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A comparison of the short-term toxicity of cadmium to indigenous and alien gammarid species

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Abstract Amphipods play an important role in many aquatic ecosystems and are commonly used in ecotoxicology and ecosystem health assessment. Several alien gammarids have been introduced in many regions of the world during the last decades. In this study, we investigated if differences in cadmium sensitivity occurred between (1) different species belonging to the family Gammaridae and (2) different populations of the same species originating from a polluted or a non-polluted site. The acute cadmium toxicity to two indigenous (*Gammarus pulex* and *Gammarus fossarum*) and four alien (*Dikerogammarus villosus*, *Echinogammarus berilloni*, *Gammarus roeseli* and *Gammarus tigrinus*) gammarids occurring in Belgium was tested. Significant differences ($P < 0.05$) in median lethal concentrations (LC_{50}) were found between the different species, with 72 h-LC50s ranging from 6.3 to 268 μg/l and 96 h-LC50s from 4.7 to 88.9 μg/l. No clear trend in Cd sensitivity was found when comparing indigenous and alien gammarids. *D. villosus*, an alien invasive species, was the most sensitive to Cd toxicity and *E. berilloni*, another alien species, the least sensitive. In addition, larger Gammarid species were more sensitive to Cd toxicity than smaller ones. No significant differences were found between populations of the same species originating from metal polluted sites or non-polluted sites. Overall, our results showed that considerable differences in Cd sensitivity exist between gammarid species, which should be taken into consideration in environmental risk assessment and water quality standard setting. Finally, our data suggest that alien gammarids would not have an advantage over indigenous gammarids in Cd contaminated environments.

Keywords Acute toxicity · Alien species · Cadmium · Gammarids

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Introduction

Amphipods of the superfamily Gammaroidea are widespread and often play an important functional role in fresh and brackish water ecosystems (Jazdzewski 1980). In the aquatic environment, gammarids are often the dominant macroinvertebrates, both in terms of biomass and density (MacNeil et al. 1997). Consequently, in these environments, they contribute significantly to the energy flow by decomposing organic material and serving as food for other organisms such as fish (MacNeil et al. 2000; Grabowski et al. 2007). Furthermore, Gammaridae are sensitive to a wide range of toxicants (Felten et al. 2008) and are increasingly being used in ecotoxicology (Kunz et al. 2010). Different gammarid species serve as important ecotoxicological test organisms, but also as valuable indicators of aquatic ecosystem health (Kunz et al. 2010). *Gammarus pulex* (Linnaeus, 1758) is the most commonly used fresh water amphipod species in ecotoxicology since it has a wide distribution area in streams of Europe and Northern Asia and is fairly easy to maintain in the laboratory (Karaman and Pinkster 1977; McCahon and Pascoe 1988a). However, the sensitivity of other freshwater gammarid species to toxicants has been studied less frequently (but for examples see, for instance, Martinez et al. 1996; Alonso and Camargo 2006; Pestana et al. 2007; Chaumot et al. 2009; Alonso et al. 2010; Sroda and Cossu-Leguille 2011).

Due to increasing world-wide trade, the number of alien species introductions is increasing gradually (Simberloff et al. 2005). Several species belonging to Gammaroidea are considered successful invaders (van der Velde et al. 2000; Grabowski et al. 2007). Their short generation time, early sexual maturity, high fecundity and low sensitivity to environmental change and stress in general are put forward as important traits contributing to their success (Grabowski et al. 2007; Statzner et al. 2008). In Belgium, seven different gammarid species occur in freshwater ecosystems, two of which are indigenous: *Gammarus pulex* and *Gammarus fossarum* Koch, 1835 and five of which are alien: *Dikerogammarus haemobaphes* (Eichwald, 1841), *Dikerogammarus villosus* (Sowinsky, 1834), *Echinogammarus berilloni* (Catta, 1878), *Gammarus roeseli* Gervais, 1835 and *Gammarus tigrinus* Sexton, 1939 (Wouters 2002; Josens et al. 2005; Messiaen et al. 2010). Each species has its own preferences regarding water chemistry and habitat (Elliot 2005; Boets et al. 2010, 2011a) and some species are geographically separated from others (Peeters and Gardeniers 1998). As many alien macroinvertebrate species in Belgium often have lower requirements than indigenous species regarding water quality (Boets et al. 2011b), we hypothesized that alien gammarids are more tolerant to chemical stress than indigenous species.

We also hypothesized that gammarid populations originating from historically metal-polluted sites are less sensitive than populations of the same species originating from non-polluted sites. Such reduced sensitivity to metal stress has been shown earlier to occur in crustaceans and may be acquired under metal-polluted conditions either by genetic adaptation (Klerks and Weis 1987; Ward and Robinson 2005) or by physiological acclimation (Howell 1985; Stuhlbacher and Maltby 1992; Muyssen et al. 2002).

