Vol. 45: 77–80, 2001 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published May 4

NOTE

Hyperventilation and loss of hemolymph Na^+ and Cl^- in the freshwater amphipod *Gammarus fossarum* exposed to acid stress: a preliminary study

V. Felten, F. Guerold*

Laboratoire EBSE, Equipe de Production des Ecosystèmes et Ecotoxicologie, Université de Metz, Boulevard du Général Délestraint, 57070 Metz, France

ABSTRACT: The effect of acidification on the acid-sensitive species *Gammarus fossarum* was investigated in the laboratory. The results showed that as mortality increased, mean hemolymph chloride and sodium concentrations decreased rapidly. Concomitantly, organisms hyperventilated during the first 24 h and then started to hypoventilate. These results demonstrated that exposure to acid stress in the acid-sensitive species *G. fossarum* led to ion-regulatory and respiratory failure as previously reported in fish and crayfish exposed to acid stress.

KEY WORDS: *Gammarus fossarum* · Acidic stress · Survival · Ion loss · Pleopod beats

One of the most striking consequences of freshwater acidification is certainly the drastic erosion of biodiversity (see Muniz 1991). In particular, various studies have shown that acidification leads to the disappearance of numerous macroinvertebrate species (Raddum & Fjellheim 1984, Guérold et al. 1995). It has been hypothesized that invertebrates should have the same physiological problems as fish and crayfish, but to date very few studies have attempted to verify this assumption. Because it has been commonly reported that amphipods are among the most acid-sensitive species (Sutcliffe & Carrick 1973, Guérold et al. 2000), the aim of this note was to investigate the effect of acidification on mortality, ventilation and hemolymph chloride and sodium concentrations in *Gammarus fossarum*.

Materials and methods. ***Organisms:*** Individuals of the freshwater amphipod *Gammarus fossarum* were collected in May 2000 using a net in a circumneutral stream (La Maix brook) from the Vosges Mountains (NE France). Only males ranging in size between 10 and 12 mm were used for exposures. In order to acclimate organisms to laboratory conditions, individuals were kept for 7 d in a 15 l polyethylene tank supplied with water from the sampling site (temperature 13 ± 1°C, photoperiod of 14 h of light and 10 h of darkness).

Survival: For this experiment, organisms were exposed to acidic water collected in the Gentil Sapin brook and in circumneutral water from the La Maix brook. Replicates (3) of 9 individuals were exposed in each medium. The chemical composition of each medium is given in Table 1. In order to avoid chemical variations, the water was changed every 12 h. After 12, 24 and 48 h of exposure in each medium, survival was evaluated in each replicate.

Hemolymph sampling and count of pleopod beats. In order to assess hemolymph Na^+ and Cl^- concentra-

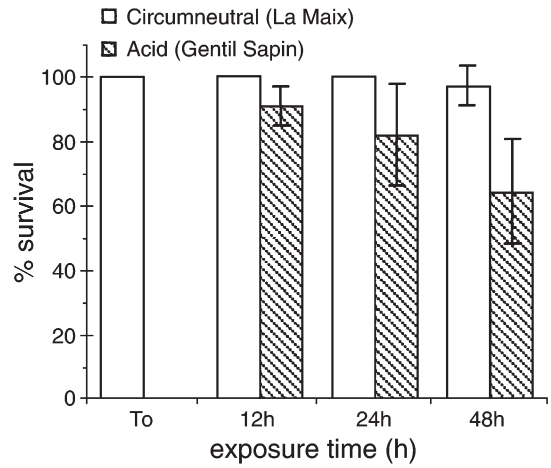
Table 1. Mean (± SD) values of chemical variables of each exposure medium. ANC: Acid Neutralizing Capacity

Variable	Units	La Maix brook Mean	SD	Gentil Sapin brook Mean	SD
pH		7.4	0.1	4.8	0.1
Conductivity	(µS cm^{-1})	75	1.4	38	0.4
ANC	(µeq l^{-1})	396	22.9	–24	3.8
Cl	(µmol l^{-1})	53	4.5	52	4.0
NO_3	(µmol l^{-1})	83	18.2	79	15.5
SO_4	(µmol l^{-1})	67	10.3	67	10.2
Na	(µmol l^{-1})	46	2.9	40	3.6
Ca	(µmol l^{-1})	176	11.4	37	3.2
Mg	(µmol l^{-1})	136	1.8	20	1.0
K	(µmol l^{-1})	39	1.1	25	1.1
Total Al	(µmol l^{-1})	5	0.4	34	1.1

