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► To cite this version:

V. Felten, F. Guerold. Short-term physiological responses to a severe acid stress in three macroinvertebrate species: A comparative study. *Chemosphere*, 2006, 63, p. 1427 - p. 1435. 10.1016/j.chemosphere.2005.10.003 . hal-00452244

HAL Id: hal-00452244

<https://hal.science/hal-00452244>

Submitted on 1 Feb 2010

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Author-produced version of the article published in Chemosphere
2006, 63, 1427-1435.

The original publication is available at [http://www.sciencedirect.com/
doi : 10.1016/j.chemosphere.2005.10.003](http://www.sciencedirect.com/doi:10.1016/j.chemosphere.2005.10.003)

Short-term physiological responses to a severe acid stress in three macroinvertebrate species: a comparative study.

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Abstract

The present study focuses on the sensitivity among freshwater invertebrate species to acidic stress. Three common macroinvertebrate species in the Vosges Mountains (North-Eastern France), *Gammarus fossarum* (Amphipoda), *Hydropsyche pellucidula* (Trichoptera) and *Dinocras cephalotes* (Plecoptera) were exposed for 24, 72 and 120 hours to natural acidified water (pH= 4.73 ± 0.08 , $[Ca^{2+}] = 39.1 \pm 0.6 \mu\text{mol.L}^{-1}$, $[Al_{\text{tot}}] = 28.4 \pm 1 \mu\text{mol.L}^{-1}$). Short-term exposure to acid stress caused significant decreases both in survival rate and haemolymph ions ($[Cl^-]$ and $[Na^+]$). The relative sensitivity to a natural acidic stress slightly differed among the species and was in the following order: *G. fossarum*, as the most sensitive, then *H. pellucidula* and *D. cephalotes*. Results of this study confirm the interest of *in situ* tests to assess the toxicity of short-term acid exposure. Finally, our results reinforce the hypothesis that transient acidification can offset the recovery of sensitive species of macroinvertebrates in streams chemically recovering from acidification either through liming or declining deposition.

Keywords: Acidification, Macroinvertebrate, Ion loss, Survival, Acid-sensitivity.

INTRODUCTION

Acidification of freshwater ecosystems related to anthropogenic emissions of SO₂ 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 at reducing 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, Evans et al., 2001, Likens et al., 2001, Folster and Wilander, 2002). Consequently, it has been assumed that the 'acidification problem' was solved. However, even if recent studies have shown that recovery of alkalinity has occurred in several areas of Europe and North America (Stoddard et al., 1999, Skjelkvale et al., 2001), acidification still occurs in many areas. (Driscoll et al., 2001, Evans et al., 2001, Wright et al., 2005).

Concomitantly, a decline of base cations (mainly Ca²⁺ and Mg²⁺) in soils and surface waters has been reported in most areas where high rates of sulphur depositions occurred previously (Likens et al., 1996, 1998, Bouchard, 1997, Lawrence et al., 1999, Castro & Morgan, 2000, Driscoll et al., 2001, Tessier et al., 2002). If such cation depletion continues, it will represent another serious threat to aquatic ecosystems. In addition, acidification of aquatic ecosystems is now reported across other large areas of the world, such as China (Thorjörn et al., 1999, Tang et al., 2001) and India (Aggarwal et al., 2001). Therefore, it appears that acidification of soils and water remains an important environmental problem.

One of the most striking consequences of freshwater acidification is the loss of biodiversity (Muniz, 1991). An ion-regulation failure leading to a severe deficiency of extracellular ions (i.e. Na⁺ and Cl⁻) has been recognised to be the major response in fish to acid stress (Neville,

1985, Booth *et al.*, 1988, Wood *et al.*, 1988, McDonald *et al.*, 1989, Potts & McWilliams, 1989, Wood, 1989, Masson *et al.*, 2002). Similar results have been also reported in crayfish (Morgan & McMahon, 1982, Wood & Ronago, 1986, McMahon & Stuart, 1989, Jensen & Malte, 1990), and molluscs (Malley *et al.*, 1988, Pynnönen, 1990, 1991).

Surprisingly, and despite the numerous papers reporting detrimental effects of acidification on macroinvertebrate communities, few realistic studies have been performed to assess the ecophysiology of smaller acid-sensitive species and finally, relatively little is known about their physiological responses to short-term acid stress. In previous studies (Felten & Guérol, 2001, 2004), we showed that *Gammarus fossarum* (Crustacea: Amphipoda), a common acid-sensitive species in west Palearctica, also sustained a severe depletion of haemolymph Na^+ and Cl^- ions when exposed to acidic conditions. Conjointly, we determined relationship between acidification level and haemolymph ion losses (Felten & Guérol, 2004).

In order to better understand the ecology of ecosystems recovering from acidification and because episodic acid-stresses generated by heavy rainfall and snowmelt continue to affect streams, the purpose of the present study was to investigate and to compare *in situ* the physiological responses and survival rates of three common acid-sensitive species to realistic episodic acid stress.

MATERIALS AND METHODS

Study sites. The study was performed in the Vosges Mountains (North-Eastern France). Two headwater streams located in the same area were selected because they presented very similar morphological characteristics (**Table 1**) but contrasted buffer capacities: a circumneutral stream (La Maix) and a strongly acidified stream (Gentil Sapin).