The aims of the present study were thus: (1) to investigate the difference in sensitivity to cadmium between different species of the family Gammaridae and (2) to test the difference in sensitivity between populations of the same species originating from a metal polluted or a non-polluted site. In addition, it was anticipated that these findings could help to explain why alien gammarid species often appear to be so successful in polluted habitats (compared to indigenous gammarids) (Grabowski et al. 2007).

Material and methods

We used five different species in total and, in addition, two different populations of two species that were collected in the field at different locations to test our hypotheses. Cadmium was used as the model toxicant.

Sampling and laboratory acclimation

Standard physical–chemical parameters (pH, conductivity, dissolved oxygen) were measured in the field with electrodes (HI 9210N, wtw cond 315i, wtw oxi 330). Hardness was analysed in the lab by means of a standard test kit (Merck Aquamerck, titrimetic method). At each site, a water sample was taken, which was afterwards filtered through a 0.45 µm filter (Acrodisc Filter, Supor Membrane, PALL, Newquay), collected in polypropylene tubes and acidified with 0.14 N HNO_3 (Normaton Ultrapure 69% HNO_3, Prolabo, VWR) prior to storage. Samples for metal analysis were stored at 4°C in the dark. The samples were analysed in the lab with GF-AAS (Thermo scientific, ICE 3000) with Zeemann background correction for dissolved Cd and dissolved Pb and with Flame-AAS (Varian, Spectra AA-100) for dissolved Zn. The detection limit was 0.1 µg/l for Cd, 10 µg/l for Zn and 0.4 µg/l for lead. Cadmium zinc and lead were analysed because it is known that these metals are the most important ones and can reach high concentrations at these sites (MIRA 2010).

Gammarids were collected using a handnet or artificial substrates similar to the sampling technique used to assess the biological water quality in Flanders. A detailed description of the sampling technique can be found in Gabriels et al. (2010). A handnet was used in shallow water (small rivers and brooks), whereas artificial substrates were used for deep water (e.g., canals). In total, six different gammarid species were collected at several locations situated in Belgium (Table 1). Site selection was based on the known occurrence of species (Messiaen et al. 2010, Boets et al. 2011a, b, Boets et al. unpublished data) combined with information on chemical and biological data retrieved from the database of the Flemish Environment Agency (VMM 2010). For two species (*Gammarus fossarum* and *Dikerogammarus villosus*), two different populations were collected: one population from a non-polluted site and one from a historically metal contaminated site (Table 1). These historical metal contaminated sites have been characterised by high metal concentrations for several decades due to mining activities or intense industry (MIRA 2010). Next to contamination with zinc and lead, high values for total cadmium (0.5–6.8 µg/l) were measured at the end of the eighties and the beginning of the nineties in the canal Ghent-Terneuzen and the Geul near Plombières (VMM 2010).

All Gammaridae were transported to the laboratory using plastic containers of 10 l, after which they were transferred to aerated aquaria of 20 l located in a temperature controlled room (13 ± 1°C) with a 14:10 h day–night cycle. Gammarids were fed with conditioned poplar (*Populus sp.*) leaves and were gradually acclimatized to the test water (moderately hard water, EPA 2002) at least 1 week prior to the tests.

Table 1 Physical-chemical parameters measured at the different sampling locations as well as concentrations of dissolved cadmium, zinc and lead measured in the water

Species	Origin	Coordinates	pH	DO (mg O_2/l)	Conductivity (µS/cm)	Hardness (mg $CaCO_3$/l)	Cd (µg/l)	Zn (µg/l)	Pb (µg/l)
Dikerogammarus villosus (NPS)	Canal Kortrijk-Bossuyt	50°49′N, 3°17′E	8.1	13.3	783	321	<0.1	16	<0.4
Dikerogammarus villosus (PS)	Canal Gent-Terneuzen	51°12′N, 3°47′E	7.9	11.5	1,977	464	<0.1	139	0.4
Echinogammarus berilloni	Lesse	50°12′N, 4°57′E	8.1	9.9	231	321	<0.1	<10	<0.4
Gammarus fossarum (NPS)	Ywoigne	50°12′N, 5°1′E	8.3	9.1	429	178	<0.1	<10	<0.4
Gammarus fossarum (PS)	Geul	50°43′N, 5°58′E	7.8	7.5	549	143	<0.1	127	33
Gammarus pulex	Verrebeek	50°46′N, 3°45′E	7.6	12.7	501	286	<0.1	<10	<0.4
Gammarus roeseli	Kleine Nete	51°12′N, 4°46′E	7.0	10.9	428	250	<0.1	16	<0.4
Gammarus tigrinus	Witte Nete	51°13′N, 5°6′E	7.6	12.3	298	143	<0.1	17	1.6

