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Do male and female gammarids defend themselves differently during chemical stress?

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ABSTRACT

To investigate xenobiotic impacts on organism physiology, several studies involve biomarker assessment. However, most studies do not take into account the toxic effect on both males and females. Here, we have investigated the influence of gender on the detoxification response (reduced glutathione, metallothionein, γ-glutamylcystein ligase and carotenoid), energy reserves (protein, lipids and glycogen) and biomarker of toxic effects (malondialdehyde) in *Gammarus roeseli* exposed to cadmium. A principal component analysis revealed that *G. roeseli* males and females were differently impacted by cadmium. We observed lower malondialdehyde levels in females than in males, whatever the condition tested (i.e. control, 2 and 8 μg Cd L^{-1}), although the pattern of responses of control and exposures to 2 or 8 μg L^{-1} was the same for both genders. Results could be linked to apparently more effective detoxification displayed by females than by males. Protein concentrations were unchanged in both genders, lipids contents were always significantly decreased and glycogen contents decreased only in females. This study supports the importance of taking into account the gender in ecotoxicological studies to have an overview of xenobiotics effects on a population.

1. Introduction

A rising anthropogenic contamination of aquatic environments is still in progress, and this disturbance is well known to induce dysfunctions in organisms, which could lead to population declines in sensitive species. To evaluate these environmental risks, ecotoxicological studies use biomarkers (Peakall, 1994) which are mainly antitoxic compounds (e.g. metallothionein, reduced glutathione) that respond to various contaminations, but also various detoxification enzymes, as for example catalase or glutathione peroxidase (Allan et al., 2006; Vasseur and Cossu-Leguille, 2003). If these tools allow to evaluate the impact of environmental contaminations on organisms, one problem they pose is that their responses are not only influenced by pollutants, but can also vary according to confounding factors such as biotic factors (e.g. age, size) (Sroda and Cossu-Leguille, 2011a; Xuereb, 2009) or abiotic factors (e.g. temperature) (Geffard et al., 2005, 2007; Gismondi et al., 2012a; Serafim et al., 2002).

Among freshwater species, those that belong to the crustacean genus *Gammarus* sp. are popular and suitable organisms for ecotoxicological assessment of environmental pollutants at a large scale, mostly because this genus is widespread throughout a large range of marine and freshwater habitats. Gammarids are known to be easy to use in both laboratory and field studies (see Kunz et al., 2010 for a review). Hence many ecotoxicological studies have been carried out using these aquatic organisms to evaluate toxic impact of xenobiotics (Adam et al., 2010; Khan et al., 2011; Krapp et al., 2009; Sornom et al., 2010; Sroda and Cossu-Leguille, 2011b; Vellinger et al., 2012; Zubrod et al., 2010). Nevertheless, to our knowledge, only a few studies have been devoted to investigate the effect of xenobiotics on both male and female gammarids. In spite of the importance of the gender as a potential confounding factor, most studies have used only males or females, to avoid this source of variation, or have not taken into account the gender and used individuals regardless of their gender. Mixing both genders is in contradiction with the fact that the few studies in which both genders have been tested separately have highlighted significant differences between the responses of both genders. For instance, Sornom et al. (2010) observed that *Gammarus roeseli* females were more sensitive than males when exposed for 72 h to salinity stress. Indeed, females LC_{50} (concentration that caused the death of 50% of individuals) value was on average of 9.28 g NaCl L^{-1} in females as compared to male LC_{50} which was on average 12.24 g NaCl L^{-1}. In addition, the authors observed higher ventilation activity in females than in males. Under copper exposure, Sroda and Cossu-Leguille (2011b) have underlined higher sensitivity in females as compared to males in two gammarid species, i.e. *G. roeseli* and the invasive amphipod *Dikerogammarus*

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villosus, although females had higher antioxidant enzyme activity (e.g. glutathione peroxidase). In fact, the LC_{50} value of *G. roeseli* females was 2.5-fold lower than that of males (22 and 53 μg Cu L^{-1}, respectively), while the LC_{50} value of *D. villosus* females was 1.2-fold lower than males (230 and 275 μg Cu L^{-1}, respectively). In the same way, McCahon and Pascoe (1988) have found that females of *Gammarus pulex* were twice as sensitive as males when exposed to cadmium for 48 h. These few studies ultimately show that understanding of xenobiotic effects on both genders is of importance to provide early warning signals of population effects and protect them.

