Changes in oxygen consumption and nitrogen metabolism in the dragonfly *Somatochlora cingulata* exposed to aluminum in acid waters

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Abstract

Oxygen consumption, ammonia excretion and changes in O:N ratios by the dragonfly *Somatochlora cingulata* were measured in four nymphal growth stages, relative to aluminum concentrations and low pH. A differential reduction in respiration and ammonia excretion rates resulted in an increase in O:N ratios for all nymphal stages. The earlier stages, however, were the most sensitive. The ratios obtained were indicative of a decreased dependence on protein reserves and increased utilization of carbohydrates or lipid reserves. Also observed was an increase in the haemolymph pH and glutamate levels with a concomitant accumulation of tissue ammonia.

Introduction

Acidification of surface waters has become a major concern in areas of the northeastern United States (Davis *et al.*, 1978). An important consequence of acidification is the mobilization of Al from the edaphic to the aquatic environment (Cronan & Schofield, 1979; Driscoll *et al.*, 1980). Elevated metal concentrations (Al, Zn, Mn) are often associated with low pH levels in lakes and streams (Schofield & Trajnar, 1980; Driscoll *et al.*, 1980). Aluminum and pH toxicity has been studied in fish (Baker & Schofield, 1982), crustaceans and some insect larvae (Bell & Neberker, 1969; Bell, 1971; Havas & Hutchinson, 1982). The effects of pH and Al on the metabolism of odonate naiads though remain undescribed. The responses in nitrogen metabolism and respiration rates, to various stresses have been reported recently in fish (Paulson, 1980; Kaushik *et al.*, 1982; Seshagiri Rao *et al.*, 1983) and in crustacean larvae (Capuzzo & Lancaster, 1982; Johns & Miller, 1982). The literature describing respiration and ammonia excretion in dragonflies, however, is sparse though ammonia is the main catabolic end-product of this taxon (Staddon, 1959).

It would seem that this omission is an oversight in our search for early-warming indices to sublethal stresses. The use of biological sensors has become an alternative of increasing importance in the prediction and control of water pollution (Cairns *et al.*, 1977). Accordingly, the application of insects as early warning indices of sublethal levels of stress has become increasingly more frequent (Julin & Sanders, 1977; Muirhead-Thomson, 1978; Greeff & VanDyk, 1978; Correa & Coler, 1983). The present paper constitutes a preliminary study of the impact of low pH and sublethal aluminum concentrations on respiration rate and ammonia accumulation and excretion in different nymphal stages of the dragonfly *Somatochlora cingulata* (de Selys) (Odonata: Anisoptera).

Methods and materials

Six hundred and forty eight *Somatochlora cingulata* naiads, varying between 0.01 and 0.5 g, were

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collected during the fall of 1982 from Cranberry Pond in Leverett, Massachusetts. The experimental animals were acclimated at 21 ± 1 °C in filtered pond water for seven days. During this period they were fed mayfly naiads. The nymphs were divided into four different stages based upon weight: I < 0.1 g, II 0.1–0.2 g, III 0.2–0.3 g, and stage IV > 0.3 g.

Respiration rates, ammonia excretion rates and O:N ratios of each stage were monitored during exposure to pH's of 6.75 (control), 4.20 and 3.59, and aluminum concentrations of 0, 10, 20 and 30 mg l^{-1} at a pH of 4.20. A flow-through system described by Burrow (1949) and modified by Correa & Coler (1983) was used. To obtain desired pH levels, standards were prepared by combining 6.5 parts of concentrated sulfuric acid to 3 parts of nitric acid. The acid was diluted with an appropriate quantity of distilled water to produce a pH of 6.75, 4.20 and 3.59 in the respective delivery bottles. The various concentrations of Al were prepared by adding an appropriate amount of $AlCl_3$ to 20 l. reservoir bottles of filtered water. Water flow was regulated with Pasteur pipettes at a rate of 10 ± 0.5 ml min^{-1}. The dissolved oxygen (DO) concentrations were determined by the Alsterberg Azide modification of the Winkler Method (APHA, 1980). Ten replicates per pH and Al concentration were performed.

