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Chemosphere 58 (2005) 1255–1267

CHEMOSPHERE

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Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates

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Received 9 December 2003; received in revised form 19 October 2004; accepted 25 October 2004

Abstract

Published data on nitrate (NO_3^-) toxicity to freshwater and marine animals are reviewed. New data on nitrate toxicity to the freshwater invertebrates *Eulimnogammarus toletanus*, *Echinogammarus echinosetosus* and *Hydropsyche exocellata* are also presented. The main toxic action of nitrate is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen. Nitrate toxicity to aquatic animals increases with increasing nitrate concentrations and exposure times. In contrast, nitrate toxicity may decrease with increasing body size, water salinity, and environmental adaptation. Freshwater animals appear to be more sensitive to nitrate than marine animals. A nitrate concentration of 10 mg NO_3-N/l (USA federal maximum level for drinking water) can adversely affect, at least during long-term exposures, freshwater invertebrates (*E. toletanus*, *E. echinosetosus*, *Cheumatopsyche pettiti*, *Hydropsyche occidentalis*), fishes (*Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, *Salmo clarki*), and amphibians (*Pseudacris triseriata*, *Rana pipiens*, *Rana temporaria*, *Bufo bufo*). Safe levels below this nitrate concentration are recommended to protect sensitive freshwater animals from nitrate pollution. Furthermore, a maximum level of 2 mg NO_3-N/l would be appropriate for protecting the most sensitive freshwater species. In the case of marine animals, a maximum level of 20 mg NO_3-N/l may in general be acceptable. However, early developmental stages of some marine invertebrates, that are well adapted to low nitrate concentrations, may be so susceptible to nitrate as sensitive freshwater invertebrates.

Keywords: Nitrate toxicity; Aquatic invertebrates; Fishes; Amphibians; Review

1. Introduction

In the aquatic environment, the most common ionic (reactive) forms of inorganic nitrogen are ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). These ions may be present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen-rich geological deposits, N_2 fixation by certain prokaryotes (cyanobacteria, particularly), and biological degradation of organic matter (Spencer, 1975; Kinne, 1984; Gleick, 1993; Wetzel, 2001; Rabalais, 2002). Ammonium tends to be oxidized to nitrate in a two-step process ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) by aerobic chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*, primarily), even if levels of dissolved oxygen decline to a value as low as 1.0 mg O_2/l (Sharma and Ahlert, 1977; Stumm and Morgan, 1996; Wetzel, 2001). In consequence, concentrations of nitrate in

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0045-6535/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2004.10.044

freshwater and marine ecosystems usually are higher than those of ammonium and nitrite (Spencer, 1975; Kinne, 1984; Gleick, 1993; Wetzel, 2001; Rabalais, 2002). Nitrate (but also ammonium and nitrite) may however be removed from water by aquatic plants, algae and bacteria which assimilate it as a source of nitrogen (Nixon, 1995; Smith et al., 1999; Wetzel, 2001). Furthermore, when concentrations of dissolved oxygen decrease to minimum values, facultative anaerobic bacteria (e.g., *Pseudomonas*, *Micrococcus*, *Bacillus*, *Achromobacter*) can utilize nitrate as a terminal acceptor of electrons, resulting in the ultimate formation of N_2 (Austin, 1988; Wetzel, 2001).

During the past two centuries, the human species has substantially altered the global nitrogen cycle, increasing both the availability and the mobility of nitrogen over large regions of Earth (Vitousek et al., 1997; Carpenter et al., 1998; Galloway and Cowling, 2002). Consequently, in addition to natural sources, inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-) can nowadays enter aquatic ecosystems via anthropogenic sources such as animal farming, urban and agricultural runoff, industrial wastes, and sewage effluents (including effluents from sewage treatment plants that are not performing tertiary treatments) (Meybeck et al., 1989; Conrad, 1990; Bouchard et al., 1992; Welch and Lindell, 1992; Gleick, 1993; Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999; Wetzel, 2001; Rabalais, 2002). Moreover, the atmospheric deposition of inorganic nitrogen (mainly in the form of NO_3^-) has dramatically increased because of the extensive use of nitrogen fertilisers and the huge combustion of fossil fuels (Vitousek et al., 1997; Carpenter et al., 1998; Moomaw, 2002; Boumans et al., 2004). As a result, concentrations of nitrate in ground and surface waters are increasing around the world, causing one of the most prevalent environmental problems responsible for water quality degradation on a worldwide scale (Meybeck et al., 1989; Conrad, 1990; Bouchard et al., 1992; Welch and Lindell, 1992; Gleick, 1993; Nixon, 1995; Smith et al., 1999; Wetzel, 2001; Rabalais, 2002; Smith, 2003). Nitrate concentrations may actually exceed values as high as 25 mg NO_3-N/l in surface waters and 100 mg NO_3-N/l in ground waters (Bogardi et al., 1991; Goodrich et al., 1991; Gleick, 1993; Ministry of Agriculture, Fisheries and Food, 1993; Steinheimer et al., 1998). On the other hand, in marine aquaria and aquaculture systems, where water is recirculating with good oxygenation, nitrate concentrations can approach values of 500 mg NO_3-N/l (De Graaf, 1964; Pierce et al., 1993).

