Bull. Environ. Contam. Toxicol. (1995) 54:403–408


Environmental Contamination and Toxicology

# Effect of Body Size on the Intraspecific Tolerance of Aquatic Insects to Low pH: A Laboratory Study

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Received: 15 February 1994/Accepted: 19 August 1994

The principal mode of toxic action of low pH on the physiology of freshwater invertebrates seems to be a failure of ionic regulation ($Na^+$ regulation, primarily). Laboratory studies (Vangenechten et al. 1979; Havas et al. 1984; Firsbie and Dunson 1988) show that, if the $H^+$ concentration in the aquatic environment is increased by lowering the pH, the $Na^+$ influx in freshwater invertebrates can be significantly reduced and, consequently, their mortality increased. In addition, several experiments on the mortality of gammarids and stoneflies (Brehm and Meijering 1982; Meinel et al. 1985; Twitchen 1987) suggest that the survivability of freshwater invertebrates at low pH could be increased by using higher concentrations of sodium ion ($Na^+$) in the aquatic environment, other cations ($K^+$, $Mg^{2+}$ or $Ca^{2+}$) apparently having no ameliorating effect.

Owing to the differential degree of morphological development between larval stages in aquatic insect species, it is logical to think that their osmoregulatory ability can also be different. However, the intraspecific size-dependent sensitivity of aquatic insects to water acidification has received limited attention. A significant exception is the experiment conducted by Allan and Burton (1986) in recirculating laboratory streams. They found that the vulnerability of the caddisfly *Lepidostoma liba* to low pH during long-term exposures was directly correlated with size, smaller individuals being much less tolerant to pH 4 depression than larger individuals. In this respect, the main purpose of this laboratory study was to examine the differential tolerance between larval stages of *Cheumatopsyche pettiti* (Banks) to low pH during short-term exposures. Larvae of *C. pettiti* (Trichoptera, Hydropsychidae) are common aquatic insects in macrobenthic communities of freshwater ecosystems in North America. These animals are filter feeders that construct retreat and capture nets on the bottom of rivers and streams to strain food particles from the current.

## MATERIALS AND METHODS

Larvae of *Cheumatopsyche pettiti* were hand-collected from an unpolluted area of the Cache la Poudre River (Colorado, USA). The average (n=4) pH value at this sampling site was 7.8. No animal died during transportation. In the laboratory, larvae were arbitrarily classified in three different size groups based on maximum head capsule width: early instar (<0.5 mm), middle instar (>0.5 - <1.0 mm), and last instar (>1.0 mm). It is interesting to note that, in natural conditions, this species can present five distinct instars. After classification, larvae were randomly distributed into test aquaria and acclimatized for 3 d prior

to toxicity tests. They built their retreat and capture nets within the early hours of their acclimatization. Animals were not fed throughout the study.

Two different static (renewal system) toxicity bioassays were performed for 4 d using glass aquaria, each containing 0.5 L of Fort Collins filtered tap water and several nontoxic pieces of PVC. Chlorine was removed during filtration, and only traces of sodium chloride (NaCl) remained in the filtered tap water. Oxygenation and slight turbulence were produced with air pumps and airstones. Chamber environmental temperature and a 12 hr photoperiod were utilized.

A control (average pH value of 7.7) and five different pH values were used per bioassay, with 10 larvae of each size class per aquarium. Mean (n=4) pH values were 2.4, 3.0, 3.4, 4.0 and 4.6 for the first bioassay, and 2.8, 3.3, 3.8, 4.4 and 5.1 for the second bioassay. Test pH solutions were made from dilute sulfuric acid ($H_2SO_4$, Fisher Scientific Co, USA) and changed daily in order to improve the constancy of pH values and water quality conditions. pH values were monitored with a portable Hach model 43800 pH meter. Average values of water quality conditions during acute toxicity bioassays were 18.4 °C for temperature, 9.5 mg $O_2$/L for dissolved oxygen, 37.3 mg $CaCO_3$/L for total hardness, and 0.1 mg N/L for nitrate. Physicochemical parameters were analyzed in accordance with standard methods described by APHA (1989).

