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Contrasting sensitivities to toxicants of the freshwater amphipods *Gammarus pulex* and *G. fossarum*

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Abstract Amphipods are an important component of freshwater ecosystems. They are very often used in ecotoxicology, particularly the freshwater amphipod *Gammarus pulex*. However, there is scarce information on the sensitivity to toxicants of other species within the genus *Gammarus*. The present study aims to: (1) to compare sensitivities to ivermectin and cadmium between two species of freshwater amphipods (*G. pulex* and *G. fossarum*); (2) to compare sensitivities to these toxicants between juveniles and adults within each species; and (3) to assess whether the sensitivity to toxicants of these co-generic species is related with the wideness of their natural distribution area. Eight independent short-term bioassays (96 h) were conducted to assess sensitivity for ivermectin and cadmium for juvenile and adult life stages for each species. The LC50 (mortality) and EC50 (mortality plus immobility) were calculated to 48 and 96 h of continuous exposure. Our results showed that *G. pulex* was less tolerant to ivermectin than *G. fossarum*, the reverse being true for cadmium. In general, juveniles of both species were less tolerant to cadmium than adults. In the case of ivermectin, only for *G. fossarum* EC50 values were different between life stages. These results suggest that the risk assessment of toxicants to freshwater amphipods should include bioassays with the most sensitive species and life stage.

Keywords Cadmium · Ivermectin · Juveniles · Adults · Toxicity

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Introduction

Amphipods are an important component of freshwater ecosystems since they play a key role in the detritus breakdown process and constitute an important source of food for predators (Forrow and Maltby 2000; MacNeil et al. 2000). They can be found in high densities and may be very sensitive to a wide range of toxicants (Felten et al. 2008). These reasons explain why amphipods are often used in ecotoxicology. The most widely employed amphipod is the common species *Gammarus pulex* (L.) (Crustacea, Amphipoda), principally because this species has a wide distribution area (Barnard and Barnard 1983). Other *Gammarus* species, like *G. fossarum* (Koch), are comparatively less employed in ecotoxicology mainly due to their narrow distribution range. Furthermore, the available information on the sensitivities of these other *Gammarus* species to toxicants is scarce compared to *G. pulex*. *Gammarus pulex* and *G. fossarum* occur both in Dutch streams with *G. pulex* having a much wider geographical distribution than *G. fossarum* (Peeters and Gardeniers 1998). Optimum ranges for several common water quality parameters, such as current flow, stream dimensions, chloride, conductivity, nutrients and dissolved

oxygen levels are much narrower for *G. fossarum* than for *G. pulex* (Peeters and Gardeniers 1998). Thus we hypothesize that *G. fossarum* is more sensitive to toxicants than *G. pulex*.

Since the selection of the most sensitive stage permits a more suitable risk assessment of toxicants (McCahon and Pascoe 1988), it is of important ecotoxicological concern whether sensitivity to toxicants depends on life stages. According to McKim (1977) early fish stages (larval and early juvenile stages) are usually the most sensitive to the toxicity of different compounds. However, studies on freshwater macroinvertebrates have shown contradictory results with earlier stages of the dipteran *Chironomus riparius* and the crustacean *Asellus aquaticus,* being more sensitive to certain metals than adults (Williams et al. 1986; Naylor et al. 1990), and no difference in sensitivity for the mollusc *Potamopyrgus antipodarum* to ammonia between contrasting sizes (Alonso and Camargo 2004a). Furthermore, adults of the crustacean *A. aquaticus* were more sensitive than juveniles to low dissolved oxygen and ammonia (Maltby 1995). The literature also reports contrasting results for *G. pulex* with higher sensitivities to cadmium for juveniles, and higher sensitivity of adults to ammonia and low dissolved oxygen (McCahon and Pascoe 1988; Maltby 1995). The latter result can be partly explained by intraspecific differences in ventilation rate (Maltby 1995). Regarding *G. fossarum,* to our knowledge no data are available to the sensitivities between contrasting life stages so far. Therefore, for each toxicant and species, specific bioassays are necessary to know the relative tolerance of each stage. In the last decades many studies on rearing and handling of amphipods have been published (McCahon and Pascoe 1988; Morritt and Spicer 1996), facilitating the use of different life stages for laboratory bioassays.