In spite of the current worldwide environmental concern about increasing nitrate concentrations in ground and surface waters, comparatively few studies have been conducted to assess nitrate toxicity to aquatic animals, probably because it has been traditionally assumed that other occurring inorganic nitrogen compounds, such as ammonia (the unionized form of NH_4^+) and nitrite, are more toxic (Russo, 1985; Meade and Watts, 1995; Wetzel, 2001; Alonso and Camargo, 2003). In fact, although safe levels of ammonia have been well established for fishes and aquatic invertebrates (Alabaster and Lloyd, 1982; US Environmental Protection Agency, 1986), no safe level of nitrate has been established for aquatic animals (US Environmental Protection Agency, 1986; Scott and Crunkilton, 2000). It however is worth mentioning that an acceptable level of nitrate for seawater culture was considered to be less than 20 mg NO_3-N/l (Spotte, 1979).

The main toxic action of nitrate on aquatic animals is due to the conversion of oxygen-carrying pigments (e.g., hemoglobin, hemocyanin) to forms that are incapable of carrying oxygen (e.g., methemoglobin) (Grabda et al., 1974; Conrad, 1990; Jensen, 1996; Scott and Crunkilton, 2000; Cheng and Chen, 2002). Nevertheless, owing to the low branchial permeability to nitrate, the NO_3^- uptake in aquatic animals seems to be more limited than the uptake of NH_4^+ and NO_2^-, contributing to the relatively low toxicity of nitrate (Russo, 1985; Meade and Watts, 1995; Jensen, 1996; Stormer et al., 1996; Cheng and Chen, 2002; Alonso and Camargo, 2003).

Elevated nitrate concentrations in drinking waters have serious risks for humans. Ingested nitrates may cause methemoglobinemia in infants through their conversion to nitrites (under anaerobic conditions in the gut) and the subsequent blockade of the oxygen-carrying capacity of hemoglobin (Sandstedt, 1990; Amdur et al., 1991; Wolfe and Patz, 2002). In addition, ingested nitrates have a potential role in developing cancers of the digestive tract through their contribution to the formation of nitrosamines, which are among the most potent of the known carcinogens in mammals (Harte et al., 1991; Nash, 1993). To prevent these deleterious effects of nitrate on human health, drinking water quality criteria have been established: the USA federal maximum contaminant level is 10 mg NO_3-N/l (US Environmental Protection Agency, 1986; Nash, 1993; Scott and Crunkilton, 2000).

The chief purpose of this paper is to review published scientific literature on the toxic effects of nitrate (NO_3^-) on freshwater and marine animals (invertebrates, fishes and amphibians) to establish preliminary safe levels of nitrate for aquatic life. To better compare toxicity data from different authors, all concentrations and levels of nitrate were expressed as mg NO_3-N/l. Additionally, we present new data on the short-term toxicity of nitrate to three species of freshwater invertebrates that are relatively common in rivers and streams of Central Spain: *Eulimnogammarus toletanus* Pinkster & Stock (Gammaridae, Amphipoda, Crustacea), *Echinogammarus echinosetosus* Pinkster (Gammaridae, Amphipoda, Crustacea), and *Hydropsyche exocellata* Dufour (Hydropsychidae, Trichoptera, Insecta). Individuals of *E. toletanus* and

E. echinosetosus are shredder and detritivorous animals that feed on coarse particulate organic matter. Caddisfly larvae of *H. exocellata* are filter-feeders that construct fixed silk retreat-nets to strain food particles from the current. These species were chosen because the information on nitrate toxicity to freshwater invertebrates, particularly to freshwater amphipods, was very limited.