*Corresponding author. E-mail: guerold@sciences.univ-metz.fr

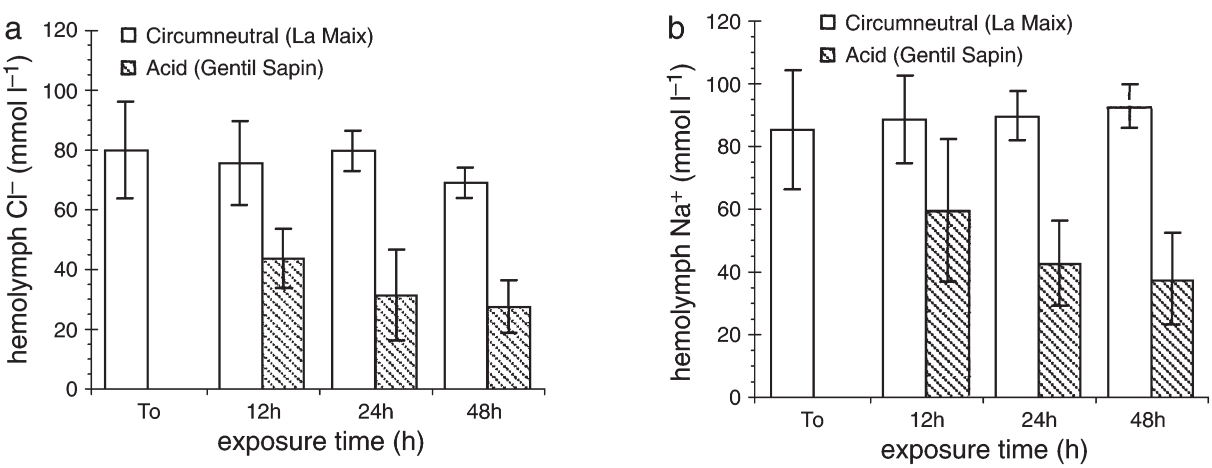
tions, an additional 27 organisms were exposed in each medium. After 12, 24 and 48 h of exposure, 3 to 9 organisms (depending on mortality) from each medium were collected and pleopod beating rates counted before hemolymph sampling. Amphipods (14) were also sampled just before the onset of the exposure (T_0). Samples of hemolymph (0.8 to 1.2 µl) were taken from the telson by a microsyringe, transferred in a gauged microcapillary tube and centrifuged for 10 min at 6596 × *g*. After centrifugation, the liquid phase was diluted in 2 ml of nanopur water in order to determine Cl^- and Na^+ concentrations by ionic chromatograghy (Dionex 4500i equipped with a Ion Pac AS4A column) and atomic absorption spectrophotometry (AAS) (Perkin Elmer Analyst 100), respectively.

Results. After 48 h, the survival of *Gammarus fossarum* exposed under acidic conditions decreased to 64% (Fig. 1). The mortality was associated with a significant loss of hemolymph Na^+ and Cl^- and with a modification of ventilation rhythm. As early as 12 h of exposure in acidic water, the mean (± SD) hemolymph Cl^- concentration decreased from 79.8 ± 16.2 to 43.4 ± 14.1 mmol l^{-1}, representing a significant loss of 46% when compared to the initial concentration (Fig. 2a). Likewise (Fig. 2b), Na^+ concentration (59.5 ± 13.7 mmol l^{-1}) showed a 30% decrease after 12 h when compared to the initial value (85.4 ± 18.8 mmol l^{-1}). For each exposure time, Cl^- and Na^+ concentrations in individu-



c 12h	n.s.					
c 24h	n.s.	n.s.				
c 48h	n.s.	n.s.	n.s.			
ac 12h	p<0.004	p<0.017	p<0.008	p<0.006		
ac 24h	p<0.000	p<0.002	p<0.002	p<0.001	n.s	
ac 48h	p<0.005	p<0.014	p<0.007	p<0.011	n.s.	n.s.
	To	**c 12h**	**c 24h**	**c 48h**	**ac 12h**	**ac 24h**

