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Short-Term Toxicity of Ammonia, Nitrite, and Nitrate to the Aquatic Snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)

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Nitrogen compounds, such as ammonia, nitrite and nitrate, are present naturally in freshwater ecosystems as biological degradation products of organic matter. These nitrogen compounds are interdependent through the nitrogen cycle. Ammonia may be present as two different chemical species, NH_3 (unionized) and NH_4^+ (ionized), whose equilibrium is affected both by water temperature and by pH (Emerson et al. 1975). Ammonia is normally oxidized by aerobic chemoautotrophic bacteria in a two-step process (Stumm and Morgan 1996), firstly to nitrite (by *Nitrosomonas*) and subsequently to nitrate (by *Nitrobacter*). Unfortunately, major anthropogenic sources, such as animal farming, agricultural runoff, industrial wastes and sewage effluents, are introducing huge amounts of organic matter and nitrogen compounds into surface waters. As a result, the concentrations of ammonia, nitrite and nitrate in aquatic ecosystems have significantly increased over background natural levels, causing a world-wide environmental problem (Scott and Crunkilton 2000).

At present, it is accepted that ammonia is more toxic than nitrite and nitrate to aquatic animals (Russo 1985; Scott and Crunkilton 2000), though the toxicity of nitrite and nitrate have been comparatively less studied than that of ammonia. The toxicity of ammonia is related to the unionized chemical species (Alabaster and Lloyd 1982; Russo 1985; Williams et al. 1986). In fish, NH_3 causes an increase in gill ventilation, hyperexcitability, convulsions and, finally, death (Alabaster and Lloyd 1982; Russo 1985). Nitrite turns hemoglobin to methemoglobin, which is unable to carry oxygen to cells, causing anoxia and death. Toxicity of nitrate may be related to *in vivo* reduction of nitrate to nitrite and a later conversion of hemoglobin to methemoglobin (Scott and Crunkilton 2000). As the sensitivity of aquatic organisms to these toxics usually differs among taxa, specific toxicity testing is necessary to know the relative tolerance of a particular taxon to the toxicity of them.

Potamopyrgus antipodarum Gray (=*P. jenkinsi* Smith) (Hydrobiidae, Mollusca) is an aquatic snail native of New Zealand, but since long established in Australia and Europe and North America as an exotic/invasive species. It is abundant in freshwater ecosystems, from fast rivers to trickles, and also in brackish water, being the most common freshwater gastropod in Britain and other regions of

Europe (Wallace 1985). Furthermore, it has been found in waters with organic pollution (Mouthon and Charvet 1999). The ecological success of *P. antipodarum* would be due to its fast parthenogenetic reproduction (Wallace 1985) and to its probable tolerance to organic pollution and nutrient enrichment. The aim of this study was to examine the tolerance of *P. antipodarum* to the toxicity of nitrogen compounds (NH_3-N, NO_2-N and NO_3-N). Our hypothesis is that this aquatic snail will have a higher tolerance to these nitrogen compounds than other aquatic invertebrates reported in bibliography.

MATERIALS AND METHODS

Snails were collected with a hand-net (250 μm) from a relatively unpolluted upper reach of the Henares river (Guadalajara, Central Spain). Physico-chemical characteristics of river water are presented in Table 1. In the laboratory, snails were randomly distributed into small aquaria (glass vessels) and acclimatized to test water (bottled drinking water without chlorine) conditions (Table 1) for 7 d prior to the start of bioassays. During acclimatization, animals were fed with aquatic plants, which were collected in the same reach of the Henares river.

Three independent static short-term bioassays (4 d), with daily water renovation, were conducted in triplicate using glass vessels, each containing 0.1 L of test water and 10 snails. Vessels were covered with a plastic foil with perforations to prevent water evaporation. No aeration was supplied during toxicity tests in order to avoid significant reductions of ammonia and nitrite concentrations by oxidation to nitrate. The first bioassay consisted in a control and five nominal concentrations of unionized ammonia (0.47, 0.95, 1.90, 3.80, 7.60 ppm NH_3-N). The control had less than 0.01 ppm NH_3-N (Table 1). Nominal concentrations were made with ammonium chloride (NH_4Cl) (PANREAC, Spain, Lot No. 149959380) with a reported purity of 99.5%. Values of water temperature and pH were used to estimate the percentage of unionized ammonia (Emerson et al. 1975). The second bioassay consisted in a control and five nominal concentrations of nitrite (80, 160, 320, 640, 1280 ppm NO_2-N). The control had less than 0.005 ppm NO_2-N (Table 1). Nominal concentrations were made with sodium nitrite ($NaNO_2$) (SIGMA, Germany, Lot No. 70K0999) with a reported purity of 99.5%. The third bioassay consisted in a control and five nominal concentrations of nitrate (640, 840, 1040, 1280, 1480 ppm NO_3-N). The control had less than 1.25 ppm NO_3-N (Table 1). Nominal concentrations were made with sodium nitrate ($NaNO_3$) (SIGMA, Germany, Lot No. 97H1563) with a reported purity of 99.0%. Nominal concentrations were the initial concentrations resulting from dissolving weighted amounts of nitrogen salts in known volumes of test (control) water. Snails were not fed during toxicity tests to prevent potential changes in the concentrations of nitrogen compounds. Dead animals were removed every day. After finishing the bioassays, the shell length of all snails was measured with an ocular micrometer.

