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Preliminary Investigation of Effects of Sublethal Acid Exposure on Maternal Behavior in the Crayfish *Orconectes virilis*

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The occurrence of crayfish inhabiting the littoral regions of many oligotrophic acid sensitive lakes makes these organisms vulnerable to spring pH decreases. Egg extrusion in Orconectes spp. found in south-central Ontario is generally synchronous and occurs during or slightly after ice melting in late April (Berrill 1978), a time at which these lakes receive between 36-77% of the year's export of H^+ from their watersheds (Jeffries et al. 1979). For those species such as Astacus astacus that oviposit in late autumn and carry their developing eggs during all phases of springmelt, it is the effect of low pH upon the embryos that may ultimately determine whether a crayfish population will survive. Placement of ovigerous A. astacus in holding containers in a river of varying acidity produced a hatching failure at pH values below 5.2 (Furst 1977).

To understand how a toxicant affects crayfish reproduction it is necessary that the maternal and embryonic responses to experimental perturbation be separated. Due to acid inhibition of O_2 uptake in crayfish eggs (Appelberg 1981), changes in maternal pleopod vibration, and therefore rate of egg oxygenation, may be critical for successful embryogenisis. When pleopod movement is retarded, egg development may be delayed or halted, and fungal infections can result (Andrews 1904, Suko 1956). In an acidified lake in the Experimental Lakes Area, northwestern Ontario, 6.9-16.9% of the egg-bearing females experienced partial mortality of their broods at pH 5.4-5.6 (France, in prep.). An ancillary experiment was undertaken here to determine if this egg mortality could be a result of low pH-induced behavioral modification of the maternal female, rather than direct acid toxicity to the developing eggs.

MATERIALS AND METHODS

Ovigerous age 2-3 (20-30 mm carapace length) Orconectes virilis (Hagen) were collected during June 1980 from Lake 240, a control (pH 6.6-6.8) basin in the Experimental Lakes Area, northwestern

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Ontario (93°30'-94°00'W and 49°30'-49°45'N). A diver-operated suction gun was used to prevent mechanical damage to specimens. Animals were held for 48 h prior to testing with pre-acidified and vigorously aerated Lake 239 water (Prokopowich 1979). Five crayfish displaying rhythmic pleopod beating of egg clusters were selected for experimental observation. Tests were performed in a 2-L glass beaker shielded on all sides except for an observation window in front of which was mounted a desk magnifying lens. Experiments were conducted in a secluded location to minimize external influences on crayfish behavior, and extreme care taken at all times to avoid startling the individual being tested.

Following placement inside the testing container, each animal was allowed 30 min to adjust to the experimental conditions and to establish a set rhythmic pattern of pleopod oscillation. Observations timed with a stopwatch were then made of vibration frequency and the duration of vibration and stationary (between beating) periods. Crayfish behavior was monitored for 15 such cycles of pleopod oscillation, which in most cases amounted to about 30 min. Toxicological modification of any aspect of behavioral patterns is likely to be subtle and sometimes difficult to interpret quantitatively. To attempt to compensate for individual variability, it was thought advisable that following each test, for the same crayfish to recover for about 4 h before further testing at a different, randomly chosen, pH concentration. Retesting of the single individual displaying the greatest response to acidified water was performed to validate the repeatibility of experimental results.

RESULTS AND DISCUSSION

Measurements of maternal behavior of 10 crayfish within the large holding aquaria, both immediately after capture, and again two days later, indicated that little stress occurred when animals were transferred to the testing vessel. O. virilis at neutral pH aerate eggs for a greater proportion of the time than Procambarus clarkii tested at the same temperature (Suko 1956), and also at a higher frequency of beats per min than several other Orconectes species when tested at comparable stages of egg development (Bechler 1981). These differences may reflect both the longer developmental period in the case of P. clarkii (4 mo), and the colder temperatures (12-16 C) used in Bechler's study.

Over the range tested, pH had no effect on either vibration frequency or duration of the vibration period (Table 1). Below pH 5.0, the length of time spent stationary with no pleopod beating increased with higher acidity, such that a smaller percentage of the total time was occupied by each crayfish in egg aeration (Fig. 1). ANOVA testing (randomized complete block design; acrsin transformed data) found that individual crayfish differed significantly ($F = 7.97$; d.f. = 4,18; $p<0.001$) in the precentage of time spent vibrating pleopods. The mean percentage of total time spent vibrating eggs was not, however, significantly different ($p>0.05$) for the crayfish retested at pH 4.0, 4.5 and 5.0, compared to

original values. The mean percentage of total time spent vibrating was also significantly different ($F = 9.15$; d.f. = 4,18; $p<0.001$) among treatments. Further analysis (LSD procedure) showed that the percentage of total time spent vibrating eggs at pH treatments 4.5 and 4.0 were significantly lower ($p<0.05$) than those at treatments pH 5.0, 5.5, and 7.0 which were not different from one another.