The aims of the present study are (1) to test experimentally the hypothesis that *G. fossarum* is more sensitive to toxicants than *G. pulex*, and (2) to evaluate how sensitivities to these compounds vary between adults and juveniles for both species. We used two toxicants (ivermectin and cadmium) that differ in their mode of action. Ivermectin is a synthetic antiparasitic compound supplied to domestic cattle on a regular basis. This compound amplifies the effect of glutamate on the chloride channels of invertebrates, causing their opening (Rohrer and Arena 1995). Cadmium is a nonessential heavy metal which can be accumulated on gills and hepatopancreas of crustaceans causing damage on cells and disruption of enzymatic reactions (Felten et al. 2008). Several studies have shown that crustaceans are very sensitive to both compounds (Garric et al. 2007; Felten et al. 2008). The high sensitivity and the contrasting mode of action were the criteria used to select these toxicants.

Materials and methods

Amphipod collection and laboratory acclimatization

Gammarus pulex was collected using two sieves (2 and 4 mm mesh size) from a relatively unpolluted reach of the Heelsum Stream (51° 58′N, 5° 45′E) near Wageningen (The Netherlands) (De Lange et al. 2006a; b). *Gammarus fossarum* was collected using a dip-net from two relatively unpolluted upper streams (Geul Partij 50° 48′N, 5° 51′E, Cottesen 50° 45′N, 5° 56′E) near Maastricht (The Netherlands), where cadmium concentrations were less than 0.4 µg/l (data from the regional Waterboard Roer en Overmaas, The Netherlands). Invertebrates were transferred to the laboratory using plastic containers (5 l), and kept in aerated aquaria in a temperature and humidity controlled room (15 ± 1°C; 60% humidity). Animals were fed with stream-conditioned poplar (*Populus* sp.) leaves and were progressively acclimatized to the test water (Dutch Standard Water-DSW; Netherlands Normalisation Institute 1980) during at least 1 week prior to the bioassays. This standarized water was used because it has similar physical–chemical properties as most natural Dutch freshwaters (Netherlands Normalisation Institute 1980). For each species two life stages were selected on the basis of body length: adults (pre-copulatory pairs were rejected) and juveniles (McCahon and Pascoe 1988; Pockl 1992).

Bioassay design

Adults and juveniles of *G. pulex* and *G. fossarum* were used to test the effects of ivermectin and Cd in independent bioassays that lasted for 4 days. Independent bioassays were used to avoid possible cannibalism of adults over juveniles, or competition between different species. Toxic solutions (Cd and Ivermectin) and controls were renewed every 2 days. Amphipods were not fed during the bioassays.

Design of bioassays is shown in Table 1. For ivermectin bioassays, each amphipod was individually placed in a glass vessel with 40 ml of toxic solution, acetone control (acetone concentration = 0.0055 ml acetone/l), or control water (Dutch Standard Water-DSW, Netherlands Normalisation Institute 1980). For each treatment a total of 30 individuals were used. In each treatment, each group of 10 individuals was considered as a replicate to calculate the percentage of individuals showing a response. Therefore, three replicates (i.e. three groups of 10 individuals per group) were used for each treatment to calculate LC and EC values. All vessels were covered with a plastic foil to reduce water evaporation. As ivermectin is known to be photodegradable (Garric et al. 2007), bioassays were conducted in darkness, in a temperature and

Table 1 Experimental designs for the bioassays with *Gammarus pulex* and *Gammarus fossarum*

Toxicant	Species	Stage	Length (±SD) (mm) ($n = 40$)	Nominal concentrations	Solution volume (ml)	Individuals per vessel	Controls
Ivermectin	*G. pulex*	Adults	11.01 ± 1.55	1.5, 2.5, 3.5, 4.5, 5.5 µg/l	40	1	Acetone (0.0055 ml/l) and DSW
	G. pulex	Juveniles	4.70 ± 0.88	1.5, 2.5, 3.5, 4.5, 5.5 µg/l	40	1	Acetone (0.0055 ml/l) and DSW
	G. fossarum	Adults	10.11 ± 0.94	1.5, 2.5, 3.5, 4.5, 5.5 µg/l	40	1	Acetone (0.0055 ml/l) and DSW
	G. fossarum	Juveniles	5.06 ± 0.90	1.5, 2.5, 3.5, 4.5, 5.5 µg/l	40	1	Acetone (0.0055 ml/l) and DSW
Cadmium	*G. pulex*	Adults	10.6 ± 1.2	0.3, 0.5, 0.7, 1.0, 2.0 mg/l	300	10	DSW
	G. pulex	Juveniles	4.0 ± 0.7	0.1, 0.3, 0.5, 0.7, 1.0 mg/l	20	10	DSW
	G. fossarum	Adults	10.5 ± 0.91	0.1, 0.3, 0.5, 0.7, 1.0 mg/l	300	10	DSW
	G. fossarum	Juveniles	2.19 ± 0.37	0.05, 0.1, 0.3, 0.5, 0.7 mg/l	20	10	DSW

