

# Effects of intra- and interspecific competition on the sensitivity of *Daphnia magna* populations to the fungicide carbendazim

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**Abstract** The ecological risk assessment of pesticides is generally based on toxicity data obtained from single-species laboratory experiments and does not take into account ecological interactions such as competition or predation. Intraspecific and interspecific competition are expected to result in additional stress and might increase the sensitivity of aquatic populations to pesticide contamination. To test this hypothesis, the effects of the fungicide carbendazim were assessed on the population dynamics of the micro-crustacean *Daphnia magna* under different levels of intraspecific and interspecific competition for an algal food resource, using the rotifer *Brachionus calyciflorus* as competing species. The experiments were performed in glass jars with three different carbendazim concentrations (i.e., 50, 100 and 150 µg/L), and had a duration of 25 days, with a 4-day pre-treatment period in which competition was allowed to take place and a 21-day exposure period. The endpoints evaluated were *D. magna* total population abundance and population structure. Results of these experiments show that competition stress on its own had a significant influence on shaping *D. magna* population's structure, however, a different response was observed in the intraspecific and interspecific competition experiments.

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The use of a 4-day pre-treatment period in the intraspecific experiment already led to an absence of interactive effects due to the quick abundance confluence between the different intraspecific treatments, thus not allowing the observation of interactive effects between competition and carbendazim stress. Results of the interspecific competition experiment showed that rotifers were quickly outcompeted by *D. magna* and that *D. magna* even profited from the rotifer presence through exploitative competition, which alleviated the original stress caused by the algal resource limitation. These experiments suggest that competition interactions play an important role in defining population-level effects of pesticides in a more complex way than was hypothesized (“increasing competition leading to a sensitivity increase”), as the interspecific experiment showed. Therefore, these should be taken into account in the extrapolation of single-species toxicity data to protect higher levels of biological organization.

**Keywords** Competition · Pesticides · Ecological interactions · Carbendazim · Ecological risk assessment

## Introduction

Toxicant pollution is a driver of environmental changes, shaping biological population responses together with other abiotic and biotic factors. The interaction between toxicant exposure and biotic factors (i.e., ecological interactions such as competition, predation and/or parasitism) influence the response of populations to toxicants (Coors and De Meester 2008; Stampfli et al. 2011; Buser et al. 2012; Scherer et al. 2013; Gergs et al. 2013). Despite the importance of such interactions, the ecological risk assessment (ERA) of pesticides is generally based on data

obtained from single-species toxicity tests in which ecological interactions such as competition are not taken into account. This limits the valid extrapolation of single-species toxicity test results to field conditions, where indirect and/or sublethal toxicant-related effects might occur (Van den Brink 2013; Brooks et al. 2009; Boone and Semlitsch 2002; Wootton 2002). For instance, a decrease of the most sensitive species after toxicant addition may result in an increase of a resistant species owing to a release from competition (Fleeger et al. 2003; Relyea and Howerman 2006). Such release of competition can also be driven by abiotic-mediated changes (i.e., salinity) influencing competition outcomes, so then shaping communities' composition (Loureiro et al. 2013). These kind of community changes may have long-term effects, e.g. to a shift to another stable state, compromising the recovery capacity of sensitive species (Scheffer et al. 2001). Driskell et al. (2001) reported how a single cohort of *Fucus gardneri* (brown algae) has dominated aquatic ecosystems after oil toxicant exposure due to a release of competition from other more sensitive cohorts; and such long-term effects on algae biomass were overseen because total algae abundance was equal in the impacted and reference sites.

Awareness about the importance of ecological interactions for toxicant-related effects is growing at both the scientific and the regulatory levels (De Laender and Janssen 2013). To date, there are only few studies that evaluated the combined effects of toxicants and ecological interactions on the sensitivity of aquatic organisms. Generally, an additive or synergic effect of competition on toxicant sensitivity is applied as the general hypothesis. For instance, Chandini (1988) reported extreme adverse effects of Cadmium on cladoceran (*Echinisca triserialis*) populations under high competition pressure created by a low food level. In a more recent study, Liess (2002) reported a decrease of survival of the trichopteran *Limnephilus lunatus* exposed to the insecticide fenvalerate as density increases. However, other studies point out that species interactions not necessarily result in higher toxicant effects and that the effects of the competition and toxicant interaction might vary according to the different competition strength defined by density pressures, behavioural aspects of competing species, and toxicant concentrations (Loureiro et al. 2013; Boone and Semlitsch 2002). Knillmann et al. (2012) applied esfenvalerate to outdoor pond microcosms and assessed its effects on several *Daphnia* spp. populations with different densities. They found that intraspecific competition had a negative effect on the sensitivity response to the toxicant. They also reported, however, that interspecific competition, imposed by harvesting 30 % of the organisms of the cosms which resulted in lower competition for food, did not show any alteration of the effect of the toxicant on *Daphnia* spp. Del Arco et al.