The 24, 48, 72 and 96 hr LC_{50}, LC_{10} and $LC_{0.01}$ values, and their respective 95% confidence limits, were calculated using the multifactor probit analysis (MPA)

software (US Environmental Protection Agency 1991). Because previous checking, following standard methods described in American Public Health Association (1992), showed that variation coefficients of nominal concentrations after 1 d were relatively low (13.9% for ammonia, 7.1% for nitrite, and 6.6% for nitrate), we used these concentrations for calculating LC values. The MPA methodology solves the concentration-time-response equation simultaneously via the iterative reweighed least squares technique (multiple linear regression). The dependent variable is the probit of the proportion responding to each concentration, and the independent variables are exposure time and toxicant concentration (nominal concentrations of nitrogen compounds). After evaluating several MPA models regarding the heterogeneity factor (chi-squared variable divided by degrees of freedom), a parallel-regression-line model was selected as the best one. In this study, 96 hr $LC_{0.01}$ values may be considered as short-term safe concentrations of ammonia, nitrite and nitrate for *P. antipodarum*, since these calculated values refer to the concentrations of nitrogen compounds affecting to 0.01% individuals of the test population. However, these calculated short-term safe concentrations (96 hr $LC_{0.01}$) should not be related to the toxicological concept of NOEL, since this concept refers to the no observed effect level. Significant ($P<0.05$) differences in toxicity between nitrogen compounds were accepted when 95% confidence limits of LC values, for the same exposure time, did not overlap (US Environmental Protection Agency 1991). Differences in mean length of shell between control and treatments for each bioassay were examined by an analysis of variance (ANOVA-Dunnett test).

RESULTS AND DISCUSSION

In all bioassays snail mortality increased with increasing toxicant concentrations and exposure times. No mortality was found in control vessels after finishing each bioassay. Mean values of snail shell length were 3.6±0.6, 3.8±0.5 and 3.2±0.6 mm for NH_3-N, NO_2-N and NO_3-N bioassays, respectively. No significant difference in mean shell length was found between control and treatments in each bioassay ($P>0.05$; Dunnett test). LC values, and their 95% confidence limits, for NH_3-N, NO_2-N and NO_3-N bioassays are presented in Table 2. The comparison of LC values showed that ammonia (as NH_3-N) was significantly more toxic to *P. antipodarum* than nitrite and nitrate ($P<0.05$; 95% confidence limits did not overlap). Similarly, this comparison showed that nitrite was significantly more toxic than nitrate ($P<0.05$; 95% confidence limits did not overlap except for an exposure of 24 hours).

When compared with bibliography, *P. antipodarum* generally showed higher tolerance to the toxicity of NH_3-N, NO_2-N and NO_3-N than other aquatic invertebrates (see Figure 1). In the case of NH_3-N, only the tubificid worm *Tubifex tubifex* had a higher 96 hr LC_{50} value (2.70 ppm; Stammer 1953). Other tested aquatic invertebrates exhibited lower 96 hr LC_{50} values: the worms *Limnodrilus hoffmeisteri* (1.58 ppm; Williams et al. 1986) and *Lumbriculus variegatus* (0.29 ppm; Besser et al. 1998); the flatworm *Dendrocoelum lactum* (1.40 ppm; Stammer 1953); the midges *Chironomus riparius* (1.36 ppm; Williams

Table 1. Physicochemical characteristics of both Henares river water and toxicity (control) tests water.