2. Materials and methods

Adults of *Eulimnogammarus toletanus* (average size of 8.5 mm in length) and *Echinogammarus echinosetosus* (average size of 11.2 mm in length), and last instar larvae of *Hydropsyche exocellata* (>1 mm head capsule width), were obtained from relatively unpolluted reaches of the Henares River (Central Spain). Invertebrates were transported to the laboratory using plastic containers with river water. No animal died during transportation. In the laboratory, invertebrates were deposited into three glass aquaria (one for each species) and acclimated to water quality conditions for seven days prior to the beginning of toxicity bioassays. During acclimation, amphipods were fed with macerated poplar leaves from the Henares river, and caddisfly larvae were fed with fine particulate dried fish food.

Invertebrate species were tested separately. Three static (with water renovation) short-term toxicity bioassays were conducted in triplicate for five days using small glass aquaria, each containing one litre of bottled drinking water (with no chlorine). A control and 5-6 different nominal nitrate concentrations were used per bioassay, with 10 animals per concentration/aquarium (including control). Test nitrate concentrations ranged from 5 to 160 mg NO_3-N/l for *E. echinosetosus*, from 15 to 480 mg NO_3-N/l for *E. toletanus*, and from 20 to 640 mg NO_3-N/l for *H. exocellata*. In all cases, nitrate solutions were made from sodium nitrate ($NaNO_3$, Merck, Germany). These nitrate solutions, together with water in control aquaria, were daily renewed. Invertebrates were not fed during bioassays to prevent changes in nitrate concentrations. Water oxygenation and turbulence were produced with air pumps and airstones. Average water quality conditions during bioassays were: 7.7 mg O_2/l for dissolved oxygen, 17.9 °C for temperature, 7.8 for pH, and 293 mg $CaCO_3$/l for total hardness. In the case of *H.* exocellata, and following previous recommendations by Camargo and Ward (1992), PVC pieces were added to quaria to facilitate net-building by net-spinning caddisfly larvae. Mortality was recorded every day, dead animals being removed.

Statistical analyses were performed using the multifactor probit analysis (MPA) software (US Environmental Protection Agency, 1991; Lee et al., 1995). 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. 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. 48, 72, 96 and 120 h LC_{10} and LC_{50} values were calculated for each test species. In addition, 120 h $LC_{0.01}$ values (lethal concentrations for 0.01% response after 120 h of exposure) were estimated for each test species as short-term safe levels of nitrate.

3. Toxicity to aquatic invertebrates

Nitrate toxicity to aquatic invertebrates increases with increasing nitrate concentrations and exposure times (Camargo and Ward, 1992, 1995; Scott and Crunkilton, 2000; Tsai and Chen, 2002; Alonso and Camargo, 2003). Conversely, nitrate toxicity decreases with increasing body size and water salinity (Camargo and Ward, 1992, 1995; Tsai and Chen, 2002). In general, freshwater invertebrates appear to be more sensitive to nitrate toxicity than marine invertebrates as a probable consequence of the ameliorating effect of water salinity on the tolerance of aquatic invertebrates to nitrate ions. However, early life stages of some marine invertebrates may be very sensitive to nitrate toxicity (Muir et al., 1991).

Camargo and Ward (1992), studying the short-term toxicity of $NaNO_3$ to the Nearctic net-spinning caddisflies *Cheumatopsyche pettiti* and *Hydropsyche occidentalis*, calculated 72, 96 and 120 h LC_{50} values of nitrate-nitrogen for early and last instar larvae of these two hydropsychid species (Table 1). In both cases, early instar larvae appeared to be more sensitive to nitrate toxicity than last instar larvae. Additionally, Camargo and Ward (1995) estimated short-term safe levels (120 h $LC_{0.01}$ values) of 6.7 and 9.6 mg NO_3-N/l for early and last instar larvae of *C. pettiti*, and 4.5 and 6.5 mg NO_3-N/l for early and last instar larvae of *H. occidentalis* (Table 1).

Meade and Watts (1995) examined the toxic effects of $NaNO_3$ on the survival and metabolic rate (oxygen consumption) in juvenile individuals (9–13 mm total length) of the Australian freshwater crayfish *Cherax quadricarinatus*. After 5 days, no mortality was observed in crayfish exposed to a nominal nitrate concentration of 1000 mg NO_3-N/l. Furthermore, no significant difference was observed in oxygen consumption between control (0 mg NO_3-N/l) and experimental (1000 mg NO_3-N/l) individuals (Table 1).