Study organisms. Three macroinvertebrate species were selected because they are known to be acid-sensitive, widespread in European inland waters and easy to collect: *Hydropsyche pellucidula* (Trichoptera, Hydropsychidae), *Dinocras cephalotes* (Plecoptera, Perlidae) and *Gammarus fossarum* (Amphipoda, Gammaridae). Organisms were collected by using a net from a circumneutral stream, La Maix.

Experimental design. A total of 1728 males of *Gammarus fossarum* with 8 to 10mm body lengths were collected. 288 organisms were placed in 2 Plexiglas flow-through enclosures each consisting of 18 compartments each with 8 organisms.

Conjointly, we used 216 *Dinocras Cephalotes* and 216 *Hydropsyche pellucidula* with body lengths of 20mm and 15 mm respectively. For both species, 36 organisms were placed in 2 Plexiglas flow-through enclosures each consisting of 18 compartments (10-cm long and 5-cm i.d.) each filled with 1 organism to avoid potential cannibalism.

The enclosures were then transferred to the 2 streams (circumneutral and strongly acid). For each stream, one enclosure was used for haemolymph analyses and the other for the assessment of survival. For each stream and species, there were 3 pairs of enclosure each corresponding to one exposure time (24, 72 and 120 h).

In order to evaluate the initial concentrations of haemolymph Cl^- and Na^+ in each species, organisms (12 individuals for *G. fossarum*, 8 individuals for *D. cephalotes* and 8 individuals for *H. pellucidula*) were sampled in the neutral “native” stream just before the onset of the experiments (T_0 , control). At 24, 72 and 120 h after exposure transfer, survival was assessed and samples of haemolymph from 8 (*G. fossarum*) or 6 (*D. cephalotes*, *H. pellucidula*) organisms were randomly collected in each stream for analysis (in enclosure corresponding to exposure time). The organisms were not fed during the experiment. However, the experimental design allowed drifting material (organic particles, algae and small invertebrates

< 1 mm) to enter the compartments. For more details concerning enclosure technique see Felten & Guérolde (2004).

Survival, haemolymph sampling and analysis. The survival was assessed in each stream for each species and exposure time (*G. fossarum*: 3 replicates of 48 organisms; *D. cephalotes* and *H. pellucidula*: 3 replicates of 6 organisms).

For *D. cephalotes* and *H. pellucidula* samples of haemolymph were taken from the base of the cephalic capsule with a 5- μ l microcapillary tube (n = 6). For *G. fossarum*, samples were taken from the telson (n = 8) using a microsyringe and then transferred to a gauged 5- μ l microcapillary. After centrifugation of the microcapillary tubes (10 min at 6596 g), the liquid phase was diluted in 2 ml of Nanopur water to determine chloride and sodium concentrations in haemolymph by ionic chromatography (Dionex 4500i with Ion Pac AS4A column) and atomic absorption spectrophotometry (AAS) (Perkin Elmer Analyst 100), respectively.

Water analysis. Water was collected at the initiation of the experiment (T_0) and at each exposure time (24, 72 and 120 h). Cations were analysed by flame AAS and anions by ionic chromatography as described previously. Total aluminium was determined by graphite furnace AAS (Varian Spectraa 300) after acidification with 0.25% HNO_3 . Acid neutralising capacity (ANC) was measured by Gran's titration and pH (glass electrode), and conductivity with a multi-parametric equipment (WTW). Chemical characteristics of water of each stream are given in **Table 2**.

Statistical analysis. All data are reported as mean $\pm$ SD. Statistical comparisons of experimental data were performed by two-way analysis of variance (ANOVA) and Fisher's Least Significant Difference test (LSD). The analyses were carried out using STATISTICA

(Microsoft), with a probability limit of $p \leq 0.05$ considered as significant.

RESULTS

To study and compare the effect of acidification on haemolymph $[\text{Na}^+]$ and $[\text{Cl}^-]$ and survival of the 3 species, specimens were transferred from the native circumneutral stream to a strongly acidified stream. Acidified stream was characterized by low pH (i.e. high $[\text{H}^+]$), low ANC, low $[\text{Mg}^{2+}]$ and low $[\text{Ca}^{2+}]$ and high $[\text{Al}_{\text{tot}}]$. Note that, small-scale differences in mineral composition of the underlying bedrock explain the marked differences in buffering capacity, ion content and acidification (**Table2**). The 2-way analysis of variance (ANOVA) indicated that the factors “exposure time” and “stream” (acidification level) exerted a significant effect on haemolymph parameters ($[\text{Na}^+]$, $[\text{Cl}^-]$) and survival (**Table 3**).

Gammarus fossarum

G. fossarum baseline levels of haemolymph $[\text{Cl}^-]$ and $[\text{Na}^+]$ on the onset of the exposure were 61.4 ± 9.2 and $77.5 \pm 15.5 \text{ mmol.L}^{-1}$ (mean $\pm$ SD), respectively, in control organisms collected in La Maix (**Fig. 1a&b**).

Haemolymph $[\text{Na}^+]$ and $[\text{Cl}^-]$ in organisms exposed in the circumneutral stream (mean pH = 7.42) remained constant over the 120-h exposure period, but significantly decreased in organisms exposed in the strongly acidic one (mean pH = 4.73) (**Fig. 1a&b**). Indeed, in the acidic stream, the loss of haemolymph Cl^- (**Fig. 1a**) ranged from about 44.4% after 72h (mean haemolymph $[\text{Cl}^-] = 33.6 \pm 4.8 \text{ mmol.L}^{-1}$) to 58.7% after 120 h of exposure (mean haemolymph $\text{Cl}^- = 23.2 \pm 3.7 \text{ mmol.L}^{-1}$) compared with the control (mean haemolymph $\text{Cl}^- = 61.4 \pm 9.2 \text{ mmol.L}^{-1}$).