PS polluted site, *NPS* non-polluted site, *DO* dissolved oxygen

Alien species are indicated in bold

Experimental design

Tests were carried out in small 200 ml translucent polyethylene cups containing 130 ml of test solution or control water (EPA 2002). Per species, only adults within the same size range (Table 2) were used to test the effect of cadmium. Each gammarid was individually placed in a cup to avoid stress and cannibalism since intraguild predation is common among gammarids (Dick and Platvoet 1996). Gammarids were not fed during the tests. Fifteen replicates were used per treatment. Tests were conducted in a climate room under controlled conditions (13 ± 1°C and day:night cycle with a photoperiod of 14:10 h). Test solutions were prepared from a stock solution of 100 mg Cd/l (added as $CdCl_2.H_2O$ in the moderately hard water). Static non-renewal acute toxicity tests were conducted for all species according to the EPA guidelines (EPA 2002). Physical–chemical parameters (water temperature, dissolved oxygen, conductivity, pH, hardness) were measured every day. In addition, ammonia and nitrite were measured daily in order to be sure that the water quality was appropriate for gammarids. Nitrite and ammonium were determined using a colorimetric method (Spectroquant, Aquamerck). Standards between 0.01 and 0.9 NO_2-N mg/l and 0.05–2.5 NH_4-N mg/l were used to calibrate absorbance in function of NO_2 and NH_4 concentration. The absorbance at 525 nm for nitrite and 690 nm for ammonium of samples and standards was determined using a spectrophotometer (Aquamate, Thermo Electron Corporation). Measured values of nitrite (mg/l) and ammonium (mg/l) were always below concentrations (<0.05 mg/l) that could be harmful to gammarids.

The nominal concentrations of cadmium ranged between 5.6 and 320 µg Cd/l. Different ranges were used for different species because range-finding tests indicated different sensitivities among species (results not shown). Five cadmium concentrations were tested per species. Tests lasted 96 h and each day, the number of dead individuals

Table 2 Average physical–chemical values (±SD) measured during the experiments and the average size of all different species and populations ([a]Significant difference in size, [b]No significant difference in size) used in the toxicity tests

Species	Origin	Size (mm)	pH	DO (mg O_2/l)	Conductivity (µS/cm)	Hardness (mg $CaCO_3$/l)
Dikerogammarus villosus (NPS)	Canal Kortrijk-Bossuyt	20 ± 1[a]	7.8 ± 0.1	10.2 ± 0.2	293 ± 7	118 ± 6
Dikerogammarus villosus (PS)	Canal Gent-Terneuzen	20 ± 2[a]	7.8 ± 0.1	9.3 ± 0.1	293 ± 10	112 ± 8
Echinogammarus berilloni	Lesse	11 ± 2[b]	7.6 ± 0.3	9.2 ± 0.5	304 ± 7	118 ± 16
Gammarus fossarum (NPS)	Ywoigne	9 ± 2[b]	7.8 ± 0.1	8.3 ± 0.5	295 ± 8	120 ± 8
Gammarus fossarum (PS)	Geul	11 ± 2[b]	7.6 ± 0.2	9.2 ± 0.9	277 ± 21	118 ± 10
Gammarus pulex	Verrebeek	11 ± 2[b]	7.8 ± 0.1	8.8 ± 0.7	280 ± 5	122 ± 6
Gammarus roeseli	Kleine Nete	15 ± 2[a]	7.9 ± 0.1	9.1 ± 0.3	285 ± 9	120 ± 6
Gammarus tigrinus	Witte Nete	9 ± 2[b]	7.5 ± 0.2	8.5 ± 1.1	330 ± 25	116 ± 12

PS polluted site, *NPS* non-polluted site, *DO* dissolved oxygen

Alien species are indicated in bold

was counted and removed after which their length was measured. Gammarids were considered to be dead when neither swimming nor movements were observed after touching the animal with a forceps. At the beginning and end of each experiment, water samples of the test solution were taken to determine the Cd concentration with GF-AAS (Thermo scientific, ICE 3000) with Zeemann background correction. At the end of the test, the length of all animals (including animals that were alive) was measured. Length was measured from base of the rostral tip to the end of the last abdominal segment.