Jensen (1996) studied the uptake and physiological effects of nitrate ions (from $NaNO_3$) in the freshwater crayfish *Astacus astacus*. The nitrate uptake was minor

Table 1
Comparative toxicity of nitrate-nitrogen (NO_3-N) to aquatic invertebrates

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
Cheumatopsyche pettiti	Early instar larvae	Freshwater	191 (72 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	113.5 (96 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	106.5 (120 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	6.7 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
	Last instar larvae	Freshwater	210 (72 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	165.5 (96 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	119 (120 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	9.6 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
Hydropsyche occidentalis	Early instar larvae	Freshwater	148.5 (72 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	97.3 (96 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	65.5 (120 h LC_{50})	Camargo and Ward (1992)
	Early instar larvae	Freshwater	4.5 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
	Last instar larvae	Freshwater	183.5 (72 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	109 (96 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	77.2 (120 h LC_{50})	Camargo and Ward (1992)
	Last instar larvae	Freshwater	6.5 (120 h $LC_{0.01}$)	Camargo and Ward (1995)
Cherax quadricarinatus	Juveniles (9–13 mm)	Freshwater	1000 (5 d NOAEL)	Meade and Watts (1995)
Astacus astacus	Juveniles	Freshwater	14 (7 d NOAEL)	Jensen (1996)
Ceriodaphnia dubia	Neonates (<24 h)	Freshwater	374 (48 h LC_{50})	Scott and Crunkilton (2000)
	Neonates (<24 h)	Freshwater	7.1–56.5 (7 d NOEC)	Scott and Crunkilton (2000)
	Neonates (<24 h)	Freshwater	14.1–113 (7d LOEC)	Scott and Crunkilton (2000)
Daphnia magna	Neonates (<48 h)	Freshwater	462 (48 h LC_{50})	Scott and Crunkilton (2000)
Potamopyrgus antipodarum	Adults (2.6–3.8 mm)	Freshwater	2009 (24 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1128 (24 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1297 (48 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	728 (48 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1121 (72 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	629 (72 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	1042 (96 h LC_{50})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	585 (96 h LC_{10})	Alonso and Camargo (2003)
	Adults (2.6–3.8 mm)	Freshwater	195 (96 h $LC_{0.01}$)	Alonso and Camargo (2003)
Crassostrea virginica	Juveniles	Seawater	3794 (96 h LC_{50})	Epifano and Srna (1975)
Penaeus spp.	Juveniles	Seawater (28‰)	3400 (48 h LC_{50})	Wickins (1976)
Haliotis tuberculata	Juveniles (12–14.4 g)	Seawater (34‰)	250 (15 d safe level)	Basuyaux and Mathieu (1999)
Paracentrotus lividus	Juveniles (2.7–5.9 g)	Seawater (34‰)	100 (15 d safe level)	Basuyaux and Mathieu (1999)
Penaeus monodon	Protozoea (I stage)	Seawater (32‰)	0.226 (31–37% mortality 40 h)	Muir et al. (1991)
	Protozoea (I stage)	Seawater (32‰)	2.26 (35–43% mortality 40 h)	Muir et al. (1991)
	Protozoea (I stage)	Seawater (32‰)	22.6 (37–58% mortality 40 h)	Muir et al. (1991)
	Juveniles (22–35 mm)	Seawater (15‰)	2876 (48 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (15‰)	1723 (72 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (15‰)	1449 (96 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (25‰)	3894 (48 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (25‰)	2506 (72 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (25‰)	1575 (96 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	4970 (48 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	3525 (72 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	2316 (96 h LC_{50})	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (15‰)	145 (safe level)	Tsai and Chen (2002)

Table 1 (*continued*)

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
	Juveniles (22–35 mm)	Seawater (25‰)	158 (safe level)	Tsai and Chen (2002)
	Juveniles (22–35 mm)	Seawater (35‰)	232 (safe level)	Tsai and Chen (2002)
Marsupenaeus japonicus	Juveniles (8.3–14.9 g)	Seawater (30‰)	105 (24 h LOEC)	Cheng and Chen (2002)

Values of toxicological parameters (LC_{50}, LC_{10}, $LC_{0.01}$, NOAEL, NOEC, LOEC) at different exposure times for several species of freshwater and marine invertebrates. In all cases, animals were exposed to sodium nitrate ($NaNO_3$).

in crayfish exposed to a nitrate concentration of 14 mg NO_3-N/l for seven days, indicating a low branchial permeability to nitrate (Table 1). This minor uptake of nitrate appeared to be passive, the haemolymph nitrate concentration staying far below the ambient nitrate concentration. In addition, nitrate exposure did not induce significant changes in haemolymph chloride, sodium or potassium concentrations, nor in divalent cations and anions, extracellular osmolality and amino acid concentrations (Table 1).