The same trend was observed for haemolymph $[\text{Na}^+]$ (**Fig. 1b**). In the acidic stream, the significant loss of haemolymph Na^+ ranged from 48.5% after 72 h of exposure (mean

150 haemolymph $\text{Na}^+ = 38.5 \pm 6.1 \text{ mmol.L}^{-1}$) to 57.4% after 120 h of exposure (mean
151 haemolymph $\text{Na}^+ = 31.1 \pm 3.5 \text{ mmol.L}^{-1}$) compared with the control (mean haemolymph Na^+
152 $= 77.5 \pm 15.5 \text{ mmol.L}^{-1}$).

153 For each exposure time, the survival rate in *G. fossarum* transferred to the circumneutral
154 stream (La Maix) remained very high and above 96%. On the contrary, the survival rate in
155 organisms exposed to acidic water significantly decreased from $79.9 \pm 10.8\%$ after 24h of
156 exposure to $20.5 \pm 6.2\%$ after 120h of exposure (**Fig. 1c**).

157

158 *Hydropsyche pellucidula*

159 As observed for *G. fossarum*, the factors “exposure time” and “stream” (acidification level)
160 exerted a significant effect on haemolymph parameters ($[\text{Na}^+]$, $[\text{Cl}^-]$) and survival (2-way
161 ANOVA, **Table 3**). *H. pellucidula* baseline levels of haemolymph $[\text{Cl}^-]$ and $[\text{Na}^+]$ on the
162 onset of the exposure were 37.2 ± 5.7 and $132.2 \pm 8.8 \text{ mmol.L}^{-1}$ (mean $\pm$ SD) respectively, in
163 control organisms collected in the neutral stream (**Fig. 2a&b**).

164 Haemolymph $[\text{Na}^+]$ and $[\text{Cl}^-]$ in organisms exposed into the circumneutral stream remained
165 constant over the 120-h exposure period, but significantly decreased in organisms exposed to
166 the strongly acidic stream. Indeed, in the acidic stream, the loss of haemolymph Cl^- ranged
167 from 33.6% after 24 h of exposure (mean haemolymph $[\text{Cl}^-] = 23.5 \pm 6.3 \text{ mmol.L}^{-1}$) to 51%
168 after 120 h of exposure (mean haemolymph $\text{Cl}^- = 16.7 \pm 5.6 \text{ mmol.L}^{-1}$) compared with the
169 control (mean haemolymph $\text{Cl}^- = 37.2 \pm 5.7 \text{ mmol.L}^{-1}$) (**Fig. 2a**).

170 A similar trend was observed for haemolymph $[\text{Na}^+]$. In the acidic stream, the significant loss
171 of haemolymph Na^+ ranged from 12.1% after 24 h of exposure (mean haemolymph $\text{Na}^+ =$
172 $120.7 \pm 5.3 \text{ mmol.L}^{-1}$) to 20.8% after 120 h of exposure (mean haemolymph $\text{Na}^+ = 100.5 \pm$
173 9.2 mmol.L^{-1}) compared with the control (mean haemolymph $\text{Na}^+ = 132.2 \pm 8.8 \text{ mmol.L}^{-1}$)
174 (**Fig. 2b**).

Throughout the exposure period the survival rate in *H. pellucidula* transferred to the circumneutral stream remained very high and above 94%. In contrast, survival decreased significantly in organisms exposed to the acidic stream, where the survival rate ranged from $72.2 \pm 9.6\%$ after 24h of exposure to $58.3 \pm 14.4 \%$ after 120h (**Fig. 2c**).

Dinocras cephalotes

The exposure time had a significant effect on haemolymph $[\text{Cl}^-]$ and $[\text{Na}^+]$ and survival, but a significant effect of stream was only observed on $[\text{Cl}^-]$ and survival (2-way ANOVA, **Table 3**). *D. cephalotes* baseline levels of haemolymph $[\text{Cl}^-]$ and $[\text{Na}^+]$ on the onset of the exposure were 126.6 ± 12.6 and $133.0 \pm 6.8 \text{ mmol.L}^{-1}$ (mean $\pm$ SD), respectively, in control organisms collected in the neutral stream (**Fig. 3a&b**).

Haemolymph $[\text{Na}^+]$ and $[\text{Cl}^-]$ in *D. cephalotes* exposed into the circumneutral stream remained constant over the 120-h exposure period. In organisms exposed into the acidic stream, a significant loss was observed only after 120h of exposure. The loss of haemolymph Cl^- and Na^+ reached respectively 20.6% (mean haemolymph $[\text{Cl}^-] = 96.7 \pm 17.3 \text{ mmol.L}^{-1}$) and 13.1% (mean haemolymph $\text{Na}^+ = 109.3 \pm 22.2 \text{ mmol.L}^{-1}$) compared with the control (mean haemolymph $\text{Cl}^- = 126.6 \pm 12.6 \text{ mmol.L}^{-1}$; mean haemolymph $\text{Na}^+ = 133.0 \pm 6.8 \text{ mmol.L}^{-1}$).

No mortality was observed in *D. cephalotes* transferred to the circumneutral stream (La Maix) during the experiment. Survival rate in organisms exposed to the acidic stream remained also high after 24h, but decreased significantly after 72h and 120h exposure (reaching $83.3 \pm 8.3\%$, **Fig. 3c**).

