* populations were recovering (day

Table 2. Measured pyrene concentrations for the 3 pyrene exposure profiles[a]

Exposure	Day 0	Day 7	Day 8	Day 10	Day 14	Day 22
Low pyrene (μg/L)	5.2 ± 0.8 (7.5)	0.2 ± 0.1	10.6 ± 1.4 (15)	3.9 ± 1	0.6 ± 0.2	0.3 ± 0.2
Medium pyrene (μg/L)	13 ± 2.2 (20)	0.6 ± 0.3	22.9 ± 4 (40)	12.8 ± 2.1	3.2 ± 0.5	0.3 ± 0.2
High pyrene (μg/L)	38.6 ± 11.9 (55)	2.3 ± 0.5	62.8 ± 26.6 (110)	44.5 ± 16.9	13 ± 2.5	1.5 ± 1.3

[a]Measured pyrene concentrations are shown with standard deviations. Nominal concentrations are shown in parentheses when pyrene was added (day 0 and day 8).

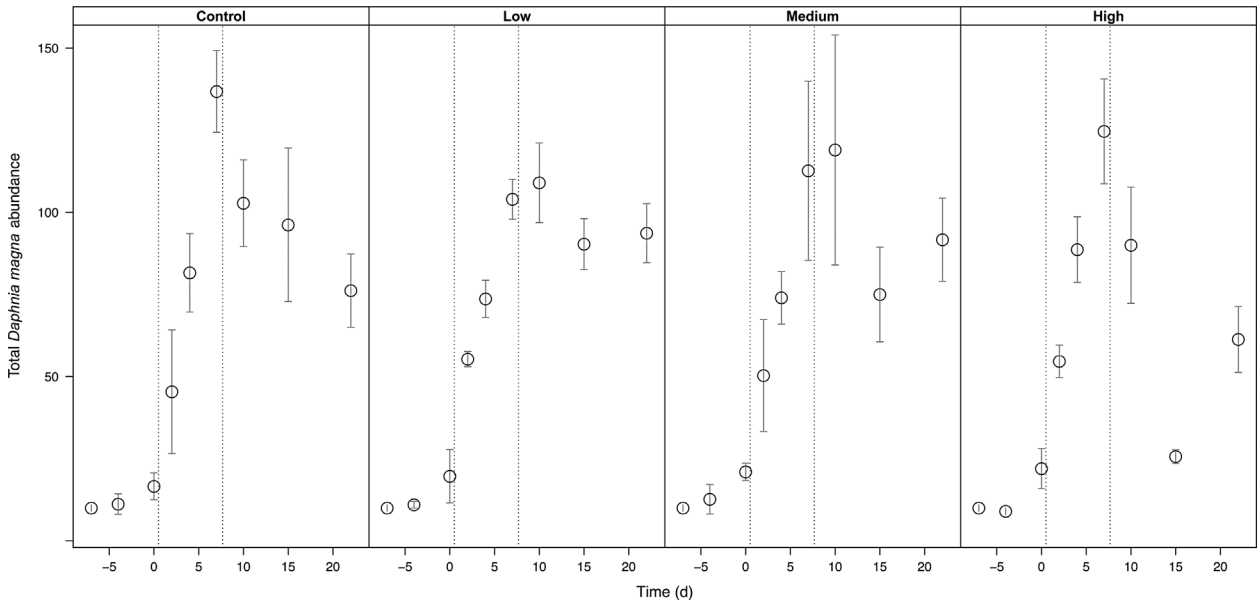

Figure 1. Total *Daphnia magna* abundances over time for 4 pyrene exposure profiles: control, low, medium, and high pyrene exposure. Data shown are the *D. magna* population densities with no additional species interactions. Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene applications.

Table 3. Generalized linear model estimates of pyrene exposure and intraspecific competition for log10-transformed total *Daphnia magna* abundance after backwards model selection[a]

	Day –4	Day 0	Day 2	Day 4	Day 7	Day 10	Day 15	Day 22
(Intercept)	1.03	1.18	1.64	1.91	2.13	2.03	1.97	1.88
Low pyrene	/	/	/	/	–0.12	/	/	0.10
Medium pyrene	/	0.19	/	/	–0.09	/	/	/
High pyrene	/	/	/	/	/	–0.07	–0.56	/
Low intraspecific	0.26	0.22	/	–0.12	–0.17	–0.09	/	–0.07
High intraspecific	0.50	0.39	0.12	–0.11	–0.11	–0.13	/	–0.11
Low pyrene × low intraspecific	/	/	/	/	0.17	/	/	/
Medium pyrene × high intraspecific	/	/	/	0.18	/	/	/	/
High pyrene × high intraspecific	/	/	/	/	/	/	0.30	/

[a]For each time point, the significant estimates of explanatory variables and their interactions are shown. Positive and negative values indicate a higher or lower abundance than the control treatment, respectively. Nonsignificant predictor variables are not shown (if never significant) or are indicated with a "/."

