ALUMINIUM IMPACT ON RESPIRATION OF LOTIC MAYFLIES AT LOW pH

Jan Herrmann and Kjell G. Andersson
Department of Animal Ecology
University of Lund
Ecology Building
S-223 62 Lund
Sweden

ABSTRACT. Three species of stream-living mayfly nymphs were exposed to total inorganic (labile) monomeric aluminium levels of 0, 0.5, and 2.0 mg L^{-1} at pH 4.0 and 4.8 for 10 days. Oxygen consumption rate of the nymphs was measured, using a closed-cylinder technique and micro-Winkler titration. The animals showed significant increases in respiration with raised Al concentrations. The mayfly species *Ephemera danica*, which is restricted to the less heavily acidified region of South Sweden, was most affected by Al. The response of the two *Heptagenia* species to elevated Al was less pronounced. The effect of pH, however, seemed less important. Two possible reasons for the respiration increase at high Al levels are: (1) A "chemical impact route", consisting of a compensatory mechanism due to decreased O_2 transport efficiency, because of impaired osmoregulation and ion transport; and/or (2) a "mechanical impact route" with Al hydroxide precipitation and mucus formation on the gills, causing lowered respiration efficiency. This stress of the increased respiration rate costs energy. Thus less will be available for growth and reproduction. A model of the proposed impact routes is presented.

1. INTRODUCTION

Acidifying precipitation has for many decades affected Sweden, and the chemical and biological effects has been especially severe in some southern and western areas with poorly buffered soils and surface waters (Johansson and Nyberg, 1981). Benthic stream invertebrate communities are known to become impoverished in acid conditions (Hall *et al.*, 1980; Singer, 1982; Sucliffe, 1983; Otto and Svensson, 1983), and especially nymphs of many mayfly species seem vulnerable (Engblom amd Lingdell, 1984). Detailed studies on the possible involved impact mechanisms for invertebrates are few, see however Sutcliffe (1983), Havas *et al.* (1984), Witters *et al.* and Appelberg (1985), and the review by Havas (1981). However, no studies of mayfly nymphs exist.

The impact of acid water on fish, a more prominent and commercially

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interesting component of the ecosystem, is much more well-known than invertebrates (e.g. Fromm, 1980; Muniz and Leivestad, 1980a, b; Baker and Schofield, 1982; Brown, 1983). Main effects of acid conditions on fish are reported to be impaired osmoregulation, leading to lowered content of Na^+, Cl^- and other ions; and precipitation of Al hydroxides and mucus on the gills, thus causing coughing and hyperventilation. To our knowledge, very little research has been conducted on the way oxygen consumption of invertebrates is affected with elevated acidity and Al impact.

This paper presents results on respiratory responses of a set of mayfly species exposed to different pH and Al conditions. Respiration reflects the extent of stress that the animal is experiencing, irrespective of its causation.

2. MATERIAL AND METHODS

Nymphs of three mayfly species were tested, all specimens being collected in fairly well buffered (pH 6 to 7) streams:

Heptagenia fuscogrisea Retz. This species is tolerant to low pH conditions and occurs almost everywhere in the acid parts of south Sweden. It can be found in streams as low as pH 4.2 (Engblom and Lingdell, 1984). The individual dry weight of the almost 1 yr old animals used was 1.5 to 4 mg.

H. sulphurea Müll. This species is more sensitive than its congener and is missing in streams with a pH less than 4.6 (Engblom and Lingdell, 1984). To judge from its total geographic range and wide tolerance limits with respect to other environmental factors, the species was probably more common some decades ago than nowadays (Svensson, B.S.: 1985, pers. comm.). Individual dry weight was 2 to 5 mg (almost 1 yr old).

Ephemera danica Müll. It occurs in less heavily acidified streams, and is fairly common at less acidic conditions (Engblom and Lingdell, 1984: lower limit at pH ca 5.5). Individual dry weight was 12 to 22 mg (almost 2 yr old).

A variety of regimes (see end of this chapter) were arranged in 2 L PVC jars. Filtrated stream water at pH 5.5 to 6.5, with very low total monomeric Al content ($\ll 0.07$ mg L^{-1}), and moderate contents of humic substance (20 to 40 mg Pt L^{-1}) and Ca (2.8 mg L^{-1}), was used for all experiments. The desired pH regimes were adjusted with H_2SO_4. Appropriate amounts of $Al_2(SO_4)_3$ were added and pH readjustments were made during two days before the experiments began.

Before the exposure began, the animals used were successively acclimatized towards their future pH conditions. The whole setup was kept at 8 to 10°C, gentle air bubbling was added and pH adjusted daily. No food was added and no natural aubstrate was used, but a piece of nylon net was provided to which the animals could cling.