Mean length (measured as length from the antennal base to the third uropod at the end of the bioassays) is shown for each amphipod stage

humidity-controlled climatic chamber (15 ± 1°C; 60% humidity). Toxic solutions were prepared from a stock DSW solution (100 µg Ivermectin/l) which came from a stock acetone pestipur grade solution of 1 mg ivermectin/ml (Ivermectin, Dr. Ehrenstorfer Gmb-H, Lot no. 50905).

In the case of cadmium bioassays, ranges of nominal concentrations were different between bioassays (Table 1) because previous pilot bioassays showed different sensitivities between life stages and species (data not shown). Ten animals were placed in a glass beaker with 300 ml of one cadmium solution or DSW (adults), or in a plastic vessel with 20 ml of solution (juveniles). Small vessels were used for juveniles in order to facilitate counting of mortality and affected individuals under stereoscopic microscope (see below). Preliminary experiments showed no difference in survival between the two test volumes (data not further shown). Each treatment was in triplicate. In the case of *G. fossarum*, 1–7 days old juveniles grown in a laboratory culture were used. This culture was obtained by placing gravid females and pairs in pre-copula in a glass aquarium (5 l DSW) with conditioned poplar leaves and adult faeces (McCahon and Pascoe 1988). After 1 week neonates were collected with a pipette and used for the bioassay. This culture was used as a source of juveniles, since not enough juveniles of *G. fossarum* were collected in the field. All vessels were covered with a plastic foil in order to reduce water evaporation. The bioassays were conducted at a photoperiod of 8:16 h of light:darkness in a climatic chamber (15 ± 1°C, 60% humidity). Toxic solutions came from a stock solution of 40 mg Cd/l, prepared by dissolving the required amount of cadmium chloride in DSW ($CdCl_2$, ALDRICH, Lot no. 188165). Physical–chemical properties (water temperature, dissolved oxygen, conductivity, and pH) were monitored every 2 days for ivermectin and cadmium bioassays.

Endpoints and statistical analysis

Two endpoints were daily monitored in all bioassays: (1) the number of dead amphipods and (2) the proportion of affected animals (including both dead and inactive animals) (Alonso and Camargo 2008). An amphipod was considered to be dead when neither swimming nor movements were observed after touching the animal with a small stick. Animals were regarded inactive when no swimming was observed but some body parts were active (e.g. pleopods, antenna, gills, or uropods). Dead amphipods were removed every day in each bioassay. Juveniles were monitored using a stereomicroscopic microscope.

LC50 (based on mortality) and EC50 (based on mortality and inactivity) values for 48 and 96 h, and their respective 95% confidence limits, were calculated for each toxicant, species and life stage using the Multifactor Probit Analysis (MPA) (US Environmental Protection Agency 1991). This methodology solves the concentration-time-response equation via an iterative reweighted least square technique, LC and EC values being calculated by a multiple linear regression. The dependent variable is the probit of the proportion of animals responding to each concentration, and the independent variables are exposure time and toxicant concentrations (Alonso and Camargo 2006a). Nominal concentrations of both toxicants were used to calculate the LC and EC values. The observed mortality and inactivity plus mortality for 24, 48, 72 and 96 h and for each toxicant concentration were used as dependent variables.

Statistical differences ($P < 0.05$) in 48 and 96 h LC50 and EC50 values between species for each compound or between life stages for each species and compound were compared by 95% confidence limits overlap test: when LC or EC values did not overlap between the corresponding

pairs, differences were considered significant ($P < 0.05$); when values overlapped, a Z test was conducted to identify significant differences that could not be detected comparing the confidence limits (US Environmental Protection Agency 1991; Wheeler et al. 2006).

