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Contrasting Sensitivities to Fluoride Toxicity between Juveniles and Adults of the Aquatic Snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)

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Abstract In contrast to aquatic vertebrates, there is scarce available information on the contrasting tolerance to fluoride of different life stages and/or sizes of aquatic invertebrates. The purpose of this study was to assess the likely differences in sensitivity between juveniles and adults of the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) to short-term (4 days) toxicity of fluoride ion (F^-). LC50 and EC50 values for juveniles were significantly lower than those for adults at 24, 48, 72 and 96 h. Based on our results, the use of fluoride data of bioassays with juveniles should provide more protective water quality criteria than data from adult stage.

Keywords Fluoride · Short-term toxicity · *Potamopyrgus antipodarum* · Life stages · Aquatic invertebrates

Fluoride ion is a common pollutant in many aquatic ecosystems (Camargo 2003). The main anthropogenic sources of fluoride to the aquatic environment include municipal waste waters, effluents from fertilizer producing plants, and aluminium refineries (Camargo 2003; Guiguère and Campbell 2004). Aquatic invertebrates living in soft waters may be more adversely affected by fluoride pollution than those living in hard or seawaters since the bioavailability of fluoride ions is reduced with the increasing of water hardness (Camargo 2003).

Natural populations of aquatic organisms are usually made up of different life stages with contrasting sensitivities to toxicants. The ecotoxicological assessment of the toxicant tolerance of different life stages can be useful to determine the sensitivity profile of species, and for an appropriate evaluation of the population-level effects (Yokohama et al. 2009). Generally, in aquatic invertebrates, larvae, juvenile stages, and/or small individuals are reported to be more sensitive to toxicants than adult stages (McCahon and Pascoe 1988; Forget et al. 1998; Coeurdassier et al. 2004; Yokohama et al. 2009). However, in the case of fluoride ion there is no previous published information focused on the assessment of its toxicity to different life stages of aquatic snails.

Potamopyrgus antipodarum Gray (= *P. jenkinsi* Smith) (Hydrobiidae, Mollusca) is an aquatic snail native to New Zeeland, but it is established in Australia, Europe, North America and Asia as an exotic/invasive species (Alonso and Castro-Díez 2008). This species is common in aquatic ecosystems, from fast rivers to trickles, and also in brackish water, being the most common aquatic gastropod in Britain and other regions of Europe (Alonso and Castro-Díez 2008). During the last years this species has been widely used for toxicity bioassays (Alonso and Camargo 2004, 2009; Duft et al. 2007).

If the toxicological information on a given toxicant is scarce, the safe levels of that toxicant must be based on the toxicological parameters of the most sensitive species and life stages. Therefore, this study was performed to asses the short-term toxicity of fluoride ions to the aquatic snail *P. antipodarum* at two contrasting life stages (juvenile and adult). We hypothesized that juveniles would be less tolerant to fluoride toxicity than adults.

Materials and Methods

Snails were obtained from our laboratory culture (at 20–22°C, in 60 L glass aquarium) at the Department of

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Ecology (University of Alcalá, Madrid, Spain). This culture was started at the beginning of 2009 with a natural population that was collected in an unpolluted reach in the upper watershed of Henares River (Guadalajara, Central Spain). The culture was established in US-EPA moderately hard water (96 mg $NaHCO_3$, 60 mg $CaSO_4*2H_2O$, 4 mg KCl, 122.2 mg $MgSO_4*7H_2O$ per one liter of deionized water) (US Environmental Protection Agency 2002). This water was enriched with calcium carbonate (10 mg $CaCO_3$ per one liter of deionized water). Animals for the bioassay were selected by size; adults (3.8 ± 0.2 mm) and juveniles (1.2 ± 0.3 mm). This snail reaches sexual maturity at 3 mm of shell length (Richards 2002, Alonso and Castro-Díez 2008). Adults were manipulated with forceps and juveniles with plastic Pasteur pipettes. Both life stages were acclimatized together (glass aquaria 1 L) in a climatic chamber to the experimental temperature (15°C) during a week prior to the bioassay. Snails were fed every 2 days with fish food (Tetra© GmbH, Germany), and after each feeding water was renewed to avoid the decomposition of non-used food.