22): no differences in total population densities were observed between pyrene exposure profiles. However, at that time, the abundances of neonates were higher in the high pyrene exposure profile compared with the control treatment (Figure 2; Supplemental Data, Table S6). Also, the negative effect of high pyrene exposure on the abundances of adults persisted on day 22, although this effect was smaller compared with day 15 (Supplemental Data, Table S4). Although the total population densities had recovered, differences in population structure thus were observed between pyrene treatments (Figures 1 and 2).

Effect of competition and predation

During the first 9 d of the experiment, the populations with a high initial population density of 40 individuals (and therefore a higher degree of intraspecific competition) remained more abundant, but this effect decreased with time (Figure 3 and Table 3). For the populations with an initial population density of 20 individuals, this effect only persisted during the first 7 d. The variance explained by intraspecific competition also decreased from 71% to 22% over this period (Supplemental Data, Table S1). A high initial density resulted in lower future population densities (starting from day 4), although this effect was limited (Table 3). The population with the lowest initial density (10 *Daphnia* per test vessel) reached the highest total *D. magna* abundance (135 individuals). The initial positive effect of a high initial density persisted longer for adult *D. magna* (until day 10; Supplemental Data Table 4) compared with the other size classes (day 2 and day 4 for juveniles and neonates, respectively; Supplemental Data, Tables S5 and S6). High initial densities resulted in a higher and more constant abundance of adults in the second half of the experiment compared with low initial densities (Figure 2).

Brachionus calyciflorus population densities decreased sharply after 1 wk, and *B. calyciflorus* completely disappeared by day 10 (Supplemental Data, Figure S14). Although *B. calyciflorus* disappeared, significant but limited differences were observed between population densities of *D. magna* of the different interspecific competition treatments starting from day 4 until day 15 (Figure 4 and Table 4). At the end of the experimental period, differences in population density were no longer observed between the different degrees of interspecific competition. Abundances of adult *D. magna* were never negatively affected by interspecific competition during the whole experiment (Supplemental Data Table 7), whereas

