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Influence of gender and season on reduced glutathione concentration and energy reserves of *Gammarus roeseli*

Eric Gismondi *, Jean-Nicolas Beisel, Carole Cossu-Leguille

Laboratoire des Interactions Ecotoxicologie Biodiversité Ecosystèmes (LIEBE), CNRS UMR 7146, Université de Lorraine (UdL), Campus Bridoux, Bât. IBiSE, 8 Rue du Général Delestraint, 57070 METZ, France

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ABSTRACT

As biomarkers are known to be influenced by biotic and abiotic factors (e.g. gender, temperature), we investigated over a one-year long sampling period, the influence of season and gender on reduced glutathione concentrations and its synthesis in the crustacean amphipod *Gammarus roeseli*. At the same time, we assessed energy reserves and malondialdehyde levels as toxic biomarker. Results have shown that, in both genders, reduced glutathione concentrations were inversely correlated to water temperature, and higher in females than in males whatever the season. Total lipid and glycogen contents were higher in females than in males, allowing females to have enough energy to assume the reproductive period and maintain high GSH concentrations for detoxification processes. Conversely, females have lower cell damages than males. These differences between genders could induce differential sensitivity in a contamination context, and thus affect the population. Females could resist better than males in contaminated environments, especially in spring when reduced glutathione concentration is the highest.

1. Introduction

Biomarkers are tools used in environmental risk assessment (Galloway, 2006; Hagger et al., 2008). Some of them belong to defense mechanisms of organisms against oxidative damage caused by the formation of reactive oxygen species (ROS) (Sies, 1986). If enzymatic activities such as catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD), glutathione peroxidase (GPx, EC 1.11.1.9), glutathione S-transferase (GST, EC 2.5.1.18), ethoxyresorufin-O-deethylase (EROD) or acetylcholinesterase (AchE, EC 3.1.1.7) are widely studied in ecotoxicology as sensitive biomarkers (Cossu et al., 1997; Doyotte et al., 1997; Sroda and Cossu-Leguille, 2011a, 2011b), the free radical and xenobiotic scavengers of the non-enzymatic system such as glutathione (GSH) are poorly studied although they constitute also sensitive biomarkers (Regoli and Principato, 1995).

Among freshwater species, *Gammarus* sp. is a suitable organism for ecotoxicological assessment of environmental pollutants at a large scale, mostly because gammarid are present in most (if not all) river in Europe (Jażdżewski, 1980). Gammarids are known to be sensitive to pollutants and can easily be used for laboratory and field studies (Kunz et al., 2010). Hence, many ecotoxicological studies were carried out using gammarids to evaluate toxic impact of xenobiotics (Sornom et al., 2010; Khan et al., 2011; Sroda and Cossu-Leguille, 2011b). However, to our knowledge, no studies have been devoted to glutathione as a biomarker in *Gammarus* sp., although it could be the limiting factor for many antioxidant enzymes that use glutathione as a substrate and which are commonly assessed in these organisms (Correia et al., 2003; Bedulina et al., 2010; Sroda and Cossu-Leguille, 2011a,b).

Glutathione is a tripeptide widely distributed in animal and plant tissues. In its reduced form, it plays a central role into the detoxification system thanks to its thiol function which acts as a scavenger of organic or metallic xenobiotics (Griffith, 1999; Vasseur and Leguille, 2004). It participates in a lot of fundamental cellular processes, including protein synthesis, amino acid transport, enzymatic activity and cell defense against a wide range of stressors (Meister and Anderson, 1983; Singhal et al., 1987; Akira et al., 1990). It also plays an important role as a reducer which could prevent the toxic effect of ROS (Meister, 1988). Its contents have therefore been assayed in ecotoxicological studies about invertebrates, especially in bivalves (Doyotte et al., 1997; Canesi et al., 1999; Romero-Ruiz et al., 2003).

Due to the fact that biomarkers are known to be influenced by biotic and abiotic factors (e.g. gender, temperature) (Geffard et al., 2007; Bigot et al., 2011; Sroda and Cossu-Leguille, 2011a), in the present study, we investigated the influence of gender and season on the GSH concentrations and its synthesis (i.e. γ-glutamylcysteine ligase activity—GCL) in *Gammarus roeseli*, a widespread

* Corresponding author. Fax: +33 3 87 37 85 12.
E-mail address: eric.gismondi@hotmail.fr (E. Gismondi).