Ammonia excretion rates of individual nymphs were monitored in a static system. Three sets of DO bottles were filled with filtered pond water saturated with O_2 at a pH of 6.75 (control), 4.20 and 3.59 respectively, and 3 other sets containing 10, 20 and 30 mg l^{-1} of Al at a pH of 4.20. The nymphs (6 sets of 10) were each blotted, weighed and placed individually in 300 ml DO bottles. Ammonia excretory rates were measured according to the Nesslerization Method (APHA, 1980) from which O:N ratios were derived. To determine tissue ammonia and glutamine levels, 168 dragonfly nymphs were exposed during 96 hours to low pH (3.59) and sublethal Al concentration (30 mg l^{-1}) + low pH (4.20) using a flow-through system. Every 24 h one set of 12 dragonfly nymphs for ammonia and one for glutamate were analyzed. The whole body of each nymph was blotted, weighed and then homogenized for 1–2 min. in a mixer with cold distilled water and 15% perchloric acid and centrifuged at 750 g for 10 min. The supernatants were decanted for the determination of ammonia and glutamate levels using the Nesslerization procedure (APHA 1980) and the Bergemeyer Method (1965) respectively.

Changes in levels of the haemolymph pH were measured in 108 dragonfly nymphs exposed in a flow-through system for 96 h both to a pH of 3.59 and to 30 mg l^{-1} of Al at a pH of 4.20. Every 0, 2, 4, 8, 12, 24, 48, 72 and 96 h one set of four nymphs per contaminant was analyzed. The pH of the haemolymph was measured directly with a needle pH microelectrode (MI-408B Microelectrodes, Inc.) using a digital ionalyzer.

Statistical differences between means of oxygen consumption and ammonia excretion rates at the designated low pH and Al concentrations were evaluated using one-way analysis of variance to determine the effects of low pH and aluminum exposure on stages of development (Sokal & Rohlf, 1969). In those instances when significant differences (at $P = 0.01$) were found among nymphs in the three treatments, a Duncan's Multipe Range test was used (Snedecor & Cochran, 1967).

Results and discussion

Oxygen consumption in *Somatochlora cingulata* decreased with increasing concentrations of aluminum and hydrogen ions (Table 1). While a reduction in respiration was associated with all nymphal stages, it was most pronounced in earlier stages.

Very significant differences ($P < 0.01$) were found between controls and experimental animals exposed to low pH and to sublethal aluminum concentrations plus low pH. When comparing reductions of the respiration rate in both treatments it was noted that sublethal aluminum concentrations at low pH were as toxic as low pH alone. Aluminum did not provoke a significant change in the respiratory rate compared to low pH alone.

Ammonia excretion levels decreased very significantly ($P < 0.01$) with increased exposure to Al concentrations and low pH (Table 2). It may be that these pollutants alter the normal deamination pathway of the excretory process. Seshagiri Rao *et al.* (1982) similarly attributed the decreased ammonia levels in fish to the decreased deamination of the free amino acids during pesticide exposure. Further Karlsson *et al.* (1975) observed that increased ammonia and lactate levels inhibit the activity of the

Table 1. Relationship of wet weight-respiration for the dragonfly *Somatochlora cingulata* exposed to low pH and different Al concentrations at 21 ± 1 °C. Each value is the mean and ± SD of 10 observations.

Stage	Mean O_2 consumption ($\mu g\ O_2\ h^{-1}\ g^{-1} \pm SD$) at different pH values			Mean O_2 consumption ($\mu g\ O_2\ h^{-1}\ g^{-1} \pm SD$) at different Al concentrations (mg l^{-1}) and pH 4.20		
	6.75[a]	4.20[b]	3.59	10	20	30
I	265.5 ± 09.8	211.6 ± 9.3	179.6 ± 19.3	145.2 ± 18.7	130.7 ± 14.5	126.6 ± 14.3
II	270.6 ± 14.8	218.1 ± 6.9	198.5 ± 13.1	180.6 ± 16.5	162.7 ± 06.6	157.8 ± 11.9
III	270.9 ± 12.1	226.6 ± 7.1	212.5 ± 09.4	188.5 ± 09.4	172.4 ± 07.2	174.6 ± 10.5
IV	263.7 ± 20.0	240.1 ± 7.7	215.8 ± 05.6	202.2 ± 15.1	184.4 ± 05.9	183.0 ± 07.6

[a] This pH was used as the control value.
[b] This pH was used as the control when organisms were exposed to Aluminum

Table 2. Relationship of wet weight-ammonia excretion for the dragonfly *Somatochlora cingulata* exposed to low pH and different Al concentrations + low pH, at 21 ± 1 °C. Each value is mean of ± SD of 10 observations.