Scott and Crunkilton (2000), examining the acute toxicity of $NaNO_3$ to neonates of the cladocerans *Ceriodaphnia dubia* (<24 h old) and *Daphnia magna* (<48 h old), estimated 48 h LC_{50} values of 374 and 462 mg NO_3-N/l (Table 1). Moreover, Scott and Crunkilton (2000) reported that the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC), for neonate production in *C. dubia* females after 7 days of exposure to nominal nitrate concentrations ranging from 2.2 to 113 mg NO_3-N/l, ranged from 7.1 to 56.5 mg NO_3-N/l (average NOEC value of 21.3 mg NO_3-N/l) and from 14.1 to 113 mg NO_3-N/l (average LOEC value of 42.6 mg NO_3-N/l) (Table 1).

Alonso and Camargo (2003), conducting laboratory experiments to examine the acute toxicity of $NaNO_3$ to the snail *Potamopyrgus antipodarum*, calculated 24, 48, 72 and 96 h LC_{10} and LC_{50} values (Table 1). This aquatic snail appeared to be relatively tolerant to nitrate toxicity, since an exposure of 4 days to a nitrate concentration as high as 585 mg NO_3-N/l (96 h LC_{10} value) could potentially cause 10% mortality in *P. antipodarum*. Alonso and Camargo (2003) also estimated a short-term safe level (96 h $LC_{0.01}$ value) of 195 mg NO_3-N/l (Table 1).

In toxicity tests with *Eulimnogammarus toletanus*, *Echinogammarus echinosetosus* and *Hydropsyche exocellata*, mortality percentages increased with increasing nitrate concentrations and exposure times. Before death, gammarids showed alterations in normal movement, and net-spinning caddisfly larvae tended to migrate from their retreat and capture nets. This sublethal effect of migration in larvae of *H. exocellata* has been previously reported in larvae of other hydropsychid species exposed to high levels of sodium nitrate (Camargo and Ward, 1992, 1995). The 48, 72, 96 and 120 h LC_{10} and LC_{50} values, and their 95% confidence limits, are presented in Table 2. From a simple comparison of LC_{50} values (Tables 1 and 2), we can see that test gammarid species (in particular *E. echinosetosus*) seem to be more sensitive to nitrate toxicity than other freshwater invertebrates, at least during short-term exposures. Furthermore, a nitrate concentration as low as 8.5 mg NO_3-N/l (120 h LC_{10} value) could potentially cause 10% mortality in *E. echinosetosus*. Short-term safe levels (120 h $LC_{0.01}$ values) of nitrate for *E. toletanus*, *E. echinosetosus* and *H. exocellata* are also presented in Table 2. 120 h $LC_{0.01}$ values for gammarid species were lower than those for hydropsychid species (Tables 1 and 2). The lowest

Table 2
LC_{50}, LC_{10} and $LC_{0.01}$ values for *Eulimnogammarus toletanus*, *Echinogammarus echinosetosus* and *Hydropsyche exocellata*

Toxicological parameter (mg NO_3-N/l)	*E. toletanus*	*E. echinosetosus*	*H. exocellata*
48 h LC_{50}	180.3 (135.6–266.4)	106.9 (86.6–140.5)	592.3 (447.5–813.1)
48 h LC_{10}	47.2 (26.5–66.4)	16.2 (11.5–20.9)	62.7 (35.0–92.8)
72 h LC_{50}	109.2 (84.9–148.1)	74.8 (61.4–96.6)	350.4 (289.6–436.6)
72 h LC_{10}	28.5 (14.9–40.9)	11.4 (7.9–14.7)	40.0 (20.9–60.5)
96 h LC_{50}	85.0 (63.6–116.8)	62.5 (50.6–81.9)	269.5 (227.4–327.8)
96 h LC_{10}	22.2 (10.9–33.0)	9.5 (6.5–12.6)	31.8 (15.7–50.2)
120 h LC_{50}	73.1 (52.6–102.8)	56.2 (44.7–74.5)	230.2 (194.3–279.4)
120 h LC_{10}	19.1 (9.0–29.3)	8.5 (5.7–11.4)	27.8 (13.2–45.2)
120 h $LC_{0.01}$	4.4 (1.6–7.9)	2.8 (1.0–5.2)	11.9 (4.6–20.8)

95% confidence limits are presented in parenthesis.