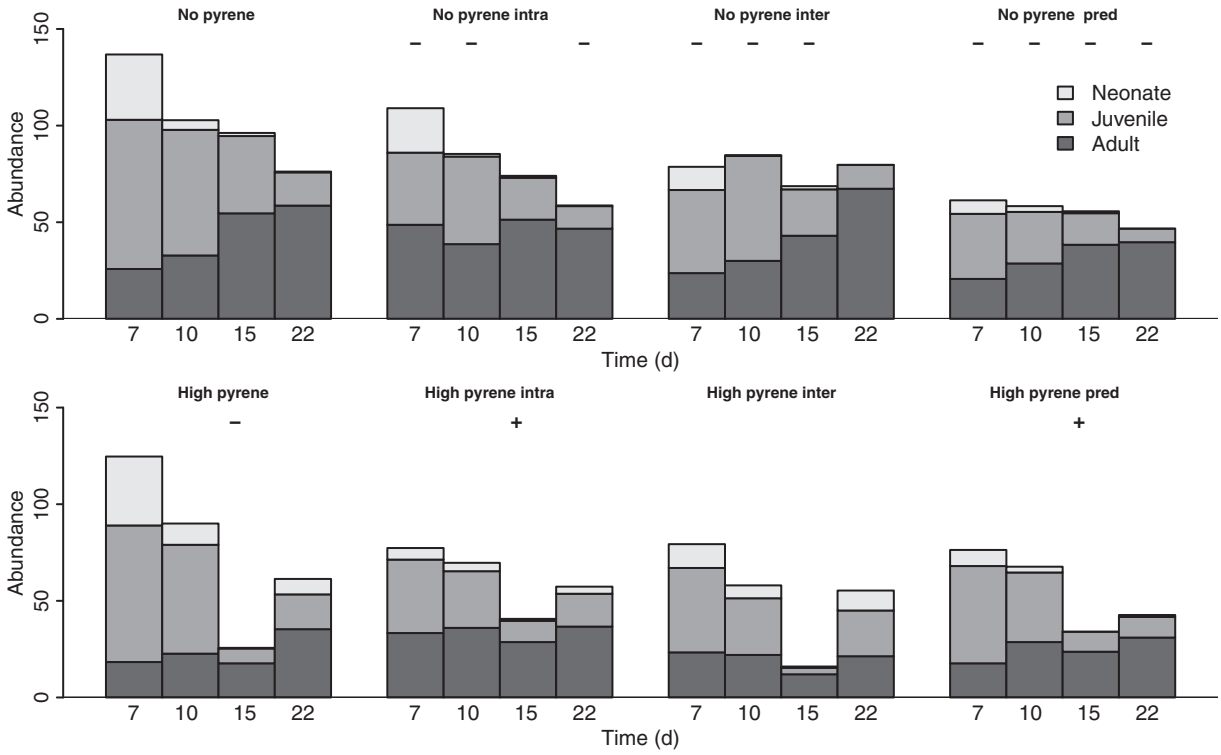

Figure 2. Average population structure of *Daphnia magna* just before and after the second pyrene application (day 8) for different treatments. Data shown are the average abundances of adults (dark gray), juveniles (medium gray), and neonates (light gray) of the specific treatments. The "−" and "+" symbols indicate a significant negative and positive effects, respectively, of that treatment or combination of treatments on total population density, compared with the control treatment ("no pyrene"). High pyrene = high pyrene exposure; intra = high intraspecific competition treatment; inter = high interspecific competition treatment.

abundances of juvenile and neonate individuals were (Supplemental Data, Tables S8 and S9). The effect of interspecific competition on the abundance of neonates was only significant up to day 10, because almost no neonates were observed in either of the pyrene exposure treatments the following sample days.

Of all species interactions studied, predation had the largest negative effect on population densities (Figure 2, Figure 5, and Table 5). Predation had a continuous negative effect on total *D. magna* abundance. The explained variance was always higher than 42%, except on day 15, when most variance was explained by pyrene exposure (Table 5). Because *Chaoborus* sp. larvae

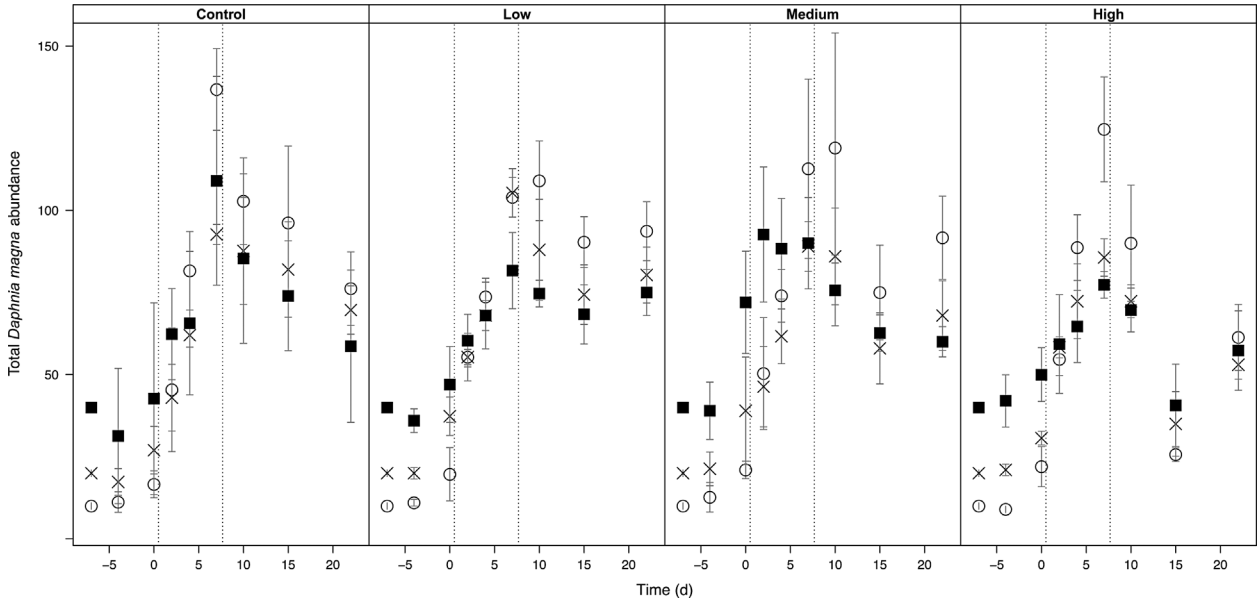

Figure 3. Total *Daphnia magna* abundance over time for 4 pyrene exposure profiles: control, low, medium, and high pyrene exposure. Data shown are the *D. magna* population densities for the treatment with no additional species interactions (circles), low intraspecific competition (crosses), and high intraspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene applications.

