Bull. Environm. Contam. Toxicol. 25, 358-363 (1980)

Respiration of Aquatic Insect Larvae (Ephemeroptera, Plecoptera) in Acid Mine Water

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Ecotoxicology (TRUHAUT 1975) is the study of the harmful effects of natural substances and artificial pollutants experienced by organisms in the environment. The degree of response exhibited by an organism toward the presence of noxious substances can often be determined by monitoring a physiological parameter. One such parameter is respiration (CHENG & RODRICK 1974; KAWATSKI et al. 1974). The majority of studies dealing with the biological impact of acid mine drainage have been ecological surveys. No studies have been reported which deal with the physiological response of an organism to acid mine water other than acute toxicities of the various components expected in an effluent stream (BELL & NEBEKER 1969; KIMMEL & HALES 1973; WARNICK & BELL 1969). These studies did not consider possible synergistic effects between individual components of acid mine water or the mode of action of pollutants involved. The work we report was undertaken to determine whether the toxic mode of action of an acid water effluent involves any aspect of the respiratory processes in three species of aquatic insect larvae. Although respirometry can be valuable in detecting signs of metabolic involvement, one must be aware that it is not a technique for the identification of specific toxic mechanisms (CHENG & RODRICK 1974).

METHODS AND MATERIALS

Mayfly larvae (Ephemeroptera; two species of the genus Ameletus) (EDMUNDS et al. 1976) were collected on a twice weekly basis for a six week period during April and May, 1978. Stonefly larvae (Plecoptera; Acroneuria xanthenes) (HITCHCOCK 1974) were collected every other day for a two week period in mid-June, 1978. Animals used in this study were collected from Trace Run, a tributary of Hewett Fork and Raccoon Creek, located near the town of Hocking in southeastern Ohio (SW 1/4, SE 1/4, Sec. 1, T12N, R16W). They were readily found in the unpolluted stream portions but not in zones below which mixing had occurred with an acid water effluent. Neither sex nor instar stage of the larvae were determined. To

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0007-4861/80/0025-0358 $01.20

minimize handling and possible damage to individuals, species identifications were not made until after rate determinations had been completed. Collections were halted by larval emergence.

In the laboratory, larvae were starved and kept in 25 ml. beakers containing nonaerated, nonagitated stream water cooled to 15 $\pm$ 0.5 ^{o}C in a constant temperature, circulating waterbath. No tests were begun before an elapsed time of 24 hours. Organisms were subjected to fluorescent room lighting for an approximately natural photoperiod (13L:11D).

Mine water used in this experiment was collected from a flooded, abandoned coal mine shaft, located 50m downstream from the collection site for larvae at Trace Run. The upper unpolluted stretch of Trace Run from which larvae were collected served as the source of control water. Analyses for pH, conductivity, and particular ions (Mn, Fe, SO_4) were done respectively with an Orion Research Ionanalyzer Model 404, YSI Model 33 S-C-T meter, and Bausch and Lomb Spectronic 20 with the aid of prepackaged reagents (Hach Chemical Co.). Alkalinity was measured through titration with 0.02N H_2SO_4 (APHA 1976).

Time to mortality studies were performed at 15 ^{o}C in fingerbowls of acid mine water on the two species of Ameletus to determine the median lethal time (LT50) for the population. An insufficient number of specimens precluded comparable tests on Acroneuria xanthenes. Animals in which a response could not be elicited by tactile stimulation over a one-min period were considered dead.

A circular Warburg apparatus was used in determining O_2 consumption rates by the method of UMBREIT et al. (1949) the apparatus being calibrated by the method of LAZAROW (1949). Gaseous volumes of manometer flasks were 18-21 ml with a fluid volume in each of 2.2 ml (2 ml of test fluid in the main compartment; 0.2 ml CO_2 absorber (10% KOH) in the center well). Experiments were conducted at 15 + 0.5 ^{o}C. Warburg flasks were kept in a constant linear shaking motion, utilizing a rate of 60 strokes/min over a cyclic distance of 2.5 cm. No substratum was employed during the measurements. Rates were determined on solitary individuals. Manometers were read to the nearest 0.5 mm.

All control respiratory rates in unpolluted water were determined from a period of measurement 3 hours in duration. After the initial 3 hour period, control water was removed from all flasks. Fresh control water was returned to half of the flasks while the remainder were supplied with acid mine water. Determination of respiratory rates was then continued for a period of 3 hours. Subsequent to this portion of the study, respiratory rate determinations were also conducted for individuals considered dead because of mine water toxicity. Time intervals between manometer readings on a particular day were set at either 10 or 15 minutes. After rate determinations had been completed, animals were sacrificed and dried at 80°C for 24 hours. Dry weights were the mean of three readings obtained on a Mettler H20T Analytic balance to the nearest 10 ug.

Calculation of LT50 values were performed through probit analysis (FINNEY 1971). Hourly rates of oxygen consumption were determined through calculation of regression coefficients by least squares. Comparison of rates in unpolluted and acid mine water was performed through the use of the F-test for differences between two regression coefficients. Significance in all tests was set at the 5% level.