Statistical analysis

LC_{50} values and their confidence intervals for 72 h exposure periods were calculated for each species using the trimmed Spearman-Karber method (Hamilton et al. 1977). Because with gammarids it is common to report 48 h and 96 h-LC_{50} values, these were also calculated if possible, i.e., for species that exhibited more than 50% mortality after 48 or 96 h in at least one experimental concentration. However, as this is a comparative study, only the 72 h-LC_{50} values are further discussed, since these could be calculated for all species. Statistical differences of 72 h-LC_{50} values between species and between populations were tested using a ratio test (Wheeler et al. 2006). Difference in size between the different gammarid species was tested using Kruskal–Wallis ANOVA, followed by post hoc-multiple comparisons (Conover 1980) using Statistica 7.0 (Statsoft Inc 2004). The correlation between size and 72 h-LC_{50} values was tested using Spearman's Rank Correlation coefficient.

Results

All measured physical–chemical parameters as well as dissolved metal concentrations of the sampled sites where the Gammaridae were collected are given in Table 1. All sites were characterized by moderately hard to hard water conditions according to EPA (2002). Water conductivity of the canal Gent-Terneuzen was relatively high compared to the other sampling locations due to brackish water conditions. Concentrations of zinc and lead were in general more than ten times higher in the polluted sites compared to the non-polluted sites (Table 1). Cadmium concentrations were below the detection limit (<0.1 μg/l) in all sites.

Physical–chemical properties recorded during the experiments as well as the size of the different species can be found in Table 2. *D. villosus* was the largest gammarid, followed by *G. roeseli, G. fossarum* and *G. pulex* and the smallest one *G. tigrinus*. Based on post hoc multiple comparisons, a significant difference ($P < 0.05$) in size was found between *D. villosus* and all other species and between *G. roeseli* and all other species (Table 2).

Average mortality in the controls was less than 10% in all experiments. Gammarid mortality increased with increasing cadmium concentration and increasing exposure duration. The LC_{50} values for cadmium (48, 72 and 96 h) indicated that *Dikerogammarus villosus* was the most sensitive species, followed by *Gammarus roeseli*, *G. pulex*, *G. fossarum*, *G. tigrinus* and *Echinogammarus berilloni* (Table 3; Fig. 1). There was a significant difference in the sensitivity to cadmium between Gammaridae (Table 4). However, alien species as a group were not more tolerant to cadmium compared to indigenous species. Indeed, two alien species (*D. villosus* and *G. roeseli*) were the most sensitive to cadmium, whereas two other alien species (*G. tigrinus* and *E. berilloni*) were the least sensitive to cadmium. Indigenous species had an intermediate sensitivity. There was a significant difference ($P < 0.05$) in sensitivity to cadmium toxicity between *D. villosus* and all other species (Table 4). The 72 h-LC_{50} values for populations of *D. villosus* and *G. fossarum* originating from polluted sites were not significantly different from

Table 3 LC_{50} values (48, 72 and 96 h) and 95% confidence intervals calculated (when possible) for all species and populations tested based on the trimmed Spearman-Karber method

Species	48 h-LC_{50} (μg Cd/l)	72 h-LC_{50} (μg Cd/l)	96 h-LC_{50} (μg Cd/l)
Dikerogammarus villosus (NPS)	25.7 (16.0–41.1)	6.3 (4.0–9.7)	–
Dikerogammarus villosus (PS)	14.2 (9.8–20.6)	7.6 (6.3–9.2)	4.7 (2.9–7.6)
Echinogammarus berilloni	601.9 (429.9–842.7)	268.1 (97.8–734.5)	88.9 (46.7–169.1)
Gammarus fossarum (NPS)	–	51.7 (20.8–128.8)	38.4 (14.8–99.8)
Gammarus fossarum (PS)	–	78.3 (56.7–108.1)	64.2 (45.4–90.9)
Gammarus pulex	–	50.2 (24.4–103.0)	20.3 (12.5–32.9)
Gammarus roeseli	–	29.0 (11.7–71.3)	8.6 (4.4–16.7)
Gammarus tigrinus	269.8 (164.7–441.9)	146.5 (65.8–326.0)	–