(2015) assessed the effect of carbendazim on *Gammarus pulex* under intraspecific and interspecific food competition with *Asellus aquaticus*. Results from this study showed that intra- and interspecific competition under specific competition and toxicant pressures could have a positive influence on the survival of the most sensitive species, which was explained by an enhanced cannibalism and predation behaviour that alleviated food limitations. Gui and Grant (2008) reported compensatory mechanisms at specific toxicant exposure and competition levels owing to density-dependent population factors in an experiment carried out with *Drosophila melanogaster* populations under food competition pressure. Therefore, because some studies have shown that the combined effects of ecological interactions and toxicant exposure can influence species sensitivity responses in a diverse way, more studies should be performed with other chemicals and species in order to assess whether general rules can be extracted from these experiments to better understand when and how such interactions should be considered for ERA (Linke-Gamenick et al. 1999; Gui and Grant 2008; Foit et al. 2012; De Laender and Jansen 2013).

The aim of this study was to evaluate the influence of intra- and interspecific competition on the sensitivity of an aquatic population under different pesticide exposure concentrations. To this end, experiments were performed in which *D. magna* was present in different densities (intraspecific competition) and in which *D. magna* (most sensitive species) and *B. calyciflorus* (resistant species) competed for food resources while being exposed to the carbamate fungicide carbendazim. Such experiments intended to establish an intermediate level of complexity between a single-species test and community/ecosystem level experiment (e.g. microcosms or mesocosms). It aimed to better understand and quantify the extent to which the combined stress changes the overall sensitivity response, provided the role that competitive interactions play in controlling population composition and dynamics (Loureiro et al. 2013).

## Materials and methods

### Test organisms and compound

*Daphnia magna* and *Brachionus calyciflorus* were chosen as the focal species and competing species, respectively, based on their difference in sensitivity to carbendazim. Carbendazim is used worldwide to control pest in diverse crops (i.e., oilseed rape, maize and rice) and found in aquatic systems mainly due to runoff events. This compound was chosen because it is indicated to be toxic to Daphniids at lower concentrations than rotifers, and a

relatively large amount of lab and semi-field toxicity data is available (Van Wijngaarden et al. 1998; Slijkerman et al. 2004; Daam et al. 2010; Cuppen et al. 2000; Van den Brink et al. 2000). Such differences allowed us to establish asymmetries on the food competition process and to better observe the combined effects of the pesticide and the competition stress on the focal species. *Daphnia magna* is known to have a relatively high sensitivity to carbendazim, with an LC50 (48 h) between 320 µg/L (Van Wijngaarden et al. 1998) and 270 µg/L (Maslankiewicz and Linders 1993). *Brachionus calyciflorus* was expected to show a higher tolerance to carbendazim than *D. magna*, according to the results of two microcosm studies (Daam et al. 2010; Slijkerman et al. 2004). Test organisms were obtained from a permanent culture at the Aquatic Ecology and Water Quality Management department of Wageningen University, and were cultured for several generations in the water bath where the experiment took place for acclimatization. During the acclimatization period, *D. magna* and *B. calyciflorus* were maintained in moderately hard synthetic freshwater (RT medium; Tollrian 1993) and were fed three times per week with a stock solution of the green algae *Scenedesmus obliquus* without any food restrictions.

## Experimental set-up

The experimental set-up consisted of sixty glass jars of 1.5 L filled with 0.5 L of RT medium and placed in a water bath with constant water temperature ( $20 \pm 0.5^\circ\text{C}$ ) and a 12:12 h (light:dark) photoperiod, provided by high pressure metal halide lamps (Philips HPI-T, 400 W, light intensity ca.500 µE/m<sup>2</sup> s at the jar's water surface).

The experiment was performed in triplicate ( $n = 3$ ), following a factorial design with five competition treatments and four carbendazim treatments, which were randomly assigned to the test jars. The five competition treatments were low, medium and high intraspecific competition, and no, medium and high interspecific competition, with the low intraspecific treatment being similar to the one with no interspecific competition. The carbendazim treatment consisted of control, low, medium and high exposure concentrations, corresponding to 0, 50, 100 and 150 µg/L of carbendazim respectively. Because *D. magna* and *B. calyciflorus* have been reported to feed and compete for *S. obliquus* (Ferrando et al. 1993), 1 mg C/L of *S. obliquus* was added as competing food resource to all jars once every 3 days. The added food quantity was established based on OECD *Daphnia* reproduction test (OECD 2008), in which a daily food of between 0.10 and 0.20 mg C/individual/day is recommended. Therefore, the food amount dose provided in our study was much lower, i.e., 0.03 mg C/individual/day, and ensured competition pressure due to food limitation. The control (intraC), medium

(intraM) and high (intraH) intraspecific competition treatments consisted of glass jars containing 20, 40 and 80 individuals of *D. magna*, respectively. The resulting initial density was 40, 80 and 160 individuals/L for the intraC, intraM and intraH treatments, respectively. Even the highest densities occur in the field but are high compared to other experimental studies (e.g. Gergs et al. 2013). We used rather high starting densities (OECD recommends 10 individuals/L) in order to impose the competition over resources from the start of the experiment. We were not interested to extract a growth rate from the experiment, which is often an important endpoint in populations studies and which can only be determined using a low initial density. The study by Buser et al. (2012) also used 90 daphnias/L to simulate high competition pressures which is similar to our simulation of high competition pressure.