A 96 h short-term bioassay was conducted in triplicate. Eight adults and eight juveniles were randomly selected for each replicate (glass vessel 150 mL). With this experimental set-up we ensured that both stages were exposed to the same actual fluoride concentration for each treatment. Vessels were covered with a perforated plastic Petri dish to reduce evaporation. Both life stages were exposed to one control and seven nominal fluoride concentrations (25, 50, 75, 100, 150, 200 and 300 mg F^-/L) in triplicate. These concentrations were selected based on previous trial bioassays. Nominal fluoride concentrations were prepared by dissolving weighted amounts of sodium fluoride (NaF) in known volumes of test water (US-EPA moderately hard water enriched with 10 mg $CaCO_3$ mg/L) (US Environmental Protection Agency 2002). Actual total fluoride concentrations before and after water renewal was measured by spectrophotometric method (Hanna®). Control and fluoride solutions were renewed at 48 h and animals were not fed during the bioassay. Actual hardness (as mg $CaCO_3$/L) and chloride concentrations (Cl^-) of the test water were measured using the EDTA titrimetric method and the argentometric method, respectively (APHA 1995). Actual chloride concentration was less than 5 mg Cl^-/L, and actual mean (n = 8) (± SD) total hardness (as $CaCO_3$) was 92.5 ± 5.4 mg $CaCO_3$/L. The mean (n = 7) (± SD) conductivity of test water was 408 ± 4 µS/cm. Water temperature and dissolved oxygen were monitored daily. Total ammonia and pH were measured at the beginning of the bioassay and every 48 h (before and after solution renewal). Dead animals were removed in each monitoring (24 h). After finishing the bioassay, the shell length of all snails was measured using a micrometer installed in a stereoscopic microscope.

LC50 (based on mortality) and EC50 (based on mortality plus immobility) values at 24, 48, 72 and 96 h after exposure were calculated using the Multifactor Probit Analysis (MPA) software (US Environmental Protection Agency 1991). This methodology solves the concentration–time-response equation via the iterative reweighted least square technique, LC and EC values being calculated by a multiple linear regression. The dependent variable is the probit of the proportion of animals responding to each concentration, and the independent variables are exposure time and toxicant concentration (Alonso and Camargo 2006). Actual mean fluoride concentrations were used to calculate LC and EC values. Significant differences ($p < 0.05$) between life stages for each LC or EC value and exposure time were accepted when 95% confidence limits of LC and EC values did not overlap.

Results and Discussion

Mean physico-chemical properties during the bioassay were: 8.07 ± 0.16 for pH, 15.6 ± 0.5°C for water temperature, 9.6 ± 0.4 mg O_2/L for dissolved oxygen and 0.19 ± 0.09 mg N-NH_4/L for total ammonia. The actual fluoride concentrations in the control were less than 0.01 mg F^-/L. Mean actual (n = 5) (± SD) fluoride concentrations (25, 50, 75, 100, 150, 200 and 300 mg F^-/L) were 17.4 ± 2.9 (25), 40.4 ± 3.6 (50), 53.9 ± 3.4 (75), 74.8 ± 4.8 (100), 103.7 ± 8.0 (150), 139 ± 27.4 (200) and 222.9 ± 18.1 (300) mg F^-/L.

Throughout the bioassay, snail mortality increased with increasing toxicant concentrations and exposure times, as same as immobility, to both adults and juveniles. No mortality or immobility was found in control vessels at any time of the bioassay.

LC50 and EC50 values for each life stages and exposure time are shown in Fig. 1. The lethal concentration (LC50) for each life stage decreased with increasing exposure time. The same result was obtained for EC50 (mortality plus immobility). Significant differences for LC50 and EC50 between adults and juveniles were observed for all exposure periods (24, 48, 72 and 96 h) ($p < 0.05$; overlap test). This difference between LC and EC values was higher at 24 h than in longer exposure periods, but always kept significant. Neither adults nor juveniles died at the lowest nominal concentration (25 mgF^-/L) but it caused immobility in juveniles. In general, mortality plus immobility (EC50) showed lower values than for mortality (LC50) (Fig. 1).