The aim of the present study was to examine the importance of gender on the responses of antitoxic defences and energy biomarkers in the amphipod *G. roeseli* exposed to cadmium for 96 h. Cd accumulates in the animal probably mostly through the gills, where metals can significantly influence osmoregulation and respiration (Vellinger et al., 2012). Usually, the internal concentration of metals in gammarids increases with exposure concentration and this bioaccumulation could induce oxidative stress due to the overproduction of reactive oxygen species (Vellinger et al., 2013). Antitoxic defences to protect cells were estimated by measuring the reduced glutathione (GSH) concentrations and the γ-glutamylcystein ligase (GCL) activity, as well as metallothionein (MT) and carotenoid concentrations. GSH is an essential tripeptide in the detoxification system: its thiol groups scavenge organic or metallic xenobiotics (Griffith, 1999; Vasseur and Leguille, 2004), but it also plays an important role as a substrate for several antioxidant enzymes like selenium-dependant glutathione peroxidase or glutathione-*S*-transferases. The γ-glutamylcystein ligase is the enzyme that limits the de novo GSH synthesis. Metallothioneins (MT) are involved in the binding of metallic compounds thanks to their thiol groups and contribute to protect tissues against oxidative damages (Bigot et al., 2011; Roesijadi, 1992). Carotenoids, which are used in antioxidant defences (Palozza and Krinsky, 1992) and involved in the reproduction (Gilchrist and Lee, 1972), were also measured. To evaluate cellular damages in organisms, we measured the malondialdehyde level (MDA). This compound is an end-product of the lipid membrane degradation and is considered as a biomarker of toxic effects. Finally, energy reserves of gammarids were estimated by measuring protein concentrations as well as total lipid and glycogen contents. We hypothesized (H1) that *G. roeseli* females are more sensitive to cadmium than males, according to most results obtained in previous studies. A second hypothesis is that a difference exists between males and females in terms of antitoxic defence mechanisms and capacities (H2), which explains the difference of sensitivity. Our third hypothesis (H3) is that the energetic cost of physiological responses to the chemical stress increases the use of body reserves in gammarids but with a different pattern between males and females for glycogen, lipids and proteins.

2. Materials and methods

2.1. Cadmium exposure

Males and non-ovigerous females of *G. roeseli* were collected using pond nets and artificial traps during the spring of 2012, in the French Nied River (Rémilly, North-eastern France, 49°00′ N and 6°23′ E) where cadmium concentrations in the water were lower than 0.2 μg L^{-1} (LADROME Laboratory, Valence, France). Non-ovigerous females were chosen to avoid the influence of the eggs (McCahon and Pascoe, 1988). Males and females were sorted out on the spot by observing gnathopods (smaller in females than in males). Gammarids were transferred to the laboratory in large containers filled with river water. In the laboratory, they were acclimated for 5 days at 15 °C in an EDTA-free Elendt M4 solution (Elendt and Bias, 1990), and fed ad libitum with alder leaves. Test solutions were prepared using EDTA-free Elendt M4 solution with $CdCl_2$ added to obtain two cadmium concentrations: 2 and 8 μg Cd L^{-1} (measured concentrations: 1.95 ± 0.03 and 7.93 ± 0.04 μg Cd L^{-1}). Controls consisted of EDTA-free Elendt M4 solution only. Cadmium concentrations were chosen according to (i) the 96 h LC_{50} obtained in our previous study which were 107 and 50 μg Cd L^{-1} for females and males, respectively (Gismondi et al., 2012b); and to (ii) the maximum admissible cadmium concentration in drinking water, which is 5 μg Cd L^{-1} (CD 98/83/EC, 1998). For each exposure condition, three replicates of 12 *G. roeseli* males and three replicates of 24 *G. roeseli* females were exposed at 15 °C for 96 h in aquaria previously saturated with the corresponding cadmium solutions for 5 days. During cadmium exposure, animals were not fed. More females were used because about 50% are infected by vertically-transmitted microsporidia, which could bias the interpretation of the biomarker responses and increase the female sensitivity (Gismondi et al., 2012c). As only females were infected by the vertically-transmitted microsporidia (Gismondi et al., 2012c), at the end of the experiment, gonadal tissues of females were dissected and the microsporidian status of females was verified using the method from Gismondi et al. (2012c) to keep only uninfected females. Then, 6 pools of 6 individuals (i.e. males and females separated) were constituted for each condition, frozen in liquid nitrogen and stored at −80 °C awaiting biomarker analyses.

