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Physiological and behavioural responses of *Gammarus pulex* exposed to acid stress

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Abstract

Physiological and behavioural responses of the acid-sensitive amphipod *Gammarus pulex* exposed to a wide range of acid conditions (pH 4.1, 5.1, 6.0) under laboratory conditions were investigated. An exposure of 38 h to acid conditions caused significant decreases in survival rate, osmolality, haemolymph Na^+ concentration, ventilatory and locomotor activity compared to organisms exposed to a circumneutral medium (pH 7.9). We highlighted the interest of using individual response distribution, since drastic effects can be detected in organisms exposed to pH 6.0, in particular for osmolality: The response can be divided into two groups, unimpacted and impacted organisms. Moreover this representation permitted to evaluate the health level of individual organisms through the determination of threshold values. Significant correlations between mean pH and mean physiological/behavioural responses were observed. The relationships between individual responses permitted not only to compare endpoints, but also to show that affected organisms were impacted by ionoregulation failure, hypoventilation and low locomotor activity. The energetic reallocation in favour of maintenance functions, such as osmoregulation, is discussed. The results of this study indicate that the values of haemolymph Na^+ concentration, osmolality and locomotor activity in *G. pulex* could be effective ecophysiological/behavioural markers to monitor freshwater ecosystems and to assess the health of organisms or populations.

Keywords: *Gammarus pulex*; Survival; Osmolality; Ion loss; Ventilation; Locomotion; Acid stress

1. Introduction

Acidification of freshwater ecosystems related to anthropogenic emissions of SO_2 and NO_X has been one of the most striking ecological problems throughout the northern hemisphere during the 20th century. National and international legislation in the 1980s and 1990s aimed to reduce the emissions of acidifying pollutants (*e.g.* Clean Air Act in the USA and the Convention on Long-Range Transboundary Air Pollution in Europe) have led to the decline in acidic depositions across wide areas of Europe and North America (Stoddard et al., 1999; Lawrence et al., 2000; Likens et al., 2001). Several recent studies have shown that recovery of alkalinity has occurred in many areas of Europe and North America (Stoddard et al., 1999; Skjelkvale et al., 2001), but acidification of freshwater ecosystems still occurs in many areas (Guérold et al., 2000; Driscoll et al., 2001; Evans et al., 2001). In addition, acidification of aquatic ecosystems is now reported across other large areas of the world where high economic and demographic growth rates occur, such as in China (Thorjørn et al., 1999; Tang et al., 2001) and India (Aggarwal et al., 2001). A major consequence of freshwater acidification is the erosion of biodiversity (Muniz, 1991), perhaps through negative interferences with the physiology of affected species. Studies have clearly demonstrated a failure to regulate blood or haemolymph Na^+ and Cl^- levels in acid-stressed fish (Massabuau, 1985; McDonald et al., 1989; Potts and McWilliams, 1989; Wood, 1989), clams (Unionidae: Pynnönen, 1991) and decapod crustaceans (Appelberg, 1985; Jensen and Malte, 1990). However, most of the studies have focused on large species, and relatively little is known about physiological responses in smaller acid-sensitive species of macroinvertebrates.

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Studies have previously shown that crustaceans exposed to water-borne pollutants, acid stress and pathological agents usually exhibit disruption of ionic and/or osmotic regulation (McMahon and Stuart, 1989; Rupprecht, 1992; Lignot et al., 2000; Felten and Guérold, 2001, 2004). Different causes include alterations in the structure and ultrastructure of the branchial and excretory organs, and changes in Na^+, K^+-ATPase activity, ion fluxes and surface permeability. Consequently, the capacity to osmoregulate has been proposed as an effective way to monitor the health status of crustaceans (review in Lignot et al., 2000).

Osmoregulation is an important and highly energy-consuming regulatory function in aquatic invertebrates. The total energy budget devoted to osmoregulation was evaluated at 11% in *Gammarus pulex* (Sutcliffe, 1984). We thus hypothesize that adverse environmental stress may impair osmoregulation as well as other functions linked to behaviour. In fact under stress conditions, energetic allocation should be in favour of maintenance function (such as osmoregulation).