	Toxicity tests	Henares river
Conductivity (μS)	784±7	987±275
pH	8.3±0.1	8.1±0.1
Calcium (ppm)	90.8±0.9	120±24
Dissolved oxygen (ppm)	6.7±0.4	12.7±1.2
Water temperature (°C)	20.4±0.9	8.0±4.2
NO_2-N (ppm)	<0.005	<0.005
NO_3-N (ppm)	1.15±0.08	1.60±0.7
NH_3-N (ppm)	<0.01	<0.01

Mean values ± standard deviations are presented for each parameter. Water analyses were performed following standard methods described by American Public Health Association (1992).

Table 2. 24, 48, 72 and 96 hr $LC_{0.01}$, LC_{10} and LC_{50} values for each bioassay on *Potamopyrgus antipodarum*.

	24	48	72	96
$LC_{0.01}$ (ppm NH_3-N)	0.20	0.17	0.16	0.16
	(0.06-0.4)	(0.05-0.33)	(0.04-0.31)	(0.04-0.30)
LC_{10} (ppm NH_3-N)	1.12	0.93	0.86	0.83
	(0.7-1.47)	(0.55-1.25)	(0.51-1.18)	(0.48-1.15)
LC_{50} (ppm NH_3-N)	2.72	2.23	2.09	2.02
	(2.28-3.14)	(1.78-2.66)	(1.63-2.52)	(1.56-2.45)
$LC_{0.01}$ (ppm NO_2-N)	132	52.4	38.5	33
	(41.6-256)	(16.1-98.5)	(11.3-74.1)	(9.5-64.6)
LC_{10} (ppm NO_2-N)	817	325	239	205
	(482-1250)	(218-412)	(154-309)	(127-272)
LC_{50} (ppm NO_2-N)	2134	848	624	535
	(1396-3598)	(717-1042)	(522-759)	(433-666)
$LC_{0.01}$ (ppm NO_3-N)	376	243	210	195
	(259-482)	(159-319)	(134-280)	(123-263)
LC_{10} (ppm NO_3-N)	1128	728	629	585
	(980-1282)	(642-794)	(540-697)	(494-656)
LC_{50} (ppm NO_3-N)	2009	1297	1121	1042
	(1736-2438)	(1218-1407)	(1052-1205)	(969-1126)

95% confidence limits are presented in parentheses.

