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Acid Effect on Ion Changes from Haemolymph of *Orthetrum sabina* Nymph

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ABSTRACT

The determination of acidification effect on haemolymph concentration of odonate nymph was studied. The experiment was conducted to evaluated Na^+ and Cl^- ion changes in the haemolymph. The Odonate nymph, *Orthetrum sabina*, was exposed to synthetically prepared acidic water (pH 4, 5) for 12 h, 24 h and 48 h. The concentrations of Na^+ and Cl^- in the haemolymph were assessed after each exposure period to the acidic water. The exposure caused significant losses of Na^+ and Cl^- concentrations in the haemolymph after 12 to 48 h of exposure. The acid water was positively correlated with the decrease in the Na^+ and Cl^- concentrations in the studies. Importantly, all aquatic organisms display direct effects to varying pH values, which presents challenges to the extracellular fluid ions and pH homeostasis. There is a need for *Orthetrum sabina* to balance the ions from environmental influences via specific channels or the carrier itself. Na^+ and Cl^- loss actually is coupled to H^+ and HCO^-_3 levels found in the surrounding environment, either by Na^+/H^+ or Cl^-/HCO^-_3 exchangers. The results indicate that Na^+ and Cl^- concentration levels in the haemolymph of *Orthetrum sabina* could be effective ecophysiological markers in the monitoring of acid freshwater ecosystems.

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INTRODUCTION

A major consequence of freshwater acidification is the erosion of biodiversity [1], perhaps through a negative interference with the physiology of the affected species. In order to evaluate the degree of acidification and the long-term trends and variations in the aquatic chemistry and aquatic biota, monitoring programs have been in place for several decades in Europe and North America. Presently, 23 countries in Europe and North America are participating in this program. Although chemical trends have been well documented at numerous sites, the effects of acidification (or recovery) on aquatic biota have been largely neglected, except when reporting the presence or absence of benthic invertebrates. For example, acidic pH values and heavy metals of AMD that have been reported up to now are considered indicators for the presence of algae, high densities of chironomids, the presence of iron hydroxide and show a correlation with pH values [2]. In addition to being important to the community of organisms, macroinvertebrates have been used as indicators of water quality for several other reasons. Different macroinvertebrate groups can survive in varying levels of water quality. Some are very intolerant of poor water quality while others not only survive, but actually thrive, when water quality levels decrease.

Acid-sensitive indicators as monitors of acidification have been useful tools [3], but they provide limited information about how organisms respond to and recover from environmental stressors. A more powerful and relevant approach is a combination of bioindicators at the population/community level, together with rapidly responding exposure biomarkers, such as alterations in haemolymph chemistry. By using an integrated approach, the link between environmental changes and observed biological effects can not only be assessed [4], but can also be applied to determine stream water quality (a combination of several chemical parameters), compare acidification levels of several brooks and then monitor the acidification state (during the year or with the passing of years) and to monitor long-term evolution periods (degradation or recovery) [5, 6].

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In recent years, there has been a growing awareness of the need to detect and assess the adverse effects of contaminants in inhabiting fauna. Among the available techniques, the integrated use of physico-chemical analyses and bio-chemical responses to pollutants is a sound procedure for the detection of the impact of contaminants on ecosystems [7, 8, 9]. An ion-regulation failure leading to a severe deficiency of extracellular ions (i.e. Na^+ and Cl^-) has been recognized as a major response in fish to acid stress levels [10, 11, 12, 13, 14, 15, 16, 17] and also reported in Amphipod *Gammarus fossarum* [18, 19, 20] and found in aquatic insect *Hydropsyche pellucidula* (Trichoptera) and *Dinocras cephalotes* (Plecoptera) [20]. Similar results have also been reported in crayfish [21, 22, 23, 24], and in molluscs [25, 26, 27]. More recent studies using two cichlid fish species of the Amazon evaluated the effects of acute exposure to low pH. [28]

The aims of this study were to determine a correlation between water acidification and ion levels in the haemolymph and to examine the physiological response and ion change of the haemolymph of odonate nymphs to acidic conditions in the laboratory.

MATERIALS AND METHODS

This study aimed to investigate the ecotoxicological effects of acid stress on the physiological characteristics of odonate nymphs. Odonate nymphs were collected from unacidic water and reared in the laboratory. Ion changes in the haemolymph were investigated over the course of the experiment. Haemolymph samples were collected for ion analysis using Ion Chromatography (IC) and involving sodium (Na^+) and chloride (Cl^-). This experiment investigated the physiological responses of odonate nymphs to acidic water. The odonate nymph samples were chosen according to species that were similar. The experimental design involved 3 different pH levels (4, 5 and 7) and odonate nymphs were reared in each pH value. Odonate nymph haemolymph samples were collected every 12 hours. After the experiment, ion changes in the haemolymph were determined by those that responded to acidic water and were indicative of a correlation between the acid value and Na^+ and Cl^- ion changes in the haemolymph. The data were used to monitor the effects from acid stress at the drainage water source.