PS polluted site, *NPS* non-polluted site

Alien species are indicated in bold

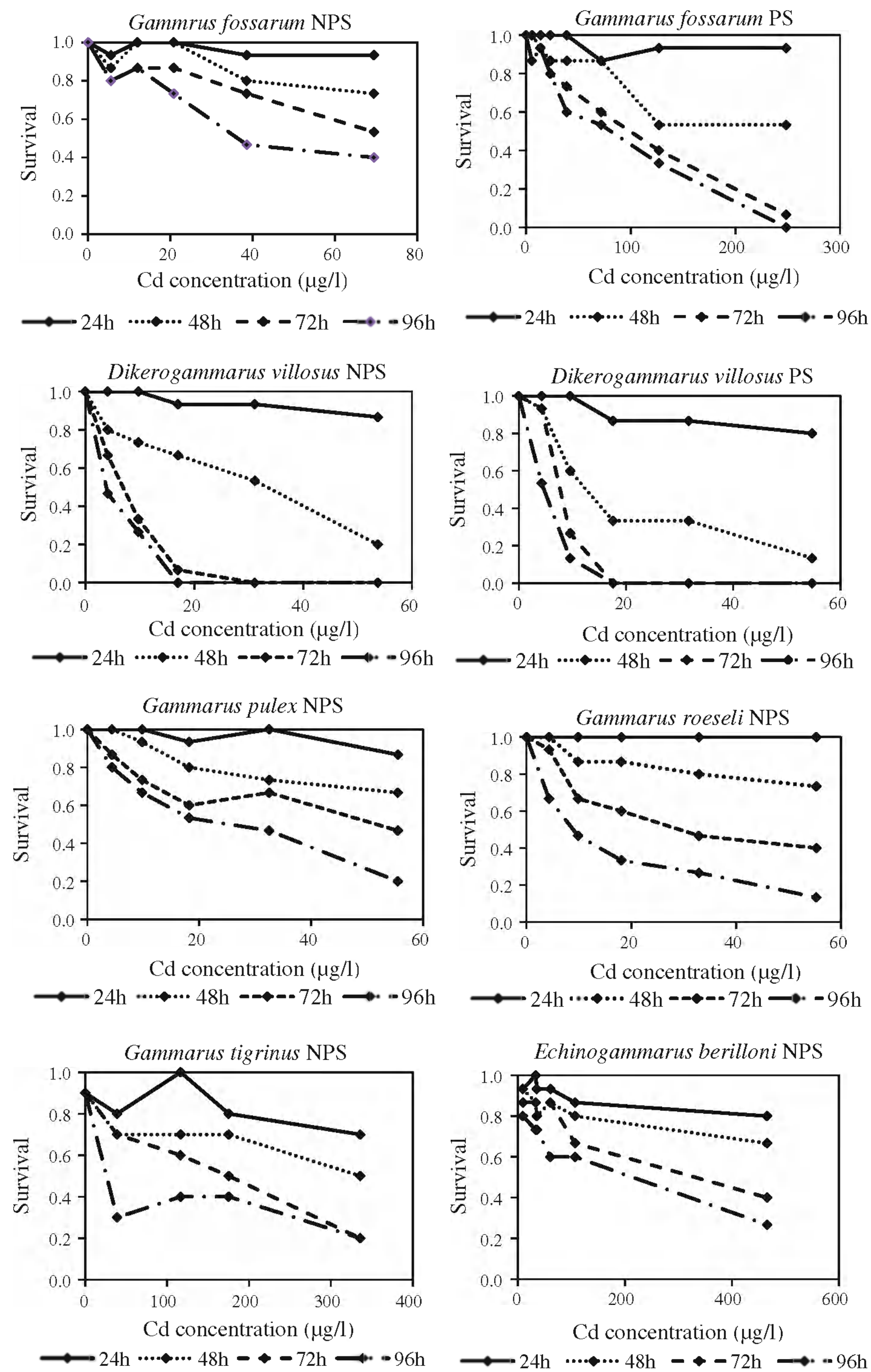

Fig. 1 Dose–response curves of cadmium toxicity for *Gammarus fossarum* from a non-polluted site, *G. fossarum* from a polluted site, *Dikerogammarus villosus* from a non-polluted site, *D. villosus* from a polluted site, *Gammarus pulex*, *Gammarus roeseli*, *Gammarus tigrinus* and *Echinogammarus berilloni* (*NPS* non-polluted site, *PS* polluted site)

those obtained with populations originating from non-polluted sites ($P > 0.05$; Table 4). A significant correlation was found between size and LC_{50} values ($R = 0.85$; $P < 0.05$), with the largest species being the most sensitive to cadmium (Fig. 2a). The two largest species (*D. villosus* and *G. roeseli*), two alien species, were most sensitive to cadmium toxicity (Table 3).

Discussion

Significant differences in Cd sensitivity between different species within the family Gammaridae were observed. The alien species *Dikerogammarus villosus* was the most sensitive, whereas *Echinogammarus berilloni* also an alien species was the least sensitive. LC_{50} values between

Table 4 Ratio test of Wheeler et al. 2006 giving *p* values for differences in 72 h-LC_{50} values between different species and different populations

	D. villosus (NPS)	***D. villosus*** (PS)	***E. berilloni***	*G. fossarum* (NPS)	*G. fossarum* (PS)	*G. pulex*	***G. roeseli***	***G. tigrinus***
D.villosus (NPS)	–							
D. villosus (PS)	NS	–						
E. berilloni	<0.001	<0.001	–					
G. fossarum (NPS)	<0.001	<0.001	0.02	–				
G. fossarum (PS)	<0.001	<0.001	0.02	NS	–			
G. pulex	<0.001	<0.001	0.01	NS	NS	–		
G. roeseli	0.003	0.003	0.002	NS	0.04	NS	–	
G. tigrinus	<0.001	<0.001	NS	NS	NS	NS	0.008	–