After an exposure time of 10 days, the respiration measurements were performed. First the O_2 bubbling was closed for 1 hr to let the water gas content stabilize. Then the O_2 content of the water was measured by means of a micro-Winkler method. Twenty mL of water was extracted with a syringe and the standard reagents were added to this syringe by means of 1 mL syringes. Titration with 0.01-M $Na_2S_2O_3$ was done from a microburette. Initial O_2 saturation was 75 to 85 %.

Subsequently, the experimental water and animals were gently transferred to small glass vials (ca 45 mL each). Change of O_2 saturation was considered to be very marginal. Lids with rubber gasket were mounted, so that no air could enter when submersed into a water bath at $10 \pm 1^{o}C$. To avoid O_2 stratification, each vial was equipped internally with a slow-speed propeller device.

After 4 to 6 hr, a micro-Winkler O_2 sample was taken directly from each vial. The animals were removed and dried at $60^{o}C$ overnight before being weighed. Content of monomeric Al of the experimental water was determined at the start and end of each 10 day period on a Varian AAS. Analyses included separation of Al into organic (non-labile) and inorganic (labile) fractions by means of a Dowex cation exchanger (for details see Nilsson and Bergkvist, 1983). In the following text, the denoted Al concentrations are total inorganic (labile) monomeric Al

The above procedure provided data for each vial that allowed calculation of the O_2 consumption per unit dry weight of animal and time. Each experimental regime comprised 5 to 10 vials (number of replicates) and each vial contained 1 to 4 animals, depending on animal size. Experiments were performed in May and June, and at six regimes, namely the Al contents of 0, 0.5 and 2.0 mg L^{-1} at both pH 4.0 and 4.8.

3. RESULTS AND DISCUSSION

In all series of experiments, comparisons between the respiration rate responses at Al concentrations 0 and 2.0 mg L^{-1} clearly showed that mayfly nymphs exhibit an increased respiration rate at a raised Al concentration (Figure 1). Also at Al = 0.5 mg L^{-1} the respiration rate levels of *H. sulphurea* and *E. danica* follows this raising pattern. For *H. fuscogrisea* we observed a "dip" at this concentration (Figure 1), which coincided with substantial problems in maintaining the correct Al levels.

Even if "the base level" (respiration rate in Al-free water) differed between the mayfly species used, all sets of data for the increase of respiration with raised Al levels showed a significant response (Table I). The effect of pH, however, seemed less important in these experiments (Figure 1).

The impact of Al on the respiration rate certainly puts a higher demand on the animal for aquiring energy, which can be at the expense of growth and reproduction. Havas (1980) suggested that the primary effect on aquatic invertebrates at acid conditions is that acid-base balance and osmoregulation are affected. She indicated that respiration effects would be less important, being less influenced at a moderately low pH levels, but also because it is a secondary effect, influenced by the earlier.

Two possible ways along which Al could physiologically affect mayfly nymphs are as follows (also Figure 2). (1). A layer of mucus is

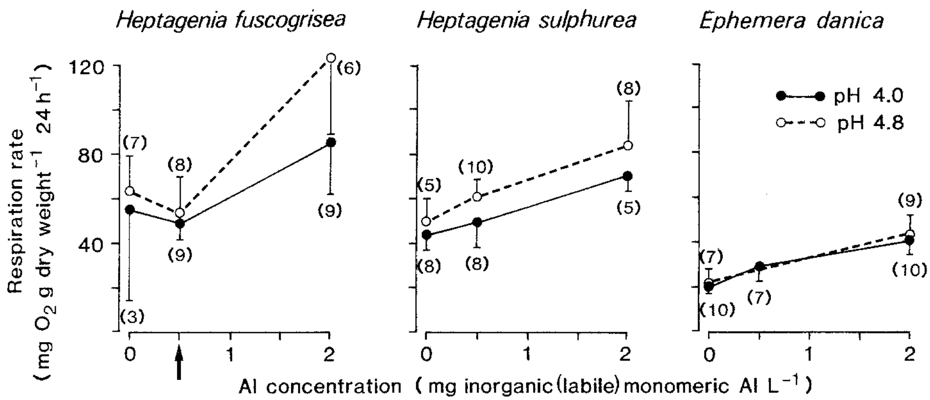

Fig. 1. Respiration rate (mg O_2 g dry weight^{-1} 24 hr^{-1}) of three species of mayfly nymphs, exposed to different Al concentrations (mg inorganic monomeric Al L^{-1}), at pH levels of 4.0 and 4.8. Number of experiments given within parentheses and 95 % confidence limits are indicated in one direction. Arrow indicates that Al levels decreased much below the denoted value.

formed, following impaired osmoregulation, and/or (2) hydroxide precipitates on the gills. Mucus or hydroxides prevents access to O_2 and

Table I. Significances (one-way ANOVA) for the data of increase in respiration rate, due to raised Al concentrations, presented in Figure 1. Also included, to the right, are the figures for the percentage increase of respiration rate, from Al = 0 mg L^{-1} to Al = 2.0 mg L^{-1}.