In the case of interspecific competition, the control (interC), medium (interM) and high (interH) interspecific competition treatments consisted of 20 individuals of *D. magna* and 0, 150 and 300 individuals of *B. calyciflorus*, respectively. Please note that the interC and intraC treatments are the same and consisted of the same jars. In order to maintain the rotifer competition pressure constant and to counterbalance high mortality in jars observed in a previous study (Viaene et al. 2015), rotifers were added weekly. Extra jars were set-up containing only 300 *B. calyciflorus* (rotifC) to monitor the survival of the competing species during the experimental period. At the start of the experiment, *D. magna* individuals were stocked attending to the following population structure: 30 % adults (>800 µm), 50 % sub-adults (800–500 µm) and 20 % juveniles (<200 µm), in all test units containing *D. magna*, this population structure was artificially defined to ensure coverage of all life-stages at the beginning of the experiment.

The experimental period consisted of a 4-day pre-treatment period, in which intra- and interspecific competition were allowed to take place; and a 21-day exposure period, in which we assessed the isolated and combined effects of competition and carbendazim exposure. Carbendazim was applied only at the beginning of the treatment period (D0). No additional pulses of pesticide were deemed necessary because of the static nature of the test and the high reported persistence of carbendazim under laboratory experimental conditions (van Wijngaarden et al. 1998). These exposure concentrations were chosen to be somewhat lower than the LC50 for *D. magna* reported by Van Wijngaarden et al. (1998) and Maslankiewicz and Linders (1993) in order to avoid elevated mortality, which could hamper the observation of competition effects.

The abundance of the different *D. magna* population cohorts (adults, sub-adults and juveniles) was monitored weekly in the test units by separating the *D. magna*

individuals into size classes using sieves with different mesh size: >800 µm for adults, 500–800 µm for sub-adults, and <200 µm for juveniles. After counting, *D. magna* individuals were returned to their original test unit. Abundance of *B. calyciflorus* in the interspecific competition treatments and in the rotifer controls (rotifC) was monitored weekly by taking 10 mL samples from each jar. During counting, individuals of *D. magna* and *B. calyciflorus* that were immobile (including dead organisms) were not taken into account. Water quality was evaluated during the experimental period in order to detect any potential effect of carbendazim or individual density on the conditions of the exposure media. Temperature, pH, dissolved oxygen concentration and conductivity were weekly monitored at the start of the photoperiod. Temperature, pH and dissolved oxygen concentration were measured with a WTW 340i multi-meter, and conductivity was measured with a WTW 315i meter.

### Carbendazim application and analysis

Carbendazim stock solutions (100 mg/L) were prepared using Derosal (50 %carbendazim, w/v). Aliquots of the carbendazim stock solution were distributed over the surface of the experimental jars belonging to the carbendazim treatments. Subsequently, the medium was gently stirred to ensure an homogeneous distribution of the carbendazim. Dosing of the experimental units was performed from low to high concentration in order to avoid any possible cross-contamination.

Experimental medium samples (2 mL) from each treatment were taken after carbendazim application and weekly after the carbendazim application. These samples were used to verify the exposure concentrations in the test medium after dosing, as well as to assess the dissipation of the compound during the experimental period. Analytical measurements of carbendazim in the collected medium samples were performed. A Perkin Elmer LC-90 UV detector was used to perform a direct analysis of the carbendazim concentrations in these samples. The mobile phase used was methanol:water (70:30), pumped at a flow rate of 0.7 mL/min with a Waters M590 pump through a Waters Novapak C-18 column. This column was set in a Waters Temperature Control Module at 40 °C and with a wavelength of 285 nm. The retention time for carbendazim was 5 min. Calculation of the concentrations was based on external standard samples. According to this method, the limit of detection was 2 µg/L.

### Statistical analyses

In order to assess the effect of competition on the sensitivity of the *D. magna* population to carbendazim, logistic

regression and general linear models (GLM) were used. EC50s were calculated based on observed abundances for each combination of sampling day and competition level separately. EC50s were calculated by means of a log-logistic regression model according to Rubach et al. (2011). GLMs were constructed for each sampling day using each replicate data in the model, using the total abundance of *D. magna*, and the adult, sub-adult and juvenile abundances separately as response variables. The GLM analyses were constructed by using a Poisson distribution and using the logarithm as the link function. The model included three explanatory factors: carbendazim concentration, competition level, and the combined effects of carbendazim and competition. The effect of those three factors upon the *D. magna* abundance were considered statistically significant when the p values were <0.05, and moderately significant when they were between 0.05 and 0.10. All calculations were performed using the GenStat 11th software (VSN International Ltd., Oxford, UK).

## Results and discussion

### Carbendazim concentrations

Measured carbendazim concentrations in the exposure media after application were, on average,  $101.2 \pm 2.0$ ,  $98.9 \pm 0.6$  and  $97.7 \pm 1.4$  % (average  $\pm$ SD; Table 1) of the intended carbendazim concentrations (50, 100 and 150 µg/L, respectively). Carbendazim was found to be very stable during the exposure period so that a dissipation rate was not calculated. High water stability of carbendazim under laboratory conditions has also been reported by Van Wijngaarden et al. (1998), Cuppen et al. (2000) and Slijkerman et al. (2004). Water quality was not affected during the course of the experiment neither by the carbendazim treatments nor by the elevated organism density, as compared to the exposure and densities in the controls, respectively. The average values of the measured physico-chemical parameters were: temperature  $20.6 \pm 1.0$  °C, pH  $7.7 \pm 0.3$ , dissolved oxygen  $7.0 \pm 0.5$  mg/L, and conductivity  $356 \pm 60$  µS/cm (average  $\pm$ SD).