Species sensitivity comparison

The baseline level of haemolymph $[\text{Cl}^-]$ in *D. cephalotes* was respectively 2 and 3.5 times

higher than in *G. fossarum* and *H. pellucidula*. Haemolymph $[Na^+]$ baseline levels in *D. cephalotes* and *H. pellucidula* were similar and 1.8 times higher than in *G. fossarum*. Haemolymph $[Na^+]$ was always higher than $[Cl^-]$ representing a difference of 5%, 21.7% and 74.4% in *D. cephalotes*, *G. fossarum* and *H. pellucidula*, respectively. Under the experimental conditions of this study, *D. cephalotes* was the least acid-sensitive species, since it presented the lowest ion losses and mortality rate. *G. fossarum* was the most acid-sensitive species, showing both the highest losses of ions and mortality.

DISCUSSION

Over recent years, the loss of biodiversity has become a major concern (see Gaston, 2000, Loreau et al., 2001, Naeem et al., 1999). Acidification of freshwater due to anthropogenic activities has been recognized as an important cause of biodiversity loss in ecosystems located in remote areas. In the Vosges Mountains, acidification of headwater streams has been identified as being the major threat to aquatic biota. Acidified streams are strongly impoverished. For example, macroinvertebrate communities have lost between 50 and 70% of the original species (Guérol et al., 2000).

Although all major taxonomic groups are affected by acidification, species sensitivity appears different among macroinvertebrates groups. Studies carried out in Europe and in North America have highlighted the high sensitivity of molluscs, Ephemeroptera and crustacean (Hall et al., 1980, Harriman & Morison, 1982, Sutcliffe, 1983, Engblom & Lingbell, 1984, Perterson et al., 1985, Guérol et al., 2000). The response of Trichoptera to acidification is more variable: several species disappear (e.g. *Hydropsyche* sp.) whereas some others exhibit higher densities (e.g. *Plectrocnemia conspersa*). Thus, many works have reported the acid-sensitivity of Hydropsychidae and Philopotamidae as well as the acid-tolerance of *Plectrocnemia*, *Rhyacophyla* and Limnephilidae (Sutcliffe & Carrick, 1973, Ziemann, 1975,

225 Harriman & Morrison, 1982, Havas & Hutchinson, 1982, Townsend et al., 1983, Raddum &
 226 Fjellheim, 1984, Simpson et al., 1985). Although Perlidae (e.g., *Dinocras cephalotes*) and, to
 227 a lesser extent, Perlodidae are recognized as being acid-sensitive, the majority of Plecoptera
 228 (especially Nemouridae and Leuctridae) are acid-tolerant and can be very abundant in acid
 229 streams (Sutcliffe & Carrick, 1973, Raddum & Fjellheim, 1984, Tixier & Gurold, 2005).
 230 The toxicity of acid water seems to be the principal hypothesis (often referred to as the
 231 ecotoxicological hypothesis) explaining the loss of species. In the present study we showed
 232 that a short-term exposure of *G. fossarum*, *H. pellucidula* and *D. cephalotes* to strongly
 233 natural acidic water caused significant losses of haemolymph $[Na^+]$ and $[Cl^-]$ accompanied by
 234 significant mortality. However, the intensity and rapidity of ion loss depends on the species
 235 considered: in *G. fossarum* and *H. pellucidula* significant ion losses occurred as early as 24h
 236 of exposure in an acidic stream whereas in *D. cephalotes* a significant decrease of
 237 haemolymph $[Cl^-]$ and $[Na^+]$ was observed only after 120h of exposure.
 238 The magnitude of ion loss also differs among species: after 120h of exposure in acidic stream,
 239 haemolymph $[Cl^-]$ and $[Na^+]$ losses reached respectively 58.7% and 57.4% in *G. fossarum*,
 240 51% and 20.8% in *H. pellucidula* and only 20.6% and 13.1% in *D. cephalotes*. Similarly, the
 241 lowest survival rate was observed for *G. fossarum* (20.5%), the highest for *D. cephalotes*
 242 (83.3%) whereas it was intermediate for *H. pellucidula* (58.3%).
 243 Although some studies have clearly identified a depletion of ion concentrations in invertebrate
 244 exposed to acidic waters, most of these studies were performed on moderately acid-sensitive
 245 species (*Corixia dentipes*, *C. punctata*: Vangenechten et al., 1989; *Cenocorixia blaisdelli*:
 246 Needham, 1990; *Libellula julia*: Rockwood & Coler, 1991; *Pteronarcys proteus*: Leichleitner
 247 et al., 1985) and/or on organisms experimentally exposed to conditions that were not
 248 environmentally meaningful; e.g. pH 2.8-3.0 (Lechleitner et al., 1985; pH 3.0, Rupprecht,
 249 1992) or aluminium concentrations of 30 mg.L⁻¹ (Rockwood & Coler, 1991). Similar results

have also been obtained from pooled samples of haemolymph (Needham, 1990) or from whole body analyses (Hermann, 1987, Rowe *et al.* 1988, 1989, Havens, 1992).

Because of the protocol and the realistic *in situ* conditions of exposure, our results study provides more rigorous results and consolidates the experimental studies previously mentioned (see above). There is no doubt that significant ion loss and mortality in acid sensitive species can be induced by short exposures to acidic stresses. This is particularly pertinent to running water ecosystems experiencing acute acid stresses that are, for most of the time, within the pH range that could sustain such taxa. For example, in the Vosges Mountains, several circumneutral streams draining sandstone are subjected to rare acid stresses. These streams exhibit communities of invertebrates that are rather typical of moderately acidified streams despite their average chemical characteristics.