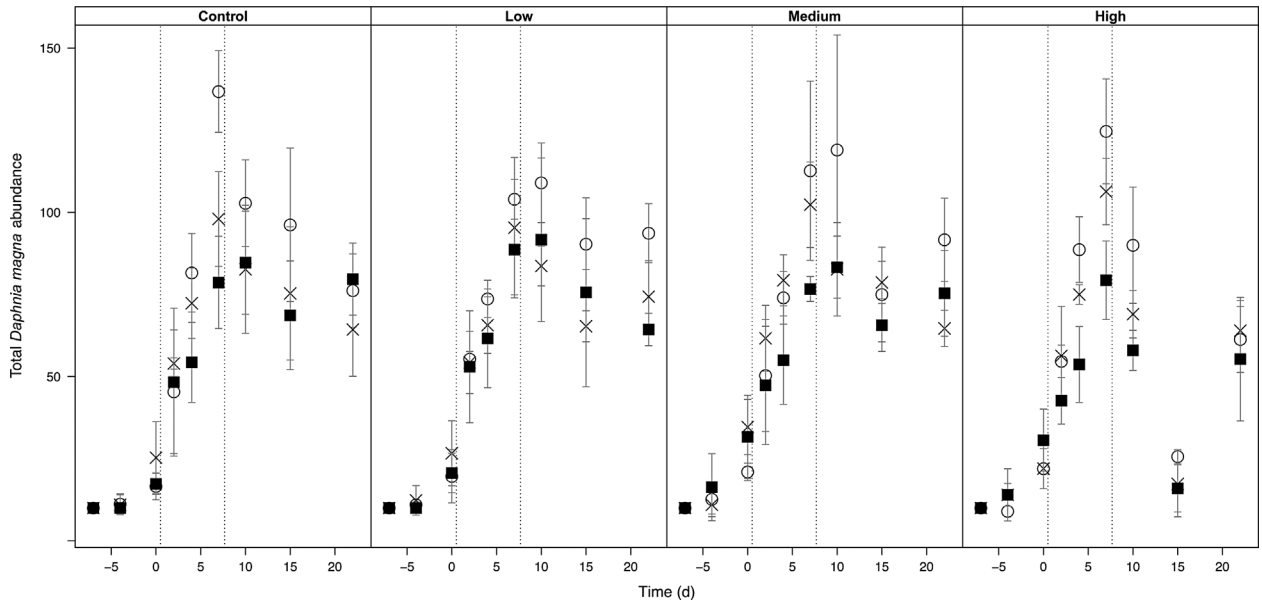

Figure 4. Total *Daphnia magna* abundance over time for 4 pyrene exposure profiles: control, low, medium, and high pyrene exposure. Data shown are the *D. magna* population densities for the treatment with no additional species interactions (circles), low interspecific competition (crosses), and high interspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene applications.

Table 4. Generalized linear model estimates of pyrene exposure and interspecific competition for log10-transformed total *Daphnia magna* abundance after backwards model selection[a]

	Day –4	Day 0	Day 2	Day 4	Day 7	Day 10	Day 15	Day 22
(Intercept)	1.06	1.20	1.70	1.90	2.13	2.02	1.97	1.88
Low pyrene	/	/	/	/	–0.12	/	/	/
Medium pyrene	/	0.17	/	/	/	/	/	/
High pyrene	/	/	/	/	/	–0.09	–0.63	/
Low interspecific	/	0.13	/	/	–0.15	–0.12	–0.11	/
High interspecific	/	/	/	–0.16	–0.24	–0.12	–0.13	/
Low pyrene × high interspecific	/	/	/	/	0.17	/	/	–0.18

[a]For each time point, the significant estimates of explanatory variables and their interactions are shown. Positive and negative values indicate a higher or lower abundance than the control treatment, respectively. Nonsignificant predictor variables are not shown (if never significant) or are indicated with a "/."

