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EFFECT OF PH AND TOTAL ION CONCENTRATION ON GROWTH RATE OF *CHIRONOMUS* NR. *MATURUS* LARVAE (DIPTERA: CHIRONOMIDAE) FROM AN ACID STRIP-MINE LAKE

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ABSTRACT

Larvae of *Chironomus* nr. *maturus* Johannsen, a species abundant in an acid (pH 3) strip-mine lake, were raised in the laboratory in sixteen solutions combining pH's of 3.2, 4.0, 5.0, and 6.6 with total ion concentrations of 2670, 1350, 690, and 30 mg/1. Larval growth rate was significantly lower at pH 6.6 than at the three lower pH's, suggesting that a low pH is optimal for this species. Larval growth rate was unaffected by total ion concentration.

INTRODUCTION

Lakes on strip-mined areas are often chemically distinctive: In Illinois about six percent are very acid, with a pH less than 4.5 (Haynes and Klimstra 1975), and most, whether acid or not, have a considerably higher total ion concentration than other lakes (Konik 1980). Chemical conditions can affect the composition and abundance of the fauna living in a lake, following Thienemann's second ecological principle: "The more the conditions in a locality deviate from normal, and hence from the normal optima of most species, the smaller is the number of species which occur there and the greater the number of individuals of each of the species which do occur" (Hynes 1970, p. 234, citing Thienemann 1954). The abundance of species in acid waters with a high ion concentration does not necessarily mean that this environment is optimal for them; their abundance could simply result from the absence of predation and ability of the species to tolerate conditions far below their optimum. In one acid strip-mine lake in southern Illinois, Bradley's Acid Pit, only two species of Chironomidae occurred, but the larvae were abundant, each with as many as 50,000 larvae/m^2 (Zullo 1981). This situation offered an opportunity to determine whether or not larvae in this lake are stressed by the low pH and high total ion concentration.

One of the species appears to be identical to *Chironomus maturus* Johannsen in larval, pupal, and adult stages as described by Sublette and Sublette (1974) but differs in the banding pattern of the giant chromosomes, so has been designated *C.* nr. *maturus* (Yamamoto 1977). From August 1977 to May 1978 the pH of Bradley's Acid Pit ranged from 2.70 to 3.85, and conductivity from 1085 to 3111 μmhos at 25°C except for one measurement of 35,943 μmhos in March 1978 (Zullo 1981). In this lake μmhos are equal to mg/liter total ion concentration (Stahl, unpublished). Sulfate varied from 800 to 1950 mg/liter except for two anomalous values.

The question is: even though the population of *C.* nr. *maturus* in Bradley's Acid Pit is high, are the larvae being stressed by either the low pH or the high ion concentration? To answer this question, growth of larvae in a range of pH's and total ion concentrations was measured.

METHODS

Sixteen solutions were made up with $CaSO_4$, $MgSO_4$, Knop's solution and phosphoric acid buffer, with combinations of four different pH's and four different total ion concentrations, namely, pH 3.2, 4.0, 5.0, and 6.6, and total ion concentrations in mg/l of 2670, 1350, 690, and 30. The aim was to make up simplified solutions having ratios of major ions similar to those in Bradley's Acid Pit water. The Ca^{++}:Mg^{++} weight ratio was kept at 1.3:1. The desired pH was obtained and maintained with 1 N HCl and 1 N NaOH, and the desired total ion concentrations with $CaSO_4$ and $MgSO_4$ as well as 14 mg/l Knop's solution and 16 mg/l phosphoric acid buffer in each 900 ml of water. Knop's contains 1 mg/l K^+ and 8 mg/l NO_3^- (Beers 1959). Originally we wanted solutions with a pH of 3.0 and 7.0, but solubility problems developed at those pH's. Total ion concentration and the concentration of Ca^{++}, Mg^{++}, and SO_4^{--} in each solution were, respectively: 2670 (420, 222, 2000); 1350 (212, 112, 1000); 690 (107, 58, 500); 30 (2, 1, 2).

The solutions were poured into one-liter mason jars having 1 cm of clean sand on the bottom. Each jar received 1.8 mg of methylene blue as a fungicide (Thornton and Wilhm 1974). The jars were kept in a water bath at a temperature of 20.5 ± 1.5°C and were continuously aerated.

Each jar received 65 to 90 eggs of *C.* nr. *maturus*, collected from Bradley's Acid Pit. If all eggs hatched, larval population density would have been 7190 to 9960/m^2. Larvae were fed dog food (Hartz Rocky Mountain Dog Kisses®) at a rate of approximately 0.31 mg food per larva each day. Procedures generally followed those described by Biever (1965, 1971).

Three runs of the sixteen different solutions were made for a total of 48 jars. Jars were checked every 12 hours for signs of pupation and for pH. A Beckman Electromate pH meter with combination probe was used to measure pH. Sulfate was measured intermittently and at the end of each experimental run using the Hach

SulfaVer IV method (Hach Chemical Company, 1973). All solutions were checked at the end of each experimental run. When a pupa was found all larvae in that jar were then preserved in 10 percent formalin. Mortality was calculated by subtracting the number of live larvae, pupae, and pupal exuviae from the number of eggs placed in each jar. Total body length was measured with an ocular micrometer and wet weight with a Sartorius balance after blotting each larva for 30 seconds. All length and weight measurements were made within two days to minimize shrinkage and weight loss (Mackey 1977). Daily growth rates were calculated by dividing length and weight by the number of days in the larval growth period.

