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Effects of Body Size and Sodium Chloride on the Tolerance of Net-Spinning Caddisfly Larvae to Fluoride Toxicity

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Fluoride (F^-) levels in unpolluted fresh waters generally range from 0.01 to 0.3 mg/L whilst in unpolluted sea waters generally range from 1.2 to 1.5 mg/L (Camargo 2003). Fluoride ions can be removed from the aquatic phase by the precipitation of calcium carbonate, calcium phosphate, calcium fluoride and, even, magnesium fluoride (Stumm and Morgan 1996). Contemporary anthropogenic sources, such as aluminium smelters, fluoridated municipal waters, fluoride containing pesticides, and plants manufacturing brick, glass, ceramics and fluoride chemicals, are however leading to increased local F^- levels (up to 100 times the natural background level) in surface waters (Camargo 2003). In spite of the fact that fluoride must be considered as a serious pollutant since its concentration in many aquatic ecosystems is significantly increasing as a consequence of man's activities, relatively little is known about the effects of biotic and abiotic factors on the toxicity of fluoride ions to aquatic animals.

To know how biotic and abiotic factors can affect the toxicity of chemicals is an important question in order to determine appropriately water quality criteria for protecting aquatic life (US Environmental Protection Agency 1986). In the case of fishes, fluoride toxicity increases with increasing fluoride concentration, exposure time, and water temperature (Angelovic et al. 1961; Wright 1977; Smith et al. 1985; Camargo 1991). Conversely, fluoride toxicity to fishes decreases with increasing intraspecific body size and water content of calcium and chloride (Neuhold and Sigler 1960, 1962; Pimentel and Bulkley, 1983; Smith et al. 1985). Nevertheless, in the case of aquatic invertebrates, the effects of body size and water ionic content on the tolerance of these animals to fluoride ions have not been examined (Camargo 2003).

The main purpose of this study was to examine the effects of body size and sodium chloride on the toxicity of fluoride ions to aquatic invertebrates. The initial hypothesis is that these biotic and abiotic factors can increase the tolerance of freshwater invertebrates to fluoride ions. Selected invertebrates were net-spinning caddisfly larvae of *Hydropsyche tibialis* McLachlan, 1884 (Hydropsychidae, Trichoptera, Insecta). Larvae of *H. tibialis*, like other net-spinning caddisfly larvae, construct retreat and capture nets on the bottom of rivers and streams to strain food particles from the current. *H. tibialis* larvae were

chosen because previous laboratory studies with other Hydropsychidae species (Camargo and Tarazona 1990; Camargo et al. 1992) had shown that net-spinning caddisfly larvae were relatively sensitive to fluoride toxicity. In addition, the taxonomic identification of *H. tibialis* larvae was clear and easy, and their acclimatization to laboratory water conditions did not result in major problems.

MATERIALS AND METHODS

Middle (0.6–0.8 mm in head capsule width) and last (>1 mm in head capsule width) instar larvae of *H. tibialis* were hand-collected from a fluoride unpolluted (<0.10 mg F^-/L) area of the siliceous Riaza river (Segovia, Central Spain). In the laboratory, larvae were randomly distributed into test aquaria and acclimatized to water conditions for 4-5 d prior to fluoride toxicity bioassays. During acclimatization, larvae built their retreat and capture nets within the first 48 hr, being fed with fine particulate dried fish food. Water oxygenation and slight turbulence were produced with air pumps and airstones.

For each instar class, two separate static toxicity bioassays (with and without addition of sodium chloride) were conducted in duplicate for 5 d using small aquaria (glass vessels covered with plastic lids). Each aquarium contained 0.5 L of Riaza-river water and several PVC pieces to facilitate net-building by caddisfly larvae. A control (<0.10 mg F^-/L) and five nominal fluoride concentrations were used per replicate (5, 10, 20, 40 and 80 mg F^-/L for the first replicate, and 7.5, 15, 30, 60 and 120 mg F^-/L for the second replicate), with 10 larvae per concentration/aquarium. Fluoride concentrations were made from sodium fluoride (NaF, Panreac, Spain), and determined on the basis of previous F^- toxicity tests with other Hydropsychidae species (Camargo and Tarazona 1990; Camargo et al. 1992). These short-term toxicity tests had shown that, when soft waters are used, nominal fluoride concentrations agree well with measured fluoride concentrations. At the initiation of the second toxicity bioassays, 50 mg of sodium chloride (NaCl, Panreac, Spain) were added to each aquarium (nominal concentration of 100 mg NaCl/L).