2.2. Sample preparation

Each pool was homogenized with a Potter Elvejhem manual tissue grinder in 50 mM phosphate buffer KH_2PO_4/K_2HPO_4 (pH 7.6) supplemented with 1 mM phenylmethylsulphonylfluoride (PMSF) and 1 mM L-serine-borate mixture as protease inhibitors, and 5 mM phenylglyoxal as a γ-glutamyl transpeptidase inhibitor. The homogenization buffer was adjusted to a volume two-fold the wet weight of the sample pool (e.g. 200 μL of homogenization buffer for 100 mg of wet weight tissue). The total homogenate was divided into seven parts to measure the different parameters. For each replicate, two independent measures were made for each biomarker.

2.3. Biomarker measurements

All biomarker measurements were performed according to Gismondi et al. (2012d). Energy reserve (lipids, glycogen, proteins) measurements were conducted with spectrophotometrical methods while antitoxic defences (GSH, GCL, MT) and biomarker of toxic effects (MDA) were measured using HPLC methods.

In parallel, carotenoid concentrations were measured by a spectrophotometrical method adapted from Rauque and Semenas (2009). A volume of 40 μL of the total homogenate was diluted in 450 μL of 96% ethanol and kept for 6 h in the dark at 4 °C, before being centrifuged 10 min at 3500 × *g*. The optical density of the resulting supernatant was measured at 422, 448 and 476 nm. A commercial carotene mixture (Sigma–Aldrich, France) was used as a standard. Carotenoid concentrations were expressed in ng carotenoids mg^{-1} lipids.

2.4. Statistical analyses

All data met normality and homogeneity of variance assumptions (Shapiro and Bartlett tests, p-values > 0.05). Our data were analyzed by using ANOVA tests with respect to "individual gender" and "cadmium exposure" as fixed factors. Then, Tukey HSD post hoc tests were used to describe significant differences. All tests were performed with a 5% type-I error risk, using R 2.9.0 software.

The relationships between biomarker responses considered altogether and cadmium exposure conditions were assessed by using a principal component analysis (PCA) using R 2.9.0 software.

3. Results

ANOVA tests (Table 1) revealed an effect of gender, cadmium exposure and interaction between gender and cadmium exposure. Indeed, except for protein concentrations, cadmium exposure impacted all biomarker responses. Similarly, gender also influenced all biomarkers. The interaction between these two factors influenced four out of eight biomarkers: glycogen content, GSH concentration, GCL activity and MDA levels.

3.1. Energy reserves

Overall, cadmium exposures influenced the energy reserves of *G. roeseli* males and females. Protein concentrations were unchanged regardless of the gender (Fig. 1A), lipid contents were always significantly decreased (Fig. 1B) and glycogen contents were only decreased in females (Fig. 1C). In detail, lipid content was decreased in males and females according to a concentration-response relationship. In both genders, lipids contents were 1.3- and 1.8-fold lower at 2 and 8 μg Cd L^{-1} respectively, compared to controls (Fig. 1B). Contrary to lipid results, glycogen content was only impacted in *G. roeseli* females. Indeed, no significant difference was observed in males whatever the cadmium exposures while in females, glycogen content was in average 1.3-fold lower in exposed females than in controls (Fig. 1C).