Several studies show that juvenile stages and/or small individuals are more sensitive to toxicants than adult stages of aquatic invertebrates. The juvenile stage of the freshwater snail *Biomphalaria tenagophila* was less tolerant to

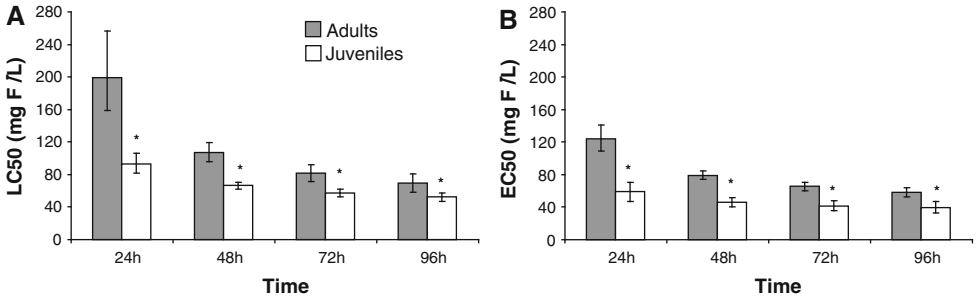

Fig. 1 LC50 (**a**) and EC50 (**b**) values of fluoride ion (mg F^-/L) for adults and juveniles of *Potamopyrgus antipodarum*. *Error bars* represent 95% confidence interval. *Asterisks* indicate significant differences between adults and juveniles for each exposure time (Overlap test; $p < 0.05$)

the insecticide endosulfan, the surfactant nonylphenol ethoxylates and ethanol than adult stage (Oliveira-Filho et al. 2005). In the freshwater pulmonate *Lymnaea stagnalis*, sensitivity to cadmium decreased in adults (Coeurdassier et al. 2004). Adults of freshwater amphipods were more tolerant to cadmium than juveniles (McCahon and Pascoe 1988; McGee et al. 1998; Alonso et al. 2010). Juveniles of the seawater copepod *Tigriopus* brevicornis were more sensitive to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos and malathion, than adults (Forget et al. 1998). In the case of aquatic insects, last instar larve of the caddisfly *Hydropsyche tibialis* exhibited a higher tolerance to fluoride toxicity than middle instar larvae (Camargo 2004).

Juvenile snails in this study were more sensitive to fluoride (LC50(96 h) = 52.2 mgF^-/L, EC50(96 h) = 39.2 mgF^-/L) than adults (LC50(96 h) = 69.9 mgF^-/L, EC50(96 h) = 58.2 mgF^-/L), for each exposure time. Explanations for this finding could include increased surface area to volume ratio, thinner body covering, higher metabolic rates and undeveloped detoxification mechanisms in young animals (McGee et al. 1998). Youngest individuals are usually more sensitive due to a higher metabolism associated with fast growth and development (DeLorenzo et al. 2006). Rac et al. (2007) found in the gastropod *Helix aspersa* that rising concentrations of fluoride lead to increase levels of adenosine monophosphate (AMP), to inhibit oxidative phosphorylation, and depress metabolic function. Several studies demonstrate that juveniles can accumulate more toxicants (cadmium, lead, zinc) quantity than adults after 96 h of exposure (Reineke et al. 2003; Pastorinho et al. 2009). The ameliorating effect of intraspecific age or/and body size might be also due to a better developed osmoregulatory ability in older organisms and, hence a better ability to eliminate fluoride ions from the body (Camargo 2003, 2004). However, available information on the physiological responses of aquatic animals to fluoride is scarce, and therefore further research in this area is recommended.

Evaluation of both lethal and sublethal endpoints is necessary for the determination of no-effect concentrations and to define a safe level for the future use or release of toxicants (Coeurdassier et al. 2004), as the lower the toxicological values included in the species sensitivity distribution, the lower the estimated safe levels to the community are (Alonso et al. 2010). In order to set limits for toxicants in ecological risk assessment it is necessary to take into account the most sensitive life stages (McCahon and Pascoe 1988).

We conclude that juvenile individuals of the aquatic snail *P. antipodarum* are more sensitive to fluoride ion toxicity than adult ones. We recommend that the most sensitive life stage for each species be used in bioassays and in the ecological risk assessment procedure. In addition, further studies are necessary to compare sublethal effects of toxicants between different life stages of aquatic snails. Differences in sensitivity between life stages may be related to the mode of action of toxicants but, due to the scarce information, it would be necessary to conduct studies on the physiological responses of aquatic animals to fluoride ion.

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