Depending on their duration, magnitude and frequency, such acid stresses can lead to the loss of sensitive species or simply impair their abundances. This has been recently evidenced by Lepori and Ormerod (2005) who have shown that the distribution and the density of the bivoltine species, *Baetis alpinus* (Ephemeroptera) in areas subjected to acidification in the Alps, depend on the toxicity of acid run-off during snowmelt. However, concerning species which are monovoltine or semi-voltine, repeated acid stresses are more likely to eradicate them from sensitive ecosystems.

Finally, our results sustain the hypothesis proposed by Bradley and Ormerod (2002) that short episodes of low pH can offset the recovery of sensitive species of macroinvertebrates in streams that have chemically recovered from acidification following catchment liming.

Understanding both the biological effect and ecological consequences of episodic acidity has real management implications in defining strategies. Given the general context of recovery from acidification, in response to reduced acidic depositions as well as following liming, the use of physiological variables such as haemolymph $[Cl^+]$ and $[Na^-]$, would be helpful to

predict whether the ecosystem meets again the chemical quality that can allow the re-colonization of acid-sensitive species. Likewise, the development and the use of methods based on macroinvertebrate communities would provide important information of ecological relevance (for example on the settlement of sensitive species populations and the sustainability of these populations). Finally, in the context of ecological toxicology (*sensu* Chapman, 2002) a combination of both approaches would greatly enhance the pertinence of biological monitoring with minimal uncertainty, especially in national or international survey programs aiming to assess the consequences of the reduction of atmospheric emissions of acidic pollutants as well as the effectiveness of liming.

Acknowledgements. We are grateful to Marie-Danielle Murard and two anonymous referees for helpful comments and linguistic corrections to improve the manuscript.

LITERATURE CITED

- Aggarwal, S.G., Chandrawanshi, K., Patel, R.M., Agarwal, S., Kamavishdar, A., Mundhara, G., 2001. Acidification of surface water in central India. *Water Air Soil Poll.* 130:855–862.
- Booth, C.E., McDonald, D.G., Simons, B.P., Wood, C.M., 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). *Can J Fish Aquat Sci* 45 (9), 1563-1574.
- Bouchard, A., 1997. Recent lakes acidification and recovery trends in southern Quebec, Canada. *Water Air Soil Poll.* 94, 225-245.
- Bradley, D.C., Ormerod, S.J., 2002. Long term effects of catchment liming on invertebrates in upland streams. *Freshwater Biol* 47, 161-171

300 Castro, M.S., Morgan, II R.P., 2000. Input–output budgets of major ions for a forested
 301 watershed in western Maryland. *Water Air Soil Poll.* 119, 121–137.

302 Chapman, P.M., 2002. Integrating toxicology and ecology: putting the “eco” into
 303 ecotoxicology. *Mar Pollut Bull* 44, 7-15.

304 Driscoll, C.T., Lawrence, G.B., Bulger, A.J., Butler, T.J., Cronan, C.S., Eagar, C., Lambert,
 305 K.F., Likens, G.E., Stoddard, J.L., Weathers, K.C., 2001. Acidic deposition in the
 306 northeastern United States: sources and inputs, ecosystem effects, and management
 307 strategies. *BioScience* 51, 180–198.

308 Engblom, E., Lingbell, P.E., 1984. The mapping of short-term acidification with the help of
 309 biological indicators. Institute of freshwater Research, Drottingholm, report 61, 60-68.

310 Evans, C.D., Harriman, R., Monteih, D.T., Jenkins, A., 2001. Assessing the suitability of acid
 311 neutralizing capacity as a measure of long-term trends in acidic waters based on two parallel
 312 datasets. *Water Air Soil Poll.* 130, 1541–1546.

313 Felten, V., Guérol, F., 2001. Hyperventilation and loss of haemolymph Na^+ and Cl^- in the
 314 freshwater amphipod *Gammarus fossarum* exposed to acid stress: a preliminary study. *Dis*
 315 *Aquat Organ* 45, 77–80.

316 Felten, V., Guérol, F., 2004. Haemolymph $[\text{Na}^+]$ and $[\text{Cl}^-]$ loss in *Gammarus fossarum*
 317 exposed *in situ* to a wide range of acidic streams. *Dis Aquat Organ* 61, 113-121.

318 Folster, J., Wilander, A., 2002. Recovery from acidification in Swedish forest streams.
 319 *Environ Pollut* 117, 379-89.

320 Gaston, K.J., 2000. Global patterns in biodiversity. *Nature* 405, 220-227.

321 Guérol, F., Boudot, J.P., Jacquemin, G., Vein, D., Merlet, D., Rouiller, J., 2000.
 322 Macroinvertebrate community loss as a result of headwater stream acidification in the
 323 Vosges Mountains (N-E France). *Biodivers Conserv* 9, 767–783.

324 Hall, R.J., Likens, G.E., Fiance, S.B. & Hendrey, G.R., 1980. Experimental acidification of a
 325 stream in the Hubbard brook experimental forest, New Hampshire. *Ecology* 61, 976-989.

326 Harriman, R., Morrison, B.R.S., 1982. Ecology of streams draining forested and non-forested
 327 catchments in an area of central Scotland subject to acid precipitation. *Hydrobiologia* 88,
 328 251-263.