3.2. Antitoxic defences

Like energy reserves, antitoxic defences were influenced by cadmium exposure in both gammarid genders (Table 1). In males, the GCL activity was significantly increased when animals were exposed to 8 μg Cd L^{-1}, while the exposure to 2 μg Cd L^{-1} did not result in a significant increase (Fig. 1D). The enzyme activity was significantly higher (1.7-fold) in males exposed at 8 μg Cd L^{-1}, as compared to unexposed ones. In females, regardless of the cadmium concentration, GCL activity was significantly higher in organisms exposed to 2 μg Cd L^{-1} (1.6-fold increase) and to 8 μg Cd L^{-1} (2.2-fold increase).

GSH concentration was also influenced by cadmium exposure since an 1.2-fold increase was observed at 2 μg Cd L^{-1} in both genders, as compared to unexposed ones (Fig. 1E). However, no significant differences were observed between the GSH concentrations measured at 2 μg Cd L^{-1} and those measured at 8 μg Cd L^{-1} in *G. roeseli* males and females.

Results of metallothionein concentrations showed a concentration-dependent increase in both genders (Fig. 1F). However, this increase was higher in males than in females. Indeed, MT concentration increased by a factor of 1.6 in males and of 1.3 in females exposed to 2 μg Cd L^{-1} as compared to respective controls. Moreover, at 8 μg Cd L^{-1}, MT concentration doubled in males and increased by a factor of 1.5 in females, as compared to respective controls.

Carotenoid levels significantly decreased in males according to a concentration-dependent relationship (Fig. 1G). Carotenoid contents were 1.2- and 1.4 lower in males exposed to 2 and 8 μg Cd L^{-1} respectively, as compared to controls. In contrast, carotenoid levels decreased significantly in females (1.5-fold decrease) exposed to 8 μg Cd L^{-1} compared to unexposed females. However, no significant difference was observed between carotenoid levels in control females and females exposed to 2 μg Cd L^{-1} (only a tendency of decrease was observed).