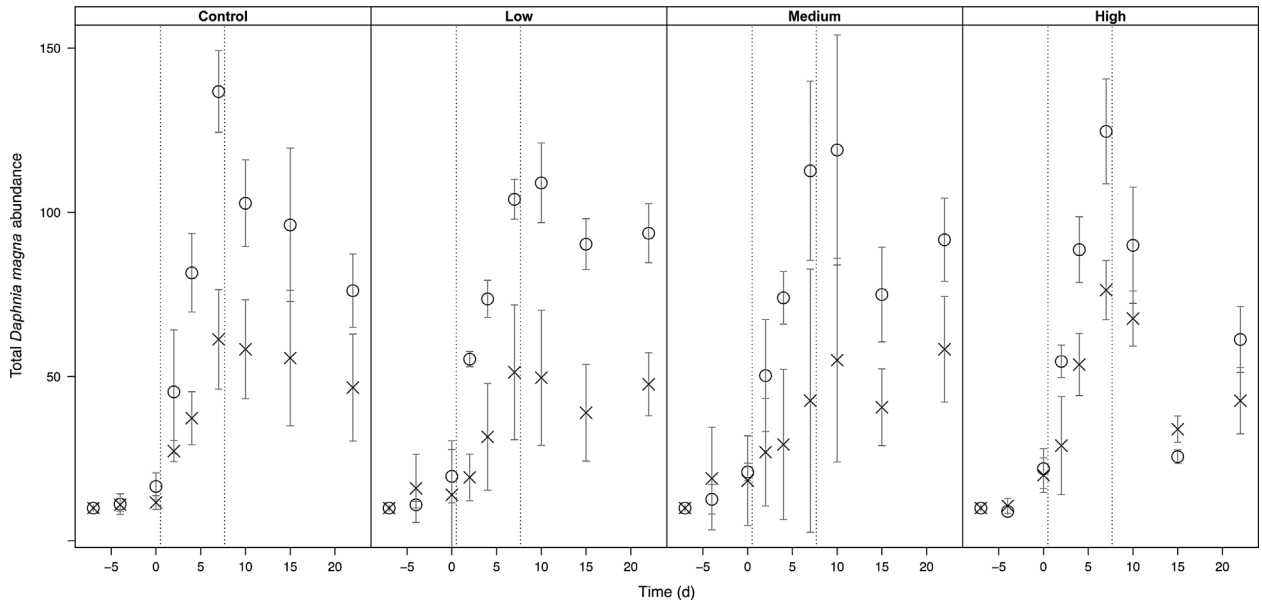

Figure 5. Total *Daphnia magna* abundance over time for 4 pyrene exposure profiles: control, low, medium, and high pyrene exposure. Data shown are the *D. magna* population densities for the treatment without (circles) and with predation (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene applications.

Table 5. Generalized linear model estimates of pyrene exposure and predation for log10-transformed total *Daphnia magna* abundance after backwards model selection[a]

	Day –4	Day 0	Day 2	Day 4	Day 7	Day 10	Day 15	Day 22
(Intercept)	1.07	1.27	1.69	1.90	2.08	2.01	1.97	1.88
High pyrene	/	/	/	/	/	/	–0.56	/
Predation	/	/	–0.32	–0.40	–0.38	–0.28	–0.25	–0.22
High pyrene × predation	/	/	/	/	/	/	0.37	/

[a]For each time point, the significant estimates of explanatory variables and their interactions are shown. Positive and negative values indicate a higher or lower abundance than the control treatment, respectively. Nonsignificant predictor variables are not shown (if never significant) or are indicated with a "/."

were added 3 d after the start of the experiment, predation was not significant at day 4. A negative effect of predation was first observed for adults (at day 0), but the largest effects were observed for the abundances of neonates and juveniles (Figure 2; Supplemental Data, Tables S10–S12).

Combined effects of pyrene and species interactions

Significant interactions between pyrene and predation or between pyrene and competition were rare and most of the time changed inconsistently with increasing pyrene exposure (Tables 3–5). However, on day 15, the interaction between high pyrene exposure and predation and between high pyrene exposure and intraspecific competition positively affected the total *D. magna* abundance. These positive interactions indicated that the negative effect of high pyrene exposure was less pronounced when the population was already exposed to predation or had experienced high intraspecific competition at the start of the experiment, suggesting antagonism between each of these 2 types of species interaction and chemical toxicity. The variance of the total abundance explained by these 2 interactions on day 15 was 8.4% and 16.8%, respectively (Supplemental Data, Tables S1 and S3).

DISCUSSION

Pyrene toxicity

In the present study, short-term effects of pyrene were limited, and the highest effects occurred 7 d after the second pyrene addition (Tables 3–5). Why the first pyrene addition had no observable effects on population densities is unclear. The highest concentration measured after the first pyrene addition (71 μg/L) was similar to the $EC50_{immobilization}$ (68 μg/L), determined in the toxicity test performed on neonate *D. magna* (<500 μm) from the same clone. However, even neonate *D. magna*—often considered the most sensitive individuals [25]—were not affected by the first pyrene addition (Supplemental Data Figure 10 and Supplemental Data Table 6). The results of the pyrene toxicity test thus did not seem applicable to the present experiment.

Whether the negative effects of pyrene on the abundances of adults after the second pyrene addition resulted from direct mortality or from a combination of direct mortality and reduced survival or growth of smaller life classes is unclear. Reduced survival and growth of earlier life stages will reduce the number of juveniles that reach the adult stage [26]. The negative effect of pyrene was largest on abundances of juveniles (Supplemental Data, Tables S4–S6). Adult *D. magna* were the only size class still affected by pyrene at the end of the experiment (Supplemental Data, Table S4). Probably, the negative effect of pyrene on the abundances of adults was thus at least partly attributable to effects on earlier life stages. Neonates were almost absent after the second pyrene addition, even in the control treatment (Supplemental Data, Figure S10), which explains the absence of significant pyrene effects for neonates. A low abundance of small individuals is not uncommon in similar experiments with *Daphnia* [15] and can be attributed to the strong competition, which reduces the energy available for reproduction.