NS non-significant, *PS* polluted site, *NPS* non-polluted site

Alien species are indicated in bold

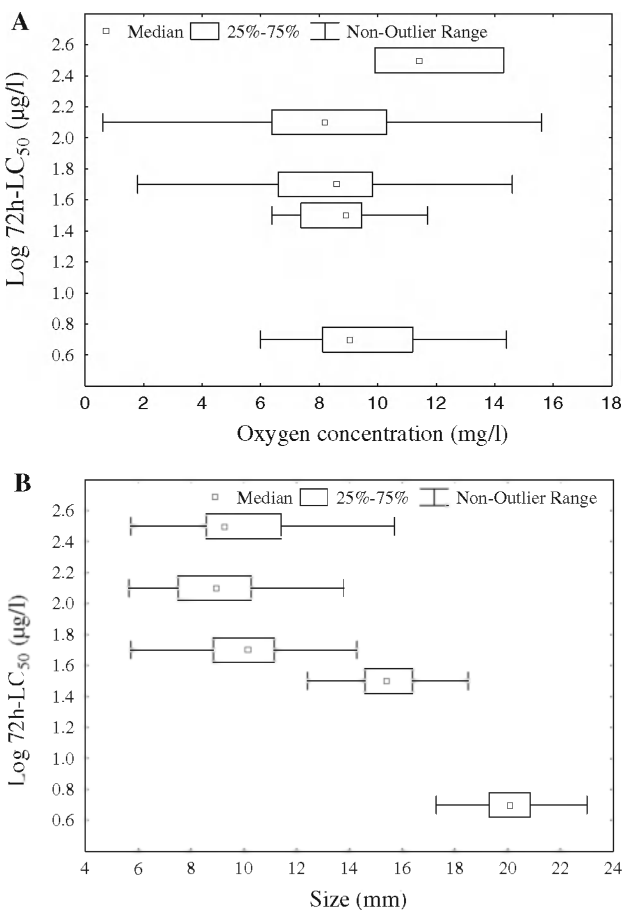

Fig. 2 Box- and whisker plots for **a** dissolved oxygen concentration and **b** size in relation to the 72 h-LC_{50} values found for the different gammarid species. Species with similar 72 h-LC_{50} values were represented by one box- and whisker plot to avoid overlap

species of the genus *Gammarus* were significantly different for the two alien species *G. roeseli* and *G. tigrinus*. The two indigenous species, *G. fossarum* and *G. pulex* had similar LC_{50} values.

As a group, alien gammarid species were not more tolerant to cadmium than indigenous species. Compared to the indigenous species, two alien species were more sensitive to cadmium toxicity, whereas the two other alien species were less sensitive. *D. villosus*, which is known to be a successful alien invasive species in many European freshwater ecosystems (Bollache et al. 2004; MacNeil et al. 2010; Messiaen et al. 2010) was the most sensitive species to cadmium toxicity of all tested species. This finding is different from the results reported by Maazouzi et al. (2008) and Boets et al. (2010, 2011) on copper toxicity to *D. villosus*, who found that this species is less sensitive to copper compared to other gammarids. They attribute its relatively low sensitivity to an efficient regulation and detoxification mechanism avoiding in this way polyunsaturated fatty acids peroxidation. Boets et al. (2010, 2011) also found that *G. roeseli* was ten times more sensitive to copper toxicity than *D. villosus*, whereas we found the opposite for Cd toxicity where *D. villosus* was five times more sensitive to Cd. The relatively low sensitivity to chemical stress of *D. villosus* is proposed by Boets et al. (2010, 2011) as a characteristic that could explain its invasive behaviour, since this species may have an advantage over other gammarids in polluted waters. This hypothesis, however, is not supported by our results with Cd. In addition, tests on fluoride toxicity to *D. villosus* indicated that this species is very sensitive to high fluoride concentrations (Gonzalo et al. 2010). Consequently, because of its high sensitivity to fluoride toxicity, Gonzalo et al. (2010) conclude that the potential risk of invasion for *D. villosus* in either natural or polluted freshwater ecosystems, exhibiting relatively high fluoride levels (>1.5 mg F^{-}/l), must be low. This shows that there is no real consistency in sensitivity to specific chemicals and that the sensitivity often depends upon the type of pollution. Comparative studies (between species) testing the effects of frequently encountered toxicants, coupled with monitoring data are therefore essential to give more conclusive insights in this matter.