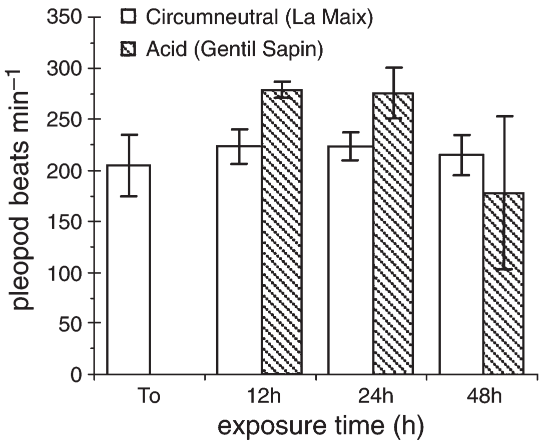
Fig. 1. Mean (± SD) survival of *Gammarus fossarum* exposed to circumneutral (c) and acidic water (ac). Matrix gives the significance of statistical comparisons (Mann-Whitney test); n.s.: non significant



c 12h	n.s.					
c 24h	n.s.	n.s.				
c 48h	n.s.	n.s.	n.s.			
ac 12h	p<0.000	p<0.001	p<0.001	p<0.003		
ac 24h	p<0.000	p<0.001	p<0.001	p<0.001	n.s	
ac 48h	p<0.005	p<0.005	p<0.005	p<0.009	n.s.	n.s.
	To	**c 12h**	**c 24h**	**c 48h**	**ac 12h**	**ac 24h**

c 12h	n.s.					
c 24h	n.s.	n.s.				
c 48h	n.s.	n.s.	n.s.			
ac 12h	p<0.004	p<0.017	p<0.008	p<0.006		
ac 24h	p<0.000	p<0.002	p<0.002	p<0.001	n.s	
ac 48h	p<0.005	p<0.014	p<0.007	p<0.011	n.s.	n.s.
	To	**c 12h**	**c 24h**	**c 48h**	**ac 12h**	**ac 24h**

Fig. 2. Mean (± SD) hemolymph (a) Cl^- or (b) Na^+ concentrations in *Gammarus fossarum* exposed to circumneutral (c) and acidic water (ac). Matrices give the significance of statistical comparisons (Mann-Whitney test); n.s.: non significant



	To	c 12h	c 24h	c 48h	ac 12h	ac 24h
c 12h	n.s.					
c 24h	n.s.	n.s.				
c 48h	n.s.	n.s.	n.s.			
ac 12h	p<0.001	p<0.001	p<0.000	p<0.001		
ac 24h	p<0.001	p<0.001	p<0.001	p<0.001	n.s.	
ac 48h	n.s.	n.s.	n.s.	p<0.011	p<0.004	n.s.

Fig. 3. Mean (± SD) number of pleopod beats min^{-1} in *Gammarus fossarum* exposed to circumneutral (c) and acidic water (ac). Matrix gives the significance of statistical comparisons (Mann-Whitney test); n.s.: non significant

als exposed to acidic water were significantly different from controls. By contrast, Cl^- and Na^+ concentrations in organisms exposed to circumneutral water did not at any time differ significantly from those recorded at T_0. The hemolymph Cl^- and Na^+ concentrations continued to decrease after 24 and 48 h of exposure to acidic water. Counting of pleopod beats (Fig. 3), showed that after 12 h of exposure under acidic conditions, the ventilation rate increased significantly from 205 pleopod beats min^{-1} at T_0 to 276 pleopod beats min^{-1} at 12 h. This hyperventilation remained stable after 24 h of exposure and then decreased to below the initial value (178 pleopod beats min^{-1}) after 48 h.