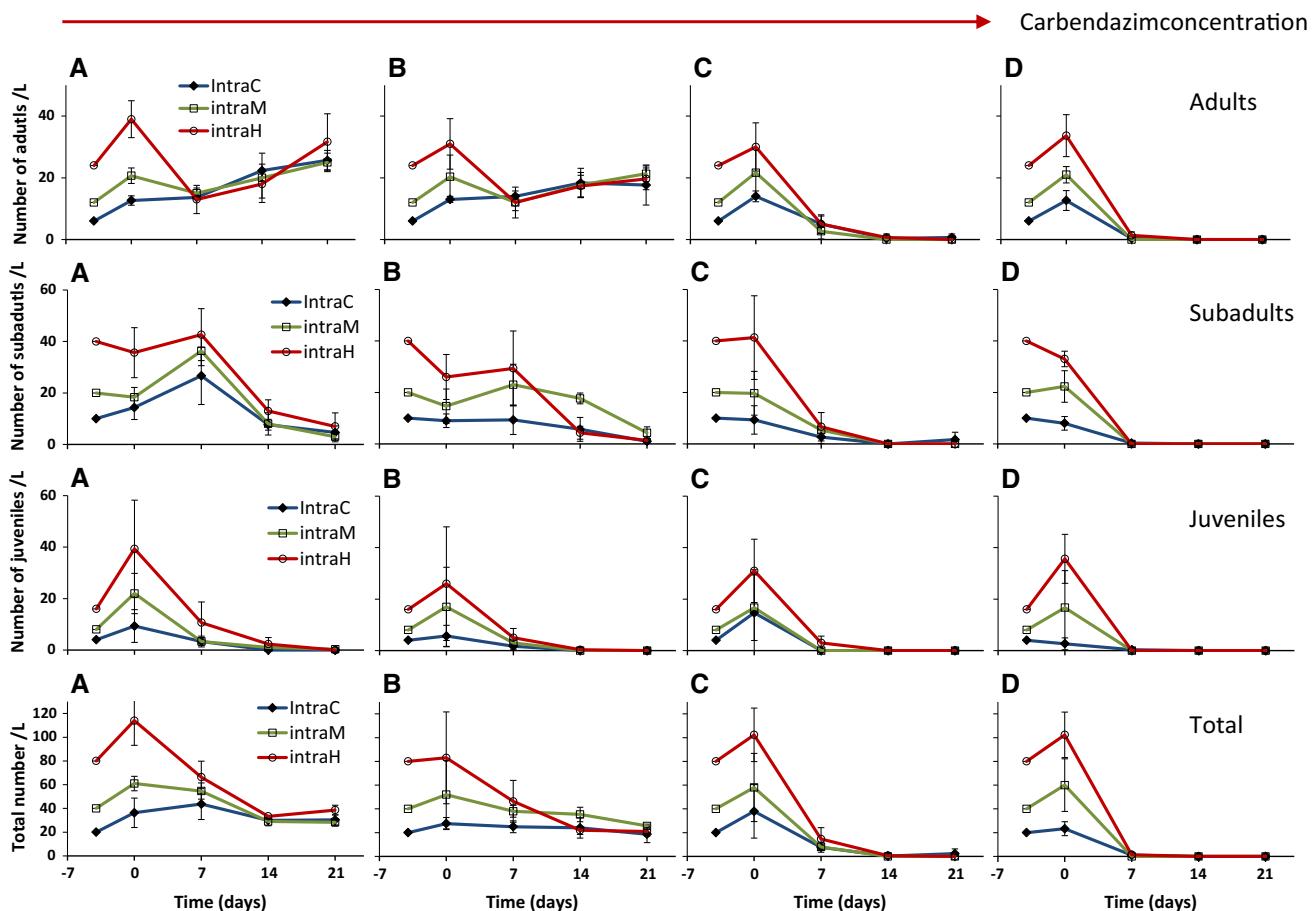
### Toxic effects of carbendazim

The total *D. magna* abundance in the controls was found to be relatively stable during the experimental period. The *D. magna* population structure changed over time, with a slight increase in the number of adults, and a decrease on the number of sub-adults and juveniles (Fig. 1). As expected, carbendazim exposure significantly reduced the abundance of *D. magna* on all life stages: adults, sub-adults and juveniles (Table 2; Figs. 1, 2). On day 7, the total

**Table 1** Measured carbendazim concentrations in the experimental media and time-weighted average concentration (TWAC) during the exposure period

| Intended concentration | Measured concentration |         |         |          | TWAC    |
|------------------------|------------------------|---------|---------|----------|---------|
|                        | Day 0                  | Day 7   | Day 14  | Day 21   |         |
| 50                     | 51 ± 1                 | 49 ± 3  | 71 ± 3  | 77 ± 6   | 62 ± 3  |
| 100                    | 99 ± 1                 | 97 ± 3  | 128 ± 7 | 117 ± 11 | 111 ± 4 |
| 150                    | 147 ± 2                | 153 ± 5 | 194 ± 7 | 189 ± 7  | 170 ± 5 |