329 Havas, K.E., Hutchinson, T.C., 1982. Aquatic invertebrate from the smoking hills: effect of
 330 pH and metals on mortality. *Can J Fish Aquat Sci* 39, 890-903.

331 Havens, K.E., 1992. Acid and aluminium effects on sodium homeostasis and survival of acid-
 332 sensitive and acid-tolerant Cladocera. *Can J Fish Aquat Sci* 49, 2392–2398.

333 Hermann, J., 1987. Sodium levels of lotic mayfly nymphs being exposed to aluminium at low
 334 pH. A preliminary report. *Ann Soc R Zool Belg* 117, 188–191.

335 Jensen, F.B., Malte, H., 1990. Acid-base and electrolyte regulation, and hemolymph gas
 336 transport in crayfish *Astacus astacus*, exposed to soft, acid water with and without
 337 aluminum. *J Comp Physiol B* 160, 483-490.

338 Lawrence, G.B., David, M.B., Lovett, G.M., Murdoch, P.S., and 5 others, 1999. Soil calcium
 339 status and the response of stream chemistry to changing acidic deposition rates in the
 340 Catskill Mountains of New York. *Ecol Appl* 9, 1059–1072.

341 Lawrence, G.B., Lovett, G.M., Baevsky, Y.H., 2000. Atmospheric deposition and watershed
 342 nitrogen export along an elevational gradient in the Catskill Mountains, New York.
 343 *Biogeochemistry* 50, 21–43.

344 Lechleitner, R., Cherry, D., Cairns, J., Stetler, D., 1985. Ionoregulatory and toxicological
 345 responses of stonefly nymphs (Plecoptera) to acidic and alkaline pH. *Arch Environ Con Tox*
 346 14, 179-185.

347 Lepori F., Ormerod S.J., 2005. Effects of spring acid episodes on macroinvertebrates revealed
 348 by population data and *in situ* toxicity tests. *Freshwater Biol* 50, 1568-1577

349 Likens, G.E., Driscoll, C.T., Buso, D.C., 1996. Long-term effects of acid rain: response and
350 recovery of a forest ecosystem. *Science* 272, 244–246.

351 Likens, G.E., Driscoll, C.T., Buso, D.C., Siccama, T.G., and 7 others, 1998. The
352 biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* 41, 89–173.

353 Likens, G.E., Butker, T.J., Buso, D.C., 2001. Long- and short-term changes in sulphate
354 deposition: effects of the 1990 Clean Air Act Amendments. *Biogeochemistry* 52, 1–11.

355 Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U.,
356 Huston, M.A., Raffaelli, D., Schmid, B., Tilman D., Wardle D.A., 2001. Biodiversity and
357 ecosystem functioning: current knowledge and future challenges. *Science* 294, 804–808.

358 Malley D.F., Huebner J.D. & Donkersloot K., 1988. Effects on ionic composition of blood
359 and tissues *Anodonta grandis grandis* (Bivalvia) of an addition of aluminum and acid to
360 lake. *Arch Environ Con Tox* 17, 479-491.

361 Masson, N., Guérol, F., Dangles, O., 2002. Use of blood parameters in fish to assess acidic
362 stress and chloride pollution in French running waters. *Chemosphere* 47, 467-473.

363 McDonald, D.G., Reader, J.P., Dalziel, T.R.K., 1989. The combined effects of pH and trace
364 metals on fish ionoregulation. In: Morris, R., Taylor, E.W., Brown, D.J.A., Brown, J.A.,
365 (Eds.) *Acid toxicity and aquatic animals*. Cambridge University Press, Cambridge, pp. 221–
366 242.

367 McMahon, B.R., Stuart, S.A., 1989. The physiological problem of crayfish in acid water. In:
368 Morris, R., Taylor, E.W., Brown, D.J.A., Brown, J.A. (Eds.) *Acid toxicity and aquatic*
369 *animals*. Cambridge University Press, Cambridge, pp. 171–200.

370 Morgan, I.J., McMahon, B.R., 1982. Acid tolerance and effects of sublethal acid exposure on
371 iono-regulation and acid-base status in two crayfish *Procambarus clarki* and *Oronectes*
372 *rusticus*. *J Exp Biol* 97, 241-252.

373 Muniz, I.P., 1991. Freshwater acidification: its effects on species and communities of
 374 freshwater microbes, plants and animals. P Roy Soc Edinb B 97, 227–254.

375 Naeem, S., Chapin III, F.S., Costanza, R., Ehrlich, P.R., Golley, F.B., Hooper, D.U., Lawton,
 376 J.H., O'Neill, R.V., Mooney, H.A., Sala, O.E., Symstad, A.J., Tilman, D., 1999.
 377 Biodiversity and ecosystem functioning: Mantaining life support processes. Issues in
 378 Ecology 4, 1-11.

379 Needham, K.M., 1990. Specific and seasonal variation in survival and sodium balance at low
 380 pH in five species of waterboatman (Hemiptera Corixidae). MS, University of British
 381 Columbia, Vancouver.

382 Neville, C.M., 1985. Phylsosiological responses of juvenile rainbow trout, *Salmo gairderi* to
 383 acid and aluminum – prediction of field responses from laboratory data. Can J Fish Aquat
 384 Sci, 42, 2004-2019.

385 Peterson R.H., Gordon D.J. & Johnston, D.J., 1985. Distribution of mayfly nymphs (Insecta
 386 Ephemeroptera) in some streams of Eastern Canada as related to stream pH. Can. Field.
 387 Nat. 99, 490-493.