Toxicity Test:

For this experiment, the nymphs of *Orthetrum sabina* were collected from a natural, neutral reservoir and were exposed to acidic water at pH values of 4, 5 and 7 and reared in a 20x30 centimeter glass aquarium. Three pH treatments of 36 individuals were exposed during each treatment. After 0, 12, 24 and 48 hours of exposure during each treatment, survival rates and haemolymph quantities were observed and collected from each replicate (3 individual each). Samples of haemolymph from the odonate nymph were randomly collected during each treatment for analysis.

Data Analysis:

The 2-way analysis of variance (ANOVA) was used to detect the effects of acidification, exposure time and the interaction of the ion concentration and pH levels on the haemolymph. The data on exposure time, parametric tests (*t*-test) was used to test for differences in mean ion concentrations of the haemolymph between the control (0 h) and the treatment organisms.

RESULTS AND DISCUSSION

Concentrations of Na^+ and Cl^- in haemolymph reduced at pH 4 and 5 values at 48 h (hours) (Table 1-2 and Figure 1-2). Acid exposure at pH 5 and pH 4 values for 48 hours reduced the Na^+ and Cl^- concentrations in haemolymph, when compared with those of a pH value of 7 (Tables 1 - 2 and Figures 1 - 2). With regard to pH 5, the mean (±SD) Na^+ concentration decreased from 183±3 mmol/L to 170±5 mmol/L over 12 h, 168±9 mmol/L in 24 h and 168±4 mmol/L over 48 h representing a significant loss of ion ($p<0.05$) between exposure times. With regard to pH 4, the mean (±SD) Na^+ concentrations decreased from 181±6 mmol/L to 163±5 mmol/L in 12 h, 154±5 mmol/L over 24 h and 149±2 mmol/L over 48 h, the results also found a significant loss of ions ($p<0.05$) between exposure times. In terms of the statistical analysis of variance, the time of exposure had a significant correlation between 0 h and the other times (12 h, 24 h and 48 h). However, a decrease in Na^+ concentrations for 12 h, 24 h and 48 h were not found to be significant, but the results from different treatments altering the pH values revealed significant differences ($p<0.05$). Differences were found between pH 7 and acidic pH values (pH 5 and 4).

The same trend was observed for Cl^- concentrations. With regard to pH 5, the mean (±SD) Cl^- concentration decreased from 136±6 mmol/L to 129±1 mmol/L over 12 h, 122±1 mmol/L over 24 h and 119±0.3 mmol/L over 48 h, while at a pH value of 4, the mean (±SD) Cl^- concentration decreased from 140±3 mmol/L to 124±4 mmol/L over 12 h, 115±4 mmol/L over 24 h and 108±4 mmol/L over 48 h representing a significant loss of ions ($p<0.05$) between exposure times. According to the statistical analysis of variance, the time of exposure between

0 h and 12 h and the other times (24 h and 48 h) revealed a significant difference. A significant difference in the Cl^- concentrations between pH treatments was also found. These losses of Na^+ and Cl^- concentrations were significantly correlated to the pH.

In this study, we demonstrated that the exposure of odonate nymphs to acidic water resulted in significantly losses of Na^+ and Cl^- concentrations in the haemolymph of *Orthetrum sabina* after 12 h to 48 h of exposure. This present study confirms the results obtained from the previous experiments, which clearly showed the effect of low pH values on Na^+ and Cl^-concentrations in the haemolymph of many animals, e.g. fish, clams, crustaceans and invertebrates. That results show that the effect of acid stress on *Gammarus fossarum* was correlated with the depletion of Na^+ and Cl^- concentrations, while similar responses had been reported among fish to acidic stress [15] and were also reported in three common macroinvertebrate species, namely *Gammarus fossarum*, *Hydropsyche pellucidula* and *Dinocrascephalotes* by Felten and Guerold [20]. This study and a previous study also agreed with the findings of Morgan and McMahon [21], which were obtained from *Procambarus clarkia* and *Orconectes rusticus* during acid exposure experimentation and has been investigated in Australian crayfish *Cherax destructer* [29]. Similarly, larvae of *Aedes aegypti* and *Culex quinquefasciatus* showed decreased Na^+ uptake rates during acute exposure to acidic water (pH 3.5) [30].

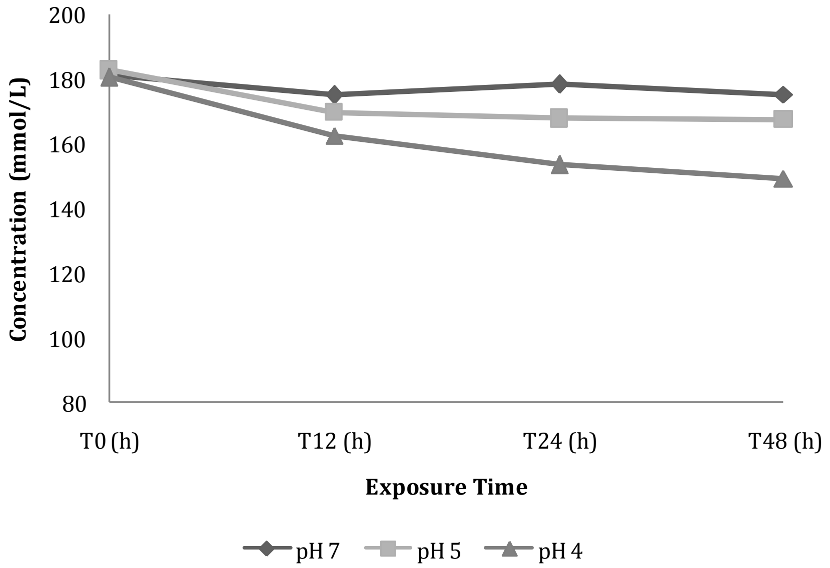

Fig. 1: Haemolymph concentrations of Na^+ in *Orthetrum sabina* during exposure times within each pH reatment (n=3).