Stage	Mean ammonia excretion ($\mu g\ NH_3\text{-}N^+\ h^{-1}\ g^{-1}$ wet weight ± SD) at different pH values			Mean ammonia excretion ($\mu g\ NH_3\text{-}N^+\ h^{-1}\ g^{-1}$ wet weight ± SD) at different Al concentrations in mg l^{-1} + pH of 4.20		
	6.75[a]	4.20[b]	3.59	10	20	30
I	15.62 ± 2.4	11.94 ± 1.2	06.92 ± 1.9	9.44 ± 0.8	7.45 ± 1.1	7.28 ± 1.9
II	13.30 ± 2.7	11.14 ± 2.9	07.24 ± 0.8	9.98 ± 1.2	6.43 ± 0.6	7.53 ± 0.5
III	15.06 ± 1.2	08.82 ± 1.0	07.91 ± 1.2	10.08 ± 1.4	7.89 ± 0.9	7.35 ± 0.6
IV	16.53 ± 2.3	13.90 ± 3.2	09.55 ± 1.6	10.38 ± 0.5	9.99 ± 1.0	8.16 ± 0.9

[a] This pH was used as control value.
[b] This pH was used as control for Aluminum exposure.

Table 3. Changes in levels of ammonia and L-glutamate in the tissues of dragonfly nymphs exposed to sublethal concentration of Aluminum (30 mg l^{-1}) and low pH (3.50) (values are expressed in $\mu g\ g^{-1}$ wet weight of tissue; each value is mean of ± SD of 4 observations.

Time (h)	Mean ammonia accumulation ($\mu g\ NH_3\text{-}N^+$: g wet weight + SD)			Mean L-glutamate accumulation (μg L-glutamate: g ± SD)		
	Control*	pH	Al + pH	Control*	pH	Al + pH
0	46.68 ± 9.85	50.08 ± 5.54	56.73 ± 5.16	65.68 ± 15.40	74.33 ± 12.87	57.42 ± 14.90
24	41.52 ± 1.60	68.59 ± 5.29	59.77 ± 5.47	69.30 ± 18.46	131.92 ± 13.42	128.56 ± 25.04
48	37.31 ± 2.76	78.25 ± 8.37	67.78 ± 9.03	61.86 ± 15.52	163.56 ± 21.18	154.63 ± 5.04
72	40.89 ± 4.40	65.86 ± 6.99	78.45 ± 9.04	54.85 ± 15.21	102.01 ± 7.69	159.61 ± 19.80
96	27.20 ± 3.45	69.45 ± 9.10	66.81 ± 6.26	55.22 ± 12.42	128.43 ± 7.69	172.93 ± 8.37

* Control (pH = 6.83).

mitochondrial enzyme (glutamine dehydrogenase) and increase the acetylcholine levels in the tissue of fish. The lowered respiration rates in dragonflies may be due not only to uptake of Al in tissue and exposure to low pH, but also to the accumulation of ammonia and/or ammonia salts in the tissues.

Ammonia accumulation in tissues increased significantly ($P < 0.01$) in both treatments compared with controls. The normal tendency observed in our controls was a decrease in ammonia with time, an obvious consequence of the starvation process. However, low pH treatment (3.59) ammonia accumulated with time reaching a high value at 48 h (78.25 $\mu g\ NH_3\text{-}N\ g^{-1}$ wet weight). The pH (4.20), Al (30 mg l^{-1}) treatment elicited the same response (78.45 $\mu g\ NH_3\text{-}N\ g^{-1}$ wet weight) in 72 h indicating

Table 4. Changes in levels of pH in the haemolymph of dragonfly nymphs exposed to sublethal concentration of Aluminum plus low pH (30 mg l^{-1}, 4.20) and low pH alone (3.50). Each value is mean of 4 observations.

Time (h)	(Mean pH of the haemolymph)		
	Control	pH 3.5	Al + pH 30 :ng l^{-1}
0	7.61	7.66	7.65
2	7.60	7.91	7.93
4	7.61	7.79	7.78
8	7.59	7.81	7.80
12	7.63	7.87	8.17
24	7.66	8.16	8.14
48	7.59	7.89	8.00
72	7.60	8.27	7.95
96	7.61	8.14	8.16

that pH alone seems to increase the rate of ammonia accumulation (Table 3). Also observed (Table 4), as a consequence of ammonia accumulation in the haemolymph were increased pH values (7.66 to 8.27 in 72 h with the pH treatment and 7.65 to 8.16 in 12 h with pH-Al treatment). The observed increase in haemolymph alkalinity remains unexplained. However, the HCO_3, PO_4 and CO_2 usually present in high concentrations in Odonota haemolymph (Stobbart & Shaw, 1965) may play an important role in this increase.