Average values of water conditions during toxicity bioassays were: 17.8±1.1 °C for temperature, 9.2±0.4 mg O_2/L for dissolved oxygen, 7.1±0.2 for pH, and 19.5±2.8 mg $CaCO_3$/L for total hardness. There were no significant (P>0.05; t-test) differences between toxicity bioassays (within and between instar classes) for these physicochemical parameters. Chloride mean concentrations (mg Cl^-/L) were 5.2±1.8 and 5.9±2.2 in the first bioassays (without NaCl addition), and 66.7±4.0 and 65.8±3.4 in the second bioassays (with NaCl addition). All physicochemical parameters were analyzed according to standard methods described by American Public Health Association (1992). Because previous F^- toxicity tests with other Hydropsychidae species (Camargo and Tarazona 1990; Camargo et al. 1992) had shown that net-spinning caddisfly larvae may tolerate the lack of food for short-term periods, larvae of *H. tibialis* were not fed during toxicity bioassays. Dead larvae were removed every day. Death was defined as larvae not moving and not reacting to gentle prodding.

The 48, 72, 96 and 120 hr LC_{50} values, and their respective 95% confidence limits, were calculated by the traditional method of Litchfield and Wilcoxon (1949), using mortalities and nominal fluoride concentrations obtained in duplicate for each instar class. According to this method, LC_{50} values are significantly ($P<0.05$) different, for a same exposure time, if their 95% confidence limits do not overlap (Litchfield and Wilcoxon 1949). In the case of overlapping, the formula of factors (Litchfield and Wilcoxon 1949; American Public Health Association 1992) was applied in order to determine significant ($P<0.05$) differences between LC_{50} values. No observed adverse effect levels (120 hr NOAELs) were also recorded on the basis of sublethal effects.

RESULTS AND DISCUSSION

In all toxicity bioassays, mortality of larvae increased with increasing fluoride concentrations and exposure times. In general, larvae of *H. tibialis* migrated from their retreat and capture nets and protruded their anal papillae before dying. Moreover, most of the protruded anal papillae were darkened in larvae exposed to the highest fluoride concentrations, mainly in the first bioassays (low NaCl condition). These sublethal effects have been observed in net-spinning caddisfly larvae of other Hydropsychidae species exposed to sodium fluoride (Camargo and Tarazona 1990; Camargo et al. 1992). The net migration of larvae is interpreted as a useful adaptation in running waters to escape from unfavorable environmental conditions, and the protrusion of anal papillae as a physiological response for improving and increasing the elimination of harmful ions (Camargo et al. 1992). No dead animal was found in control aquaria, and no sublethal effect was observed in control larvae.