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amphipod crustacean which originates from the Balkan area and has colonized several Western European rivers (Barnard and Barnard, 1983). At the same time, we measured the energy reserves by assaying glycogen and lipid contents. Glycogen represents a short-term energy source available for current activities (Sparkes et al., 1996), whereas lipids are used for long-term storage to be used as nutrients during starvation or reproduction periods (Cargill et al., 1985). Finally, we measured the levels of malondialdehyde (MDA), a product of lipid peroxidation considered as a toxicity biomarker which reflects cellular damage.

2. Material and methods

2.1. Sampling and maintenance of G. roeseli

Males and females *G. roeseli* were sampled monthly from October 2008 to September 2009 with a hand net in the French Nied River (Laquenexy, North-eastern France, 49°05′ N and 6°16′ E). Each month, water parameters such as temperature, pH, conductivity, dissolved oxygen and oxygen saturation were measured in the field with a WTW Multi 340i/set measurement (Germany). Male and female gammarids were sorted on the spot according to gnathopod size, a sexual dimorphism character. They were then transported to the laboratory in river water, where four pools of seven males and four pools of ten females were formed to measure biomarkers and energy reserves. Each pool was frozen in nitrogen liquid and maintained four weeks at −80 °C awaiting biomarker analyses. Prior to analysis, *G. roeseli* gender was checked by observing genital papillas, which are present in males only, on the 7th ventral segment.

2.2. Biomarkers measurement

2.2.1. Sample preparation

Each pool was homogenized with a manual Potter Elvejhem tissue grinder in a 50 mM phosphate buffer KH_2PO_4/K_2HPO_4 (pH 7.6) supplemented with 1 mM phenylmethyl sulphonylfluoride (PMSF), and 1 mM L-serine-borate mixture as proteases 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. 400 µL of homogenization buffer for 200 mg of wet weight tissue) (adapted from Cossu et al. (1997) and Doyotte et al. (1997)). The tissue was crushed by four turns of Potter followed by a homogenization of the mixture with a vortex and another 4 turns of Potter to obtain the homogenate. The homogenate was divided into five parts to measure the different parameters. For each pool, two independent measurements were performed for each biomarker, and the mean was used for statistical analyses.

2.2.2. Biomarkers assays

GSH concentrations measurement was adapted from Leroy et al. (1993) using High-Pressure Liquid Chromatography (HPLC) separation. The proteins of 40 µL of the total homogenate were precipitated with 10% perchloric acid (v/v). After a 10-min centrifugation at 20,000 × *g* at 4 °C, the resulting supernatant was diluted 40-fold in 0.1 M hydrochloric acid (HCl). 20 µL of the diluted supernatant were injected in a reverse-phase LiChrospher 100 RP18-encapped column (125 mm × 4 mm, 5 µm) and separation was carried out at 25 °C. Elution was performed with 7% acetonitrile (Chromanorm, 95%) in a 0.01 M phosphate buffer (pH 2.5) containing 0.5 mM *n*-decylsodiumsulfate as an ion-pairing reagent. Commercial GSH diluted in 0.1 M HCl was used as a standard and GSH concentrations were expressed in nmol mg^{-1} protein.

γ-glutamylcysteine ligase activity (GCL) was assayed using an HPLC method adapted from Parmentier et al. (1998). Measurements were carried out on the S12000 fraction obtained after centrifuging 40 µL of the total homogenate for 15 min at 500 × *g* and then centrifuging the resulting supernatant at 12,000 × g and 4 °C for 30 min. The resulting S12000 fraction was diluted 20-fold in the homogenization buffer and 40 µL of this diluted solution were added to 112 µL of incubation cocktail (0.5 M Tris–HCl, 200 mM $MgCl_2 \cdot 6H_2O$, 500 mM KCl, 45 mM glutamic acid, 90 mM cysteine, 1 mM DTT, 90 mM ATP, 0.5 mM phenylglyoxal, pH 8.25) to initiate the reaction. After a 20 min-incubation period at 25 °C, the reaction was stopped by a four-fold dilution with 0.1 M HCl. 20 µL of the resulting solution were injected into a LiChrospher 100 RP18-encapped HPLC column (125 mm × 4 mm, 5 µm). Commercial glutamylcysteine (GC) solution was used as a standard and GCL activity was expressed in nmol GC min^{-1} mg^{-1} protein.