In the past few years, studies have used behavioural responses as tools for ecotoxicity testing in crustaceans (Wallace and Estephan, 2004; Mills et al., 2006, De Lange et al., 2006) and other invertebrates such as bivalve mollusks (Legeay et al., 2005; Duquesne et al., 2004) and water quality monitoring. Thus, Wallace and Estephan (2004) showed that a 72 h-exposure of *Gammarus lawrencianus* to [Cd] ≥ 500 µg L^{-1} lead to a significant reduction of horizontal and vertical swimming. Other studies carried on copper and pharmaceuticals also report a reduction in locomotor activity (Mills et al., 2006; De Lange et al., 2006). The development of this kind of test is of interest for ecotoxicology because in addition to being sensitive, fast, simple to perform and cheap, they allow to link toxic effects obtained at biochemical/cellular levels to impacts observed on populations and communities (Wallace and Estephan, 2004).

The freshwater gammarid *G. pulex* was selected as the test species because it 1) is widespread and common in West Paleartica (Barnard and Barnard, 1983), 2) is known to be sensitive to a range of stresses (acid-sensitive: Sutcliffe and Carrick, 1973), 3) is often occurring in high density, 4) is easy to identify to species level, 5) is currently used in ecotoxicological test, 6) is an important reserve of food for macroinvertebrates, fish, bird and amphibian species (Welton, 1979; Friberg et al., 1994; MacNeil et al., 2000), and 7) is playing a major role in the leaf litter breakdown process and consequently in the entire food web (Forrow and Maltby, 2000).

The objectives of the present study were 1) to investigate physiological and behavioural responses of *G. pulex* exposed to acid stress which is known to induce selective impact on iono and osmoregulation, 2) to compare acid stress effects observed on physiological and behavioural responses and 3) to define physiological and behavioural thresholds predictive of health impairment. In this context measurements of osmolality, haemolymph Na^+ concentration, ventilation (pleopod beat number), and locomotor activity were performed in *G. pulex* exposed to different levels of acid stress under laboratory conditions.

2. Materials and methods

2.1. Collection and maintenance of animals

G. pulex were collected using a hand net from the neutral and unpolluted stream, La Boubre (near Lyon, France; pH of the water 7.8 ± 0.1). The organisms were quickly transported (< 1 h) in plastic vessels to the laboratory, where they were acclimated at 12 °C in natural drilling water (composition in Table 1) for 10 days before being used in experiments. Animals were fed on alder leaves (*Alnus glutinosa*).

2.2. Experimental design

Test solutions were prepared using natural water (pH 7.9 ± 0.01, Table 1). Sulfuric acid (96%) was added to obtain pH 4, pH 5 and pH 6 (actual pH, mean ± SD: 4.1 ± 0.26; 5.1 ± 0.15; 6.0 ± 0.05). Male *G. pulex* in similar intermoult C stage with 7–9 mm body size were selected for the study. Moult stages were determined by microscopical observation of the tip of posterior pereopods according to Blanchet-Tournier (1980). Three replicates of 30 individuals were exposed at 12 °C for 38 h to the 4 different pH conditions in 500-mL-glass tank. In order to avoid chemical variations, the water was renewed every 12 h after daily pH measurements. Animals were not fed during the experiment.

At the end of the exposure period, survival, locomotor and ventilatory activities were assessed, and samples of haemolymph were punctured from 10 and 15 organisms randomly chosen in each condition for haemolymph Na^+ concentration and osmolality measurements, respectively. Thus, a total of 25 individuals were randomly selected among the 90 gammarids (3 * 30) exposed to each treatment: In order to assess the survival, the number of gammarids exposed was higher than the number of organisms needed for the experiment. Locomotor activity, then ventilatory activity and then haemolymph Na^+ concentration were measured on the same organisms allowing the analysis of individual responses relationship (see Fig. 3). Handling and methods may induce stress in organisms, however identical process has been used in all cases, allowing to assess the impact of acid stress in exposed organisms.

Table 1
Mean (±SD) values of various chemicals of the water used in the experiments (n=4)

	Mean ± SD
Conductivity (µS cm^{-1})	569 ± 1
pH	7.9 ± 0.01
Temperature (°C)	12.03 ± 0.06
[Cl^-] (mg L^{-1})	66.7 ± 3.5
[NO_3^-] (mg L^{-1})	27 ± 1
[PO_4^{3-}] (mg L^{-1})	< 0.03
[SO_4^{2-}] (mg L^{-1})	68.3 ± 3.5
[Na^+] (mg L^{-1})	29.7 ± 1.5
[K^+] (mg L^{-1})	2.43 ± 0.21
[Ca^{2+}] (mg L^{-1})	99.3 ± 3.1
[Mg^{2+}] (mg L^{-1})	5.17 ± 0.29