Concentrations are expressed as mean ± SD ( $\mu\text{g/L}$ )



**Fig. 1** *Daphnia magna* abundance dynamics for different size classes and the totals in the intraspecific competition experiment at different carbendazim exposure concentrations. **A** control treatment, **B** 50  $\mu\text{g/L}$ , **C** 100  $\mu\text{g/L}$ , **D** 150  $\mu\text{g/L}$ . Errors bars represent the standard deviation

abundance of *D. magna* individuals in the low, medium and highest exposure concentrations was reduced by 43, 82 and 98 %, respectively, as compared to the controls. On day 14, the *D. magna* population became extinct at the highest carbendazim treatment (Figs. 1, 2). The calculated EC50 values for the total *D. magna* abundance in the competition controls (interC and intraC) on day 7 and 21 were, 55 (42–73) and 56 (46–67)  $\mu\text{g/L}$ , respectively. The calculated toxicity data for the studied *D. magna* population is similar to the values reported by other studies. For example, Van Wijngaarden et al. (1998) carried out laboratory single species toxicity tests and reported an LC50 (96 h) of 87  $\mu\text{g/L}$  for *D. magna* for carbendazim. Van den Brink et al. (2000) assessed the effects of the chronic exposure to carbendazim in indoor macrophyte-dominated freshwater microcosms and reported LC10 (96 h) and LC50 (96 h) values of 65 and 113  $\mu\text{g/L}$ , respectively; and LC10 (21 days) and LC50 (21 days) values of 25 and 41  $\mu\text{g/L}$ , respectively.

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#### Effects of intra- and interspecific competition

As expected from the design of the experiment, intraspecific competition significantly influenced the total abundance of

**Table 2** Results of the GLM analysis showing the effects of carbendazim, competition, and their interaction on *D. magna* abundance during the experimental period

| Experiment                |                          | Independent variable | <i>D. magna</i> abundance |                     |                     |                     |
|---------------------------|--------------------------|----------------------|---------------------------|---------------------|---------------------|---------------------|
|                           |                          |                      | Day 0                     | Day 7               | Day 14              | Day 21              |
| Intraspecific competition | <i>D. magna</i> total    | Carbendazim          | p = 0.41                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> |
|                           |                          | Competition          | <b>p &lt; 0.001</b>       | <b>p = 0.04</b>     | p = 0.86            | p = 0.87            |
|                           |                          | Interaction          | p = 0.81                  | p = 0.89            | p = 0.84            | p = 0.73            |
|                           | <i>D. magna</i> adult    | Carbendazim          | p = 0.29                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> |
|                           |                          | Competition          | <b>p &lt; 0.001</b>       | p = 0.87            | p = 0.88            | p = 0.87            |
|                           |                          | Interaction          | p = 0.55                  | p = 0.50            | p = 0.98            | p = 0.90            |
|                           | <i>D. magna</i> sub-ad.  | Carbendazim          | p = 0.88                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> |
|                           |                          | Competition          | <b>p &lt; 0.001</b>       | <b>p = 0.02</b>     | p = 0.22            | p = 0.94            |
|                           |                          | Interaction          | p = 0.21                  | p = 0.80            | p = 0.25            | p = 0.16            |
|                           | <i>D. magna</i> juvenile | Carbendazim          | p = 0.41                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | NC                  |
|                           |                          | Competition          | <b>p &lt; 0.001</b>       | <b>p = 0.001</b>    | <b>p &lt; 0.001</b> | NC                  |
|                           |                          | Interaction          | p = 0.92                  | p = 0.94            | p = 0.46            | NC                  |
| Interspecific competition | <i>D. magna</i> total    | Carbendazim          | p = 0.75                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> |
|                           |                          | Competition          | p = 0.52                  | <b>p = 0.09</b>     | <b>p = 0.02</b>     | <b>p = 0.01</b>     |
|                           |                          | Interaction          | p = 0.79                  | p = 0.42            | p = 0.73            | p = 0.59            |
|                           | <i>D. magna</i> adult    | Carbendazim          | p = 0.33                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> |
|                           |                          | Competition          | p = 0.23                  | p = 0.18            | p = 0.42            | <b>p = 0.07</b>     |
|                           |                          | Interaction          | p = 0.79                  | p = 0.68            | p = 0.34            | p = 0.49            |
|                           | <i>D. magna</i> sub-ad.  | Carbendazim          | p = 0.31                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> |
|                           |                          | Competition          | p = 0.76                  | p = 0.17            | <b>p = 0.002</b>    | <b>p = 0.003</b>    |
|                           |                          | Interaction          | p = 0.77                  | p = 0.35            | p = 0.98            | p = 0.57            |
|                           | <i>D. magna</i> juvenile | Carbendazim          | p = 0.89                  | <b>p &lt; 0.001</b> | <b>p &lt; 0.001</b> | NC                  |
|                           |                          | Competition          | p = 0.62                  | p = 0.10            | <b>p &lt; 0.001</b> | NC                  |
|                           |                          | Interaction          | p = 0.79                  | p = 0.20            | p = 0.40            | NC                  |