Mayfly species	pH level	F-value	df	significance	% increase
H. fuscogrisea	4.0	5.80	2/17	$p < 0.05$	54
	4.8	11.98	2/18	$p < 0.001$	95
H. sulphurea	4.0	9.24	2/18	$p < 0.01$	59
	4.8	7.21	2/20	$p < 0.01$	68
E. danica	4.0	16.27	2/24	$p < 0.001$	101
	4.8	19.98	1/13	$p < 0.001$	100

thus lowers the respiration efficiency. The animal would compensate this by increased respiration rate, thus reflecting a stressed situation. This response could be named the "mechanical impact route". However, a second type of response would include impaired osmoregulation and ion transport as well as other toxic effects, and could be called a "chemical impact route". This route would impede the O_2 transport and create a similarly increased compensatory respiration rate. In both cases, the effect would be an increased energy cost, resulting in less energy being available for growth and reproduction (cf. Sutcliffe, 1984). This model of proposed impact routes is inspired by and partly based on Muniz and Leivestad (1980a, b) and Havas (1981).

It should be added that a changed and disadvantageous behavior could be expected due to some of the impacts outlined above. Further, Al changes could also alter the bottom substrate composition in a stream, with respect to algae and humic substances (Dickson, 1978), as well as Al precipitates (McKnight and Feder, 1984), thereby indirectly affecting scraping invertebrates like mayfly nymphs.

The observed increase of O_2 consumption of the investigated mayfly nymphs at higher Al levels (Figure 1) may be due to mainly osmoregula-

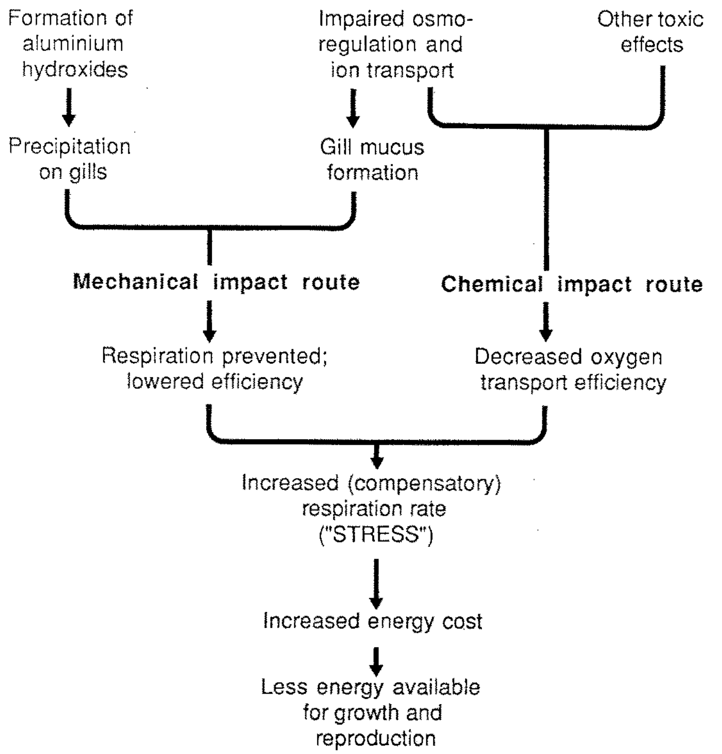

Fig. 2. A proposed model for the possible physiological impact routes for the effects of Al on respiration of mayfly nymphs at low pH conditions. See text for further explanation.

tory and ion transport problems (cf. Havas, 1981), thus lending support to the "chemical impact route" (Figure 2). Scattered observations of opaque material on gills and other parts of the mayfly nymphs during the experiments indicated that one or both of the "mechanical impact routes" also could be important. Hall *et al.* (1985) suggested that Al hydroxides could be toxic for invertebrates, but also that Al results in a disadvantageously decreased water surface tension.

Even if all series of experiments showed a significant response of increased respiration due to raised Al levels, there were differences in the degree of significance (Table I). Also, the percentage of increase was greater for *E. danica* (Table I). Thus, it is inferred that the respiration response was most pronounced and consistent for *E. danica*, but less so for *H. sulphurea* and *H. fuscogrisea*. This observation fits the fact that *E. danica* is mainly restricted to streams exhibiting relatively high pH and low Al levels. *H. fuscogrisea* is found in low pH/high Al streams, whereas *H. sulphurea* is intermediate in both these respects.

