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Comprehensive biological effects of a complex field poly-metallic pollution gradient on the New Zealand mudsnail *Potamopyrgus antipodarum* (Gray)

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ABSTRACT

The Lot River is known to be contaminated by metals, mainly cadmium and zinc, due to a former Zn ore treatment plant in the watershed of the Riou-Mort, a tributary of the Lot River. Many studies have been performed to characterize contamination, but few have assessed its consequences on the biological responses of organisms along the gradient. We exposed adult and juvenile New Zealand freshwater mudsnails *Potamopyrgus antipodarum* at several sites along the gradient of metal contamination for 28 days. Biological responses were monitored at different levels: individual (survival, growth and fecundity), tissue and biochemical (energy status and vertebrate-like sex steroid levels) to better understand the toxicity mechanisms involved. Accumulation of Cd and Zn was high during exposure. Most of the biological effects observed could be linked to this contamination and were concentration-dependent. Histological lesions of the digestive gland were observed, with hypertrophy of calcium cells and vacuolization of digestive cells. Such effects are likely to explain the decrease of energy status (triglycerides and proteins), juvenile growth and adult fecundity observed at the most polluted site. However the magnitude of the fall in fecundity cannot be attributed only to these tissular effects, indicating another mode of action of Cd or possible confounding factors. Steroid accumulation in snails indicated only organic pollution. Histopathological effects proved the most sensitive endpoint to metal (Cd and Zn) contamination.

1. Introduction

The Lot River and Gironde estuaries are known to be contaminated by metals, mainly cadmium and zinc. The main source of contamination is located in the former Zn ore treatment plant at Decazeville, in the watershed of the Riou-Mort, a tributary of the Lot River. Zn and Cd concentrations in water and suspended particulate matter are high (Coynel et al., 2007) and reach 30 μg Cd/L and 1500 μg Zn/L at the most contaminated site (Morin et al., 2008), i.e. a hundred times the European Environmental Quality Standard (EQS) for bodies of surface water for Cd (European Union, 2008).

As remedial action has just begun to decrease this waterborne metal concentration, studies have been performed to assess the current impact of this contamination on the polluted hydrosystems. Such impact assessments on freshwater ecosystem quality are usually based on chemical data and compliance with EQS, and on biological indicators such as biotic index macroinvertebrates. Experimental approaches designed to give specific insights into chemical stressors likely to cause biological disturbances and monitor the effect of specific remedial actions are seldom used. However, *in situ* approach based on the translocation of organisms to investigate selected sites is well suited to this task, providing a time-integrated assessment of environmental quality and reflecting exposure over several weeks (Regoli et al., 2006). Thus our study aims at assessing the responses of adult and juvenile mudsnails *Potamopyrgus antipodarum* exposed to such a metallic pollution gradient in a complex field situation.

We selected the New Zealand mudsnail *P. antipodarum* (Gray) because it is a sensitive test organism already used in laboratory testing, and experimental stream and field studies (Brown, 1980; Duft et al., 2003; Jobling et al., 2003; Gust et al., 2010a). It is not sensitive to abiotic factor variations (Lassen and Kristensen,

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1978) and its reproduction and steroid levels seemed unaffected by the high range of conductivities and flows measured in the other *in situ* exposures performed previously (Gust et al., 2010a). It allows measuring effect data at sub–individual and individual levels simultaneously, in particular effects on growth and reproduction. It is an invasive parthenogenetic ovoviviparous freshwater mudsnail now common in European countries that thrives in running waters from small creeks to streams, lakes and estuaries, in mud and sand, and on rocks, gravel and aquatic plants (Fretter and Graham, 1994).

The toxic effects of Cd on freshwater invertebrates have been largely documented. Among the phyla represented, gastropods have been studied, though attention has mainly been given to terrestrial and pulmonate species (Laskowski and Hopkin, 1996; Gomot, 1998; Gomot-de Vaufleury and Kerhoas, 2000; Coeurdassier et al., 2003, 2004; Notten et al., 2006; Gimbert et al., 2008; Ansaldo et al., 2009). In these taxa, Cd impaired energy status, decreased fecundity, embryonic survival and growth, and increased time to hatching. Cd can also affect the digestive gland of mollusks (Najle et al., 2000). Some studies were also performed on *P. antipodarum*: effects on size at birth, time to first reproduction, growth rate, reproductive output and reduced feeding rates at concentrations above 1 μg/L have been described (Jensen et al., 2001).

Consequently, several endpoints were measured in order to build a response profile of snails to water quality and provide evidence of the toxicity of metal contamination.