A two-way analysis of covariance (ANCOVA) was used to assess the effects of species (*G. pulex* and *G. fossarum*), life stage (adults and juveniles) and the interaction between these factors on mortality and number of affected animals (dependent variables) for each toxicant. Exposure concentrations of ivermectin or cadmium were used as covariate. One-two-way ANCOVA analysis was used for each toxicant and each endpoint. Data were tested for heterogeneity of variance using the Levene Test. When necessary, data were log-transformed to ensure the homogeneity of variance.

Results

Mean physical–chemical properties for each bioassay ($n = 4$–6) are shown in Table 2. No significant differences were found between bioassays ($P > 0.05$; ANOVA). All cadmium and ivermectin concentrations caused mortality in all bioassays, which was higher at increasing concentration and exposure time. Mean mortality in controls was less than 7% in all bioassays.

LC50 and EC50 values for ivermectin were lower for *G. pulex* than for *G. fossarum* (Fig. 1) indicating that *G. pulex* was the most sensitive species (either juveniles or adults). The only exception was juvenile 96 h EC50 values, which did not differ between species (Fig. 1). Life stages of each species did not differ in sensitivity to ivermectin except for *G. fossarum*, whose EC50 value was lower for juveniles than for adults (Fig. 1).

LC50 and EC50 values of *G. fossarum* for cadmium were lower than for *G. pulex* (Fig. 2). Therefore, *G. fossarum* was the most sensitivity species (both juveniles and adults). Juveniles were the most sensitive life stage for both species (Fig. 2).

The effects of toxicant (covariate), species (*G. pulex* and *G. fossarum*), life stage (adults and juveniles) and the interaction of both (species × life stage) are shown in Table 3 for each toxicant and endpoint. The two-way ANCOVA showed that toxicants (ivermectin and cadmium) had a significant effect on both monitored parameters (mortality and affected animals) ($P < 0.05$). The toxicity of ivermectin and cadmium differed between species ($P < 0.05$) for both monitored endpoints. Cadmium caused a contrasting effect between life stages (for both endpoints), whereas ivermectin only affected the proportion of affected animals in a different way in each life stages. The interaction between both independent variables was only significant for the ivermectin effect on the proportion of affected animals.

Discussion

Gammarus fossarum has in general much narrower optimum ranges for environmental variables than *G. pulex* in Dutch streams (Peeters and Gardeniers 1998), but according to our results this does not coincide with a higher sensitivity to toxicants. Our results demonstrated that *G. fossarum* was more sensitive to cadmium whereas *G. pulex* was more sensitive to ivermectin. Therefore, sensitivity to toxicants in general can not be derived or predicted from sensitivity to general environmental conditions. However, the higher tolerance of *G. pulex* to cadmium may be related with its wider tolerance range (including low concentrations) to dissolved oxygen as compared to *G. fossarum* (Meijerin 1991; Peeters and Gardeniers 1998). Cadmium can be accumulated in crustaceans damaging the gill cells (Felten et al. 2008) and hindering gas uptake capacity. Therefore the species with a higher innate tolerance to low dissolved oxygen

Table 2 Mean ($n = 4$–6) physical–chemical properties for each bioassay and overall bioassays ($n = 37$)

	Gammarus pulex				*Gammarus fossarum*				Overall
	Ivermectin		Cadmium		Ivermectin		Cadmium		
	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	
Water temperature (°C)	14.6 ± 0.48	15.1 ± 0.37	15.0 ± 0.10	14.9 ± 0.26	14.8 ± 0.39	15.2 ± 0.41	15.2 ± 0.21	14.7 ± 0.23	15.0 ± 0.35
Dissolved oxygen (mg O_2/l)	7.7 ± 1.25	7.9 ± 0.69	7.8 ± 0.85	7.7 ± 0.61	7.8 ± 0.60	8.1 ± 0.48	8.8 ± 0.45	8.5 ± 0.43	8.0 ± 0.72
Conductivity (µS/cm)	747 ± 26.3	722 ± 34.2	700 ± 9.1	702 ± 11	735 ± 23.8	712 ± 17.2	705 ± 12.9	716 ± 40.5	717 ± 27
pH	8.1 ± 0.17	8.1 ± 0.19	8.0 ± 0.18	8.0 ± 0.19	7.9 ± 0.26	8.0 ± 0.15	8.2 ± 0.10	8.1 ± 0.17	8.1 ± 0.18

