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Acanthocephalan parasites: help or burden in gammarid amphipods exposed to cadmium?

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Abstract We investigated the influence of the acanthocephalan parasite *Polymorphus minutus* on the mortality of its intermediate host, *Gammarus roeseli*, exposed to cadmium, by the measure of LC_{50-96h} values as well as the bioaccumulation of cadmium both in the host and in its parasite. LC_{50} results revealed that infected *G. roeseli* males died less under cadmium stress than uninfected ones; while the converse has been observed in females. Cadmium resistance of infected males could be explained by a weaker bioconcentration factor (BCF) than in females. The lower BCF in infected individuals was closely related with an uptake of cadmium by *P. minutus* in its host. Nevertheless, although infected females had both weaker BCF and cadmium concentration in their body, the presence of *P. minutus* did not induce lower mortality than uninfected females. On the contrary, their sensitivity to cadmium was increased by the presence of *P. minutus*. We discuss the hypothesis that differences of mortality between uninfected and infected gammarids could be explained by a difference of cadmium bioconcentration in host, and by the cadmium bioaccumulation in the parasite. Indeed, results suggested that *P. minutus* could help *G. roeseli* to face with stress, what contributed to keep the host alive and favour the parasite transmission.

Keywords Cadmium · Parasitism · *Gammarus roeseli* · *Polymorphus minutus* · LC_{50} · BCF

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Introduction

In recent years, there has been an increase of interest about the influence of parasite in their host under environmental contaminations. Parasites are known to disrupt behavioural and physiological host responses (Sures 2004; Marcogliese and Pietrock 2011), and several studies underlined the parasite influence on host responses when exposed to pollutants. Marcogliese et al. (2005) described an increase of antitoxic enzymes activities (i.e. catalase, glutathione *S*-transferase) in the yellow perch infected by nematode and digenean parasites in river impacted by several metals. In contrast, a depletion of the antitoxic defences has been observed in digenean-infected cockles exposed to cadmium (Baudrimont et al. 2006) or in zebra mussels infected by *Ophryoglena* sp. exposed to a multistress environment (Minguez et al. 2009). This decrease of antitoxic defence compounds could lead to an increase of host damage as it was the case in *Gammarus roeseli* infected by vertically-transmitted microsporidia parasites exposed to cadmium (Gismondi et al. 2012).

In the case of complex life cycles, parasite uses at least one intermediate host to its larval development and a final host to mature and reproduce. Among the numerous complex life cycle parasites, acanthocephalans are known to alter the phenotype of their intermediate hosts in a way that make it more prone to predation and thus, favour its transmission to the final vertebrate host (Kennedy 2006; Lagrue et al. 2007). If behavioural changes induced by the parasite have been intensively studied (Cézilly et al. 2000; Bauer et al. 2005; Médoc et al. 2006), the modifications of host antitoxic responses has become a topic of interest in a context of rising pollution in freshwater ecosystems. Sures and Radszuweit (2007) described that the cystacanth stage of *Polymorphus minutus* prevented the synthesis of heat shock protein 70 in *G. roeseli*, when subjected to a thermal disturbance or a palladium exposure. Cornet et al. (2009) have shown a decrease of the prophenoloxidase system and the haemocyte concentration, two major parameters of crustacean immunity, in *Gammarus pulex* infected by three acanthocephalan parasites: *Pomphorhynchus laevis*, *P. tereticollis* and *P. minutus*.

Because acanthocephalan parasites need their host to survive both for their own development and for their transmission to a definitive host (Plaistow et al. 2001), we hypothesized that parasite could help its intermediate host to cope with stress conditions, what favours its transmission to the final host. We also hypothesized that the decrease of antitoxic defences already shown in previous ecotoxicological studies could be due to the fact that the host could need less defences to face with stress.

We tested our hypothesis in the widespread amphipod *G. roeseli*, an intermediate host for the acanthocephalan parasite *P. minutus*, which modifies the gammarid behaviour (Médoc et al. 2006; Médoc and Beisel 2009). We first studied the influence of the acanthocephalan parasite *P. minutus* on the lethal concentration 50 of cadmium (LC_{50}—concentration that caused the death of 50% of individuals) reflecting the resistance of the gammarid host. Then, we compared the cadmium accumulation in the body-whole of uninfected and infected *G. roeseli* after having removed parasites. The accumulation of cadmium was also measured in the body-whole of *P. minutus* extracted from its host.

