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Biochemical, physiological and behavioural markers in the endobenthic bivalve *Scrobicularia plana* as tools for the assessment of estuarine sediment quality

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ABSTRACT

The aim of this study was to link the responses at different levels of biological organisation of the endobenthic bivalve *Scrobicularia plana* differentially exposed to anthropogenic pressure. Clams were collected in April 2008 from three estuaries along a pollution gradient (Goyen < Loire < Seine). Biomarkers of defence (metallothionein concentration and glutathione-*S*-transferase activity) were activated in the Loire and the Seine. Biomarkers of damage revealed neurotoxicity (decreased AChE activity) and impairment of digestive enzyme activities (cellulase or amylase) in these estuaries. The highest lactate dehydrogenase activity was registered in the Loire estuary, in parallel with enhanced levels of vanadium (a metal present in petroleum), likely as a consequence of a small oil spill that occurred one month before the sampling collection. Physiological biomarkers (energy reserves as glycogen, lipids and proteins, condition and gonado-somatic indices) showed a few intersite differences. However, the median size was significantly lower in clams exposed to direct (chemicals) or indirect (available food) effects in the most contaminated site. Burrowing behaviour was disturbed in clams from both of the Loire and Seine estuaries, a response probably due to physiological impairment rather than to avoidance of contaminated sediment. The activation of defence mechanisms towards metals (metallothionein) and other classes of contaminants (the biotransformation enzyme glutathione-*S*-transferase) do not ensure a total protection since a number of impairments were observed at the infra-organismal (AChE and digestive enzyme activities) and individual (burrowing behaviour) levels in relation to the degree of anthropogenic pressure. However, even in the most contaminated estuary (Seine), historical records do not show a consistent decrease of *S. plana* populations.

1. Introduction

Historically, estuaries have been areas of settlement for many human populations, resulting in decreased water quality as a consequence of inputs of chemicals associated with industrial and domestic activities, pesticides and fertilizers derived from agricultural activities. In addition to such local contamination, estuarine ecosystems are exposed to toxic anthropogenic effluents transported by rivers from the whole river basin. Despite most of the chemicals introduced in aquatic environments by humans are strongly accumulated in sediments, biomonitoring programmes are generally based on sentinel species representative of the water column, such as mussels widely used for the assessment of environmental levels of contaminants (Mussel Watch programmes) as well as for examining biological effects of contaminants (WGBEC, 2006). However, a number of recent works highlight the interest of endobenthic invertebrates as sentinel species (e.g. Durou et al., 2007; Solé et al., 2009).

To-date, biochemical or physiological biomarker responses have not yet been able to provide useful predictions of effects at higher levels of biological organisation (Forbes et al., 2006) thus hampering ecological risk assessment. More recently, strategies were proposed with the aim of linking chemical stress to a risk of local extinction (termed "ecological death" by Kruzynski and Birtwell (1994)) in estuarine and coastal areas, considering cascading effects: from impairments of biological responses at the infra-individual level – as early and sensitive indices – to individual and

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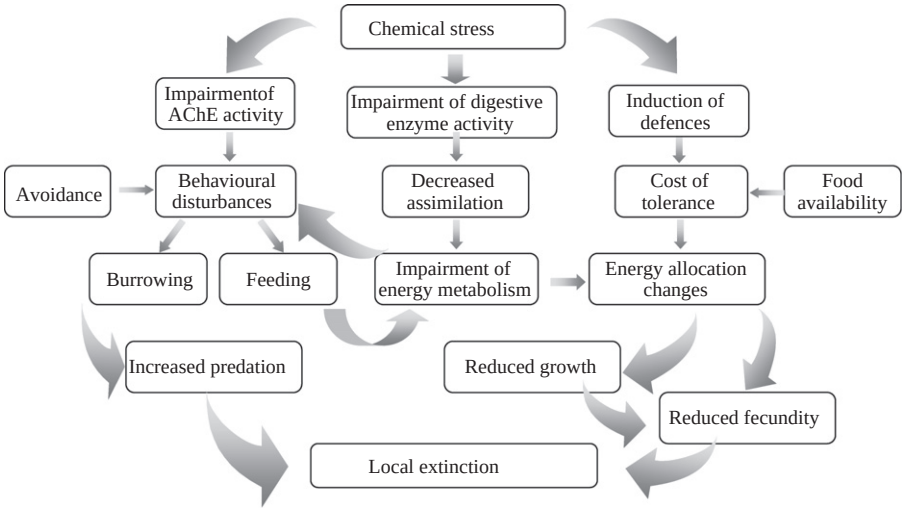

