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Seasonal variability and inter-species comparison of metal bioaccumulation in caged gammarids under urban diffuse contamination gradient: Implications for biomonitoring investigations

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HIGHLIGHTS

- Gammarids were transplanted along a multi-metallic gradient during different seasons.
- Two *Gammarus* species were used to compare their responsiveness to metal exposures.
- Metal contents in caged gammarids increase with the river contamination gradient.
- Season influences the bioaccumulation of essential metals in both species.
- Similar abilities to regulate metals between species suggest a generic responsiveness.

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ABSTRACT

Although caging of *Gammarus* species offers promising lines of inquiry to monitor metal bioavailability in freshwaters, the interspecies responsiveness to metal exposures is still unclear. In addition, abiotic factors inherent to transplantation can hamper the interpretation of field bioaccumulation data. To assess the relevance of using gammarids as biomonitors, we investigated the seasonal influence on metal bioaccumulation in two common species, *Gammarus pulex* and *Gammarus fossarum*. During four seasons, caged gammarids were deployed on three sites along the Seine River exhibiting a diffuse gradient of multi-metal contamination: a site upstream and two sites downstream from the Paris megacity. For each seasonal deployment, metal concentrations in animals were determined after 7d-exposure in situ (Ag, Cd, Co, Cu, Mn, Ni, Pb and Zn). Results show that the seasonal patterns of metal contaminations are similar between both *Gammarus* species, and closely related to the river axis' contamination gradient. Statistical analyses indicate that bioaccumulation of essential metals in both species is influenced by season, especially by water temperature. This highlights the necessity to consider this climatic factor inherent to the deployment period for a reliable interpretation of bioaccumulation data in the field. The comparison of accumulation factors suggests that these two species coming from different geochemical origins display similar abilities to internalize metals. This generic responsiveness of caged gammarids supports their use as sentinel organisms to quantify low spatiotemporal variations in metal bioavailabilities.

1. Introduction

Although metals are naturally released by geochemical sources, human activities result in their significant discharge into the aquatic environment and modify their speciation towards chemical forms more mobile for biota (Priadi et al., 2011; Tusseau-Vuillemin et al., 2007). Even at low concentrations, this diffuse multi-metallic contamination negatively affects aquatic organisms, alters community structures and ecosystem functioning (Bourgeault et al., 2010; Fechner et al., 2012; Roussel et al., 2008). Chemical analyses on water column give a snapshot of the occurrence of metals in the environment but they do not give information about their bioavailability, which is the fraction potentially toxic for biota. Alternatively, metal determination in organisms provides an integrative measure of their exposure to ecotoxicologically

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relevant fractions of ambient metals over a recent period in the local habitat (Rainbow, 2007; Verschoor et al., 2012). Over the last decades, the use of macrobenthic invertebrates as biomonitoring tools has thus gained importance for environmental risk assessment (Besse et al., 2012).

Among potential biomonitors, a recent interest has focused on the genus *Gammarus* due to its widespread distribution in Europe, high density in various habitats and ease to sample in the field and to handle. These ubiquitous amphipods are a key component of freshwater ecosystems insofar as they are involved in litter decomposition and microbial turnover, and constitute a major supply of food for various predators such as birds, amphibians and fish (Dedourge-Geffard et al., 2013; Roussel et al., 2008). Thus, the behavior and physiology of gammarids have been well studied for the development of biomarkers in ecotoxicology (Felten et al., 2008; Sroda and Cossu-Leguille, 2011). Furthermore, gammarids are known as net accumulators of metals, whether or not essential, for environmental exposure levels (Fialkowski and Rainbow, 2006; Lebrun et al., 2014). Gammarids constitute thus relevant candidates for biomonitoring the bioavailable fraction of metals in freshwater aquatic media.

Unlike passive biomonitoring that involves the sampling of indigenous organisms, active biomonitoring based on the caging of organisms offers the possibility to assess chemical contamination on sites devoid of indigenous organisms and to control the exposure time, providing thus comparable measures in time and space (Besse et al., 2012). Caging for monitoring of metal contamination has been shown to be relevant in common *Gammarus* species of European temperate freshwaters, i.e. *Gammarus fossarum* and *Gammarus pulex* (Besse et al., 2013; Maltby et al., 2002). However, even if these two species are closely related, the contamination levels can vary from one species to another because of contrasting abilities to accumulate and eliminate metals, and physiological characteristics specific to the species (gill size, ventilation rate, ionoregulatory…) (Alonso et al., 2010; Rainbow, 2007). For example, significant differences in Cd and Zn uptakes were found between stream insects and species of the same genus, which were related to numbers of membrane transporters (Buchwalter and Luoma, 2005). To date, the interspecies responsiveness within the genus *Gammarus* to metal exposures is still rarely assessed, especially regarding bioaccumulation, hence limiting a possible derivation of generic responses.