The total protein content of each sample was quantified according to Bradford (1976) with bovine serum albumin (BSA) as a standard. Results were expressed in mg mL^{-1}.

2.2.3. Lipid peroxidation

MDA levels were measured with an HPLC method adapted from Behrens and Madère (1991) with UV detection at 267 nm. 70 µL of the total homogenate were diluted four-fold with 95% ethanol (HPLC grade) and cooled on ice for 1.5 hrs to deproteinize them. The mixture was then centrifuged at 18,000 × *g* for 30 min at 4 °C. 100 µL of the resulting supernatant were injected directly into a reversed-phase LiChrospher 100RP18-encapped HPLC column. The separation was conducted at 25 °C and elution was carried out with sodium phosphate buffer (pH 6.5) containing 25% ethanol and 0.5 mM tetradecylmethylammonium bromide as an ion-paring reagent. MDA standard was prepared by hydrolyzing 24 µL of 1,1,3,3-tetraethoxy-propan 97% (TEP) in 10 mL of sulfuric acid 1% for 2 h in the dark. The hydrolyzed solution was then diluted 20-fold in sulfuric acid, and diluted once more 10-fold in phosphate buffer (pH 7.6). Optical density of this diluted solution was measured at 245 nm to estimate the concentration of the solution thanks to the molar extinction coefficient (ε = 13,700 L mol^{-1} cm^{-1}). The MDA standard curve was composed of 5 concentrations: 0.125, 0.25, 0.5, 1 and 2 µM obtained by dilution of the prepared solution. MDA levels were expressed in ng MDA mg^{-1} lipid.

2.2.4. Energy reserves

The measurement of total lipid and glycogen contents was adapted from Plaistow et al. (2001). 20 µL of 2% sodium sulfate (w/v) and 540 µL of chloroform/methanol 1:2 (v/v) were added to 40 µL of total homogenate. After 1 h on ice, the samples were centrifuged at 3000 × *g* for 5 min at 4 °C. The resulting supernatant and the pellet were used to determine the total lipid and glycogen contents, respectively.

100 µL of the supernatant were transferred into culture tubes and placed in a dry bath at 95 °C to evaporate the solvent. Then, 200 µL of 95% sulfuric acid were added in each tube and left for 10 min. The culture tubes were cooled on ice and then 4.8 mL of a vanillin-phosphoric acid reagent were added. After a 10-min reaction, the optical density was measured at 535 nm. Commercial cholesterol was used as a standard and lipid contents were expressed in mg mL^{-1}.

Total dissolution of the pellet was performed in 400 µL of deionized water for 10 mi in an ultrasonic bath. 100 µL of each sample were placed in culture tubes and 4.9 mL of anthrone reagent were added. The mixture was placed in a dry bath at 95 °C for 17 min and then cooled on ice. Optical density was measured at 625 nm. Glucose was used as a standard and concentrations were expressed in µg mg^{-1} wet weight.

2.2.5. Statistical analysis

Data analysis was performed using a multivariate analysis of variance (MANOVA, Pillai's trace) with respect to "gender" and "sampling month" as fixed factors. All data met normality and homogeneity of variance assumptions. Since the MANOVA test was significant, each biomarker was then analyzed using ANOVA tests, followed by the TukeyHSD post-hoc test. All tests were performed with a 5% type I error risk, using R 2.9.0 Software.

3. Results

3.1. Water parameters

Monthly measured physico-chemical parameters are summarized in Table 1. Among the different parameters we measured, temperature was the most variable with a coefficient of variation of 55.2%, whereas pH was the least variable. Indeed, temperature ranged from 2.3 °C in January to 20 °C in June. All other parameters were stable over time and mean values for pH, conductivity, dissolved oxygen and oxygen saturation were respectively 8.1, 1403.8 µs cm^{-1}, 10.3 mg L^{-1} and 95.4%.