388 Potts, W.T.W., McWilliams, P.G., 1989. The effect of hydrogen and aluminum ions on fish
 389 gills. In: Morris, R., Taylor, E.W., Brown, D.J.A., Brown, J.A. (Eds.) Acid toxicity and
 390 aquatic animals. Cambridge University Press, Cambridge, pp. 201–220.

391 Pynnönen, K., 1990. Physiological responses to severe acid stress in four species of
 392 freshwater Clams (Unionidae). Arch Environ Con Tox 19, 471-478.

393 Pynnönen, K., 1991. Influence of aluminum and protons on the electrolyte homeostasis in the
 394 Unionidae *Anodonta anatina* and *Unio pictorum*. Arch. Environ Cont Tox 20, 218–225.

395 Raddum, G.G., Fjellheim, A., 1984. Acidification and early warning organisms in freshwater
 396 in western Norway. Verh. Internat. Verein. Limnol. 22, 1973-1980.

397 Rockwood, J.P., Coler, R.A., 1991. The effect of aluminum in soft water at low pH on water
 398 balance and hemolymph ionic and acid–base regulation in the dragonfly *Libellula julia*
 399 Uhler. *Hydrobiologia* 215, 243–250.

400 Rowe, L., Berrill, M., Hollett, L., 1988. The influence of season and pH on mortality, molting
 401 and whole-body ion concentrations in nymphs of the mayfly *Stenonema femoratum*.- *Comp.*
 402 *Biochem. Physiol.* 90 A (3), 405-408.

403 Rowe, L., Berrill, M., Hollett, L., Hall, R.J., 1989. The effects of short-term laboratory pH
 404 depressions on molting, mortality and major ion concentrations in the mayflies *Stenonema*
 405 *femoratum* and *Leptophlebia cupida*. *Hydrobiologia* 184, 89-97.

406 Rupprecht, R., 1992. Zu den Ursachen der unterschiedlichen pH-Empfindlichkeit von
 407 *Gammarus pulex* L., *G. fossarum* Koch (Amphipoda) und *Asellus aquaticus* L. (Isopoda).
 408 *Int. Rev. Gesamt. Hydrobiol.* 77(2), 255–264.

409 Simpson, K.W., Bode, R.W., Colquhoun, J.R., 1985. The macroinvertebrate fauna of an acid
 410 stream system in the Adirondack Mountains, New York. *Freshwater Biol.* 15, 671-681.

411 Skjelkvale, B.L., Torseth, K., Aas, W., Andersen, T., 2001. Decrease in acid deposition-
 412 recovery in Norwegian waters. *Water Air Soil Poll.* 130, 1433–1438.

413 Stoddart, J.L., Jeffries, D.S., Lukewille, A., Clair, T.A., and 19 others, 1999. Regional trends
 414 in aquatic recovery from acidification in North America and Europe. *Nature* 401(6753),
 415 575–578.

416 Sutcliffe, D.W., Carrick, T.R., 1973. Studies on mountain streams in the English Lake
 417 District. I. PH, calcium and the distribution if invertebrates in the River Duddon. *Freshwater*
 418 *Biol* 3, 437–462.

419 Sutcliffe, D.W., 1983. Acid precipitation and its effects on aquatic systems in the English
 420 Lake District (Cumbria).- *Freshwater Biological Association Annual Report* 51, 30-62.

421 Tang, D., Lydersen, E., Seip, H.M., Angell, V., and 11 others, 2001. Integrated monitoring
 422 program on acidification of Chinese terrestrial systems (impacts)—A Chinese–Norwegian
 423 collaboration project. *Water Air Soil Poll.* 130, 1073–1078.

424 Tessier, J.T., Masters, R.D., Raynal, D.J., 2002. Changes in base cation deposition across
 425 New York State adjacent New England following implementation of the 1990 Clean Air
 426 Act amendments. *Atmos Environ* 36, 1645–1648.

427 Thorjörn, L., Seip, H.M., Semb, A., Mulder, J., and 6 others, 1999. Acid deposition and its
 428 effects in China: an overview. *Environ Sci Policy* 2, 9–24.

429 Tixier G., Guérol F. 2005. Plecoptera Response to acidification in several headwater streams
 430 in the Vosges Mountains (North-Eastern France). *Biodivers Conserv* 14 : 1525-1539

431 Townsend, C.R., Hildrew, A.G., Francis, J.E., 1983. Community structure in some southern
 432 English streams: the influence of physicochemical factors. *Freshwater Biol.* 13, 521-544.

433 Vangenechten, J.H.D., Witters, H., Vanderborcht, O.L.J., 1989. Laboratory studies on
 434 invertebrate survival and physiology in acid waters. In: Morris R, Taylor EW, Brown DJA,
 435 Brown JA (Eds.) *Acid toxicity and aquatic animals*. Cambridge University Press,
 436 Cambridge, pp. 153–170.

437 Wood, C.M., Rogano, M.S., 1986. Physiological responses to acid stress in crayfish
 438 (*Orconectes*): Hemolymph ions, acid-base status, and exchanges with the environment.
 439 *Can J Fish Aquat Sci* 43, 1017-1026.

440 Wood, C.M., Playle, R.L., Simons, B.P., Goss, G.C., McDonald, D.G., 1988. Blood gases,
 441 acid base status, ions and haematology in adult brook trout (*Salvelinus fontinalis*) under
 442 acid/aluminum exposure. *Can J Fish Aquat Sci* 45, 1575-1586.