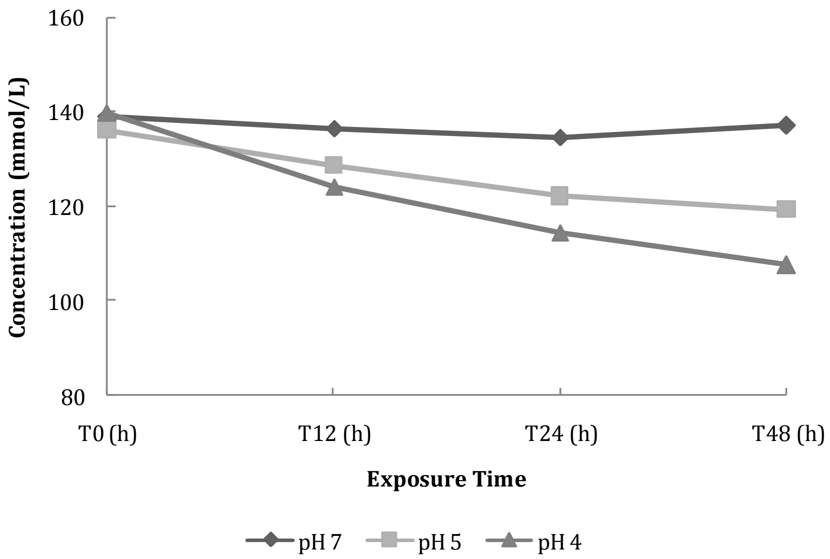

Fig. 2: Haemolymph concentrations of Cl^- in *Orthetrum sabina* during exposure times within each pH treatment (n=3).

Table 1: Mean and SD of Haemolymph concentrations of Na^+ in *Orthetrum sabina* during exposure times within each pH treatment (n=3).

pH	Na^+ concentration (mmol/L)				
	Time	T0 (h)	T12 (h)	T24 (h)	T48 (h)
7	mean ± sd	181 ± 3	175 ± 6	178 ± 8	175 ± 7
5	mean ± sd	183 ± 3	170 ± 5	168 ± 9	168 ± 4
4	mean ± sd	181 ± 6	163 ± 5	154 ± 5	149 ± 2

Table 2: Mean and SD of Haemolymph concentrations of Cl^- in *Orthetrum sabina* during exposure times within each pH treatment (n=3).

pH	Cl^- concentration (mmol/L)				
	Time	T0 (h)	T12 (h)	T24 (h)	T48 (h)
7	mean ± sd	139 ± 6	136 ± 3	135 ± 1	137 ± 8
5	mean ± sd	136 ± 6	129 ± 1	122 ± 1	119 ± 0.3
4	mean ± sd	140 ± 3	124 ± 4	115 ± 4	108 ± 4

Therefore, pH is an extremely important physical factor that may limit the distribution and abundance of aquatic animals. Accordingly, pH value will have a direct effect on aquatic organisms due to challenges to extracellular fluid ion and pH homeostasis. To keep pH values close to the neutral point seems to be most important for intracellular metabolisms, which are highly sensitive to pH levels. To maintain ion homeostasis, organisms need to balance the ion from the environment via specific channels or the carrier. Consideration of Na^+ and Cl^- loss actually is coupled to H^+ and HCO^-_3 from the surrounding environment, either by Na^+/H^+ and Cl^-/HCO^-_3 exchangers [31, 32, 33, 34]. When the ambient H^+ and HCO^-_3 are increased by lower pH, the haemolymph Na^+ and Cl^- will be decreased. That are the mechanism of aquatic insects exchange ions and water with the medium.

Conclusion:

The exposure of Odonate nymphs to acidic water caused significant losses of Na^+ and Cl^- concentrations in the haemolymph of *Orthetrum sabina* after 12 h to 48 h of exposure. These losses were significantly correlated to the pH values. This present study confirms the results obtained in a previous experiment, which clearly revealed the effect of low pH to Na^+ and Cl^- concentrations in the haemolymph of many animals. In this study, we recommend that further studies be conducted to deal with the effects of acidification on population structure, in relation to certain physiological parameters (e.g. growth) in order to better understand population regression and recovery rates.

The Na^+ and Cl^- ions change of odonate nymph has been evaluated in terms of physiological response which widespread used in biomonitoring or as a biomarker of exposure. These physiological responses are useful for monitoring fluctuation exposures, or acting as early warning of aquatic stress.

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