Fig. 1. Cascading effects of chemical stress at different levels of biological organisation.

supra-individual levels for their high ecological relevance (Weis et al., 2001; Amiard-Triquet and Rainbow, 2009) (Fig. 1). Reduced feeding and impairment of digestive enzymes can lead to reduced energy intake. In addition to this impairment of energy metabolism, activation of defences (metallothionein induction, increased activity of glutathione-*S*-transferase (Hoarau et al., 2001; Amiard et al., 2006)) can result in a cost of tolerance, leading to changes in energy allocation with implications for growth and reproduction and potential effects at the population level (Calow, 1991). In parallel, neurotoxic effects may be revealed by determining levels of neurotransmitters such as AChE activity, a core biomarker the relationship of which with behavioural disturbances is well-documented (Amiard-Triquet, 2009). In addition to feeding, burrowing of endobenthic species is also a behaviour of interest, controlling the relationships between predators and preys. Such a comprehensive approach would allow deciding protective procedures well before the situation has reached a point at which remediation is nearly impossible.

The present study was designed taking into account most of the concepts described above. We have selected the endobenthic bivalve *Scrobicularia plana* which play a key role in the structure and functioning of estuarine ecosystems. It is a dominant species in estuarine and coastal mudflats, a major link in estuarine food webs and it plays a role in biogeochemical cycles of both nutrients and contaminants as a consequence of its bioturbation activity. Another interest of this species is the strong basis available in the literature about its biology and ecology (Hughes, 1969; Mouneyrac et al., 2008; Worrall and Widdows, 1983; Worrall et al., 1983; Rodriguez-Rùa et al., 2003). Clams were collected from three estuaries along a pollution gradient according to the results of biomonitoring networks (AELB, 2004; RNO, 2006). Mechanisms of defence were examined through MT concentrations and GST activities in specimens from the different estuaries. Neurotoxicity was assessed by using AChE activity and associated with determinations of burrowing rates.

In bivalves, digestion takes place through two steps, with first an extracellular digestion and then an intracellular digestion (Morton, 1983). The crystalline style begins the digestion by mechanical and chemical actions. This extracellular phase is then completed in the digestive diverticula of the digestive gland where intracellular digestion occurs, within phagocytic digestive cells. Potential assimilation impairments were thus examined taking into account activities of cellulase and amylase in the digestive gland and the crystalline style, these two enzymes being important in a species feeding mainly on deposits (including microphytobenthos) at low tide and seston (including phytoplankton) at high tide (Hughes, 1969). Observations on intersite differences in energy metabolism were based on the measurement of the energy reserves (as proteins, lipids and glycogen) and a special attention was devoted to lactate dehydrogenase (LDH) activity because of its important function in anaerobic metabolism. Consequences in terms of fitness impairment were investigated by determining condition and gonado-somatic indices in clams from the different sites. Intersite variations of effects at different levels of biological organisation are interpreted taking into account anthropogenic pressures particularly in terms of metal contamination.

2. Material and methods

2.1. Characterisation of sampling sites

Clams were collected in April 2008 at 3 sites (Fig. 2) differentially exposed to anthropogenic pressure. Because it is impossible to analyse all the chemicals able to impair biological responses, the choice of these sites took into account informations

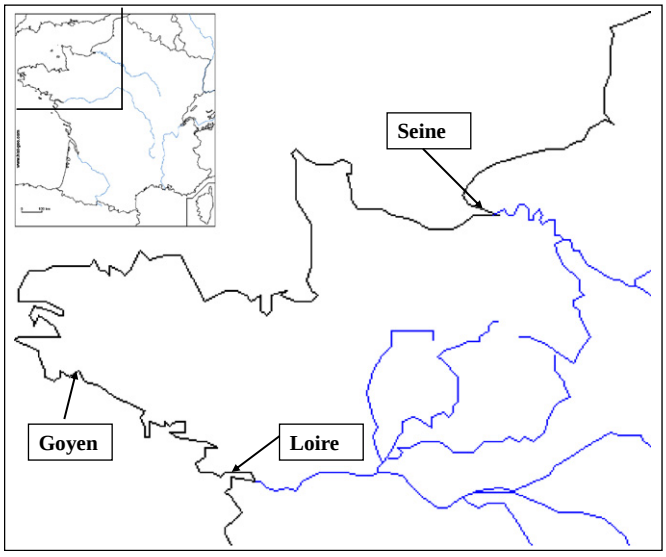

Fig. 2. Sampling sites.