3.2. Seasonal variations

Global MANOVA analysis revealed an effect of individual gender, sampling month, and of their interactions on the variations of biomarker levels (Table 2). The results are detailed below for each biomarker category.

3.2.1. GSH concentration, GCL activity and biomarker of toxicity

Reduced glutathione concentrations measured over one year in *G. roeseli* were influenced by gender and sampling month (Table 2). For both genders, GSH concentrations were higher in winter (from November to February) and lower in summer (from May to August) (Fig. 1). Moreover, overall, females had higher GSH concentrations

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Table 1
Physico-chemical parameters of the Nied River at Laquenexy measured at each sampling date. Means, standard deviations and coefficient of variation are presented at the end of the table.

		Temperature (°C)	pH	Conductivity (µS cm^{-1})	Dissolved oxygen (mg L^{-1})	Oxygen saturation (%)
2008	**Oct**	9.6	8.1	1722.0	11.0	97.8
	Nov	8.6	8.0	1460.0	10.0	84.4
	Dec	3.6	7.9	1136.0	10.8	81.4
2009	**Jan**	2.3	8.0	1135.0	11.1	81.5
	Feb	4.9	8.3	1051.0	10.7	84.7
	Mar	9.0	8.3	1091.0	12.5	108.6
	Apr	12.7	8.2	1353.0	9.9	97.3
	May	17.3	8.0	1463.0	9.1	96.0
	Jun	20.0	8.4	1580.0	11.0	123.8
	Jul	19.6	7.8	1171.0	6.4	71.9
	Aug	20.5	8.2	1685.0	10.4	105.3
	Sep	17.1	8.1	1998.0	10.5	111.9
	Mean	**12.1**	**8.1**	**1387.1**	**10.3**	**95.4**
	SD	**6.7**	**0.2**	**326.4**	**1.5**	**15.2**
	VC%	**55.2**	**2.1**	**23.5**	**14.2**	**15.9**

Table 2
Multivariate (Pillai's trace) and univariate analyses of variance investigating variation in energy reserves (protein, lipid, glycogen), defense capacities (GSH, GCL) and in biomarker of toxicity (MDA) of *G. roeseli*, as a function of gender and sampling month.

Models	Parameter	Source of variation	num d.f.[a], den d.f.[b]	F	*P*
MANOVA		Whole model	138, 432	17.44	<**0.0001**[c]
		Gender	6, 67	47.46	<**0.0001**
		Month	66, 432	24.04	<**0.0001**
		Gender: Month	66, 432	5.61	<**0.0001**
ANOVAs	Proteins	Whole model	23, 72	18.85	<**0.0001**
		Gender	1, 72	92.61	<**0.0001**
		Month	11, 72	27.85	<**0.0001**
		Gender: Month	11, 72	3.15	**0.0016**
	Total lipids	Whole model	23, 72	38.73	<**0.0001**
		Gender	1, 72	214.61	<**0.0001**
		Month	11, 72	42.01	<**0.0001**
		Gender: Month	11, 72	19.46	<**0.0001**
	Glycogen	Whole model	23, 72	13.26	<**0.0001**
		Gender	1, 72	22.82	<**0.0001**
		Month	11, 72	23.74	<**0.0001**
		Gender: Month	11, 72	1.90	**0.0520**
	GSH	Whole model	23, 72	15.45	<**0.0001**
		Gender	1, 72	39.04	<**0.0001**
		Month	11, 72	23.94	<**0.0001**
		Gender: Month	11, 72	4.81	<**0.0001**
	GCL	Whole model	23, 72	30.64	<**0.0001**
		Gender	1, 72	0.20	0.6544
		Month	11, 72	50.88	<**0.0001**
		Gender: Month	11, 72	13.16	<**0.0001**
	MDA	Whole model	23, 72	24.56	<**0.0001**
		Gender	1, 72	95.28	<**0.0001**
		Month	11, 72	29.14	<**0.0001**
		Gender: Month	11, 72	13.54	<**0.0001**

[a] Numerator degrees of freedom
[b] Denominator degrees of freedom
[c] Significant values shown in bold

than males. In females, the highest values were measured in March and April with an average 4.5 nmol GSH mg^{-1} protein, while in males, the highest GSH concentrations were observed in January (3.7 nmol GSH mg^{-1} protein). Finally, in both genders, GSH concentrations were negatively correlated to water temperature ($r=-0.68$, p-value <0.001; $r=-0.79$, p-value <0.001, for females and males respectively, Pearson correlation).