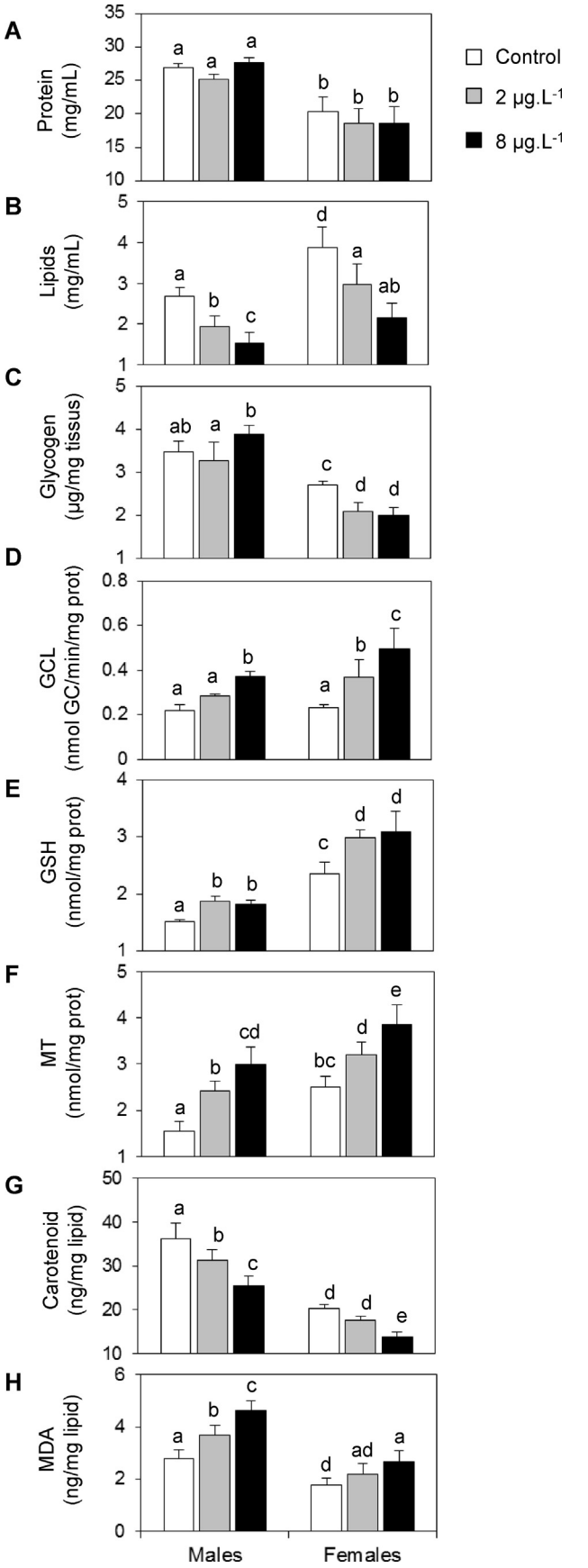

Fig. 1. Energy reserves (protein, lipids and glycogen), antitoxic defence (GSH, GCL, MT and carotenoids) and toxic effects biomarker (MDA) responses in *G. roeseli* males and females exposed to 2 and 8 μg Cd L^{-1} for 96 h. Letters above the bars indicate significantly different values (Tukey's HSD test, p-values < 0.05).

Table 1
Univariate analyses of variance (ANOVA) investigating variations in energy reserves (lipid, proteins, and glycogen), defence capacity (GSH, GCL, MT, carotenoids), and toxicity biomarker (MDA), in *Gammarus roeseli*, according to gender and cadmium exposure. Significant differences are indicated in bold ($p < 0.05$).

		Sum of square	d.f.	*F*	*p*-Value
Proteins	Cadmium	18.37	2	3.21	0.054
	Gender	492.77	1	172.36	**<0.001**
	Cadmium × gender	13.3	2	2.33	0.115
Lipids	Cadmium	12.58	2	53.12	**<0.001**
	Gender	8.09	1	68.35	**<0.001**
	Cadmium × gender	0.43	2	2.04	0.148
Glycogen	Cadmium	0.98	2	8.22	**0.001**
	Gender	14.77	1	247.72	**<0.001**
	Cadmium × gender	1.83	2	15.36	**<0.001**
GSH	Cadmium	2.14	2	31.69	**<0.001**
	Gender	10.57	1	313.84	**<0.001**
	Cadmium × gender	0.29	2	4.36	**0.022**
GCL	Cadmium	0.27	2	49.41	**<0.001**
	Gender	0.05	1	17.66	**<0.001**
	Cadmium × gender	0.02	2	3.56	**0.041**
MT	Cadmium	3.19	2	29.31	**<0.001**
	Gender	69.11	1	1267.38	**<0.001**
	Cadmium × gender	2.25	2	20.64	0.78
Carotenoid	Cadmium	449.12	2	51.77	**<0.001**
	Gender	1730.56	1	398.98	**<0.001**
	Cadmium × gender	27.01	2	3.11	0.059
MDA	Cadmium	11.23	2	38.83	**<0.001**
	Gender	19.73	1	136.48	**<0.001**
	Cadmium × gender	1.38	2	4.77	**0.016**

3.3. Biomarker of toxic effects

MDA level, reflecting cell damage, was influenced by cadmium exposure (Table 1 and Fig. 1H). Whatever the gender, an increase in MDA levels was observed. In males, MDA levels were increased according to a concentration-dependent response. Indeed, we observed a 1.3- and 1.7-fold increase in males exposed to 2 and 8 $\mu g\,Cd\,L^{-1}$, respectively. In contrast, a significant increase was observed only in females exposed to the highest cadmium concentration. No significant difference was observed in females neither between control and the exposition at 2 $\mu g\,Cd\,L^{-1}$, nor between individuals exposed at the two cadmium concentrations.