No significant differences were found between bioassays for each parameter ($P > 0.05$; ANOVA)

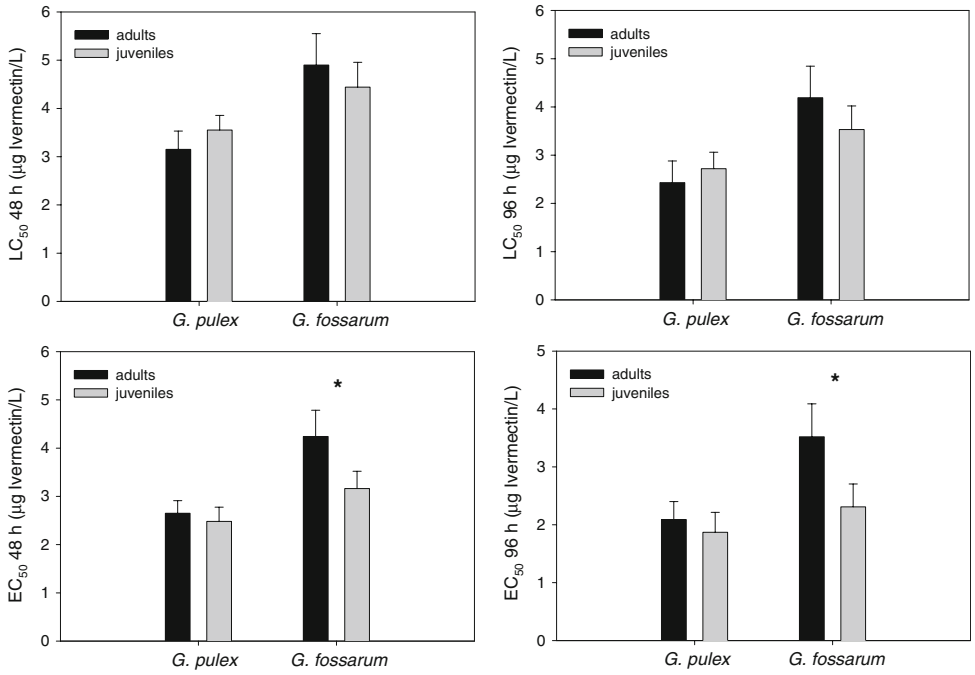

Fig. 1 LC50 (*upper panels*) and EC50 (*lower panels*) values for ivermectin, calculated after 48 h (*left hand side panels*) and 96 h (*right hand side panels*) exposure. *Error bars* represent 95% confidence interval. *Asterisks* indicate significant difference within a species between sizes. Differences in sensitivities between species for each size were significant for all comparisons, except 96 h EC50 for juveniles