The exposure levels used, 0.5 and 2.0 mg Al L^{-1}, were not unrealistically high. In some streams in the investigated region of southern Sweden, stream water contents of 0.3 to 0.7 mg Al L^{-1} are not unusual at spring and autumn, but also levels $>$ 1.0 mg Al L^{-1} has occasionally been measured. However, in the streams with Al levels of ca 1 mg L^{-1} (at pH around or below 4), no mayfly nymphs seem to exist, not even *Leptophlebia spp.* (Herrmann, unpubl.). Species of this genus are otherwise tolerant to low pH conditions (Otto and Svensson, 1983; Engblom and Lingdell, 1984; Herrmann, unpubl.).

Interstitial water often show a higher pH (and then probably lower Al levels) than that in the water column (Herrmann, unpubl.). Therefore, the conditions for the animals are usually "better" in or on the surface of the bottom sediment of an acid stream than above it, probably related to the buffering action of the organic and inorganic material in the sediment.

Havas and Hutchinson (1982) suggested that Al, besides the pH itself, is the key additional toxic factor for crustaceans in acid waters. They did not propose a particular mechanism, but Al-specific staining has indicated that this metal is related to the osmotic functions (Havas, 1986). Witters *et al.* (1984) have shown impaired Na fluxes at increased Al levels for corixids.

In the present study, the mayfly nymphs showed an evident respiration increase at raised Al levels. The main reason for this increase may be difficulty with ion regulation and transport. Further knowledge on the impact of Al and pH on mayfly osmoregulation is therefore needed.

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REFERENCES

Appelberg, M.: 1985, *Hydrobiologia* **121**, 19.

Baker, J.P. and Schofield, C.L.: 1982, *Water, Air and Soil Pollut.* **18**, 289.

Brown, D.J.A.: 1983, *Bull. Environ. Contam. Toxicol.* **30**, 582.

Dickson, W.: 1978, *Verh. Internat. Verein. Limnol.* **20**, 851.

Engblom, E. and Lingdell, P.-E.: 1984, *Rep. Inst. Freshw. Res., Drottningholm* **61**, 60.

Fromm, P.O.: 1980, *Env. Biol. Fish.* **5**, 79.

Hall, R.J., Likens, G.E., Fiance, S.B. and Hendrey, G.R.: 1980, *Ecology* **61**, 976.

Hall, R.J., Driscoll, C.T., Likens, G.E. and Pratt, J.M.: 1985, *Limnol. Oceanogr.* **30**, 212.

Havas, M.: 1981, *Effects of Acidic Precipitation on Benthos*, R. Singer (ed.), North American Benthological Society, Springfield, p. 49.

Havas, M. and Hutchinson, T.C.: 1982, *Can. J. Fish. Aquat. Sci.* **39**, 890.

Havas, M., Hutchinson, T.C. and Likens, G.E.: 1984, *Can. J. Zool.* **62**, 1965.

Havas, M.: 1986, *Water, Air and Soil Pollut.*, this volume.

Johansson, K. and Nyberg, P.: 1980, *Inform. Inst. Freshw. Res., Drottningholm* **6**, 118 pp (In Swedish with English Summary)

McKnight, D.M. and Feder, G.L.: 1984, *Hydrobiologia* **119**, 129.

Muniz, I.P. and Leivestad, H.: 1980a, *Proc. Int. Conf. Ecol. Impact Acid Precip.*, D. Drabløs and A. Tollan (eds), Norway 1980, SNSF project, p. 84.

Muniz, I.P. and Leivestad, H.: 1980b, *Proc. Int. Conf. Ecol. Impact Acid Precip.*, D. Drabløs and A. Tollan (eds), Norway 1980, SNSF project, p. 320.

Nilsson, S.I. and Bergkvist, B.: 1983, *Water, Air and Soil Pollut.* **20**,311.

Otto, C. and Svensson, B.S.: 1983, *Arch. Hydrobiol.* **99**, 15.

Singer, R.: 1982, *Acid Precipitation - Effects on Ecological Systems*, F.M. D'Itri (ed.), Ann Arbor Science, p. 329.

Sutcliffe, D.W.: 1983, *Rep. Freshw. Biol. Ass.* **51**, 30.

Sutcliffe, D.W.: 1984, *Freshw. Biol.* **14**, 443.

Witters, H., Vangenechten, J.H.D., van Puymbroeck, S. and Vanderborght, O.L.J.: 1984, *Bull. Environ. Contam. Toxicol.* **32**, 575.