3.4. Principal component analysis

The PCA performed on all biomarker measurements resulted in a first factorial plan which explained 89.9% of the total inertia (Fig. 2). The F1-axis explained 61.5% of the total inertia whereas the second principal component (F2) accounted for 28.3%. F1 was positively correlated to carotenoids, glycogen and protein concentrations and negatively correlated to GSH, and in lesser extent to GCL activity and MT concentrations. F2 was positively correlated to lipids content and negatively correlated to MDA levels.

F1-axis allowed the separation of males and females according to antitoxic defences. Females were observed with the highest GSH and MT concentrations as well as GCL activity, whereas males presented highest levels of carotenoids. Similarly, F2 has allowed differentiating between exposure conditions within male and female groups according a concentration-dependent response (control on the negative part of the F2-axis, C2 at the opposite along this axis). Lower intra-group variations (i.e. small ellipses on the PCA projection) were observed in males according to cadmium exposure, while in females, the intra-group variation increased depending on the cadmium concentration.

4. Discussion

4.1. Validation of our ecotoxicological approach: biomarker responses under cadmium stress

All biomarkers measured in *G. roeseli* males and females were influenced by the exposure to cadmium.

MDA levels increased in exposed gammarids according to a concentration-dependent response, reflecting the toxic effect of cadmium at the tested concentrations. This result is in accordance with previous studies such as those of Correia et al. (2002), and Sroda and Cossu-Leguille (2011a) who have observed an increase of MDA levels in *G. locusta* or *G. roeseli*, respectively, after a copper exposure.

Within the antitoxic defences measured, the pattern of GSH concentrations is the most difficult to interpret. The increase of GSH in *G. roeseli* exposed to 2 $\mu g\,Cd\,L^{-1}$ could result from the increase of the GCL activity, which is the enzyme limiting the de novo GSH synthesis (Volohonsky et al., 2002). It is now admitted that an increase of GSH concentration following GCL activation is the result of an oxidative stress (Dickinson et al., 2004). In our study, the absence of GSH concentration increase in *G. roeseli* exposed to 8 $\mu g\,Cd\,L^{-1}$ compared with gammarids exposed to 2 $\mu g\,Cd\,L^{-1}$, while GCL activity was increased, could be explained by the use of GSH as a scavenger of reactive oxygen species produced by cadmium (Vasseur and Leguille, 2004), and also by the fact that GSH is used as a substrate by some antioxidant enzymes such as glutathione-*S*-transferases or glutathione peroxidases (Saez et al., 1990).

With the same pattern as that of GSH, MT concentrations were increased in cadmium-exposed *G. roeseli*. This rise could be linked with the MT synthesis induced by the exposure to several metals including cadmium (Bigot et al., 2011; Stillman, 1995), as observed by Martinez et al. (1996) in the amphipod *Echinogammarus echinosetosus* exposed for 24 h to 100 $\mu g\,Cd\,L^{-1}$. Similar results were