Table 1. Influence of pH upon maternal O. virilis pleopod vibration. Results of each treatment are means ± S.E. for n = 5 animals. Temperature was 17.3-20.5°C, and oxygen at saturation for all tests. Control water was pH 6.8.

Treatment pH	Vibration frequency	Duration of vibration period (secs)	Duration of stationary period (secs)
Control	69.7 ± 2.6	26 ± 4	60 ± 13
5.5	68.6 ± 3.4	35 ± 3	75 ± 8
5.0	81.1 ± 7.6	25 ± 8	62 ± 9
4.5	74.1 ± 8.7	24 ± 6	106 ± 13
4.0	62.4 ± 2.0	18 ± 3	138 ± 19

The mechanism producing decreased egg aeration at low pH is not clear, but one possibility can be considered. Oxygen consumption increases under exercise or stress, and animals that are exercised are less resistant to acid than are those at rest (Graham & Wood 1981). Crayfish can regulate their internal state by adjusting their metabolic rate in response to low environmental O_2 concentrations (Wiens & Armitage 1961). It is possible, as has been demonstrated for Gammarus (Rees 1962), that exposure to hypoxic water, in the present case low pH-induced (cf. Hiestand 1931, Tyagi 1973, Fromm 1980), may result in a decrease in pleopod beating in attempt to regulate rate of O_2 consumption to a critical level below which uptake is reduced. The decrease in mean percentage of total time spent vibrating from 30% at pH 5.0 to 10% at pH 4.0, suggests that when encountering acidified water, the need of ovigerous females to satisfy their own increased O_2 demand may override maternal behavior to developing eggs. Further physiological investigations are of course needed to test this hypothesis.

The mean percentage of total time which O. virilis spent aerating eggs was significantly lower at pH levels 4.5 and 4.0. Egg mortality and fungal infections in the population inhabiting acidified Lake 223, however, occurred at pH 5.4-5.6 (France, in prep.), acidity concentrations which did not reduce pleopod

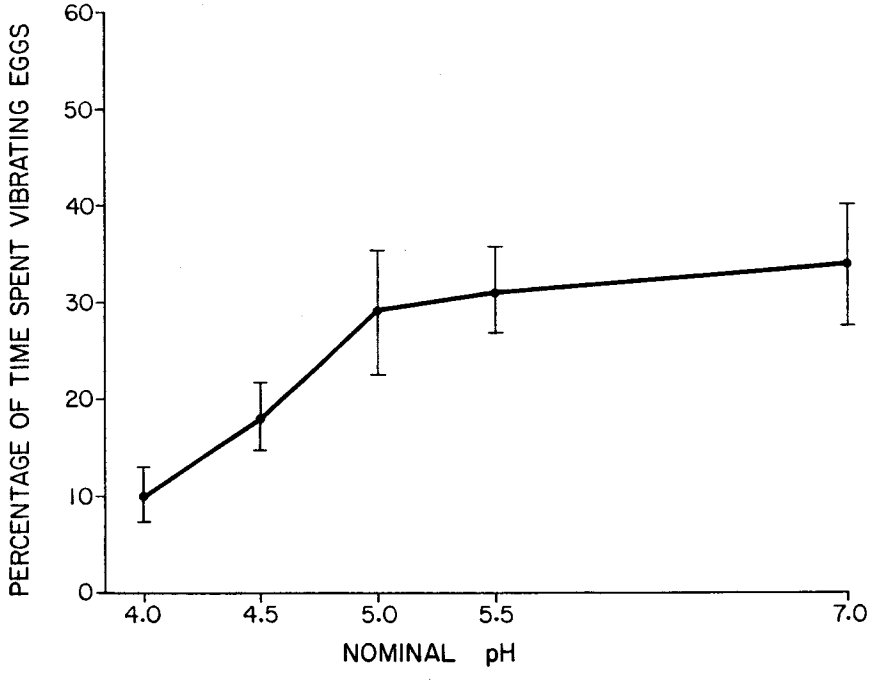

Figure 1. Behavioral responses of pleopod vibration of developing eggs in ovigerous O. virilis exposed to low pH. Symbols represent mean ±S.E. of 5 animals.

oscillation of five ovigerous females in this experiment. Consequently, low pH may be expected to directly affect crayfish reproduction before such modifications of maternal behavior become important.

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