GCL activity variations were significantly dependent of sampling month (Table 2, Fig. 2). No significant difference was determined according to gender: the annual mean value of GCL activity was 0.34 and 0.35 nmol GC min^{-1} mg^{-1} protein for females and males, respectively. In both genders, GCL activity was lower from October to April (winter), except in January, with an average 0.22 and 0.26 nmol GC min^{-1} mg^{-1} protein in males and females, respectively. The highest activities were measured from May to September (summer) and were about 0.41 and 0.43 nmol GC min^{-1} mg^{-1} protein for females and males, respectively. Finally, it was defined that in females, GCL activity was positively correlated with water temperature ($r=0.38$, p-value <0.001, Pearson correlation).

MDA levels were influenced by gender, sampling month and the interactions between these two factors (Table 2, Fig. 3). Males had higher MDA levels than females, regardless of the month (Fig. 3). Indeed, the annual MDA mean level in males was 6.60 ng mg^{-1} lipid and ranged from 2.74 ng mg^{-1} lipid in August to 14.44 ng mg^{-1} lipid in March, whereas females exhibited an average 4.26 ng mg^{-1} lipid and ranged from 2.00 ng mg^{-1} lipid in February to 7.78 ng mg^{-1} lipid in June. Significant differences between males and females were mainly observed in spring (from February to May).

3.2.2. Energy reserves

Total lipid contents were influenced by gender, sampling month and the interactions between these two factors (Table 2). Total lipid contents were higher in females than in males (Table 3) with annual mean lipid contents of 4.91 mg mL^{-1} and 3.33 mg mL^{-1}, respectively. Significant differences between males and females were observed all year long, except in November and January. For both genders, the lowest total lipid content was measured in September with 2.91 mg mL^{-1} and 1.52 mg mL^{-1} in females and males, respectively; while the highest values were observed in February for females (8.36 mg mL^{-1}) and in August for males (5.72 mg mL^{-1}).

Glycogen content was influenced by gender and the interaction between gender and sampling month (Tables 2 and 3). Significant differences between males and females were observed during the spring period, from March to June. In males, glycogen contents ranged from 0.79 µg mg^{-1} tissue in March to 7.04 µg mg^{-1} tissue in December; while in females, it ranged from 2.58 µg mg^{-1} tissue in October to 7.54 µg mg^{-1} tissue in December.

4. Discussion

This study was carried out to investigate the influence of the gender and the season on antitoxic defense capacities of the amphipod *G. roeseli*, especially on the GSH concentration and its synthesis. At the same time, variations of lipid and glycogen contents as well as MDA levels, according to gender and season, were assessed.

Results highlighted a seasonal variation in GSH concentrations, since they were higher in autumn and winter, then decreased in spring and finally reached their lowest in summer, in both genders. This decrease in GSH concentration could be explained by the rise in temperatures, resulting in an increase in oxygen consumption, as observed in *G. limnaeus* (Krog, 1954) and in *G. fossarum* (Issartel et al., 2005). In fact, an increase in oxygen consumption could favor the formation of ROS as described by