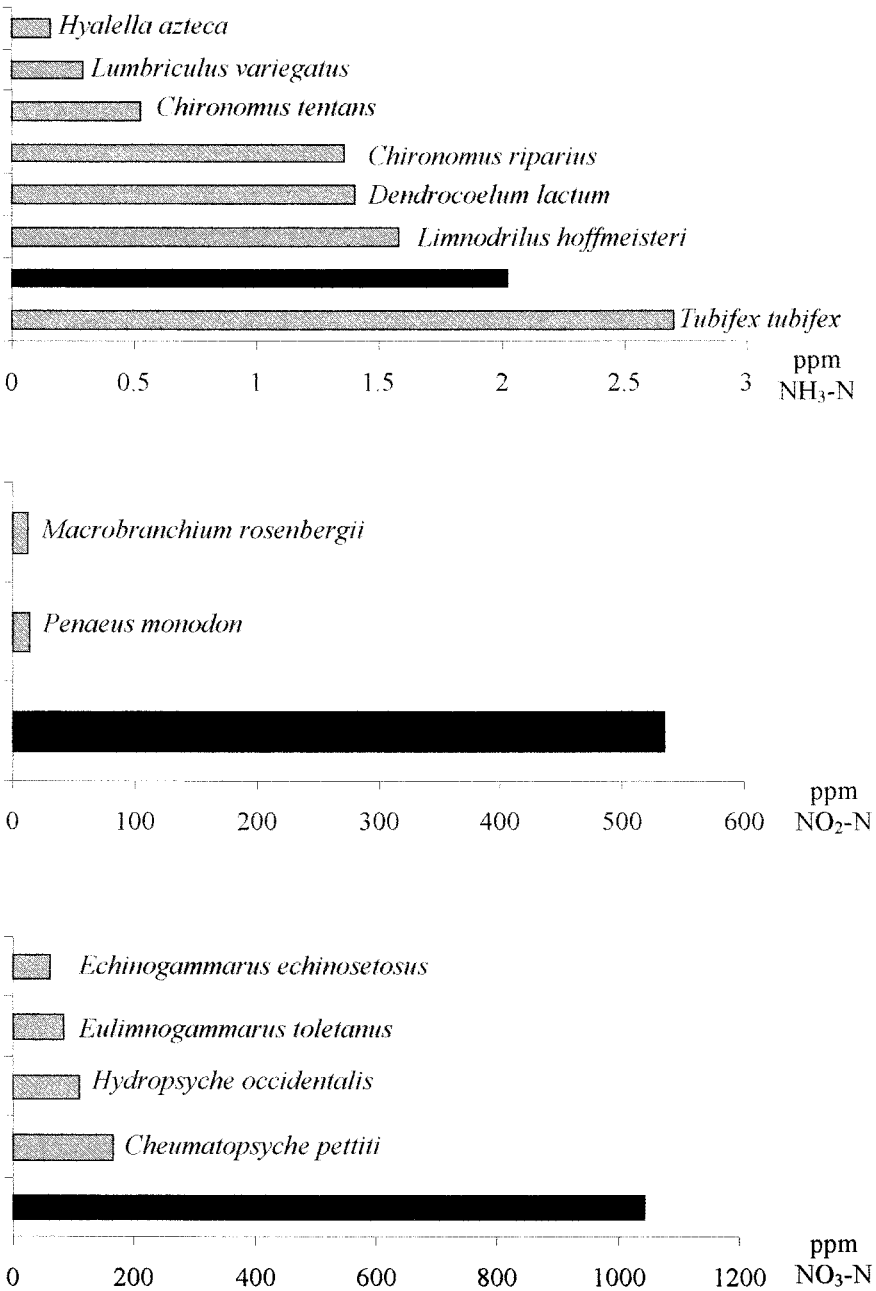

Figure 1. Comparison of 96hr LC_{50} values of unionized ammonia, nitrite and nitrate for *Potamopyrgus antipodarum* (in black) and other aquatic invertebrates, according to data published by Stammer (1953), Williams et al. (1986), Chen and Chin (1988), Camargo and Ward (1992), Chen and Lee (1997), Besser et al. (1998), and Alonso and Camargo (2001).

et al. 1986) and *Chironomus tentans* (0.53 ppm; Besser et al. 1998); the crustacean *Hyalella azteca* (0.16 ppm; Besser et al. 1998). It is interesting to note that Hickey and Vickers (1994), testing a New Zealand native population of *P. antipodarum*, estimated a 96 hr LC_{50} value of 0.31 (0.26-0.37) ppm NH_3-N. This result is much lower than the 96 hr LC_{50} value estimated in this study for a Spanish exotic population of *P. antipodarum* (Table 2). The likely causes of this significant difference between 96 hr LC_{50} values would be the mean size of snails (2.5 mm against 3.6 mm) and the distinct origin of populations. On the other hand, the 24 hr LC_{50} value of *P. antipodarum* (2.72 ppm NH_3-N; Table 2) was lower than the 24 hr LC_{50} values for other aquatic invertebrates: 7.79 ppm for the crustacean *Asellus aquaticus* (Maltby 1995), 6.76 ppm for the ephemeropteran *Baetis rhodani* (Khatami et al. 1998), and 3.56 ppm for the crustacean *Gammarus pulex* (Maltby 1995).

In the case of NO_2-N, *P. antipodarum* exhibited a higher tolerance to nitrite toxicity than other aquatic invertebrates (96 hr LC_{50} of 535 ppm; Table 2). The 96 hr LC_{50} values for the giant freshwater prawn *Macrobranchium rosenbergii* and for the tiger prawn *Penaeus monodon* were 12.87 ppm and 13.55 ppm, respectively. (Chen and Chin 1988; Chen and Lee 1997). Regarding NO_3-N, *P. antipodarum* exhibited a lower sensitivity to nitrate toxicity than other aquatic invertebrates reported in bibliography (48 and 96 hr LC_{50} values of 1297 and 1042 ppm, respectively; Table 2). Calculated 48 hr LC_{50} values were 374 and 462 ppm for the cladocerans *Ceriodaphnia dubia* and *Daphnia magna*, respectively (Scott and Crunkilton 2000). The 96 hr LC_{50} values reported for the crustaceans *Echinogammarus echinosetosus* and *Eulimnogammarus toletanus* were 62.5 and 85.0 ppm, respectively (Alonso and Camargo 2001), for the trichopterans *Hydropsyche occidentalis* and *Cheumatopsyche pettiti* were 109.0 and 165.5 ppm, respectively (Camargo and Ward 1992).

Overall, it is concluded that *P. antipodarum* is one of the most tolerant aquatic invertebrates to the short-term toxicity of NH_3-N, NO_2-N and NO_3-N. This relatively high tolerance to nitrogen compounds could partly explain its ecological success as an exotic/invasive species in many polluted aquatic ecosystems around the world.

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