Interestingly, abundances of neonates were significantly higher on day 22 in the high pyrene exposure profile compared with the control treatment (Figure 2 and Supplemental Data Table 6). As a result, total population densities were not significantly different between the different pyrene treatments at the end of the experiment (Table 3), leading to the conclusion that total population density was recovered. However, the final *D. magna* populations in the high pyrene exposure profile, consisting mainly of neonates, were probably more susceptible to new chemical stress events compared with those in the other pyrene treatments. This illustrates that population structure needs to be accounted for when assessing a population's response to and recovery from (chemical) stress [13].

Species interactions

Of all species interactions studied, predation had the largest effect on total *D. magna* abundance. Effects were visible 6 d after the addition of *Chaoborus* sp. larvae (starting from day 2), and the highest effects were observed for the abundances of juveniles and neonates (Figure 2; Supplemental Data, Tables S10–S12). This indicated a feeding preference: *Chaoborus* sp. larvae preferred to prey on smaller juvenile and neonate *D. magna* rather than on adult *D. magna*. Size-selective feeding by *Chaoborus* sp. larvae has been observed before [27]. Surprisingly, however, a significant negative effect of predation was first observed for adults (on day 0) and not for juveniles or neonates. Abundances of juveniles and neonates were very low (juveniles) or zero (neonates) until day 0, so probably the *Chaoborus* larvae were forced to feed on the larger *D. magna* adults. At later time points, neonates and juveniles were more abundant, and *Chaoborus* larvae fed on these size classes, leading to a reduced or absent effect of predation on adults. These data show that feeding preferences depend on the ecological context shaped by the prey's population structure.

Assessing the effects of interspecific competition for the full duration of the experiment is difficult, because *B. calyciflorus* were reduced to low densities (<10%) after day 7 and completely disappeared after day 10 (Supplemental Data Figure 14). The effects of interspecific competition on total population densities were therefore limited (Table 3). Posterior tests performed with the same conditions showed that even in the highest pyrene concentration, *B. calyciflorus* was able to survive for at least 24 d (Supplemental Data Figure 15). The rotifers were thus outcompeted by *D. magna*, as previously observed in interaction experiments between *B. calyciflorus* and *D. pulex* without chemical stress [28]. Gilbert [28] observed limited to no

effects of the competition with *B. calyciflorus* on population densities of *D. pulex*, similar to the results of the present study. Continuous competition pressure from *B. calyciflorus,* however, could lead to different results, and future efforts should focus on experimental designs promoting prolonged coexistence between these 2 zooplankton taxa.

Both intraspecific and interspecific competition seemed to result in effects on reproductive output. Negative effects of different initial densities on the abundances of neonates and juveniles were observed starting from day 2, whereas these were absent for adults (Supplemental Data, Tables S4–S6). Similarly, negative effects of interspecific competition were observed for juveniles and neonates from day 4 onward, whereas adults were not affected (Supplemental Data, Tables S7–S9). High initial competition thus mainly affected early life stages at later time points, suggesting competition-induced effects on *D. magna* reproduction over direct competition effects. According to the dynamic energy budget theory, the competition with *B. calyciflorus* or other *D. magna* individuals could reduce the amount of energy that could be allocated to reproduction, resulting in fewer offspring [29]. However, direct competition, through starvation, probably also contributed to the results. Young *D. magna* life stages are more prone to starvation compared with adults [15]. Under high competition conditions, less food is available per capita, possibly leading to starvation of smaller individuals and contributing to the lower proportion of young life stages in the population.

Reduced effect of pyrene when combined with predation and competition

On day 15, when the pyrene effect was largest, predation and intraspecific competition reduced the negative effect of pyrene on population densities (Figure 2). Contrary to the antagonism observed in the present study, species interactions often lead to greater effects of chemical stress. For example, the combination of predation by *Notonecta maculata* and exposure to nonylphenol led to loss of resilience in *Daphnia magna* populations, whereas individual stressors failed to affect population densities [30]. Synergistic effects of competition and chemical stress have been reported for *D. magna* [13] and other *Daphnia* species [31]. Next to synergistic effects, antagonistic effects have been reported as well. For example, exposure to predator kairomones led to antagonistic interactions with carbaryl exposure on reproduction of *Daphnia magna* [32]. This was attributed to larger-sized and thus more tolerant offspring when predation cues were present. Two mechanisms are proposed to explain the antagonism we observed in the present study: differences in population structure and pyrene-induced alterations in species interactions. First, the structure of the populations exposed to predation or to high intraspecific competition differed from that of the populations experiencing low intraspecific competition and populations not exposed to predation. On day 7, immediately before the second pyrene addition, a large negative effect of intraspecific competition and predation on the abundances of juveniles and neonates was observed although the abundances of adults were less affected (Figure 2; Supplemental Data, Tables S4–S6, S10–S12). Differences in sensitivity for different *D. magna* size classes have been shown before, for example, for 4 metals [33] or carbaryl [32]. We argue that because of the lower proportion of small and thus more sensitive life stages in populations with predation or high (initial) intraspecific competition, pyrene effects were smaller. Second, the feeding rate of *Chaoborus* sp. larvae was possibly inhibited by pyrene exposure, leading to reduced predation losses. Indeed, the estimated effect of predation (Table 5) was lower on day 15. The effect of pyrene on the feeding rates of *Chaoborus* sp. larvae was not tested in the present study, but chemicals have been shown to alter feeding behavior of fish [34] and invertebrates [35].