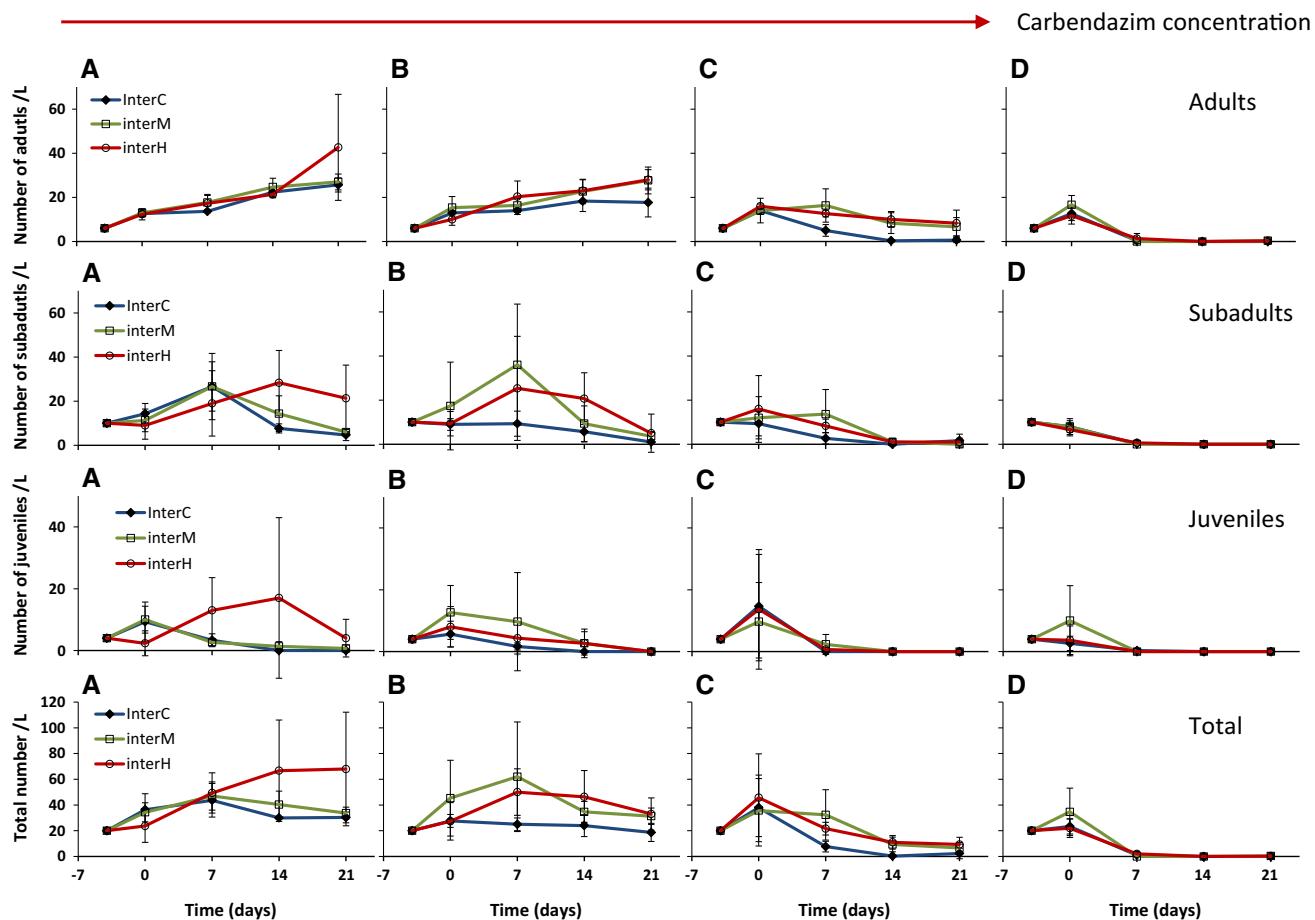
Bold *p* values indicate (marginally) statistically significant differences

NC Not calculated due to many zero values. On this sampling day, *D. magna* individuals were extinct in all but one competition and carbendazim treatment

the *D. magna* population on day 0 and 7 (Table 2). 14 days after the start of the exposure period, the total *D. magna* abundance was reduced to similar levels in all intraspecific competition treatments, apparently reaching the carrying capacity (Fig. 1). Similarly, significant differences between intraC and the intraspecific competition treatments for the *D. magna* adults and sub-adults were only observed at the beginning of the experiment. In contrast, the abundance of juveniles in the medium and high intraspecific treatments (intraM and intraH) was found to be significantly higher than in the intraC during the whole experimental period, except in the last sampling date when the juveniles were absent (Table 2; Fig. 1). This can be partially explained by the growth of some individuals from juveniles to adults life stages. Figure 1 it is relatively clear that the juveniles and sub-adults were outcompeted by the adults since at the end of the experiment almost 90 % of all individuals were

adults. Unfortunately the study did not last long enough to see the population either level-off at a certain level or collapse because of sub-optimal food conditions. It is, however, clear from the decreasing abundances in the carbendazim controls that food was limited (Fig. 1).