RESULTS

Table 1 provides chemical characteristics of the control and treatment fluids utilized in these experiments. Severity of pollution represented by the treatment fluid is indicated by a partial list of chemical parameters used as guidelines in classifying acid mine pollution.

TABLE 1

Chemical charactistics of Control and Treatment Fluids

	Control	Acid Mind Water	Typical Acid Water Levels[a]
pH	8.3	2.5	< 6.0
Conductivity (umhos/cm)	280	4400	-
Mn(mg/l)	< 0.01	6.95	> 0.5
Fe (mg/l)	0.0	1.17	> 0.5
SO_4 (mg/l)	60.4	3110	>75.0
Alk (mg/l)	85.0	0.0	-

[a] Cited in NICHOLS & BULOW (1973). U.S. Government classification criteria for acid mine water drainage.

TABLE 2

Tabular presentation of the outcome of statistical comparisons of sequentially determined respiratory rates in unpolluted and acid mine water

	Acroneuria xanthenes Control[a]	*Acroneuria xanthenes* Treatment[b]	*Ameletus lineatus* / *Ameletus* sp. Control[c]	*Ameletus lineatus* / *Ameletus* sp. Treatment[c]
Significant Increase	1	1	1	1
N. S.	6	6	3	10
Significant Decrease	3	3	0	1

[a] Signifies those numbers of individuals whose rates were determined in control water for both periods of measurement.

[b] Signifies those numbers of individuals whose rates were first determined in control water and subsequently in acid mine water.

[c] Pooled data for genus *Ameletus.*

Time to mortality studies on *Ameletus* indicated LT50 levels of 21.4 hours for *A. lineatus* and 29.0 hours for *Ameletus* sp. No correlation was found between time of death and weight of individuals for either species.

In each species it was found that log transformation of O_2 consumption (ul O_2/g hr) decreased as individual dry weight (mg) increased. The regression coefficient b, representing relative O_2 uptake per unit weight, was -0.074 (s_b = 0.021, n=14) for *Ameletus lineatus*, -0.022 (s_b = 0.012, n = 11) for *Ameletus* sp., and -0.0056 (s_b = 0.0026, n = 19) for *Acroneuria xanthenes*. from 14 to 50 mg. Only rates of the first and third species differed significantly from zero.

The outcome of statistical comparisons between regression coefficients obtained from sequential determinations of respiratory rates on solitary individuals is presented in Table 2. Results indicate that a majority of larvae exhibited similar sequentially determined respiratory rates regardless of the solution, control or acid mine water, that was utilized in the second rate determination. Similar patterns of respiratory rate increases and decreases between control and treatment groups suggest intervention in the respiratory process of extraneous factors not under the scope of these experiments.

Respiratory rates for individuals considered dead by the criteria given above decreased gradually with time, but for the first few hours still lay within the 95% confidence limits of the regression lines. Oxygen consumption rates for control and acid waters in the absence of test animals were negligible, indicating no bacterial contamination.

DISCUSSION

BELL & NEBEKER (1969) have shown that aquatic insects are tolerant of acidic conditions down to pH 4 for short periods of time. Tolerance below this level is dependent both on species and stage of life history involved (KIMMEL & HALES 1973). The absence of Ephemeroptera and Plecoptera from zones of Trace Run polluted by acid mine water and the results of laboratory tolerance tests both indicate that high concentrations of mine acid are toxic to these animals.

The toxic mode of action of acid mine water has received much discussion. One theory suggests that mucus normally present on the gills of fish is precipitated in solutions of high acidity or heavy metal concentrations. Permeability of the gill membrane system is thus presumably impaired, the fish eventually succumbing to anoxia (WESTFALL 1945; SKIDMORE 1970; NICHOLS & BULOW 1973). A similar mechanism has been suggested for benthic organisms (NICHOLS & BULOW 1973), high levels of acidity affecting animals directly through attack on their gills. An alternate theory of toxicity involves displacement of functional cations from active sites of enzymes by heavy metal ions, resulting in failure of life processes such as respiration (EICHORN 1975). Various studies (JACKIM et al. 1970; BROWN 1976) have reported the deleterious effects of heavy metals on the activities of liver and on nitrogen metabolizing enzymes, while HILTIBRAN (1971) illustrated alterations in oxygen and phosphate metabolism of bluegill liver mitochondria by heavy metals. Results of our study support neither of these theories for aquatic insect larvae. Acid mine water did not consistently alter the respiratory rates of species studied. Observations on individuals, sacrificed in acid mine water and subsequently monitored, indicated a gradual decrease in respiratory rate with the loss of life processes, supporting a claim of non-interference by noxious ions. Had anoxia due to ionic interference been a major cause of death, the decrease in respiratory rate would have been more precipitous. We conclude, therefore, that the toxic mode of action of acid mine water on these aquatic insect larvae does not operate through mechanisms that can be detected by means of respirometry.

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