The 48, 72, 96 and 120 hr LC_{50} values, with their respective 95% confidence limits, are presented in Tables 1 and 2. For both middle and last instar larvae, 48, 72, 96 and 120 hr LC_{50} values in the second bioassay (high NaCl condition) were much higher than 48, 72, 96 and 120 hr LC_{50} values in the first bioassay (low NaCl condition). Indeed, 95% confidence limits of LC_{50} values in the second bioassay do not overlap with 95% confidence limits of LC_{50} values in the first bioassay. An exception is the overlap between 95% confidence limits of 48 hr LC_{50} values for last instar larvae (Table 2). However, 48 hr LC_{50} values were significantly ($P<0.05$; formula of factors) different. In addition, NOAEL values in the second bioassays were higher than NOAEL values in the first bioassays (Tables 1 and 2). On the other hand, there was a differential response to fluoride toxicity between middle and last instar larvae. In the first and second bioassays, 48, 72, 96 and 120 hr LC_{50} values for last instar larvae (Table 2) were higher than 48, 72, 96 and 120 hr LC_{50} values for middle instar larvae (Table 1). Although 95% confidence limits of LC_{50} values for last instar larvae overlap with 95% confidence limits of LC_{50} values for middle instar larvae, differences between LC_{50} values were significant ($P<0.05$; formula of factors) in the first toxicity bioassay (low NaCl condition). In this first bioassay, the NOAEL value for last instar larvae was higher than the NOAEL value for middle instar larvae (Tables 1 and 2). In contrast, in the second toxicity bioassay (high NaCl condition), LC_{50}

Table 1. LC_{50} and NOAEL values (mg F^-/L) estimated for middle (0.6–0.8 mm in head capsule width) instar larvae of *H. tibialis* after finishing the first (low NaCl condition) and second (high NaCl condition) short-term toxicity bioassays.

Toxicological parameter	Low NaCl condition (5.2±1.8 mg Cl^-/L)	High NaCl condition (66.7±4.0 mg Cl^-/L)
48 hr LC_{50}	54.2 (42.1 – 69.4)	110.5 (82.4 – 148.1)
72 hr LC_{50}	38.5 (31.0 – 47.7)	89.2 (67.6 – 117.8)
96 hr LC_{50}	30.6 (25.3 – 37.0)	77.3 (59.5 – 100.5)
120 hr LC_{50}	27.5 (23.1 – 32.7)	71.8 (55.7 – 92.6)
120 hr NOAEL	5.0	15.0

95% confidence limits are presented in parenthesis

Table 2. LC_{50} and NOAEL values (mg F^-/L) estimated for last (>1 mm in head capsule width) instar larvae of *H. tibialis* after finishing the first (low NaCl condition) and second (high NaCl condition) short-term toxicity bioassays.

Toxicological parameter	Low NaCl condition (5.9±2.2 mg Cl^-/L)	High NaCl condition (65.8±3.4 mg Cl^-/L)
48 hr LC_{50}	80.5 (61.9 – 104.7)	131.0 (96.3 – 178.2)
72 hr LC_{50}	57.0 (45.6 – 71.3)	102.5 (77.1 – 136.4)
96 hr LC_{50}	43.8 (35.9 – 53.4)	84.7 (62.7 – 114.3)
120 hr LC_{50}	36.7 (30.5 – 44.0)	76.0 (58.0 – 99.6)
120 hr NOAEL	10.0	15.0

95% confidence limits are presented in parenthesis

values for last instar larvae were not significantly (P>0.05; formula of factors) different from LC_{50} values for middle instar larvae. Furthermore, in this second bioassay, the NOAEL value for last instar larvae was equal to the NOAEL value for middle instar larvae (Tables 1 and 2).

It is evident that sodium chloride increases the tolerance of *H. tibialis* larvae to fluoride toxicity, at least during short-term exposures, since 48, 72, 96 and 120 hr LC_{50} values (for both middle and last instar larvae) are significantly lower in the first bioassays (with mean chloride concentrations of 5.2 and 5.9 mg Cl^-/L) than in the second bioassays (with mean chloride concentrations of 66.7 and 65.8 mg Cl^-/L). This ameliorating effect of sodium chloride might be due to a constraint in the incorporation of fluoride ions into the cytosolic side of cell membrane because of the presence of chloride ions at the external side of cell membrane. If we assume that fluoride ions can enter ion-uptake cells in chloride epithelia by active transport, which is accomplished by carriers that have affinity for chloride ions, then an elevated Cl^- level in the aquatic environment would induce an increased competition between Cl^- and F^- ions at the external side and, consequently, a decreased fluoride influx at the cytosolic side. Chloride cells and chloride epithelia, as ion-uptake sites, are common in many aquatic insect species (Komnick 1977). A similar physiological mechanism, based on competitive inhibition, was proposed (Vangenechten et al. 1979) and, subsequently, demonstrated (Twitchen 1987) for the effect of Na^+ ions on the resistance of aquatic insects (waterbugs and stoneflies) to water acidification (i.e., increased H^+ concentration). In fish, Neuhold and Sigler (1962) reported that the toxicity of fluoride ions to rainbow trout (*Oncorhynchus mykiss*), exposed to a maximum fluoride concentration of 25 mg F/L, decreased with increasing water content of chloride (range 0-9 mg Cl^-/L). They concluded that a moderate increase in the chloride concentration might elicit a response in trout for fluoride excretion.