Materials and methods

Sampling collection and maintenance

Gammarus roeseli uninfected and infected by *P. minutus* were collected in April 2010 with a pond net in the French Nied River (Rémilly, North-eastern France, 49°00′N and 6°23′E), where cadmium concentrations were less than 0.2 μg L^{-1} (unpublished data). Infected *G. roeseli* were easily identified thanks to the cystacanth stage of *P. minutus* appearing as an intense orange dot through the cuticle. Individual gammarids were sorted in field after observation of the gnathopod size, a sexually dimorphic character, males having gnathopod size larger than females. Gammarids were transferred to the laboratory in containers filled with river water, and then acclimated at 15°C in an EDTA-free Elendt M4 solution. Animals were fed ad libitum with alder leaves and acclimated 5 days in these conditions before being used in experiments. This acclimation period allows selecting only healthy individuals for the experiment.

LC_{50} assessment

This experimentation aimed at determining the cadmium LC_{50} values of *G. roeseli* uninfected and infected by *P. minutus*. Four conditions were tested according to the gender and the infection status (i.e. uninfected versus *P. minutus*-infected). Five different cadmium concentrations were prepared using a 12 mg L^{-1} of $CdCl_2$ stock solution and Elendt M4 modified solution: 0, 6, 18, 54 and 162 μg Cd L^{-1}. These concentrations were chosen according to a preliminary cadmium exposition (data not shown) and the LC_{50-96h} observed by Felten et al. (2008) in *G. pulex* (82.1 μg Cd L^{-1}). Three replicates of 10 gammarids were exposed at 15°C for 96 h to the 5 cadmium concentrations in 500 mL glass tank with a photoperiod of 14:10 (d:n) and without alimentary resource. Dead individuals were removed each 24 h to avoid cannibalism and survivors were counted at the end of the experimentation.

Cadmium accumulation

At the end of the exposure, alive uninfected and infected *G. roeseli* of each gender, exposed to each cadmium condition, were immediately frozen at −196°C and stored at −80°C awaiting the cadmium analysis, taking care to remove the cystacanth from the infected hosts. Similarly, five pools of five cystacanths were carried out being careful to separate the parasites from infected males and infected females, and stored as above prior to cadmium analysis. Due to high mortality rates in the highest cadmium concentrations, the cadmium analysis was conducted only on *G. roeseli* exposed to the first three concentrations: 0, 6 and 18 μg Cd L^{-1}.

To analyze cadmium concentration, samples were dried at 100°C during 48 h, and weighted. Then, they were digested with 50 μL Suprapur nitric acid (HNO_3) at 80°C for a minimum of 12 h until a limpid solution was obtained. The volume was then adjusted to 1 mL with deionized water. Cadmium was measured in these acid solutions by flameless atomic absorption spectrophotometry (AAS). Cadmium controls were based on the Spectrapure standards AS-SPS quality control material (Oslo, Norway). The cadmium accumulation was expressed in ng Cd mg^{-1} dry weight.

The bioconcentration factors (BCF) were calculated using the method of Taylor (1983) described in Duquesne et al. (2000). BCFs were only estimated for *G. roeseli* due to the fact that *P. minutus* are located in haemolymph of the gammarid and thus, not exposed to cadmium concentration in the water. Moreover, the bioaccumulation of cadmium was measured in the body-whole of *G. roeseli*, so we could not estimate that this value also corresponded to the concentration of cadmium in the haemolymph, concentration at which the cystacanth was exposed.

Statistical analysis

LC_{50-96h} values were estimated by using the Excel macro REGTOX according to the method of Vindimian et al. (1999). Statistical comparisons of cadmium accumulation data were performed using a two way-ANOVAs following by the post hoc HSD Tukey tests, after having log transformed data to meet the normality and the variance homogeneity conditions. Similarly, significant differences of BCFs were tested using three way-ANOVAs followed by post hoc HSD Tukey tests. All tests were two-tailed with significant differences considered at the level of $p \leq 0.05$.

