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Caged mudsnail *Potamopyrgus antipodarum* (Gray) as an integrated field biomonitoring tool: Exposure assessment and reprotoxic effects of water column contamination

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ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form
22 January 2014
Accepted 24 January 2014
Available online 5 February 2014

Keywords:
Gastropods
Caging experiments
Effluent
Bioaccumulation
Reprotoxic
Oxazepam

ABSTRACT

This study highlights the usefulness of gastropods for water quality monitoring. Gastropods were caged upstream and downstream of an effluent discharge. Exposure was assessed by measurement of organic contaminants in water. Contamination of the *Potamopyrgus antipodarum* mudsnail was also measured using innovative techniques at the end of the 42 days of exposure. Biological effects were measured at the individual level (growth, reproduction) and subindividual level (energy reserves, vitellin-like proteins, steroid levels, expression of genes involved in estrogen signaling pathways), thus providing a better understanding of reprotoxic effects. The effluent was mainly contaminated by pharmaceutical compounds, as was the mudsnail. The highest concentrations were measured for oxazepam and were higher than 2 mg/kg downstream of the effluent discharge. Alkylphenols, bisphenol A, and vertebrate-like sex-steroid hormones were also bioaccumulated by the mudsnail downstream of the effluent. The combined use of water and snail contamination provided a complete exposure assessment. Exposure was further linked to biological effects.

The mudsnail was shown to be a better adapted species for *in situ* exposures than *Valvata piscinalis*. Reproduction was sharply decreased after 6 weeks of exposure in the mudsnail. Feeding issues were excluded, confirming the toxic origin. These effects were related to estrogen signaling pathways using genomic analysis. Genes coding for proteins involved in nongenomic signaling pathways were inhibited, and those of genomic pathway repressors were induced. These results suggest that the chemical contamination due to the effluent discharge altered steroid control of reproduction and blocked the transition between oocyte and unshelled embryo, resulting in a drastic decrease of embryo production, while survival was not affected.

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http://dx.doi.org/10.1016/j.watres.2014.01.057

1. Introduction

Within the Water Framework Directive (WFD); there are now regulatory arguments in favor of using biota for assessing contamination trends in water bodies, particularly for hydrophobic organic substances. Relevance of caged invertebrates within this context has recently been highlighted (Besse et al., 2012).

Moreover, for risk assessment purposes, biological effects on organisms partly depend on the concentration of active toxicant that reaches the target site. Thus, the internal concentration at the target site, or whole body concentration measurement (as a surrogate of target-site concentration) may provide valuable information for understanding the intrinsic toxicological sensitivity of selected species (Meador et al., 2008). To the best of our knowledge, although recent studies have highlighted the bioaccumulation of organic pollutants in wild fishes, invertebrates, and algae (Brooks et al., 2005; Coogan and La Point, 2008; Mimeault et al., 2005), no link has ever been made between pollutant bioaccumulation and biological effects in freshwater aquatic gastropods under field exposure.

The use of gastropods in ecotoxicology has steadily increased over the last few years, most particularly the invasive parthenogenetic New Zealand mudsnail, *Potamopyrgus antipodarum* (Matthiessen, 2008). Its sensitivity to endocrine disruptive compounds/chemicals (EDCs) such as Bisphenol A, UV filters, aromatase inhibitors, etc., has made it a promising model (Gust et al., 2010b; Matthiessen, 2008; Sieratowicz et al., 2011). Therefore, it has been recommended as a laboratory reproduction test within the OECD "Ad hoc Expert Group on Invertebrate Testing" guidelines (OECD, 2010).

In laboratory experiments, the sensitivity of the mudsnail to various pollutants has been explored and compared to the sensitivity of another gastropod: the hermaphrodite oviparous European valve snail *Valvata piscinalis* (unpublished results and Gagnaire et al., 2009; Gust et al., 2009). Both species showed different sensitivity levels. This could also occur in field exposures given that both freshwater snails thrive in the same habitat (Fretter and Graham, 1994). This study therefore focuses on the reprotoxic effects of a WWTP effluent on both snail species and was also the opportunity to investigate the suitability of the mudsnail to bioaccumulate contaminants from the water column.