It also is evident that body size increases the tolerance of *H. tibialis* larvae to fluoride toxicity, at least during short-term exposures, since 48, 72, 96 and 120 hr LC_{50} values for last instar larvae are significantly higher than those for middle instar larvae in the first bioassay (low NaCl condition). This ameliorating effect of body size might be due to a better developed osmoregulatory ability in last instar larvae and, thereby, a better ability to eliminate harmful ions. Studies on ion regulatory organs in aquatic insects indicate that their osmoregulatory ability may improve with progressing the developmental stage of animals (Komnick 1977). The fact that the difference in sensitivity to fluoride toxicity between middle and last instar larvae was more evident (significant) in the first bioassays than in the second bioassays would be a consequence of the ameliorating effect caused by the much higher concentration of sodium chloride in the second bioassays. In fish, Neuhold and Sigler (1960) reported higher tolerance to fluoride toxicity in larger individuals of rainbow trout (*O. mykiss*) and common carp (*Cyprinus carpio*).

Comparing with other freshwater animals, larvae of *H. tibialis* appear to be more sensitive to fluoride toxicity than fishes and cladocerans, at least during short-term exposures. The lowest 96 hr LC_{50} values calculated for fish species are 51 mg F/L for the rainbow trout *O. mykiss* (Pimentel and Bulkley 1983), 164.5 mg F/L for the brown trout *Salmo trutta* (Camargo 1991), 315 mg F/L for the fathead minnow *Pimephales promelas* (Smith et al. 1985), and 340 mg F/L for the threespine stickleback *Gasterosteus aculeatus* (Smith et al. 1985). For the water flea *Daphnia magna*, 48 hr LC_{50} values range from 98 to 304 mg F/L

(Dave 1984; Fieser et al. 1986), depending greatly upon water temperature. With regard to other net-spinning caddisfly species, larvae of *H. tibialis* seem to exhibit similar sensitivity to fluoride toxicity. 96 hr LC_{50} values calculated for other Hydropsychidae species are 17.0 mg F^-/L for *H. bronta* (Camargo et al. 1992), 26.3 mg F^-/L for *H. bulbifera* (Camargo and Tarazona 1990), 26.5 mg F^-/L for *H. exocellata* (Camargo and Tarazona 1990), 34.7 mg F^-/L for *H. occidentalis* (Camargo et al. 1992), 38.5 mg F^-/L for *H. pellucidula* (Camargo and Tarazona 1990), 42.5 mg F^-/L for *Cheumatopsyche pettiti* (Camargo et al. 1992), and 48.2 mg F^-/L for *H. lobata* (Camargo and Tarazona 1990).

This study has clearly shown that, like in fishes (Neuhold and Sigler 1960, 1962), intraspecific body size and water content of sodium chloride may increase the tolerance of freshwater invertebrates (net-spinning caddisfly larvae) to fluoride ions. Overall it is concluded that any future investigation, designed to determine water quality criteria for protecting freshwater animals from fluoride pollution, must include toxicity testing with individuals of smaller size inhabiting soft waters with low ionic content (low Ca^{2+} and Cl^- concentrations, primarily). Furthermore, because net-spinning caddisfly larvae appear to be more sensitive to fluoride toxicity than other freshwater animals, these aquatic invertebrates should be used as test organisms in future laboratory studies.

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