120 h $LC_{0.01}$ value was for *E. echinosetosus* (2.8 mg NO_3-N/l).

Regarding marine invertebrates, Epifano and Srna (1975), studying the acute toxicity of $NaNO_3$ to juveniles of the American oyster *Crassostrea virginica*, estimated a 96 h LC_{50} value of 3794 mg NO_3-N/l (Table 1). Wickins (1976), examining the acute toxicity of $NaNO_3$ to combined species of penaeid shrimps (*Penaeus aztecus*, *P. japonicus*, *P. occidentalis*, *P. orientalis*, *P. schmitti* and *P. setiferus*), estimated a 48 h LC_{50} value as high as 3400 mg NO_3-N/l in 28‰ seawater (Table 1). Basuyaux and Mathieu (1999), studying the effect of elevated nitrate concentrations on growth of the abalone *Haliotis tuberculata* and the sea urchin *Paracentrotus lividus* during 15 days of exposure, reported maximum safe levels of 100 mg NO_3-N/l for *P. lividus* and 250 mg NO_3-N/l for *H. tuberculata* (Table 1).

Cheng and Chen (2002) found that a nitrate concentration of 105 mg NO_3-N/l caused reduction of oxyhemocyanin and protein in individuals (wet weight of 8.28–14.85 g) of the Kuruma shrimp *Marsupenaeus japonicus* (Table 1). Similarly, Cheng et al. (2002) studied nitrate accumulation (from $NaNO_3$) in tissues of the penaeid shrimp *Penaeus monodon*, and found that nitrate accumulated in muscle, hepatopancreas, foregut, heart, gill, hemolymph, midgut and eyestalk by factors of 0.16, 0.20, 0.26, 0.45, 0.60, 0.61, 0.83 and 1.32 over the ambient nitrate concentration. In addition, Tsai and Chen (2002), examining the acute toxicity of $NaNO_3$ on juveniles (average length 28.4 mm) of *P. monodon* at different salinity levels, reported that 48, 72 and 96 h LC_{50} values were: 2876, 1723 and 1449 mg NO_3-N/l in 15‰ seawater (Table 1); 3894, 2506 and 1575 mg NO_3-N/l in 25‰ seawater (Table 1); and 4970, 3525 and 2316 mg NO_3-N/l in 35‰ seawater (Table 1). Safe levels for rearing *P. monodon* juveniles were estimated to be 145, 158 and 232 mg NO_3-N/l at salinity levels of 15‰, 25‰ and 35‰ (Table 1).

In contrast, Muir et al. (1991) reported much lower levels of nitrate toxicity in *P. monodon*. They studied the tolerance of larvae at the Protozoea I stage (55–60 h after hatching) to $NaNO_3$, and found that significant mortality (31–37%) occurred within 40 h at a nitrate concentration as low as 0.226 mg NO_3-N/l (Table 1). Examination of surviving larvae from nitrate treatments indicated sublethal histopatological changes including vacuolation and shrinkage of the ganglionic neuropiles, and minor muscle fragmentation and shrinkage. At higher nitrate concentrations (2.26 and 22.6 mg NO_3-N/l), larval mortality increased (35–43% and 37–58%; Table 1) and additional tissues were affected: vacuolation and splitting of the hypodermis from the cuticle, and cytoplasmatic vacuolation of cells in the midgut and proventriculus. Because *P. monodon* larvae moulted from Protozoea I to Protozoea II stage during the experimental study, and because *P. monodon* larvae occur naturally in offshore, tropical regions which typically contain extremely low levels of dissolved nitrate (<0.05 mg NO_3-N/l; see Spencer, 1975; Kinne, 1984; Motoh, 1985), Muir et al. (1991) concluded that the relatively great sensitivity of *P. monodon* larvae to nitrate toxicity might be related to ontogeny and natural habitat: on the one hand, it is likely that larvae are more susceptible to nitrate during ecdysis; on the other hand, it is possible that larvae are well adapted to natural conditions (very low nitrate concentrations) and, consequently, are intolerant of elevated nitrate concentrations.