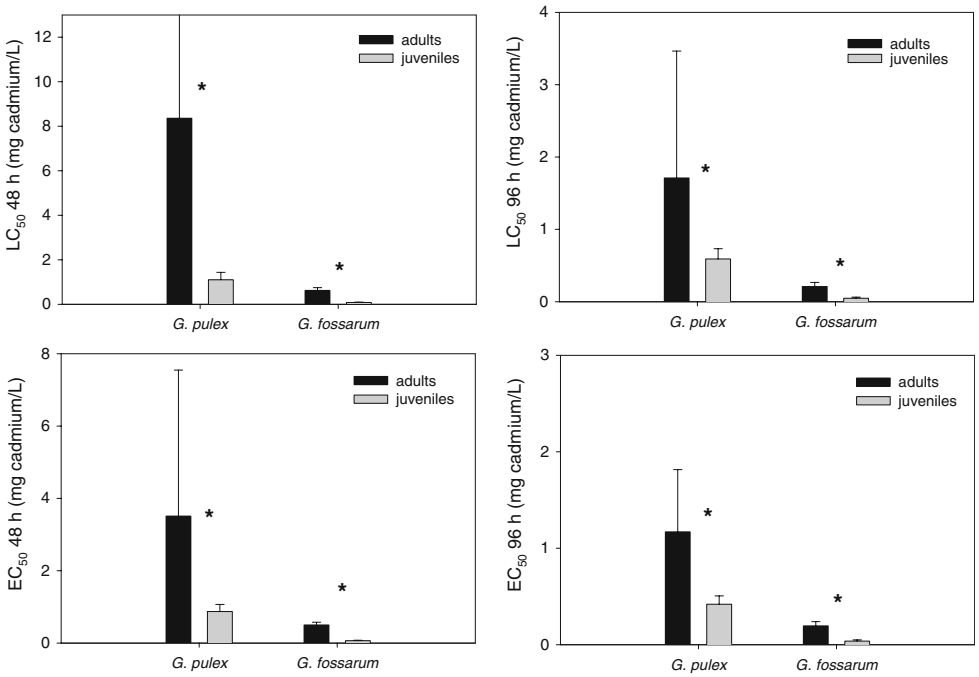

Fig. 2 LC50 (*upper panels*) and EC50 (*lower panels*) values for cadmium, calculated after 48 h (*left hand side panels*) and 96 h (*right hand side panels*) exposure. *Error bars* represent 95% confidence interval. *Asterisks* indicate significant difference within a species between sizes. Differences in sensitivities between species for each size were significant for all comparisons

(e.g. *G. pulex*) may have more chances to survive under this circumstance. A similar trend was also found by Maltby (1995) for *G. pulex* and the isopod *Asellus aquaticus.* In her study *A. aquaticus* was five times more tolerant to low dissolved oxygen than *G. pulex* and the isopod was also more tolerant to ammonia than *G. pulex*. This toxic compound also affects the oxygen uptake and gills (Rebelo et al. 2000). Therefore, species sensitivity to toxicants that affect oxygen uptake still may be predicted from the optimum ranges for oxygen conditions, but it should be investigated further.

Juveniles of both species were more sensitive than adults to exposure to cadmium. This has been observed previously for the toxicity of cadmium to *G. pulex* (McCahon and Pascoe 1988), and for other invertebrates (first instars larvae vs. last instars larvae in *C. riparius* and *Agapetus fasciatus*; Williams et al. 1986; McCahon et al. 1989; juveniles vs. adults in *Lymnaea stagnalis*; Coeurdassier et al. 2004). This

Table 3 Results of the two-way analysis of covariance (ANCOVA) using as dependent variables the mortality or affected animals (dead and inactive animals) and as independent variables the species (*G. pulex* and *G. fossarum*) and life stages (adults and juveniles)

Source of variation	DF	*F*	*P*
Mortality			
Ivermectin	1	189,489	**0.000**
Species	1	39,966	**0.000**
Stage	1	0.493	0.485
Species × stage	1	3,635	0.062
Cadmium	1	96,203	**0.000**
Species	1	91,332	**0.000**
Stage	1	27,606	**0.000**
Species × stage	1	0.259	0.613
Affected animals			
Ivermectin	1	157,701	**0.000**
Species	1	36,331	**0.000**
Stage	1	6,673	**0.012**
Species × stage	1	9,083	**0.004**
Cadmium	1	55,921	**0.000**
Species	1	49,616	**0.000**
Stage	1	25,041	**0.000**
Species × stage	1	0.232	0.632

The covariate was the toxicant concentrations

Bold letters show the *P* values that are significant, all *P* values are less than 0.05

shows that the selected life stage is a very important issue in the ecological risk assessment of cadmium (McCahon and Pascoe 1988). For instance, if cadmium tolerance is compared across amphipod populations, the population structure must be taken into account, as a higher proportion of juveniles will result in higher population sensitivity. Therefore, in order to set quality standards, ecotoxicological assays have to be carried out on the most sensitive life stage for each species (McCahon and Pascoe 1988). For instance, if we base a cadmium ecological risk assessment (ERA) on adult data we probably underestimate the toxicity to amphipods. The hazardous concentration derived from this ERA is not suitable for streams or reaches where amphipods are dominant, especially in seasons with higher abundance of juveniles. Additionally, as rearing *G. fossarum* under laboratory conditions is possible, juveniles for ecotoxicological bioassays can be easily obtained. Under a population point of view, the critical life stage is the most important one (e.g. the most sensitivity life stage).