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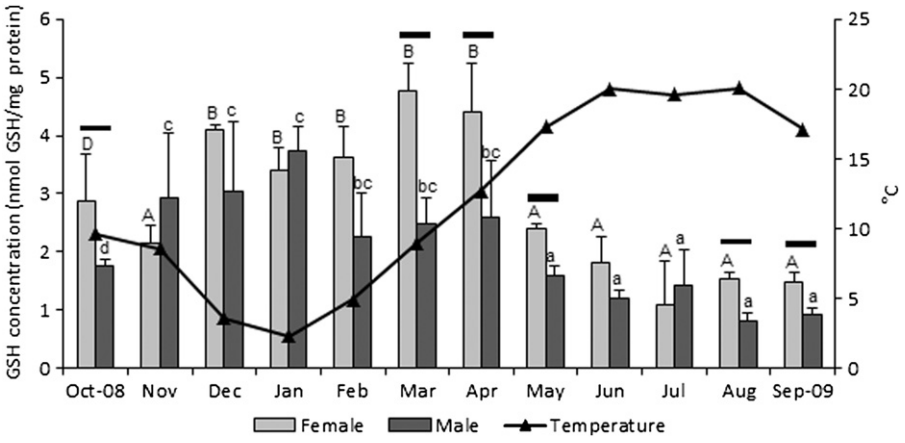

Fig. 1. Seasonal variations in GSH concentrations in males and females *G. roeseli* and in temperatures from October 2008 to September 2009. Capital and lower-case letters indicate significant differences in males and females, respectively. Horizontal bars indicate differences between males and females (Tukey's HSD test, p-values < 0.05).

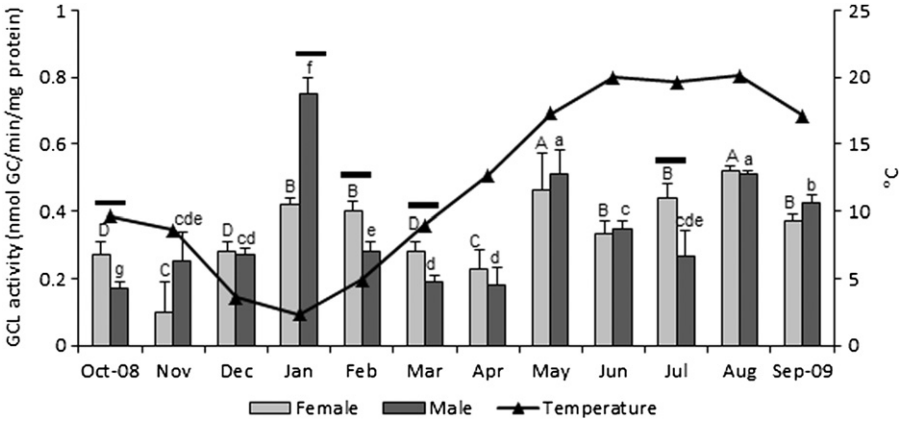

Fig. 2. Seasonal variations in GCL activity in males and females *G. roeseli* and in temperature from October 2008 to September 2009. Capital and lower-case letters indicate significant differences in males and females, respectively. Horizontal bars indicate differences between males and females (Tukey's HSD test, p-values < 0.05).

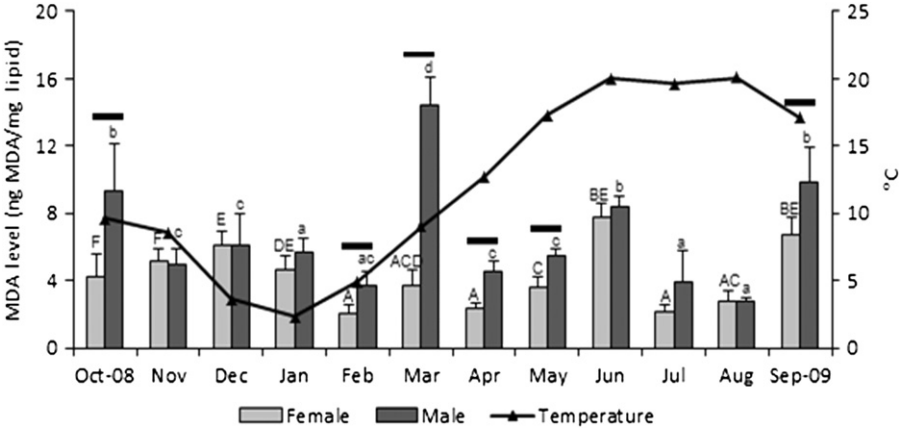

Fig. 3. Seasonal variations in MDA levels in males and females *G. roeseli* and in temperature from October 2008 to September 2009. Capital and lower-case letters indicate significant differences in males and females, respectively. Horizontal bars indicate differences between males and females (Tukey's HSD test, p-values < 0.05).