Populations of the recently invading *Dikerogammarus* species are characterised by low genetic diversity, particularly of the mitochondrial genome (Müller et al. 2002). This limited genetic variability that is often observed in alien species may be suggested to explain the high sensitivity of *Dikerogammarus villosus*, a species that has recently invaded Flanders (Messiaen et al. 2010). Genetic variation in natural populations is influenced by four micro-evolutionary processes: selection, mutation, drift and migration (Templeton 2006). The invasion by alien species often involves a bottle-neck of the population because the number of individuals introduced is often limited. Smaller populations have a higher chance of inbreeding and genetic drift (Allendorf and Lundquist 2003). Consequently, this newly established populations are often genetically less diverse compared to the resident populations (Allendorf and Lundquist 2003). A study by Nowak et al. (2007) indicates that populations characterized by a low genetic diversity are more sensitive to chemical stress. Hence, alien species are expected to be more sensitive to toxicants. Despite a possible reduced diversity, it is observed that alien species are very opportunistic and can easily take in available niches (Boets et al. 2011b) and continue their expansion (Dlugosch and Parker 2008). Except for genetic diversity, probably other characteristics (e.g., high fecundity, high tolerance to fluctuations in salinity) inherent to alien species can contribute to their success (Grabowski et al. 2007).

In this study, we performed short-term toxicity tests to evaluate the differences in Cd sensitivity between different gammarid species. While concentrations tested in these experiments are higher than typically measured environmental concentrations, measured concentrations of Cd in the vicinity of point sources have been reported to reach levels between 1 and 18 μg Cd/l (ECB, 2007). This is in the range of the lowest 96 h LC_{50} value observed in the present study, i.e., 4.7 μg Cd/l for *Dikerogammarus villosus*. Nonetheless, it would certainly be useful to investigate long-term (sub-lethal) effects of lower (more commonly occurring) concentrations of Cd, such as effects on feeding activity, reproduction, etc. In future studies it may also be interesting to evaluate the possible effects of combined stress (e.g., salinity or other toxicants and metal stress) on the competitive advantage of alien gammarids.

Several studies indicate that populations that have been pre-exposed to toxicants have an increased tolerance compared to non-exposed populations (Clements 1999; Muyssen et al. 2002; Annabi et al. 2009). Two explanations for this increased tolerance are put forward: physiological acclimation (Howell 1985; Stuhlbacher and Maltby 1992) and genetic adaptation (Barata et al. 2002; Ward and Robinson 2005). In our experiments, no significant differences in cadmium sensitivity were observed between populations originating from historically metal polluted sites compared to those originating from a non-polluted site. Historically, there has been pollution with Cd present at the selected sites which has been reported by the Flemish Environment Agency, environmental reports and earlier conducted studies (VMM 2010; Lock et al. 2003). In line with these findings, Chaumot et al. (2009) recently found, in their study on a natural *Gammarus* population, that additive genetic variation (heritability in the narrow sense) for tolerance to cadmium was very weak and they therefore suggested that the evolution of this population to increased tolerance by genetic adaptation was not possible. Further, the recent concentrations of metals measured in the field at the polluted sites were elevated for zinc and lead but not for cadmium (all sites below detection limit). Thus, even if there would have been adaptation to high Cd concentrations in our tested populations in the past, the recent return of the environment to lower Cd concentrations could have resulted in a loss of their increased tolerance. Regardless, it is clear that the metal pollution at these sites is no longer affecting these populations' tolerances to cadmium. Nevertheless, given the currently still elevated concentrations of Zn and Pb in these sites, it might be worthwhile to investigate if these populations exhibit increased zinc and/or lead tolerance.