443 Wood, C.M., 1989. The physiological problems of fish in acid water. In: Morris, R., Taylor,
 444 E.W., Brown, D.J.A., Brown, J.A. (Eds.) *Acid toxicity and aquatic animals*. Cambridge
 445 University Press, Cambridge, pp. 125–152.

Wright, R.F., Larssen, T., Camarero, L., Cosby, B.J., Ferrier, R.C., Helliwell, R., Forsius, M.,
Jenkins, A., Kopáček, J., Majer, V., Moldan, F., Posch, M., Rogora, M., Schöpp, W., 2005.
Recovery of acidified European Surface waters. *Environ Sci and Technol* 39, 64-72.
Ziemann, H., 1975. Über den Einfluss des Wasserstoffionenkonzentration und des
Hydrogenkarbonatgehaltes auf die Ausbildung von Berbachbiozönosen. *Int. Rev. Ges.*
Hydrobiol. 60 : 523-555.

Figure 1. Mean ($\pm$ SD) [Cl⁻] haemolymph (a), [Na⁺] haemolymph (b), and survival (c) of *Gammarus fossarum* exposed to circumneutral and acidic water. Significant differences against T₀ are indicated by asterisks (Fischer's Least Significant Difference test; * : p < 0.05; ** : p < 0.01; *** : p < 0.001).

Figure 2. Mean ($\pm$ SD) $[\text{Cl}^-]$ haemolymph (a), $[\text{Na}^+]$ haemolymph (b), and survival (c) of *Hydropsyche pellucidula* exposed to circumneutral and acidic water. Significant differences against T_0 are indicated by asterisks (Fischer's Least Significant Difference test; * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$).

Figure 3. Mean ($\pm$ SD) [Cl⁻] haemolymph (a), [Na⁺] haemolymph (b), and survival (c) of *Dinocras cephalotes* exposed to circumneutral and acidic waters. Significant differences against T₀ are indicated by asterisks (Fischer's Least Significant Difference test; * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$).

Table 1. Description of the two study sites.

stream name	La Maix	Gentil Sapin
stream order	2	2
elevation (m)	439	501
distance from spring (m)	3870	3280
latitude N	48°29'02.1"	48°26'56.8"
longitude E	007°04'08.5"	007°03'33.5"
width (m)	1.8	2.3
depth (m)	0.35	0.30

Table 2. Mean ($\pm$ SD) values of various chemicals of each exposure stream (n=4). ANC: Acid Neutralizing Capacity.

	La Maix		Gentil Sapin	
	Mean	SD	Mean	SD
pH	7,42	0,13	4,73	0,08
Temperature ($^{\circ}$ C)	11,9	0,7	14,0	1,5
conductivity (μ S.cm $^{-1}$)	74,3	4,3	31,5	2,4
ANC (μ eq.L $^{-1}$)	618,3	13,5	-15,5	7,0
Ca $^{2+}$ (μ mol.L $^{-1}$)	222,9	3,1	39,1	0,6
Mg $^{2+}$ (μ mol.L $^{-1}$)	188,3	0,8	28,6	0,2
Na $^{+}$ (μ mol.L $^{-1}$)	64,1	0,4	49,4	1,4
K $^{+}$ (μ mol.L $^{-1}$)	56,4	0,3	35,4	1,3
SO $_4^{2-}$ (μ mol.L $^{-1}$)	75,7	0,6	62,5	0,8
NO $_3^{-}$ (μ mol.L $^{-1}$)	60,0	0,9	88,5	1,1
Cl $^{-}$ (μ mol.L $^{-1}$)	54,0	0,6	44,9	0,7
Total Al (μ mol.L $^{-1}$)	2,1	0,4	28,4	1,0

600 **Table 3.** Summary of the 2-way analysis of variance. F : F ratio ; p : probability; d.f. : degrees
601 of freedom.

	[Cl ⁻] haemolymph			[Na ⁺] haemolymph			Survival		
	d.f.	F	p	d.f.	F	p	d.f.	F	p
a) <i>Gammarus fossarum</i>									
Factor a (Stream)	1	79,4	< 10 ⁻³	1	99,3	< 10 ⁻³	1	228,3	< 10 ⁻³
Factor b (Exposure time)	3	18,1	< 10 ⁻³	3	20,1	< 10 ⁻³	3	54,2	< 10 ⁻³
Interaction	3	10,7	< 10 ⁻³	3	14,2	< 10 ⁻³	3	47,2	< 10 ⁻³
b) <i>Hydropsyche pellucidula</i>									
Factor a (Stream)	1	32,3	< 10 ⁻³	1	28,9	< 10 ⁻³	1	33,0	< 10 ⁻³
Factor b (Exposure time)	3	12,9	< 10 ⁻³	3	9,3	< 10 ⁻³	3	6,7	< 5*10 ⁻⁵
Interaction	3	6,8	< 10 ⁻³	3	5,9	< 5*10 ⁻⁵	3	3,9	< 10 ⁻²
c) <i>Dinocras cephalotes</i>									
Factor a (Stream)	1	5,4	< 10 ⁻²	1	3,0	n.s.	1	16,8	< 10 ⁻³
Factor b (Exposure time)	3	4,9	< 5*10 ⁻⁵	3	3,7	< 10 ⁻²	3	3,5	< 10 ⁻²
Interaction	3	2,0	n.s.	3	0,8	n.s.	3	3,5	< 10 ⁻²

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