The 72 and 96-hr LC50 values, their 95% confidence limits, and $\chi^2$ values were calculated by the noncomputerized method of Litchfield and Wilcoxon (1949), using mortalities and mean pH values obtained in both bioassays for each size class. Death was defined as larvae not moving and not reacting to gentle prodding. Dead animals were removed daily. In addition, the formula of factors (Litchfield and Wilcoxon 1949; APHA 1989) was applied to estimate significant differences between larval instars with regard to their tolerance to low pH. According to that formula, sensitivities of test species (or size classes) to poisons are significantly ($P < 0.05$) different if their 95% confidence limits do not overlap significantly ($P > 0.05$).

On the other hand, maximum acceptable toxicant concentrations (MATCs) were calculated using the multifactor probit analysis software (US EPA 1991). This methodology uses data derived from acute toxicity bioassays (at least two different exposure times) to predict the concentration of toxic substance that can exist in a laboratory environment for an extended exposure time (average life time or infinite hours) causing mortality or sublethality at 0.01% test population. The independent variables are exposure time and toxicant concentration, and the dependent variable is the probit of the proportion responding to each concentration. Because pH is a log function, pH values were converted to $[H^+]$ before all statistical analyses.

## RESULTS AND DISCUSSION

Mortality percentages and mean pH values for each size class of hydropsychid larvae are shown in Figure 1. Standard deviations of mean pH values were lower than 0.13. The mortality increased with decreasing pH values and with increasing exposures times. In general, larvae of *Cheumatopsyche pettiti* migrated from their retreat and capture nets and protruded their anal papillae before dying. Neither dead animals nor sublethal effects were observed in control aquaria.