Verlecar et al. (2007) in the brown mussel *Perna viridis*. Thus, to prevent the cellular damage caused by high ROS levels in summer, GSH could be used for detoxification, resulting in its decrease. Similar variations were observed in other organism such as the mussel *P. viridis* (Verlecar et al., 2008); nevertheless, inverse variations have been observed as for example in the bivalves *Mytilus edulis* and *P. perna* (Power and Sheehan, 1996; Wilhelm Filho et al., 2001).

The hypothesis of a higher GSH mobilization during summer in *G. roeseli* males and females is supported by the fact that GCL activity was higher in summer in both genders compared to autumn and winter. This high GCL activity reflects high GSH production. Consequently, our results suggest that high levels of GSH are produced in summer and are likely to be used for cell defense, suggesting that the conversion of oxidized glutathione (GSSG) to reduced form by the glutathione reductase (GR, 1.8.1.7)

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Table 3
Monthly variations in energy reserves (lipid, glycogen) of males and females *G. roeseli*. Capital and lower-case letters indicate significant differences in males and females, respectively. Asterisks highlight differences between males and females for each month (p-values < 0.05). The minimum and the maximum of each parameter for each gender are indicated in bold type.

Month (°C)	Total lipid content		Glycogen content	
	Males	Females	Males	Females
Oct (9.6)	2.67 ± 0.52^{ab}	$5.45 \pm 0.96^{A*}$	3.34 ± 0.43^{a}	$\mathbf{2.58 \pm 0.63^{A}}$
Nov (8.6)	3.27 ± 1.00^{bc}	3.54 ± 0.98^{B}	2.51 ± 0.72^{a}	2.59 ± 1.41^{A}
Dec (3.6)	2.54 ± 0.54^{ab}	$4.97 \pm 0.59^{AC*}$	$\mathbf{7.04 \pm 1.90^{b}}$	$\mathbf{7.54 \pm 2.38^{B}}$
Jan (2.3)	3.56 ± 0.09^{bcd}	3.74 ± 0.32^{ABC}	3.53 ± 0.66^{a}	4.28 ± 0.50^{AC}
Feb (4.9)	$\mathbf{4.92 \pm 0.92^{d}}$	$\mathbf{8.36 \pm 0.59^{D*}}$	3.62 ± 0.33^{a}	4.03 ± 0.20^{AC}
Mar (9.0)	1.55 ± 0.11^{a}	$3.76 \pm 0.37^{BC*}$	$\mathbf{0.79 \pm 0.28^{c}}$	$2.60 \pm 0.32^{A*}$
Apr (12.7)	3.38 ± 0.24^{bc}	$5.83 \pm 0.43^{ACE*}$	2.19 ± 0.43^{a}	$3.13 \pm 0.32^{A*}$
May (17.3)	4.16 ± 0.16^{cd}	$7.35 \pm 0.63^{D*}$	1.94 ± 0.43^{a}	$4.23 \pm 0.62^{AC*}$
Jun (20.0)	3.07 ± 0.20^{b}	$4.85 \pm 0.35^{ACE*}$	2.18 ± 0.24^{a}	$3.27 \pm 0.33^{A*}$
Jul (19.6)	3.62 ± 0.48^{bcd}	$4.94 \pm 0.55^{ACE*}$	4.06 ± 0.73^{a}	4.95 ± 0.45^{C}
Aug (20.5)	3.22 ± 0.22^{bcd}	$5.72 \pm 0.13^{ACE*}$	2.49 ± 0.09^{a}	2.79 ± 0.74^{A}
Sep (17.1)	$\mathbf{1.52 \pm 0.10^{a}}$	$\mathbf{2.91 \pm 0.20^{B*}}$	2.67 ± 0.71^{a}	4.04 ± 0.38^{AC}
Annual mean	3.12 ± 0.38	$5.12 \pm 0.51^{*}$	3.03 ± 0.58	3.83 ± 0.69

activity is not sufficient enough to maintain the GSH concentration needed.