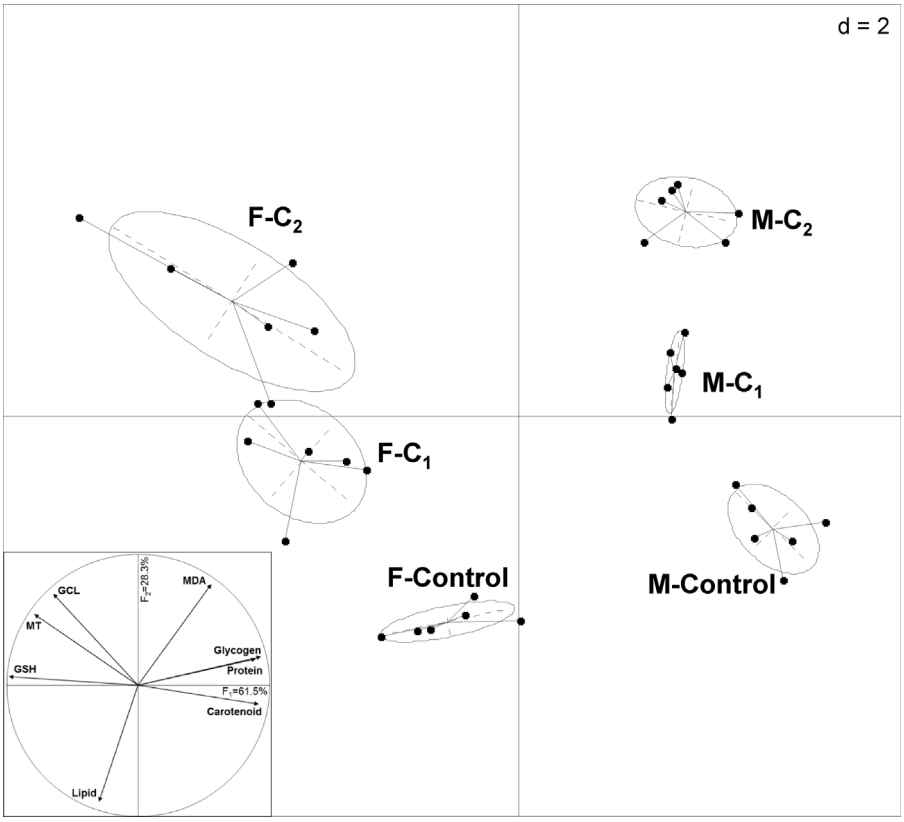

Fig. 2. Results of the correlation circle and the principal component analysis on biomarker responses of *Gammarus roeseli* males and females exposed to cadmium for 96 h. F: females, M: males, C1: 2 μg Cd L^{-1} and C2: 8 μg Cd L^{-1}.

also described in bivalves such as in *Mytilus edulis* exposed at 400 μg Cd L^{-1} for 65 days (Bebianno and Langston, 1991).

Carotenoid levels were also impacted by cadmium exposure due to the fact that they play an antitoxicant role by scavenging free radicals (Krinsky and Deneke, 1982; Krinsky, 1989). Indeed, by scavenging free radicals, carotenoids could reduce lipid peroxidation and thus, maintain the cellular integrity (Jörgensen and Skibsted, 1993).

Concerning energy reserves, protein concentrations did not vary after cadmium exposure but lipid and glycogen contents were reduced in exposed gammarids. These results could be explained by the mobilization of energy reserves to establish antitoxic defences. Indeed, lipids and glycogen are known to be mobilized in exposed organisms (Durou et al., 2008), and thus rapidly impacted by toxic stress, even during a short-term exposure such as in our study. In contrast, protein concentrations are principally mobilized in long term exposure, which could explain that we did not observe protein concentration decrease. However, the lipid content decrease could be linked to the glycogen content decrease. Sancho et al. (1998) and Dutra et al. (2009) have suggested that glycogen decrease, observed in *Anguilla anguilla* exposed to fenitrothion and *Hyallela castroi* exposed to carbofuran, respectively, could be due to pollutant stress cost, while the decrease in lipid content could be explained by the use of lipids as an energy source to compensate for the glycogen lost.

4.2. Differences between genders

Overall, the PCA used in our study highlighted differences in the responses of *G. roeseli* according to gender.