4. Toxicity to fishes

Nitrate toxicity to freshwater and marine fishes increases with increasing nitrate concentrations and exposure times (Trama, 1954; Westin, 1974; Colt and Tchobanoglous, 1976; Rubin and Elmaraghy, 1977; Kincheloe et al., 1979; Brownell, 1980; Tomasso and Carmichael, 1986; Pierce et al., 1993; Scott and Crunkilton, 2000). Furthermore, nitrate toxicity can depend greatly upon the cationic composition of the solution (Dowden and Bennett, 1965). As in the case of aquatic invertebrates, freshwater fishes appear to be more sensitive to nitrate toxicity than marine fishes.

Trama (1954) found that the common bluegill *Lepomis macrochirus* was able to tolerate elevated nitrate levels during short-term exposures: a 96 h LC_{50} value of 1975 mg NO_3-N/l was estimated for this fish species (Table 3). Dowden and Bennett (1965) reported that the 24 h LC_{50} values of $NaNO_3$ and KNO_3 for *L. macrochirus* were 2110 and 761 mg NO_3-N/l (Table 3).

Knepp and Arkin (1973) reported that the channel catfish *Ictalurus punctatus* was able to tolerate a nitrate concentration of 90 mg NO_3-N/l without affecting their growth and feeding activity after an exposure of 164 days (Table 3). Colt and Tchobanoglous (1976), evaluating the short-term toxicity of $NaNO_3$ to fingerlings (50–76 mm total length) of *I. punctatus* at 22, 26 and 30 °C, calculated 96 h LC_{50} values of 1355, 1423 and 1400 mg NO_3-N/l (Table 3). They concluded that the acute toxicity of nitrate to *I. punctatus* was independent of water temperature.

Westin (1974) reported that the 96 h LC_{50} values of nitrate for the rainbow trout *Oncorhynchus mykiss* (*Salmo gairdneri*, previously) and the chinook salmon *Oncorhynchus tshawytscha* were 1355 and 1310 mg NO_3-N/l (Table 3). Stormer et al. (1996) exposed fingerlings of *O. mykiss* to a nitrate concentration of 14 mg NO_3-N/l for 8 days. They found that NO_3^- ions were taken up passively, with plasma concentrations remaining below the ambient nitrate concentration. This limited uptake appeared central to the low toxicity of nitrate, and did not measurably influence electrolyte balance or haematology (Table 3).