The specific purposes of this study were to assess (i) the bioaccumulation and the effects of a metal gradient in a complex field situation on the life traits of the mudsnail (survival, juvenile growth, fecundity), the histopathology of the gonads and digestive gland, energetic status and vertebrate-like sex-steroids levels and (ii) the different sensitivities of the measured endpoints of this gradient.

2. Materials and methods

Adult *P. antipodarum* were collected from a known natural population in St Savin (France) one month before the experiments started. As juveniles of same size could not been sampled in sufficient number at this period of the year, they were obtained from long term cultures established in our laboratory (Cemagref, Lyon, France).

2.1. Study site

The Decazeville area is known for its polymetallic pollution due to former open-cast coal mining and Zn ore treatment and is drained by a tributary of the Lot River (Riou-Mort). Since 1971 remediation works have been carried out on waste deposits, by collecting and treating the water leaching through the mineral ore, and by confining part of the waste deposits in storage basins with underlying and overlying mud. Nevertheless, large quantities of these waste deposits are still exposed to rainwater and thus leached and eroded (Audry et al., 2004; Morin et al., 2008). Five sites were selected for our study (Fig. 1): (1) Moulin on the Riou-Viou River, upstream of the smelting plant but still slightly contaminated by Cd, (2) Decazeville and (3) Joanis, respectively, upstream and downstream of the plant on the Riou-Mort River, and (4) Lot upstream and (5) downstream of the Riou-Mort River confluence. The Joanis site is that most contaminated by Cd and Zn (respectively, around 30 and 1500 μg/L in Morin et al., 2008), and also by organic and domestic pollutants (Lemaire et al., 2006), while both the Lot Upstream and Decazeville sites were considered as reference sites, with relatively low metal concentrations (Andres et al., 1999; Morin et al., 2008). Intermediate metal contamination was measured at the Lot downstream and Moulin sites, the latter being the more contaminated of the two (Grousset et al., 1999). According to the European Directive 2008/105/CE (European Union, 2008) on EQS for bodies of surface water, Decazeville and Lot upstream are listed as class 1 sites (best chemical status), Lot downstream as class 4, and Moulin and Joanis as class 5 (worst chemical status).

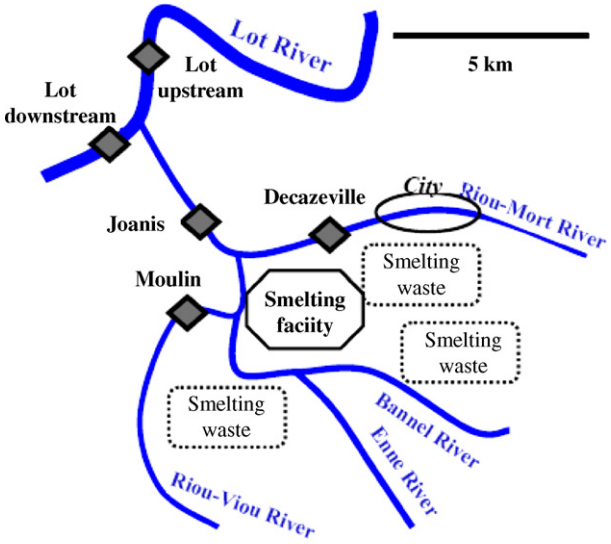

Fig. 1. Site localization. Diamonds represent the five sites.

2.2. In situ exposure of snails

Adult and juvenile snails were placed in rigid plastic containers with both extremities replaced by mesh (500 μm). Each cage, specifically designed for exposure in rivers, contained 5 replicates, with 25–80 adults and 25 juveniles. The remaining space in the cages was loaded with rocks to ballast them. In addition, rocks covered with biofilm collected from a reference site (Morcille, Rhone, France) were introduced in the containers to provide food. Temperature monitor probes were fixed to one cage at each site. Conductivity was measured weekly simultaneously with the biological measurements. The daily flows of the Lot and Riou-Mort were provided by water body authorities (http://www.hydro.eaufrance.fr/). The size of the adults (4.4 ± 0.4 mm) and juveniles (2.1 ± 0.2 mm) were measured one day before being placed in the exposure chambers. The exposures lasted 28 days, from 19 May to 17 June 2009.

2.3. Biological measurements

Mortalities of adults and juveniles were counted every week directly in the field. Fecundity was assessed on days 0, 21 and 28 on 30 adults taken randomly in the replicates from each site and preserved in the river water until the measurements were performed. The embryos in the brood pouch were counted at the laboratory using a technique adapted from Duft et al. (2003). Shells were measured for length and then cracked using a vice. The shell parts were removed in order to observe the embryos through the epithelia. After carefully opening the brood pouch, the number of shelled embryos and unshelled new embryos was counted under a binocular microscope. On day 28 the size of the juveniles was measured under a binocular microscope.