RESULTS AND DISCUSSION

Mortality as calculated was erratic (Table 1). Two-way ANOVA indicated no significant effects of either pH or total ion concentration on mortality (Table 2). Evidently much of the apparent mortality was caused by eggs not hatching, presumably because of infertility. It would have been better to have added newly hatched larvae to the experimental jars rather than the unhatched eggs. Analysis of variance of weighted means of length and wet weight showed that much of the variation was between the jars (MS value 0.0331) and little within each jar (MS value 0.0017). This implied that two-way ANOVA tests on the unweighted means of the growth rates would be more appropriate (Starks, pers. comm.).

A two-way ANOVA showed that length growth rate was significantly affected by pH but was not significantly affected by total ion concentration (Table 3). Analysis of the data on wet weight, the other measure of growth, confirmed this finding (Table 4). Analysis of the pH data by a modified Duncan's multiple range test showed that only at pH 6.6 were the growth rates in length and wet weight significantly different (Table 5). Since they were slower at pH 6.6 this implies that the more acid environments are more optimal for *C.* nr. *maturus* larvae. Thus this species seems not to be stressed by either the low pH or the high total ion concentrations characteristic of strip-mine lakes.

There have been a few studies of the effect of low pH on larval or nymphal mortality and emergence success in aquatic insects (Harp and Campbell 1967, Bell and Nebeker 1969, Bell 1970, and Bell 1971). The only chironomids evaluated were an undescribed species of *Chironomus* by Harp and Campbell (1967) (see Harp and Hubbard 1972 for a clarification of the identification) and *Tanytarsus dissimilis* Johannsen by Bell (1970). Emergence of *C.* sp. was inhibited by a pH of 2.8 to 3.0, and all larvae of *T. dissimilis* died in 5 to 10 days at pH's of 3.0 and 4.0. To our knowledge there are no other studies of the effects of pH on growth rates of chironomid larvae. Indeed, apparently only Fiance (1978) measured growth rate in an aquatic insect at low pH. In a section of stream acidified to pH 4 for three months the larvae of the mayfly *Ephemerella funeralis* were 29 per cent smaller than in the reference section of the stream. In all the studies cited the effect of low pH was adverse. However, none of these studies used our approach of testing the response to higher pH's of a species found in an acid environment. Our approach might reveal other species that are favored by the low pH of acid strip-mine lakes. In Bradley's Acid Pit the other abundant chironomid species, *Tanytarsus dendyi* Sublette, is known from circumneutral water (Buckley and Sublette 1964), so it might either be favored or inhibited by a highly acid environment.

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Table 1. Percent mortality of *C*. nr. *maturus* larvae in experimental solutions, runs 1, 2 and 3.

	Total ion concentration (mg/l)			
pH	2670	1350	690	30
3.2	11, 100, 40	13, 93, 25	96, 100, 44	86, 43, 15
4.0	11, 100, 17	15, 100, 11	97, 65, 60	58, 100, 24
5.0	12, 20, 16	6, 35, 41	21, 87, 40	28, 10, 0
6.6	100, 70, 35	9, 38, 27	11, 7, 77	31, 53, 43

Table 2. Two way ANOVA of the effects of pH and total ion concentration on mortality of *C*. nr. *maturus* larvae grown in experimental solutions.

Source	df	SS	MS	F value
Model	15	17,168	1,144	1.04[a]
pH	3	3,810	1,270	1.15[a]
total ion concentration	3	6,783	2,261	2.05[a]
pH × total ion concentration	9	6,573	730	0.66[a]
Error	32	35,383	1,105	
Total	47	52,551		

[a]Not significant at the 0.05 level.
df = degrees of freedom
SS = sum of squares
MS = mean square
F = variance ratio
Identical abbreviations are to be found on Tables 3 and 4.

Table 3. Two way ANOVA of the effects of pH and total ion concentration on the unweighted means of the length growth rate (mm/day) of *C*. nr. *maturus* larvae in experimental solutions.

Source	df	SS	MS	F value
Model	8	0.1220	0.0152	6.75***
replicate	2	0.0150	0.0075	3.33
pH	3	0.1086	0.0362	16.03***
total ion concentration	3	0.0034	0.0011	0.51
Error	33	0.0745	0.0023	
Total	41	0.1965		

***Significant at the 0.001 level.

Table 4. Two way ANOVA of the effects of pH and total ion concentration on the unweighted means of the wet weight growth rate (mg/day) of *C.* nr. *maturus* larvae in experimental solutions.

Source	df	SS	MS	F value
Model	8	0.000040	0.000005	3.44***
replicate	2	0.000005	0.000003	2.55
pH	3	0.000030	0.000010	7.09***
total ion concentration	3	0.000005	0.000002	0.92
Error	33	0.000050	0.000001	
Total	41	0.000090		

**Significant at the 0.01 level.
***Significant at the 0.001 level.

Table 5. Analysis by a modified Duncan's multiple range test of the effect of pH on unweighted means of length growth rate (mm/day) and wet weight growth rate (mg/day) of *C.* nr. *maturus* larvae in experimental solutions.

		Growth rate	
pH	No. observations	Length Mean*	Wet weight Mean*
3.2	10	0.3225[a]	0.1380[a]
4.0	9	0.3401[a]	0.1280[a]
5.0	12	0.3243[a]	0.1250[a]
6.6	11	0.2164[b]	0.0740[b]

*Means with the same letter are not significantly different at the 0.05 level.