Since ammonia is a toxic metabolite, tissues may reduce it by incorporating it into the formation of glutamine. Indeed, we observed elevated glutamate levels in dragonfly nymphs exposed to low pH and Al (Table 3). L-glutamic acid is oxidized by a specific enzyme L-glutamic acid dehydrogenase, which is one of the few enzymes oxidizing amino acids requiring NAD^+. The oxidation of glutamate by glutamic acid dehydrogenase also provides a means of regenerating α-ketoglutarate which normally would serve as an amino acceptor for amino groups during transamination. The fact that α-ketoglutarate is also oxidized by the citric acid cycle confers upon dehydrogenase the role of link between two important metabolic systems (Chefurka, 1965). Another pathway for the metabolism of glutamate is by decarboxylation to γ-amino butyric acid. Glutamic acid is also converted to glutamine in insects by glutamine synthetase. This enzyme requires ATP, Mg^{2+}, and NH_3^+ to function. If any one of these is limiting glutamine synthesis is restricted.

Levenbook (1962) suggested this synthesis as a possible trapping system for traces of metabolic NH_3^+. Its activity in the fat-body may, in part, account for the high levels of glutamine in larval haemolymph and tissue.

In the present experiments the increase of glutamate varied directly with time. The higher value of glutamate found after 96 h (172.93 μg L-glutamate g^{-1}) of exposure to Al and low pH suggest an increase in toxicity occurs when compared to that from low pH alone. Our results support, in part, the observations of Seshagiri Rao *et al.* (1983), who found that pesticide exposure increased ammonia levels in fish causing a shift in nitrogen metabolism toward synthesis of glutamine. Inhibition of glutamate oxidation to ammonia and α-ketoglutarate by glutamate dehydrogenase suggests an adaptive mechanism to reduce ammonia toxicity by minimizing the addition of further ammonia to the existing elevated ammonia levels.

The O:N ratio has been used in several studies (Conover & Corner, 1968; Corner & Cowey, 1968) as an index of substrate utilization of energy production and has been shown to vary with stage of development, diet, and degree of physiological stress. Bayne & Scullard (1977) found that nitrogen excretion and oxygen consumption do not always vary in the same direction, nor to the same extent, in response to changes in the environment. This fact is of significance, not only for understanding the rates of protein catabolism in general, but also for our interpretation of the balance in catabolism between the different nutrient reserves in the tissues. They concluded that the O:N ratio has proved useful in assessing the physiological responses of bivalves to various stressful environments.

Pandian (1970) suggested that the principal source of energy during embryonic development of the American lobster was lipid oxidation and this might be carried over to some degree in the early stages of post-embryonic development. The observed decrease in percentage lipid content of the final larval stages was an indication that utilization of lipid reserves provided an additional source of energy during larval and early postlarval development.

Exposing the larvae of the crab *Cancer irroratus* to Cu and Ca, Johns & Miller (1982) noted that the ratio of oxygen consumed to nitrogen excreted, (O:N), fluctuated during the nymphal stage be-

Table 5. O:N ratios of the dragonfly *Somatochlora cingulata* exposed to low pH and different Al concentration of 21 ± 1 °C.

Stage	Mean O:N ratios at low pH			Mean O:N ratios at different A conc. mg l^{-1} + pH of 4.20		
	6.75[a]	4.20[b]	3.59	10	20	30
I	16.9	17.7	25.9	15.3	17.5	17.3
II	20.3	19.5	27.4	18.0	25.2	20.9
III	17.9	25.6	26.8	18.6	21.8	23.7
IV	15.9	17.2	22.5	19.4	18.4	22.4

[a] This pH was used as control value.
[b] This pH was used as control for Aluminum concentration.

tween 28.3 and 35.4. They concluded that oxygen to nitrogen ratios of approximately 7 indicate that protein is the sole substrate used for energy production, and that increasing values can be interpreted as an increased reliance on carbohydrates and/or lipids for this purpose.

Upon exposing larval lobsters to crude oil Cappuzzo & Lancaster (1982) observed delayed molting and reduced respiration rates and O:N ratios as a result of inhibition of lipid utilization. This is consistent with their earlier thesis (1981). Changes in metabolic activity and energy utilization during those intense morphological and behavioral changes associated with metamorphosis are interesting phenomena to consider as indications of stress.

In our experience no O:N ratios below 10 were observed during nymphal stages in any of the treatments (Table 5). The average ratio in the different stages ranged between 15.3 and 27.4. This O:N ratio is indicative of decreased dependence on protein reserves, because protein catabolism leads to the production of ammonia in addition to water and CO_2.

The authors conclude that *S. cingulata* partially compensates for the disruption of its excretory processes, induced by exposures to Al and low pH, by reducing the quantity of accumulated metabolites. This is accomplished through:

1) depressed oxygen consumption
2) restricted utilization of protein as fuel
3) shifting intermediate metabolism toward the synthesis of glutamate

Contrary to fish studies, Al does not seem to exert a toxic effect, for low pH alone seems to elicit the same response as Al and low pH together. Because the above physiological changes are relatively easy to monitor, we suggest considering their incorporation into a bioassay procedure for acid rain stress.

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