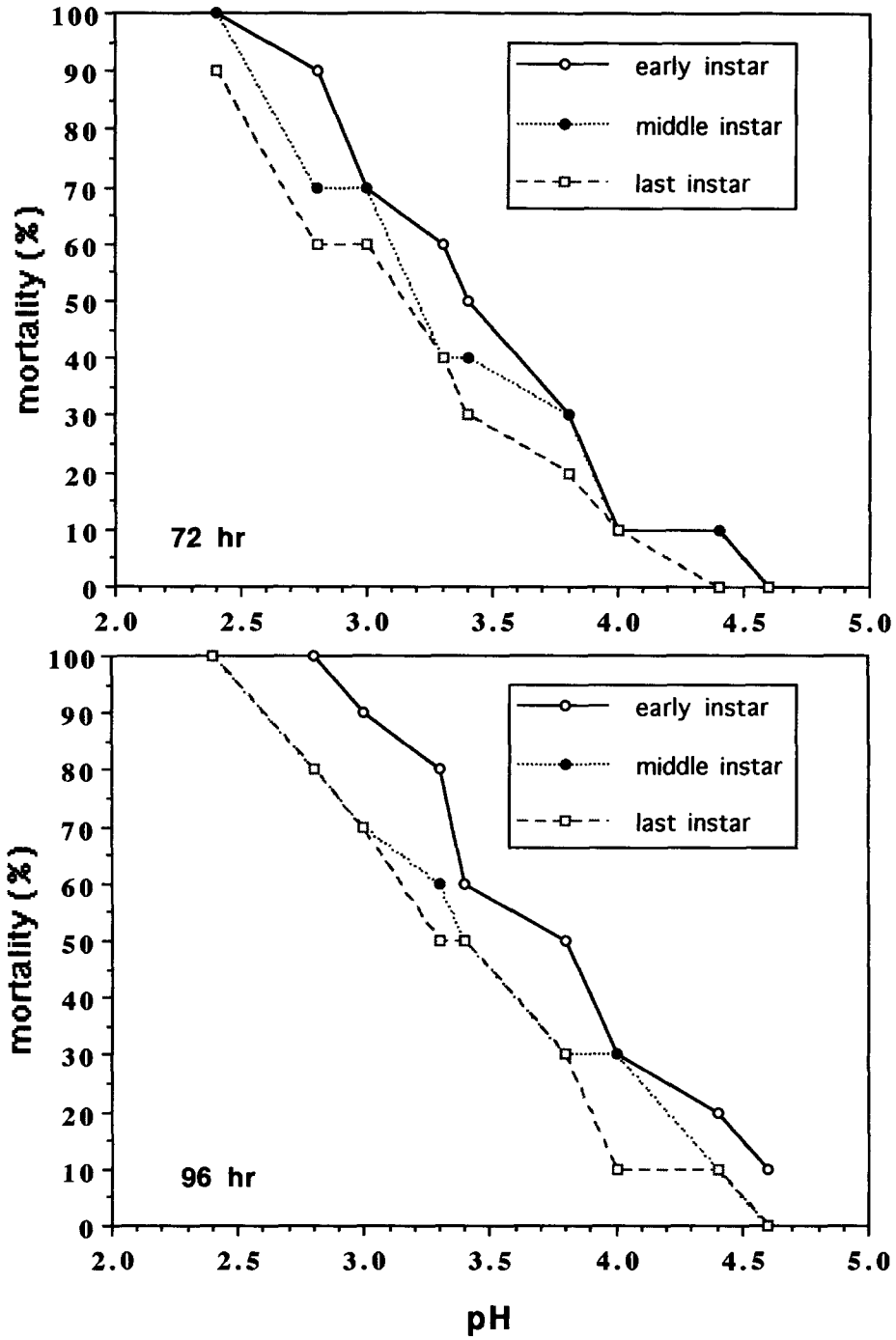


Figure 1. Mortality percentages after 72 and 96 hr of exposure to mean pH values for each larval instar of *Cheumatopsyche pettiti* (Banks) during short-term toxicity bioassays.

Table 1. LC50s, their 95% confidence limits, and MATCs (as pH values) for each larval instar of *Cheumatopsyche pettiti* after 72 and 96 hr of exposure to water acidification. MATC is defined as the lowest pH value that would have no statistically significant observed adverse effect on the survival of each larval instar during continuous exposure throughout a life-cycle toxicity bioassay ("safe" concentration of H+). It was assumed that slopes for each exposure time were similar.

| | 72-hr LC50 | 96-hr LC50 | MATC |
|---|---|---|---|
| early instar | 3.49 (3.26 - 3.73) | 3.71 (3.44 - 3.95) | 6.62 |
| middle instar | 3.25 (3.00 - 3.51) | 3.48 (3.24 - 3.73) | 5.80 |
| last instar | 3.12 (2.87 - 3.38) | 3.35 (3.11 - 3.59) | 5.34 |

There was a differential response between larval instars to water acidification. After 72 hr of exposure to low pH, both early and middle instars exhibited mortalities of 10% and 100% at mean pH values of 4.4 and 2.4 respectively, whereas the last instar exhibited mortalities of 0% and 90% at those pH values. Similarly, after 96 hr of exposure to low pH, both middle and last instars exhibited mortalities of 0% and 10% at mean pH values of 4.6 and 4.4 respectively, whereas the early instar exhibited mortalities of 10% and 20% at those pH values. Nevertheless, all larval instars exhibited 100% mortality at a mean pH value of 2.4 after 96 hr of exposure, and no larval instar exhibited mortality at a mean pH value of 5.1 during the second replicate.

Median lethal concentrations (LC50s) and maximum acceptable toxicant concentrations (MATCs) for each larval instar are presented in Table 1. All $\chi^2$ values were lower than those for $P = 0.05$, indicating that data are not significantly heterogeneous. From a simple comparison of LC50s and MATCs (as pH values), we can see that early instar larvae (the smallest size class) seem to be more sensitive to water acidification because their LC50 and MATC values are highest. Furthermore, the 95% confidence limits of early instar larvae do not overlap significantly ($P > 0.05$) with the 95% confidence limits of last instar larvae, and therefore early instar larvae are significantly ($P < 0.05$; formula of factors) more sensitive to low pH than last instar larvae (the greatest size class). However, 95% confidence limits of the early and middle instars overlap significantly ($P < 0.05$) with 95% confidence limits of the middle and last instars, respectively.