Unexpectedly, interspecific competition resulted in a large and significant increase of the total *D. magna* abundance in the higher competition treatment (interH) without carbendazim exposure during the last 2 weeks of the experiment (day 14 and 21; Table 2; Fig. 2). Such significant differences were also observed for the different size classes (Table 2; Fig. 2). At the same time, the number of rotifers in the interspecific treatments without carbendazim exposure showed a notable decline, becoming totally extinct in the medium and high competition treatment. The rotifer abundance in the rotifC, a treatment without *Daphnia*, however, increased by a factor of



**Fig. 2** *Daphnia magna* abundance dynamics for different size classes and the totals in the interspecific competition experiment at different carbendazim exposure concentrations. A control treatment, B 50 µg/L, C 100 µg/L, D 150 µg/L. Errors bars represent the standard deviation

50 during the experimental period. This indicates that without carbendazim pressure, *D. magna* is able to outcompete the rotifer population. Ha and Hanazato (2009) studied the interaction between daphnids and rotifers in freshwater microcosms under excess food conditions. They found that rotifers were rapidly outcompeted in the microcosms containing six individuals per litre of *Daphnia galeata* and argued that rotifer's damage could have been incurred by being swept into the branchial chambers of the daphnids. This had been previously demonstrated in the competition experiments between *B. calyciflorus* and *D. pulex* performed by Gilbert (1985), who also pointed out that ingestion of small rotifers may be possible by large daphnids. This suggests that besides the exploitative competition created by the limited *S. obliquus* abundance in the test systems, interference competition could also have been a major cause explaining the rotifer decline. In addition, the accumulation and damage of rotifers in the *D. magna* chambers could have contributed to an alleviation of the stress caused by food limitation, either by the direct rotifer ingestion, by the intake of suspended organic

material generated by dead rotifer individuals, by a higher grazing capacity of *D. magna*, or by all.

#### Combined effects of carbendazim and competition

In contrast to our original hypothesis, the carbendazim and the intraspecific competition treatments did not result in significant interaction effects on the *D. magna* total abundance, neither on the population structure, while the control response clearly showed a food limitation at the two intraspecific treatments. This finding indicates that differences in abundance due to competition stress disappeared too quickly to allow a significantly interaction with the carbendazim exposure and that a shorter pre-treatment period could be used in further experiments when using a fast reproducing species like *D. magna*. In our experiment, competition only affected the total *D. magna* population abundance during the first week of the experiment (Table 2), and the carrying capacity was rapidly reached afterwards (Fig. 1). This is in line with the population dynamics theory, which suggest that as density increases and competition

intensifies, population growth rate will decline until the system's carrying capacity is reached (Forbes et al. 2001). Another potential explanation could be that carbendazim exposure was too high to allow the detection of competition stress effects. For instance, Antunes et al. (2004) pointed out that exposure to the pesticide lindane had higher effects on life history endpoints of *Daphnia longispina* than the food stress, even though sensitivity was also altered by the combined effect of both. Knillmann et al. (2012) studied how intraspecific competition influenced toxicant effect on freshwater invertebrate communities and found that increasing population density can have adverse effects on the community response to a toxicant, but in their experiment the intraspecific competition was imposed constantly by periodic harvesting. The importance of whether the intraspecific competition is only present at the start or imposed by periodic harvesting is confirmed by the findings of Liess and Foit (2012), who exposed *D. magna* to a toxicant under two different competition treatment levels and found a negative influence of competition on the effects of the toxicant, and also delaying population recovery. In the same line, Barata et al. (2002) studied the influence of food supply and density on the response of cladocerans to cadmium exposure using life table response experiments. They found that under high competition pressure and low food availability there will be more compensation for cadmium toxicity in the form of that density dependent effects on mortality and reproductive rate, decreasing extinction possibilities; while at lower densities and higher food availability an increase of density will exacerbate the effect of the toxicant. This shows that the effect of intraspecific competition on the toxicity of a chemical is not always straightforward, as is also shown in this paper and in our earlier findings (Del Arco et al. 2015). Del Arco et al. (2015) report results of a similar experiment to the one presented here but using macroinvertebrate species. Their main finding was that intraspecific competition induces cannibalistic and predation compensatory mechanisms, especially under food limiting conditions. The study shows that intra- and interspecific competition pressure may influence the response of sensitive aquatic organisms in a more complex way (positive, non-significant and negative effects were observed) than just increasing the sensitivity of the studied species, as has generally been hypothesized. The advantage of the approach of Barata et al. (2002) of combining life table response experiments with a demographic analysis of the data is that more summary endpoints (life history traits like growth rate) than abundance of the total population and of different size classes alone, can be evaluated.

Combined effects of interspecific competition and carbendazim stress on the *D. magna* population were not significant (Table 2). However, medium and high rotifer competition treatments (interM and interH) seemed to result