Although caging offers promising lines of inquiry to monitor metal bioavailability in freshwaters, some abiotic factors inherent to transplantation periods can hinder the interpretation of bioaccumulation data in field. In particular, it is known that seasons influence the physiological status of gammarids, e.g. energy reserves, digestive metabolism or antitoxic defenses and subsequently, their biological responses to chemical exposures (Becker et al., 2013; Dedourge-Geffard et al., 2013; Sroda and Cossu-Leguille, 2011). Regarding bioaccumulation, seasonal variations in Cu levels have been reported in Talitrid amphipods due to variations in their metabolic activities (Fialkowski et al., 2003). Therefore, it is important to assess the influence of such confounding factors on metal bioaccumulation for reliable information of contamination levels of caged gammarids.

In this present study, we investigated the seasonal influence on metal bioaccumulation in two closely related species of gammarids that are largely widespread in Europe, *G. pulex* and *G. fossarum*. Calibrated gammarids were collected in two metal-unpolluted freshwaters coming from different river basins of France. During four seasonal experiments, caged gammarids were deployed on three sites along the Seine River axis exhibiting a diffuse gradient of multi-metallic contamination: a site upstream and two sites downstream from the Paris megacity. For each seasonal deployment, metal concentrations were determined in caged animals, i.e. Ag, Cd, Co, Cu, Mn, Ni, Pb and Zn, after a 7d-exposure under field conditions. To characterize the growing anthropogenic pressure along the Seine axis, total and dissolved metals were determined in the water column during exposures at each site as well as the main physicochemical parameters.

2. Materials and methods

2.1. Study area

In the North of France, the Seine river basin covers 68,000 km^2 with approximately 16 million inhabitants (i.e. about 25% of the French population). The Seine crosses the Paris megacity with its very high population density (>4000 people/km^2) and significant industrial activities (30% of French industry), resulting in metallic discharges from urban, domestic and industrial effluents (Thevenot et al., 2007).

Three sites were studied along the Seine River axis: Marnay (48°30′53.8″N 3°33′29.6″E), Bougival (48°52′11.2″N 2°07′47.1″E) and Triel (48°58′55.5″N 1°59′53.1″E) (Fig. 1). Marnay is located 200 km upstream from the Paris megacity and is not directly impacted by either urban or industrial activities. This site is hence considered as a reference site in this biomonitoring investigation. Bougival and Triel, respectively 40 and 80 km downstream from Paris, are known to be subjected to diffuse metal contamination due to atmospheric fallouts, intensive traffic, wastewater discharges of domestic and industrial activities (Bourgeault et al., 2011; Priadi et al., 2011).

2.2. Seasonal deployments of caged gammarids

The two control populations of gammarids used in this biomonitoring investigation came from two different river basins: *G. pulex* (Seine basin in the North of France — Ru de l'Etang, Doue) and *G. fossarum* (Rhône basin in the Southeast of France — La Bourbe, Tour du Pin). These two species were distinguished through taxonomic identification (Piscart and Bollache, 2012). The sampling sites were small streams at the head of watersheds, known to be uncontaminated by metals and to display good physicochemical quality (Geffard et al., 2010; Lebrun et al., 2012). Main physiochemical characteristics of sampling sites are given in supplementary information (Table S1).

On both sampling sites, adult gammarids were selected according to their body diameter using a series of sieves (2–2.5 mm), so that the length of animals was about 1 cm. The animals were transported in pails of local water and introduced in a cool box for return to the laboratory. Then, 20 gammarids were caged in polypropylene cylinders (length, 10 cm; diameter, 5.5 cm) capped at their ends with pieces of net (mesh: 1 mm) in the presence of alder leaves (*Alnus glutinosa*) as food resource. These caging systems were placed into perforated plastic pails to protect them and to ensure free flow of water. The leaves were previously collected at a pristine site and conditioned for a week in a groundwater before their adding in excess into the caging systems to avoid metal uptake from leaves and starvation (Besse et al., 2013).

Both caged species of gammarids were deployed on the same day at the three sites of the Seine axis during four seasonal campaigns – i.e. autumn (September 2011), spring (March–April 2012), summer (June 2012) and winter (December 2012) – namely S1, S2, S3 and S4 respectively. After 7 days of in situ exposure, caged gammarids were collected to determine metal concentrations accumulated in their bodies at the laboratory. During the first seasonal campaign, gammarids were also deployed for 2 weeks. No major differences in metal contents in gammarids were observed between both deployment periods (data not shown). We thus considered that a week of exposure was sufficient to reach the equilibrium of metallic exchanges.

For each seasonal campaign, water samples were collected at the beginning and end of deployments to monitor global physicochemical parameters (Table 1) and to determine total and dissolved metal concentrations. For the total metal fraction, 50 mL of raw water was sampled in a polypropylene tube (SCP Science). For the dissolved metal fraction, 30 mL of raw water was filtered through a 0.45-µm PES syringe filter (Millipore) and transferred to a 50-mL polypropylene tube.