Discussion. This preliminary study suggests that *Gammarus fossarum* exposed to low pH, low calcium and high aluminum concentrations suffered from ion-regulatory and respiratory failure. Loss of extracellular ions (i.e., Na^+ and Cl^-) has been recognized as a major response in fish to acidic stress (Wood 1989). With respect to invertebrate organisms, similar responses have been reported in crayfish (McMahon & Stuart 1989, Jensen & Malte 1990) and molluscs (Pynnönen 1991). In spite of the numerous papers reporting detrimental effects of acidification on invertebrate communities, surprisingly few studies have been carried out on the ecophysiology of smaller acid-sensitive macroinvertebrate species. Although some studies have clearly shown a loss of ion concentrations in invertebrates exposed to acidic waters, most have been performed with moderately acid-sensitive species (e.g., *Corixia dentipes*, *C. punctata*: Vangenechten et al. 1989) and/or organisms exposed to non-realistic concentrations of aluminum (e.g., up to 30 mg l^{-1}: Rockwood & Coler 1991). Moreover, similar results have been obtained with pooled samples of hemolymph or from whole body analyses (Hermann 1987, Havens 1992). Impairment of respiratory function has also been reported in acid-sensitive species of fish (Laitinen & Valtonen 1995) and crayfish (Patterson & DeFur 1988). According to these authors, the hyperventilatory response observed during the first hours of acid stress served to increase O_2 delivery and CO_2 removal from the respiratory surface.

In conclusion, this preliminary study indicated that the disappearance of small acid-sensitive species such as amphipods probably results from ion-regulatory and respiratory failure which can be easily and rapidly assessed by simple hemolymph sampling and pleopod beat counting.

LITERATURE CITED

Guérold F, Vein D, Jacquemin G, Pihan JC (1995) The macroinvertebrates communities of streams draining a small granitic catchment exposed to acidic precipitations (Vosges Mountains, Northeastern France). Hydrobiologia 300/301:141–148

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

Havens KE (1992) Acid and aluminium effects on sodium homeostasis and survival of acid-sensitive and acid-tolerant Cladocera. Can J Fish Aquat Sci 49(11):2392–2398

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

Jensen FB, Malte H (1990) Acid-base and electrolyte regulation, and hemolymph gas transport in crayfish *Astacus astacus* exposed to soft, acid water with and without aluminium. J Comp Physiol B 160:483–490

Laitinen M, Valtonen T (1995) Cardiovascular, ventilatory and haematological responses of brown trout (*Salmo trutta* L.), to the combined effect of the acidity and aluminium in humic water at winter temperatures. Aquat Toxicol 31:99–112

McMahon BR, Stuart SA (1989) The physiological problem of crayfish in acid water. In: Morris R, Taylor EW, Brown JA (eds) Acid toxicity and aquatic animals. Cambridge University Press, Cambridge, p 171–200

Muniz IP (1991) Freshwater acidification: its effects on species and communities of freshwater microbes, plants and animals. Proc R Soc Edinb Sect B (Biol) 97:227–254

Patterson NE, DeFur PL (1988) Ventilatory and circulatory responses of the crayfish, *Procambarus clarki*, to low environmental pH. Physiol Zool 61(5):396–406

Pynnönen K (1991) Influence of aluminium and protons on the electrolyte homeostasis in the Unionidae *Anodonta anatina* and *Unio pictorum*. Arch Environ Contam Toxicol 20: 218–225

Raddum GG, Fjellheim A (1984) Acidification and early warning organisms in freshwater in Western Norway. Verh Int Ver Limnol 22:1972–1979

Rockwood JP, Coler RA (1991) The effect of aluminium in soft water at low pH on water balance and hemolymph ionic and acid-base regulation in the dragonfly *Libellula julia* Uhler. Hydrobiologia 215:243–250

Sutcliffe DW, Carrick TR (1973) Studies on mountain streams in the English Lake District. I. pH, calcium and the distribution if invertebrates in the River Duddon. Freshw Biol 3: 437–462

Vangenechten JHD, Witters H, Vanderborght OLJ (1989) Laboratory studies on invertebrate survival and physiology in acid waters. In: Morris R, Taylor EW, Brown JA (eds) Acid toxicity and aquatic animals. Cambridge University Press, Cambridge, p 153–170

Wood CM (1989) The physiological problems of fish in acid water. In: Morris R, Taylor EW, Brown JA (eds) Acid toxicity and aquatic animals. Cambridge University Press, Cambridge, p 125–152

Editorial responsibility: Timothy Flegel, Bangkok, Thailand

Submitted: October 12, 2000; Accepted: February 28, 2001
Proofs received from author(s): April 23, 2001