Cell damage is less important in females than in males according to the lower MDA levels regardless of the cadmium concentration tested, suggesting than females are less sensitive to cadmium than males (H1 refuted). This result contrasts with several other studies such as those of Sornom et al. (2010) about *G. roeseli* exposed to a salinity stress, Sroda and Cossu-Leguille (2011b) about *G. roeseli* and the invasive *D. villosus* under copper exposure, McCahon and Pascoe (1988) concerning *G. pulex* and a cadmium exposure for 48 h. This difference could be explained by the different type of stress used (e.g. salinity, copper) or the different species used (e.g. *G. pulex*, *G. roeseli*). Nevertheless, our results confirm those observed in a previous study where we found that the 96 h LC_{50} of *G. roeseli* females exposed to cadmium was higher than the 96 h LC_{50} of males (Gismondi et al., 2012b).

Differences of cell damage between males and females could be the consequence of a difference in antitoxic compound concentrations. Our study highlighted higher GSH and MT concentrations and higher GCL activity in females (H2 validated), allowing them to have a better antitoxic defence when exposed to pollutants. This is in agreement with other investigations on amphipods which underlined higher antioxidant enzymes activity in females as for example in *G. roeseli* (Sroda and Cossu-Leguille, 2011a), or lower metallothionein concentrations in males such as in *Gammarus locustra* (Correia et al., 2004).

The different antitoxicant molecule levels could be linked to energy reserves and energy metabolism. In controls, we observed that females have higher lipid but lower glycogen and protein content than males. The differences in energy reserves between genders could be explained by differences in feeding as observed by Dick et al. (2005). Indeed these authors examined guts of *Echinogammarus marinus* and concluded that females consumed significantly more animals than males, resulting probably in a higher energy intake. This difference in energy content could also be explained by the high needs of energy in females for oogenesis (Sutcliffe, 1993). When individuals were exposed to cadmium, protein concentrations were unchanged regardless of the gender,

lipid contents were always significantly decreased and glycogen contents were only decreased in females (H3 validated). The difference between males and females is in apparent contradiction with their respective investments in reproduction. Oogenesis and egg incubation require high lipid synthesis and mobilization, in comparison to processes of spermatogenesis, which are less demanding in terms of energy (Buikema and Benfield, 1979). Males might also have a more constant physiological state than females due to the asymmetry of the energy required in gamete production. If this resource allocation between genders is generally assumed to lead to a better physiological state for males (the opposite of our results), a complementary view is that females have higher energy reserves as compared to males that can be reallocated. Thus, the difference in reproduction costs could allow females to have a better metabolism to assume antitoxic defence mechanisms when they are exposed to a chemical stressor. Two other hypotheses have been advanced to explain a better resistance of females by comparison with males (McClellan-Green et al., 2007). First, high female resistance as compared to males could be linked to a higher molting rate, the process of exoskeleton shedding being a potential removal pathway of pollutants. This hypothesis supports the high intra-group variation we observed in females exposed to the highest concentration of cadmium. Second, ovodeposition involves the transfer of contaminants into the eggs which confers to females a better metabolism to eliminate environmental contaminants (Dipinto et al., 1993; Oberdörster et al., 2000). Pöckl et al. (2003) showed that *G. roeseli* females can reproduce during their entire life-span of 1.5–2 years about six to eight successive broods, which add-up to the production of at least 200 eggs. Hence, reproduction could decrease body burdens of contaminants since many chemicals are associated with lipids and transferred to oocytes. However, further studies are needed to investigate the importance of these processes (molting rate, level of detoxification due to exuvia and egg production) in gender differences.

5. Conclusions

This work underlined the differential antitoxic defence responses in gammarids according to gender. Our results revealed that *G. roeseli* females seem to be less sensitive to cadmium than males probably due to a better mobilization of antitoxic defences. Studies on the influence of gender, especially females, under stress conditions seem to be important to have an overview of xenobiotics effects on the population. In anthropogenic contexts where females are more sensitive to certain pollutants than males, population declines can take place faster in environments contaminated by these compounds. As *Gammarus* sp. is an important compartment in the ecosystem, the decline of gammarids in these disturbed environments can have serious effects on the cycle of organic matter and the overall foodweb.

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