Laboratory studies are very far from realistic field situations, where organisms are also subjected to variations in abiotic factors and changeable pollutant mixtures, and are exposed throughout their life cycle. Therefore, caging experiments have recently been implemented using *P. antipodarum*, either to assess contamination of the water column (Gust et al., 2011a, 2010a) or sediment (Schmitt et al., 2010b). Caged mudsnails have proven to be relevant for such experiments: both sensitive to pollutants and unaffected by variations in abiotic factors. In field studies, several endpoints can be assessed. Reproduction, which was shown to be impaired in a contaminated environment (Gust et al., 2010a), is particularly relevant because it is an important parameter to monitor for population dynamics. Other linked biomarkers, known to be affected by wastewater treatment plant (WWTP) effluent can also be assessed in the mudsnail: energy reserves (Gust et al., 2011a), vitellin-like proteins (Gagnaire et al., 2009), and vertebrate-like sex steroids (Gust et al., 2010a).

As for the latter, a recent study described estradiol (E2) signaling pathways at a molecular level (Stange and Oehlmann, 2012), but alterations of the hormonal signaling pathway have never been studied simultaneously with reproductive effects. Evidence also indicates that the signaling pathway in invertebrates may be a target of estrogenic chemicals (Castro et al., 2007); however, the issue of the involvement of steroids in mollusc reproduction remains very controversial (Scott, 2013). Nevertheless, estrogen-like (E2-like) chemicals or xenobiotics can trigger an estrogen-receptor-like (ER-like) activity and induce either genomic effects or nongenomic effects (Janer and Porte, 2007) using preserved pathways (Fig. 1). Genomic effects involve a nuclear ER, constitutively active in molluscs (Keay et al., 2006), which translocates into the nucleus and induces transcription of target genes (vitellogenin [VTG] potentially being a target gene). Prohibitin-2 (PHB) is an ER-coregulator that represses the genomic effects of estrogens (Mishra et al., 2006). Nongenomic effects are mediated via membrane or cytosolic ER (Canesi et al., 2004). Striatin (STN), a scaffold protein, assembles a membrane signaling complex (involving the Gαi protein) necessary for rapid, nongenomic activation of nitric oxide (NO) synthase by ERα (Qing et al., 2004). The release of NO induces rapid cellular effects. Other nuclear receptors are potentially involved in reproduction control such as COUP-TF (chicken ovalbumin upstream promoter-transcription factor), which is a preserved orphan receptor binding to various response elements including ER elements. It acts as a negative signaling regulator by many nuclear receptors such as ER (Tsai and Tsai, 1997). E75 (ecdysone-induced protein) is a nuclear receptor binding the insect molting hormone, ecdysone, which has been identified in molluscs, even if its function in reproduction control is unknown (Laguerre and Veenstra, 2010).

Therefore, the general aim of this study was to assess the relevance of gastropod species as biomonitoring tools to i) monitor chemical contamination and ii) assess toxic effects of water column pollution (survival, reproduction, growth). Specifically, snails were exposed *in situ* to a WWTP effluent discharge over 42 days. Within the French Program on Endocrine Disruptors, organic substances (pesticides, pharmaceuticals, and endocrine disruptors) were measured both in water and mudsnails. The effects on adult reproduction and juvenile growth were evaluated. Both chemicals and biological data were compared to yeast estrogen screening assay results. Feeding issues were validated by energy reserve measurement, and endocrine disruption was appraised by vitellin-like proteins and vertebrate-like sex-steroid measurements as well as E2 signaling pathway gene expression. Finally, the sensitivity of the mudsnail and the valve snail were compared.

2. Material and methods

Adult *P. antipodarum* were obtained from natural populations collected in clean sediment in the Rhone River 1 month before the beginning of the experiments. The juvenile *P. antipodarum* and adult *V. piscinalis* were obtained from long-term cultures