Data from this laboratory study indicate that there exists an intraspecific size-dependent tolerance to water acidification in hydropsychid larvae of *Cheumatopsyche pettiti*, at least during short-term exposures, the tolerance increasing with body size. The fact that this differential sensitivity to low pH is significant ($P < 0.05$) only between early intar larvae and last instar larvae would be due to the methodology utilized. After all, the selection of size classes was arbitrary. Because mortality was checked every 24 hr, shorter differences in survivability between early and middle instars and between middle and last instars were not recorded.

The principal mode of toxic action of low pH on the physiology of freshwater invertebrates appears to be a failure of $Na^+$ regulation and, consequently, a disruption in sodium balance (Vangenechten et al. 1979; Brehm and Meijering 1982; Havas et al. 1984; Meinel et al. 1985; Twitchen 1987; Frisbie and Dunson 1988). Vangenechten et al. (1979), working with the waterbugs *Corixa dentipes* and *C. punctata*, have suggested that sodium uptake at the site of active transport (chloride cells) is accomplished by a carrier-mediated $Na^+/H^+$ or $Na^+/NH_4^+$ exchange mechanism, the affinity of the carrier being high for $Na^+$ at the external side of the membrane and high for $H^+$ at the cytosolic side. And Twitchen (1987) has shown that in stonefly nymphs the reduction of sodium uptake at low pH is due to competitive inhibition between $Na^+$ and $H^+$, the inhibition being greater at low sodium concentrations than at high sodium concentrations.

Chloride cells and chloride epithelia (as sodium-uptake sites) are common in many aquatic insect species (Komnick 1977). Therefore, we can conjecture that the differential sensitivity between early instar larvae and last instar larvae of *C. pettiti* to water acidification would be due fundamentally to a better developed osmoregulatory ability in last instars. It is probable that the affinity of the carrier for $Na^+$ ions at the external side of sodium-uptake cells improves with increasing body size (or maturity), and thereby decreasing competitive inhibition by $H^+$ at the expense of sodium cations.

On the other hand, the migration of *Cheumatopsyche* larvae exposed to low pH from their retreat and capture nets may be interpreted as a useful adaptation in running waters to escape from unfavorable environmental conditions, and the protrusion of their anal papillae may be interpreted as a physiological response to eliminate harmful ions (primarily $H^+$). It has been reported that anal papillae can act as important osmoregulatory organs in other aquatic insect species (Nuske and Wichard 1972; Komnick 1977). These two sublethal effects have been also observed in larvae of *C. pettiti* exposed to sodium nitrate (Camargo and Ward 1992) and sodium fluoride (Camargo et al. 1992).

The range of pH quality criteria for aquatic life has been established as 6.5-8.5 (US EPA 1986). This range seems to be sufficient for preventing acute and chronic mortalities (according to MATC values) in natural populations of hydropsychid larvae of *C. pettiti* exposed to acidified waters. Therefore, any investigation designed to evaluate water quality criteria for aquatic life should include testing of several life stages of aquatic insect species, with special emphasis on smaller size classes. In this respect, it is concluded that the use of the multifactor probit analysis software (US EPA 1991) to estimate MATCs can be a valuable tool for environmental toxicologists since this method does not require the performance of long-term toxicity bioassays, which is time consuming and expensive. Yet, this methodology should not be regarded as a perfect alternative to chronic toxicity testing.

*Acknowledgments.* I am particularly grateful to Professor JV Ward for his scientific assistance during this laboratory study, which was carried out at the Department of Biology (Colorado State University) while I was working as a Postdoctoral Associate. This manuscript was prepared at the Department of Environmental Toxicology (TIWET, Clemson University) while I was working as an Adjunct Professor and supported by a postdoctoral grant from the National Institute for Agrarian Research (INIA) of Spain.

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