In parallel of the seasonal influence, results highlighted gender-based GSH concentrations, as *G. roeseli* females had higher GSH concentrations than males. This higher GSH content in females, as compared to males, could be linked to higher activities of antioxidant enzymes using GSH as a substrate (e.g. selenium-dependent glutathione peroxidase—Sroda and Cossu-Leguille (2011a)). The difference in GSH concentrations between males and females could also be caused by the physiological status of the organisms. The GSH variations could be related to the changing metabolic status of the organism. Indeed, Viarengo et al. (1991) and Wilhelm Filho et al. (2001) have shown a close link between GSH concentrations and the reproduction cycle in *Mytilus* sp. and *P. perna*, respectively. In *G. roeseli*, Pöckl (1992) showed that sexual maturity was reached faster at 16–20 °C, which corresponds to the temperatures measured from May to September on our study site. Therefore, the higher GSH concentrations measured in March and April, especially in females, could be an adaptation of the organism to increase their defense capacities before the breeding period (Knapen et al., 1999). Higher defense capacities observed in females allow us to hypothesize that females could be less sensitive than males in a contamination context. This hypothesis is supported by the measurement of lower MDA levels in *G. roeseli* females than in males ones (this study; Sroda and Cossu-Leguille, 2011a), reflecting lower cell damage, but also by the fact that in our previous study, we demonstrated that the LC_{50} value (lethal concentration 50) was higher in females exposed to cadmium stress than males, suggesting higher resistance of females (Gismondi et al., 2012).

Energy reserves in *G. roeseli* were influenced by season and gender. Indeed, in both genders, higher energy reserves were measured in autumn and winter, and lower energy reserves were measured in spring and summer. The highest energy reserves in autumn could be the consequence of the leaf-fall which increased the food-source for gammarids, as suggested by Sutcliffe, 1993, while the decrease in summer could be explain by the energy mobilization for the reproductive period. Indeed, in females, the decrease of lipid and glycogen contents were measured from June to September, which corresponds to the reproductive period, linked to the oogenesis process. This process involves the vitellus production (required for eggs development) which uses lipids (Rosa and Nunes, 2002), and pleopod beats (considered as brood care for juveniles oxygenation—Dick et al., 1998) which could use glycogen (short-term energy—Sparkes et al., 1996). Conversely, the high lipid content measured in February in females could be explained by the maturation period. This hypothesis is supported by Clarke et al. (1985) who observed an increase in lipid contents during the different maturation stages of female in *G. oceanicus*. Although in females, the decrease of energy reserves could be explain by the breeding period; in males, the lowest glycogen contents measured in spring could be explained by an increase of the locomotor activity to seek for receptive females for breeding, as supposed by Plaistow et al. (2003) and Sroda and Cossu-Leguille (2011a). In opposition, the higher glycogen contents in winter (i.e. December) in both genders could be the consequence to a slower metabolism (e.g. a weak locomotor activity), due to low temperature.

In our study, the difference in energy reserves between males and females could explain the gender-based GSH concentrations. This hypothesis was supported by the fact that the energy reserves depletion observed in spring corresponds to an increase of the GCL activity. Indeed, GSH synthesis is performed by two successive ATP-dependent reactions (Meister and Anderson, 1983), which consume energy coming from lipids and glycogen degradation. Thus, higher energy reserves in females allows them to have a higher activity of GCL and therefore produce higher concentrations of GSH, as it was also observed for antioxidant enzyme activities in *G. roeseli* (Sroda and Cossu-Leguille, 2011a).

5. Conclusion

The present work highlighted variations in GSH concentrations, GCL activity and energy reserves in *G. roeseli* according to gender and season. All parameters were higher in females compared to males suggesting that (i) they could have a better antitoxic defense, and (ii) could assume the antitoxic defense and reproduction costs. Moreover, considering our results, it would therefore be cautious to consider gender and season as confounding factors in ecotoxicological studies using gammarids as biological models, in order to interpret biomarker responses more relevantly as possible. However, it could also be interesting to compare our population's results to another population of gammarid, since previous studies highlighted a variation in the detoxification response according to the population sampled (Damiens et al., 2006).

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