Contrary to a similar study with *D. magna* populations exposed to fenvalerate [26], we observed no prolonged dominance of smaller organisms after chemical stress in the treatment with predation. After high pyrene exposure, the proportion of small individuals was higher in the populations not exposed to predation (Figure 2). These contradicting results can be explained by how predation was applied in the 2 studies. Liess and Foit [26] simulated predation by removing individuals nonselective on size, but *Chaoborus* sp. larvae preferred to prey on smaller individuals, leading to lower abundances of neonates in the predation treatments at the end of the experiment. This highlights the complexity of assessing how ecological interactions alter the response of a population to chemical stress and the need for ecologically realistic tools [36,37].

The present study is an example of how species interactions can lead to a priori unpredictable effects of chemicals. Predation and intraspecific competition were shown to interact antagonistically with pyrene when the effect of pyrene was most pronounced. The present study also highlights the need to consider the effects of a chemical not only on population density but also on population structure when assessing the risk of chemicals for populations and communities. These types of studies, in combination with approaches such as mechanistic ecological models [1,38,39], could be used to integrate species interactions at the same time assessing the long-term ecological risk of a chemical. In addition, such models could help to infer what processes contribute most to the patterns observed in experimental studies.

SUPPLEMENTAL DATA

Tables S1–S12.
Figures S1–S15. (556 KB DOC).

Acknowledgment—The Chimera project is financed by the Long Range Initiative of CEFIC (project code: LRI-ECO19).

Disclaimer—The authors confirm that there are no known conflicts of interests associated with the present study.

Data availability—Raw data have been made publicly available on Researchgate (https://www.researchgate.net) by the main author (Karel P J Viaene).

REFERENCES

1. De Laender F, De Schamphelaere KAC, Vanrolleghem PA, Janssen CR. 2008. Validation of an ecosystem modelling approach as a tool for ecological effect assessments. *Chemosphere* 71:529–545.
2. Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Scientific Committee on Consumer Safety (SCCS). 2013. Addressing the new challenges for risk assessment. European Commission, Brussels, Belgium.
3. De Laender F, De Schamphelaere KAC, Vanrolleghem PA, Janssen CR. 2008. Do we have to incorporate ecological interactions in the sensitivity assessment of ecosystems? An examination of a theoretical assumption underlying species sensitivity distribution models. *Environ Int* 34:390–396.
4. Hanazato T. 2001. Pesticide effects on freshwater zooplankton: An ecological perspective. *Environ Pollut* 112:1–10.
5. De Hoop L, De Troch M, Hendriks a J, De Laender F. 2013. Modeling toxic stress by atrazine in a marine consumer-resource system. *Environ Toxicol Chem* 32:1088–1095.