in higher *D. magna* total abundances, and in increasing abundances at all life stages, as compared to the competition controls (Table 2; Fig. 2). This was observed at all carbendazim treatments except at the higher exposure concentration, in which competition effects were less visible due to the strong toxic effect of carbendazim on *D. magna*. In fact EC50 values were found to be slightly higher at the end of the experiment in the medium competition level (interM) as compared to the low and high competition levels (interC and interH, Table 3). In the 50 and 100 µg/L carbendazim treatments, the rotifer population was outcompeted by *D. magna* within the first week, but numbers increased to values around 10 individuals/L towards the end of the experiment (Fig. 3). The rotifer population in the highest exposure concentration (150 µg/L) was reduced to about 10 % during the first week, but increased exponentially after the *D. magna* population became extinct, reaching a full recovery (1258 individuals/L on day 21) within the 2 weeks after the decline and herewith showing a low sensitivity to carbendazim (Fig. 3). These findings are in contrast to the hypothesis of a negative effect of interspecific competition on the toxicity of the chemical which was expected since this has been previously reported by other authors. For instance, Foit et al. (2012) found a negative effect of interspecific competition on the *D. magna* population response when competing with *Culex pipiens molestus*, leading to an increase of toxicant sensitivity and a delay of the *D. magna* population recovery. Again, the use of a 4-day pre-treatment period could be the reason for the absence of interaction effects, as the rotifers were quickly outcompeted by *D. magna*, while *D. magna* even profited from the presence of the rotifers through exploitative competition.

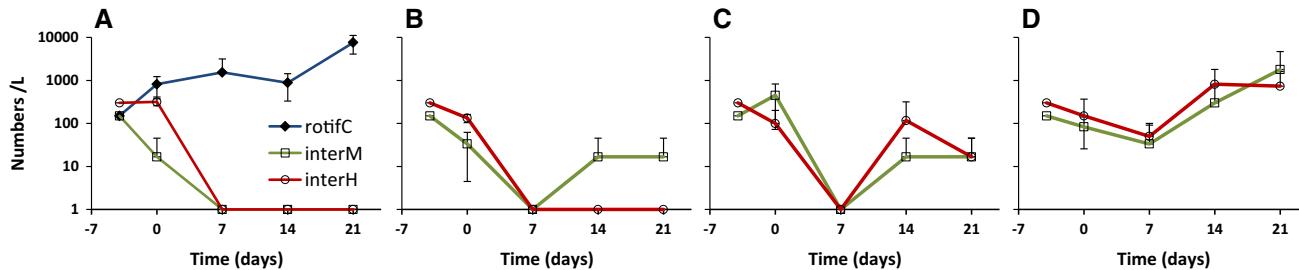
### The importance of ecological interactions for risk assessment

The European Union encourages establishing maximum environmental concentrations for chemicals in order to protect ecosystems based on toxicity data for sensitive species. In order to achieve this goal, ERA should become more ecologically realistic and science-based (De Laender and Janssen 2013; Van den Brink 2008). A step forward towards this objective would be to include ecological interactions such as competition in toxicity testing. The results of this experiment highlight the complexity of the influence of competition on species sensitivity response. First of all, intra- and interspecific competition have different impact on species' responses. The results of this experiment show that intraspecific competition occurs. However, it is only of importance when it is present at the time of chemical exposure; because we found that the use of a 4-day pre-treatment period already led to an absence of

**Table 3** Calculated EC50 (total abundance) values for each sampling day for *D. magna* ( $\mu\text{g/L}$ ). Values within brackets represent the 95 % Confidence Intervals

| Day | interC and intraC | Intraspecific competition |            | Interspecific competition |             |
|-----|-------------------|---------------------------|------------|---------------------------|-------------|
|     |                   | intraM                    | intraH     | interM                    | interH      |
| 7   | 55 (42–73)        | 62 (55–69)                | >150       | >150                      | >150        |
| 14  | 59 (39–88)        | >150                      | 55 (51–60) | 76 (58–100)               | 63 (39–101) |
| 21  | 56 (46–67)        | 53 (52–54)                | 50 (49–52) | 79 (63–99)                | 49 (27–89)  |

The values “<50” mean that the maximum effect was already present at the lowest concentration (50  $\mu\text{g/L}$ ) so the EC50 is somewhere between 0 and 50  $\mu\text{g/L}$ , and the values “>150” mean that no effects were observed at the highest tested concentration (150  $\mu\text{g/L}$ )



**Fig. 3** Results of the measured abundance of rotifers in the interspecific competition experiment at different carbendazim exposure concentrations. **a** control treatment, **b** 50  $\mu\text{g/L}$ , **c** 100  $\mu\text{g/L}$ ,

**d** 150  $\mu\text{g/L}$ . Errors bars represent the standard deviation. A log scale for the y axis was used because of the high abundance of the rotifers

interactive effects. It is most likely due to the quick confluence of the abundance between the different intraspecific treatments towards the system's carrying capacity (Fig. 1). In the case of interspecific competition, we observed a positive effect of interspecific competition on the abundance of *D. magna*, which is most probably related to the superior grazing capacity of *D. magna* versus *B. calyciflorus* and the uptake of rotifers by *D. magna* in food limiting conditions. Therefore, species selection is a key point to establish the target competition pressure and also the strength of the ecological interactions under study. Toxicant concentration is also a critical factor in detecting competition pressure effects, and a shortcoming of this experiment could be that we used too high toxicant exposures. Population structure will also influence the sensitivity response because some life stages could be more sensitive, which would alter the population structure dynamics (i.e., low juvenile development). In conclusion, this experiment gives empirical evidence of species interactions resulting in indirect effects on sensitivity responses. Competition effects are shown to be not always easily classified as antagonistic, additive or synergistic and are highly dependent on the environmental conditions and the community structure. Consequently, more studies are needed to better characterize ecological interactions and to decide on which key species and interactions should be taken into account in ERA to better predict population's responses.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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