Comparing our toxicity data with previously published data, following observations were made. First, the 96 h-LC_{50} values for the indigenous *G. pulex* reported by Williams et al. (1985) and Felten et al. (2008) were 20 and 80 μg Cd/l, respectively and are in the same range as our observation (20.3 μg Cd/l). However, our 96 h-LC_{50} value for *G. pulex* and *G. fossarum* is different from recent work done on Cd toxicity to Gammaridae by Alonso et al. (2010), who report 96 h-LC_{50} values above 1.5 mg Cd/l for adults of *G. pulex* and above 0.2 mg Cd/l for adults of *G. fossarum*. Our values are 85 times and 5 times lower for *G. pulex* and *G. fossarum*, respectively. These high values reported by Alonso et al. (2010) are not in line with the earlier findings of Williams et al. (1985) and Felten et al. (2008). It is well-known that animals, including Gammaridae (Wright and Frain 1981), exposed in water with a high hardness show a lower sensitivity to Cd compared to those exposed in water with a low hardness (Tan and Wang 2011). However, although there was a difference in hardness between our test water (120 mg $CaCO_3$/l) and the Dutch Standard Water (210 mg $CaCO_3$/l) used by Alonso et al. (2010), it is very unlikely that these differences could explain the large differences found in LC_{50} values. When taking into account the US-EPA hardness correction for Cd toxicity data only a difference of a factor two could be explained (USGS 2006). A more likely explanation is that their reported effect concentrations are nominal concentrations. Hence, their actual exposure concentrations may have been much lower than the nominal concentrations (e.g., by adsorption to test vessel walls or particulate matter). In addition, differences found between different studies testing the sensitivity to cadmium toxicity may be due to differences in the life cycle stage (juvenile-adult), moult cycle stage, reproductive period or gender of the organism (McCahon and Pascoe 1988a, b, c; Sornom et al. 2010). Differences in other experimental conditions such as, temperature, salinity (Wright and Frain 1981; Martin and Holdich 1986; Roast et al. 2001) and pre-exposure to other toxicants (Howell 1985; Stuhlbacher and Maltby 1992) could also explain the differences observed in LC_{50} values.

Alonso et al. (2010) found that *G. fossarum* is significantly more sensitive (eight times) to Cd compared to *G. pulex*, whereas we found no significant difference between both species. They attribute the lower sensitivity of *G. pulex* to Cd toxicity to its wider tolerance range to factors such as reduced dissolved oxygen concentrations as compared to *G. fossarum*. Cadmium can be accumulated in crustaceans damaging the gills (Felten et al. 2008) and hindering gas uptake capacity. Therefore, species with a higher tolerance to low dissolved oxygen may have more chances to survive higher concentrations of cadmium (Alonso et al. 2010). All different gammarids tested occur at a range of different dissolved oxygen concentrations (Messiaen et al. 2010; Boets et al. 2010, 2011a). In general, species occurring at a narrow range of higher dissolved oxygen concentrations had lower LC_{50} values (Fig. 2b). *D. villosus* and *G. roeseli*, which occur at higher oxygen concentrations and have a narrow tolerance range for dissolved oxygen concentrations, had lower LC_{50} values, whereas *G. tigrinus* has a larger range of occurrence explaining its lower Cd sensitivity. *E. berilloni* and *G. fossarum* did not follow this trend, but the number of data regarding preference for dissolved oxygen concentration is much more limited for these two species. A larger dataset would be required for these species to determine if they do or do not fit within the trend observed for the other four species.

The European Framework Directive states that all surface waters in Europe should have a good water quality by 2015 (Herring et al. 2010; Directive 2000/60/EC). This entails that in the future not only indigenous species may profit from an improving water quality, but also alien species may potentially extend their distribution. This is in line with earlier findings of Boets et al. (2010), who found that *D. villosus* easily spreads in artificial rivers with a good chemical water quality and hard substrates. Since we found some alien species being less sensitive to cadmium compared to indigenous species, it is possible that in the future alien species sensitive to toxicants will continue their spread in Belgium.

A final observation of interest is that the smaller gammarid species investigated here were less sensitive to acute Cd stress than the larger ones (Fig. 2a). This seems to be in contrast with Grosell et al. (2002), who found that smaller freshwater organisms were more sensitive to acute Cu and Ag exposure. They related this to the mechanism of acute toxicity of these metals, i.e., an iono-regulatory disturbance of Na homeostasis by these two metals (both Na antagonists). The latter is more pronounced in smaller organisms, due to their larger surface to volume ratios. However, Cd is a Ca antagonist rather than a Na antagonist, and is not known if the mechanism of Ca balance disturbance could lead to a similar size-sensitivity relationship as those observed for Cu and Ag. Furthermore, the relationship by Grosell et al. (2002) covers a much wider range of sizes (10 orders of magnitude) than the range investigated here (<1 order of magnitude). Hence, the size-metal sensitivity relationship may not be applicable when only species within a more narrow size range are considered. In this context, Bossuyt and Janssen (2005) also did not find a relation between size and acute copper sensitivity for cladoceran species covering a threefold variation in size (length).

Conclusion

Overall, our results clearly show differences (factor 40) in cadmium toxicity between different species belonging to the family Gammaridae. Some alien species seemed to be more sensitive to Cd toxicity than indigenous species and they would probably not have a competitive advantage in Cd contaminated environments, compared to indigenous species. The indigenous species tested had a similar cadmium sensitivity and had LC_{50} values situated between those of the four alien species. Larger gammarid species were more sensitive to cadmium. Further, those species occurring at a wider range of oxygen concentrations in the field were less sensitive to cadmium toxicity. Finally, no significant differences in cadmium toxicity were found between populations of the same species originating from metal polluted or non-polluted sites.

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