6. Rohr JR, Crumrine PW. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecol Appl* 15:1135–1147.
7. Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* 317:207–233.
8. Preston BL. 2002. Indirect effects in aquatic ecotoxicology: Implications for ecological risk assessment. *Environ Manage* 29:311–323.
9. Liebig M, Schmidt G, Bontje D, Kooi BW, Streck G, Traunspurger W, Knacker T. 2008. Direct and indirect effects of pollutants on algae and algivorous ciliates in an aquatic indoor microcosm. *Aquat Toxicol* 88:102–110.
10. Van den Brink PJ, Hattink J, Bransen F, Donk E Van, Brock TCM. 2000. Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquat Toxicol* 48:251–264.
11. De Laender F, van den Brink PJ, Janssen CR, Di Guardo A. 2014. The ChimERA project: Coupling mechanistic exposure and effect models into an integrated platform for ecological risk assessment. *Environ Sci Pollut Res Int* 21:6263–6267.
12. Knillmann S, Stampfli NC, Noskov YA, Beketov MA, Liess M. 2012. Interspecific competition delays recovery of *Daphnia* spp. populations from pesticide stress. *Ecotoxicology* 21:1039–1049.
13. Foit K, Kaske O, Liess M. 2012. Competition increases toxicant sensitivity and delays the recovery of two interacting populations. *Aquat Toxicol* 106–107:25–31.
14. Tollrian R. 1993. Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: Morphological effects of *Chaoborus* kairomone concentration and their quantification. *J Plankton Res* 15:1309–1318.
15. Preuss TG, Hammers-Wirtz M, Hommen U, Rubach MN, Ratte HT. 2009. Development and validation of an individual based *Daphnia magna* population model: The influence of crowding on population dynamics. *Ecol Model* 220:310–329.
16. Di Toro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and Tissue. *Environ Toxicol Chem* 19:1951–1970.
17. Bellas J, Saco-Alvarez L, Nieto O, Beiras R. 2008. Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo-larval bioassays. *Mar Pollut Bull* 57:493–502.
18. Organisation for Economic Co-operation and Development. 2004. Test No. 202: *Daphnia* sp. acute immobilisation test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
19. Morselli M, Semplice M, Villa S, Di Guardo A. 2014. Evaluating the temporal variability of concentrations of POPs in a glacier-fed stream food chain using a combined modelling approach. *Sci Total Environ* 493:571–579.
20. Dumont HJ, Velde I, Dumont S. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. *Oecologia* 19:75–97.
21. Dumont HJ, Balvay G. 1979. The dry weight estimate of *Chaoborus flavicans* (Meigen) as a function of length and instars. *Hydrobiologia* 64:139–145.
22. Mackay D, Shiu WY, Ma KC. 1992. *Illustrated Handbook of Physical Chemical Properties and Environmental Fate for Organic Chemicals*. Lewis Publishers, Chelsea, MI, USA.
23. R Core Development Team. 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
24. Zuur AF, Elena NI, Walker NJ, Saveliev AA, Smith GM. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer Science +Business Media, New York, NY, USA.
25. Muyssen BTA, Janssen CR. 2007. Age and exposure duration as a factor influencing Cu and Zn toxicity toward *Daphnia magna*. *Ecotoxicol Environ Saf* 68:436–442.
26. Liess M, Foit K. 2010. Intraspecific competition delays recovery of population structure. *Aquat Toxicol* 97:15–22.
27. Swift MC. 1992. Prey capture by the four larval instars of *Chaoborus crystallinus*. *Limnol Oceanogr* 37:14–24.
28. Gilbert JJ. 1985. Competition between rotifers and daphnia. *Ecology* 66:1943–1950.
29. Kooijman SALM. 2010. *Dynamic Energy Budget Theory for Metabolic Organisation*. Cambridge University Press, Cambridge, UK.
30. Gergs A, Zenker A, Grimm V, Preuss TG. 2013. Chemical and natural stressors combined: From cryptic effects to population extinction. *Scientific Reports* 3. DOI: 10.1038/srep02036.
31. Knillmann S, Stampfli NC, Beketov MA, Liess M. 2012. Intraspecific competition increases toxicant effects in outdoor pond microcosms. *Ecotoxicology* 21:1857–1866.
32. Coors A, De Meester L. 2008. Synergistic, antagonistic and additive effects of multiple stressors: Predation threat, parasitism and pesticide exposure in *Daphnia magna*. *J Appl Ecol* 45:1820–1828.
33. Hoang TC, Klaine SJ. 2007. Influence of organism age on metal toxicity to *Daphnia magna*. *Environ Toxicol Chem* 26:1198–1204.
34. Weis JS, Smith G, Zhou T, Santiago-Bass C, Weis P. 2001. Effects of contaminants on behavior: Biochemical mechanisms and ecological consequences. *Bioscience* 51:209.
35. Maltby L, Hills L. 2008. Spray drift of pesticides and stream macroinvertebrates: Experimental evidence of impacts and effectiveness of mitigation measures. *Environ Pollut* 156:1112–1120.
36. De Laender F, Janssen CR. 2013. The ecosystem perspective in ecotoxicology as a way forward for the ecological risk assessment of chemicals. *Integr Environ Assess Manag* 9:e34–e38.
37. Gabsi F, Schäffer A, Preuss TG. 2014. Predicting the sensitivity of populations from individual exposure to chemicals: The role of ecological interactions. *Environ Toxicol Chem* 33:1449–1457.
38. Galic N, Hommen U, Baveco JMH, van den Brink PJ. 2010. Potential application of population models in the European ecological risk assessment of chemicals. II. Review of models and their potential to address environmental protection aims. *Integr Environ Assess Manag* 6:338–360.
39. Bontje D, Kooi BW, Liebig M, Kooijman SALM. 2009. Modelling long-term ecotoxicological effects on an algal population under dynamic nutrient stress. *Water Res* 43:3292–3300.