Results

LC_{50} assessment

Results of LC_{50-96h} values according to gender and infection status of *G. roeseli* were summarized in Table 1. Cadmium sensitivity was different according to gender. Indeed, uninfected females were twice more resistant to cadmium than uninfected males. However, when *G. roeseli* were infected by *P. minutus*, males were four times more resistant to cadmium than females. Finally, results shown that *P. minutus*-infected males were twice less sensitive to cadmium than uninfected ones; while *P. minutus*-infected females were three times more sensitive than uninfected ones.

Cadmium accumulation

After 96 h of cadmium exposure, there was a dose–response relationship for the cadmium accumulation in *G. roeseli*, whatever the gender and infection status (Fig. 1). Indeed, whatsoever the gender, cadmium concentrations in uninfected gammarids exposed at 6 and 18 μg Cd L^{-1} were on average 12 and 24 times higher than controls. When *P. minutus* was present, cadmium concentrations in exposed males were 4 and 10-fold higher than their controls; while in exposed females, they were 12 and 20-fold higher than unexposed ones. Results have also shown that cadmium accumulation in gammarids was higher in absence of *P. minutus* whatever the cadmium exposure. In

Table 1 LC_{50-96h} values (±95% confidence interval) of *Gammarus roeseli* according to gender and the presence/absence of *Polymorphus minutus*, after 96 h of cadmium exposure ($n = 30$ for each condition)

LC_{50-96h} (μg Cd L^{-1})			
Males		Females	
Uninfected	Infected	Uninfected	Infected
50 (26–92)	146 (94–269)	107 (72–167)	35 (25–50)

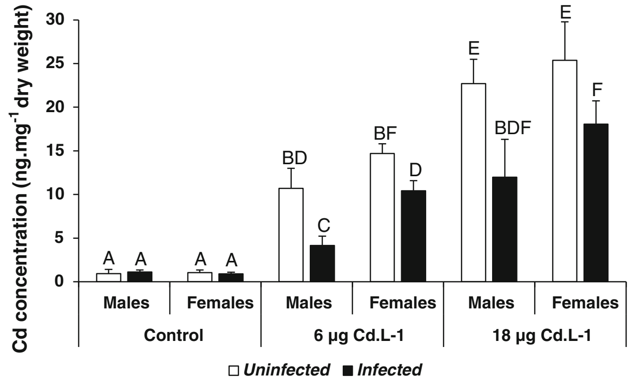

Fig. 1 Concentrations of cadmium measured in *G. roeseli* exposed at 6 and 18 μg Cd L^{-1} according to the gender and the infected status. Five individuals were analyzed for each condition. *Different letters* above the bars indicate significantly different values (p values < 0.05)

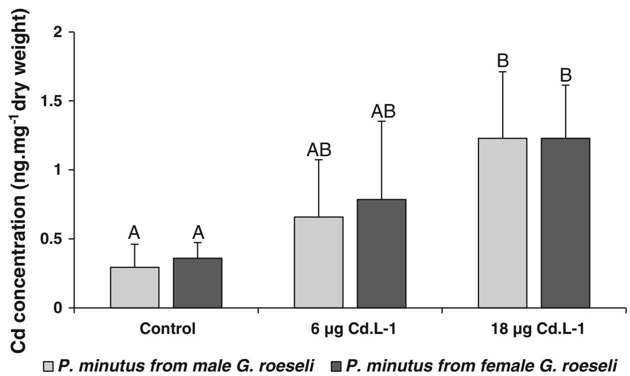

Fig. 2 Concentrations of cadmium determined in *P. minutus* from *G. roeseli* males and females exposed at 6 and 18 μg Cd L^{-1}. Five pools of five cystacanths were analyzed for each condition. *Different letters* above the bars indicate significantly different values (p values < 0.05)

fact, infected males had accumulated in average twice less cadmium than uninfected ones; while infected female had in average accumulated 1.5-fold less than uninfected ones.

In the cystacanth of *P. minutus*, the cadmium accumulation was also described by a dose–response relationship (Fig. 2). Indeed, *P. minutus* cystacanths from gammarids exposed to 6 μg Cd L^{-1} tended to accumulate more cadmium than their respective controls. Moreover, *P. minutus* from individuals exposed to 18 μg Cd L^{-1} showed significantly higher cadmium concentrations than their respective control. No difference was observed in the cystacanth accumulation according to the host gender.