The results showed a contrasting effect between life stages to cadmium toxicity. Cadmium can be taken up by crustaceans through the gut and the gills (Wright 1980; Marsdena and Rainbow 2004), and this uptake can be passive (by simple diffusion) or active (mainly by accidental cadmium uptake through calcium pump) (Wright 1980; Rainbow and Dallinger 1993). The higher sensitivity to cadmium of juveniles found in both species may be attributed to their higher surface area to volume ratio as compared to adults, to a higher active calcium uptake in juveniles to supply growing necessities, and/or to the thinner body covering of juveniles (Wright 1980; Rand 1995; Pastorinho et al. 2009). A recent study (Pastorinho et al. 2009) showed that neonates and juveniles of the estuarine amphipod *Echinogammarus marinus* can bioaccumulate higher body burdens of cadmium than adults after 96 h of exposure to 1 mg Cd\l. A similar trend was observed for other groups of invertebrates (Tessier et al. 1996; Reinecke et al. 2003; Marsdena and Rainbow 2004). Additionally, other factors such as contrasting ventilation rate and numbers of ionoregulatory cells, different moulting rate, and contrasting detoxification mechanisms can also contribute to explain the observed differences between life stages (Marsdena and Rainbow 2004; Buchwalter and Luoma 2005; Veltman et al. 2008).

In our study ivermectin toxicity is independent of the life stages, except for the EC values of *G. fossarum*. This shows that for ivermectin, species has higher influence on contrasting sensitivities than life stages. Therefore, toxicological results from amphipod populations seem to depend more of species innate properties than on population structure. Therefore, we conclude that for ivermectin risk assessment the chosen species is more important than selected life stage for bioassays.

The comparison of monitored endpoints (mortality versus mortality plus immobility) has shown lower values for immobility (EC values) than for the classical mortality endpoint (LC values). The same result has been previously found for other toxicants (ammonia and nitrite) and species (the planarian *Polycelis felina*, and the amphipods *Echinogammarus echinosetosus* and *Eulimnogammarus toletanus*) (Alonso and Camargo 2006b; 2008). The differences between both endpoints were especially contrasting for ivermectin, where juveniles showed higher sensitivities for immobility than adults. However, the contrasting difference between both endpoints has not been found by other authors for the mollusc *Lampsilis cardium* (Newton et al. 2003). We suggest that, for amphipods, the use of mortality plus immobility endpoints (EC and LC values) in ecological risk assessment can be a step forward, as the lower the toxicological values included in the species sensitivity distribution (SSD), the lower the estimated safe levels to the community are (Posthuma et al. 2002). Additionally, EC values have been previously found to be a good level to avoid short-term mortality or sub-lethal effects (e.g. egestion rate, movement) to other species of invertebrates (e.g. mollusc, amphipod, planarian) and toxicants (e.g. ammonia, nitrite) (Alonso and Camargo 2004a; b; 2006b; 2008). Our study highlights the contrasting sensitivity to

toxicants between species of the same genus. However, these short-term toxicity tests usually employ high non-realistic environmental concentrations (Alonso et al. 2009). Therefore, to get a more realistic approach, further studies are necessary to compare sub-lethal effects of toxicants (e.g. feeding activity, behaviour, etc.) between different species of freshwater amphipods. Several methods have been developed for that purpose during last decades (Taylor et al. 1993; Gerhardt 1995; Alonso et al. 2009).

Our results are expressed as nominal concentrations of ivermectin and cadmium, so these concentrations could have been reduced by adsorption or dissipation processes during bioassays. Therefore, our LC/EC values can not be directly compared with previous studies where LC values are expressed as actual concentrations or nominal concentrations were confirmed (Stuhlbacher and Maltby 1992: Felten et al. 2008). However, given the similar protocol across all bioassays, the likely reduction of toxicant concentrations had to be similar among them, allowing a reliable comparison of sensitivities between species/life stages.

We conclude that the toxicity of cadmium and ivermectin was different between both species of amphipods. None of the species showed higher sensitivity for both toxicants. Differences in sensitivity to toxicants between life stages seem to be related to the mode of action of toxicants, rather than to the species. Cadmium was more toxic to juveniles and ivermectin showed the same toxicity for both stages. For improving the ecological risk assessment, we suggest that bioassays have to be conducted on the most sensitive life stage for each selected species, and on the highest number of species affordable. Additionally, we recommend the use of juveniles for cadmium and ivermectin bioassays since they show the same or lower tolerance than adults, and can be easily reared under laboratory conditions.

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