Results revealed that *P. minutus* cystacanth accumulated less cadmium concentration than *G. roeseli* host. In fact, according to the cadmium concentration in the exposure medium, gammarids had on average from 3 to 15 times more cadmium than their respective cystacanths. A linear relationship between both cadmium concentrations in *G. roeseli* and *P. minutus* was observed (Fig. 3). Slopes of relationships between the cadmium concentrations in

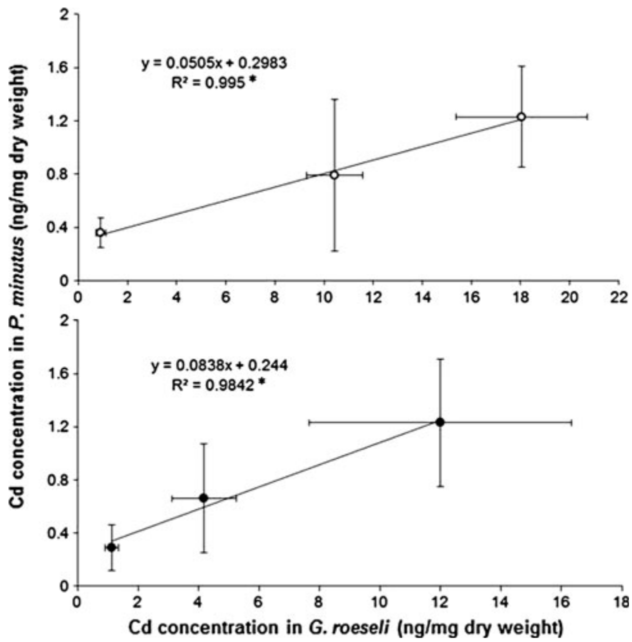

Fig. 3 Relationship between cadmium concentrations in *P. minutus* body tissues and *G. roeseli* females (*open circle*) and males (*filled circle*) body tissues after 96 h of exposure. *Asterisks* indicate p value < 0.015

P. minutus and its host were approximately the same for both genders (ANCOVA test, $p = 0.21$).

Bioconcentration factors

BCFs were evaluated in *G. roeseli* males and females, uninfected or infected and exposed to 6 and 18 µg Cd L^{-1}. Results confirmed that uninfected gammarids accumulated more cadmium than infected ones (Fig. 4). Whatever the individual gender and the cadmium concentration, uninfected *G. roeseli* had a BCF value higher than infected ones. When infected, BCFs were not different according to the cadmium concentration; while BCFs were higher for uninfected gammarid exposed at 6 µg Cd L^{-1} (130 on average) than for uninfected ones exposed to 18 µg Cd L^{-1} (85 on average).

Discussion

This study was carried out to investigate the effect of the acanthocephalan parasite *P. minutus* on the mortality of its intermediate host *G. roeseli*, exposed to cadmium, especially on its $LC_{50–96h}$ value and cadmium bioaccumulation.

The $LC_{50–96h}$ values showed that gammarids have different mortality depending on gender, and infection status. Indeed, in the absence of *P. minutus*, females were two times less sensitive than males. This could be due to higher

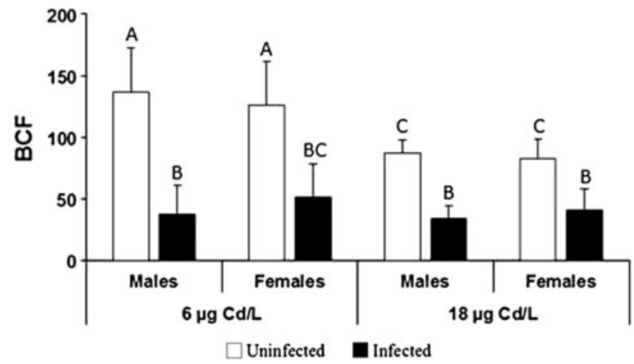

Fig. 4 Bioconcentration factors (BCFs) of *G. roeseli* according to the gender, the infection status and the cadmium exposure. Five individuals were analyzed for each condition. *Different letters* above the bars indicate significantly different values (p values < 0.05)

antitoxic defences reflecting by higher antioxidant enzyme activities (Sroda and Cossu-Leguille 2011a) or higher reduced glutathione concentration (Gismondi et al. unpublished data), but also due to higher energy reserves in females, for reproductive demand, and mobilized if necessary for antitoxic defence syntheses. However, previous studies reported different mortality between genders in a pollutant context. Sroda and Cossu-Leguille (2011b) demonstrated that females of *G. roeseli* and *Dikerogammarus villosus* were more sensitive to copper than respective males, the converse of our results with cadmium. *G. roeseli* submitted to a salinity stress revealed a significant higher sensitivity of females in survival, ventilation and ionoregulation (Sornom et al. 2010). When authors have reported a lower mortality of females in comparison to males (Emery 1970; Benoit et al. 1976; Heit and Fingerman 1977, our study), the tested substances were always a non-essential molecule (e.g. cadmium, mercury, cresol).

In our study, cadmium $LC_{50–96h}$ of uninfected *G. roeseli* was in the same range that those obtained by Felten et al. (2008), 82 µg Cd L^{-1} for *G. pulex*. This highlighted that male gammarids infected by *P. minutus* were more resistant to cadmium exposure than uninfected ones, while infected females were more sensitive than uninfected ones. Female results were in agreement with McCahon et al. (1988) who had determined cadmium LC_{50} value higher for uninfected *G. pulex* than *P. laevis*-infected ones. Similarly, Brown and Pascoe (1989) showed that *P. laevis*-infected *G. pulex* exposed to 2.1 µg Cd L^{-1} died faster than uninfected ones. In the present study, the difference observed between cadmium $LC_{50–96h}$ of uninfected and infected males illustrates a help from the parasite to its host to face with cadmium stress, what contributes to keep it alive. The parasite help could be due to its antitoxic defences such as heat shock protein 70 increased in

P. minutus exposed to palladium (Sures and Radszuweit 2007), or catalase activity present in secretion products and total extracts as observed in nematode or helminth parasites (Dzik 2006; Morassutti et al. 2011). By this action, *P. minutus* could increase its own survival and favour its transmission to its final vertebrate host, a water bird.

The gammarid death could be closely related to a modification of cadmium accumulation. Cadmium accumulation in gammarids has been described in the literature for *G. pulex* (Stuhlbacher and Maltby 1992; Felten et al. 2008; Vellinger et al. 2012), *G. locustra* (Clason and Zauke 2000), or *G. oceanicus* (Zauke et al. 2003), but no study has been devoted to *G. roeseli* and only a few ones have investigated the influence of parasite on the metal bioaccumulation in the host. The present work highlighted that infected *G. roeseli* accumulated less cadmium in their body-whole than uninfected ones, conducting to the weakest BCF in *P. minutus*-infected gammarids. This result was in agreement with Heinonen (2000) in *Pisidium amnicum* clams who observed lower BCF in trematode-infected individuals than in uninfected ones. Our results have shown that *P. minutus* had absorbed cadmium. The cystacanth lives in the haemocoel of *G. roeseli*, surrounded by the haemolymph which contain toxic metals when individuals are exposed to pollutants (Xu and Pascoe (1993): *G. pulex* exposed to zinc). The cadmium accumulation by an acanthocephalan parasite has already been determined in *P. laevis* infecting *G. pulex* (Brown and Pascoe 1989). Moreover, other metal pollutants can be accumulated by acanthocephalan cystacanth, as palladium in *P. minutus* cystacanth (Sures and Radszuweit 2007).

Regarding results of this work, *P. minutus* could influence the mortality of *G. roeseli* males by influencing the cadmium bioaccumulation. Indeed, a weak uptake of cadmium by the host could lead to a weak formation of reactive oxygen species, responsible of cellular damage in organisms (Sies 1986).

In contrast of *G. roeseli* male results, *G. roeseli* females were more sensitive to cadmium when *P. minutus* was present, although their BCF and the cadmium concentration in the body-whole were weaker compared to uninfected ones. This higher mortality could be explained by the presence of other parasites as vertically-transmitted microsporidia parasites which are found in many arthropod species (Terry et al. 1999; Ironside et al. 2003; Weedall et al. 2006; Haine et al. 2007), especially in females (Haine et al. 2004). In a previous study, we demonstrated that these microsporidia parasites accentuated oxidative stress in *G. roeseli* females exposed to cadmium (Gismondi et al. 2012). Results obtained in the present study suggested that the potential presence of two parasites (i.e. *P. minutus* and vertically-transmitted microsporidia) could weaken the host survival and accentuate the cadmium stress.

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