Table 3
Comparative toxicity of nitrate-nitrogen (NO_3-N) to fishes

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
Lepomis macrochirus	Fingerlings	Freshwater	1975 (96 h LC_{50})[a]	Trama (1954)
	Fingerlings	Freshwater	2110 (24 h LC_{50})[a]	Dowden and Bennett (1965)
	Fingerlings	Freshwater	761 (24 h LC_{50})[b]	Dowden and Bennett (1965)
Ictalurus punctatus	Fingerlings	Freshwater	90 (164 d NOAEL)[a]	Knepp and Arkin (1973)
	Fingerlings (50–76 mm)	Freshwater (22 °C)	1355 (96 h LC_{50})[a]	Colt and Tchobanoglous (1976)
	Fingerlings (50–76 mm)	Freshwater (26 °C)	1423 (96 h LC_{50})[a]	Colt and Tchobanoglous (1976)
	Fingerlings (50–76 mm)	Freshwater (30 °C)	1400 (96 h LC_{50})[a]	Colt and Tchobanoglous (1976)
Oncorhynchus mykiss	Fingerlings	Freshwater	1355 (96 h LC_{50})[a]	Westin (1974)
	Eggs (anadromous)	Freshwater	1.1 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fry (anadromous)	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Eggs (nonanadromous)	Freshwater	1.1 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Eggs (nonanadromous)	Freshwater	2.3 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fry (nonanadromous)	Freshwater	1.1 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry (nonanadromous)	Freshwater	2.3 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fingerlings	Freshwater	14.0 (8 d NOAEL)[a]	Stormer et al. (1996)
Oncorhynchus tshawytscha	Fingerlings	Freshwater	1310 (96 h LC_{50})[a]	Westin (1974)
	Eggs	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	2.3 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	4.5 (30 d LOEC)[a]	Kincheloe et al. (1979)
Salmo clarki	Eggs	Freshwater	2.3 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Eggs	Freshwater	4.5 (30 d LOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	7.6 (30 d LOEC)[a]	Kincheloe et al. (1979)
Oncorhynchus kisutch	Eggs	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
	Fry	Freshwater	4.5 (30 d NOEC)[a]	Kincheloe et al. (1979)
Poecilia reticulatus	Fry	Freshwater	267 (24 h LC_{50})[b]	Rubin and Elmaraghy (1977)
	Fry	Freshwater	219 (48 h LC_{50})[b]	Rubin and Elmaraghy (1977)
	Fry	Freshwater	199 (72 h LC_{50})[b]	Rubin and Elmaraghy (1977)
	Fry	Freshwater	191 (96 h LC_{50})[b]	Rubin and Elmaraghy (1977)
Micropterus treculi	Fingerlings	Freshwater	1261 (96 h LC_{50})[a]	Tomasso and Carmichael (1986)
Pimephales promelas	Larvae (<8 d)	Freshwater	1010–1607 (96 h LC_{50})[a]	Scott and Crunkilton (2000)
	Larvae (<24 h)	Freshwater	358 (7 d NOEC)[a]	Scott and Crunkilton (2000)
	Larvae (<24 h)	Freshwater	717 (7d LOEC)[a]	Scott and Crunkilton (2000)
Catla catla	Fry (static system)	Freshwater	1565 (24 h LC_{50})[a]	Tilak et al. (2002)
	Fry (flow through system)	Freshwater	1484 (24 h LC_{50})[a]	Tilak et al. (2002)
Lithognathus mormyrus	Fingerlings	Seawater (34‰)	3450 (24 h LC_{50})[a]	Brownell (1980)
Diplodus saegus	Fingerlings	Seawater (34‰)	3560 (24 h LC_{50})[a]	Brownell (1980)
Heteromycteris capensis	Fingerlings	Seawater (34‰)	5050 (24 h LC_{50})[a]	Brownell (1980)
Pomacentrus leucostritus	Fingerlings (59–85 mm)	Seawater (32‰)	>3000 (96 h LC_{50})[a]	Pierce et al. (1993)
Centropristis striata	Fingerlings (106–168 mm)	Seawater (32‰)	2400 (96 h LC_{50})[a]	Pierce et al. (1993)
Trachinotus carolinus	Fingerlings (69–115 mm)	Seawater (32‰)	1000 (96 h LC_{50})[a]	Pierce et al. (1993)
Raja eglanteria	Fingerlings (75–125 mm)	Seawater (32‰)	>960 (96 h LC_{50})[a]	Pierce et al. (1993)
Monocanthus hispidus	Fingerlings (39–55 mm)	Seawater (32‰)	573 (96 h LC_{50})[a]	Pierce et al. (1993)

Values of toxicological parameters (LC_{50}, NOAEL, NOEC, LOEC) at different exposure times for several species of freshwater and marine fishes.

[a] Animals were exposed to sodium nitrate ($NaNO_3$).

[b] Animals were exposed to potassium nitrate (KNO_3).

The first indication that relatively low concentrations of nitrate might be harmful to fish came from Grabda et al. (1974). They reported that fry of rainbow trout, exposed to 5–6 mg NO_3-N/l for several days, displayed increased blood levels of ferrihemoglobin, alterations in the peripheral blood and hematopoietic centres, and liver damage. In addition, Kincheloe et al. (1979), examining the tolerance of several salmonid species to nitrate toxicity after an exposure of 30 days, reported that developing eggs and early fry stages of *O. mykiss*, *O. tshawytscha* and the (Lahontan) cutthroat trout *Salmo clarki* exhibited significant increases in mortality at nitrate concentrations from 1.1 to 4.5 mg NO_3-N/l (Table 3). In the case of the coho salmon *Oncorhynchus kisutch*, eggs and fry were not affected at the highest nitrate concentration of 4.5 mg NO_3-N/l (Table 3). Kincheloe et al. (1979) concluded that a nitrate level as low as 2.0 mg NO_3-N/l in surface waters of low total hardness (<40 mg $CaCO_3$/l) would be expected to limit survival of some salmonid fish populations because of impaired reproductive success.

Rubin and Elmaraghy (1977), after examining the acute toxicity of KNO_3 to guppy (*Poecilia reticulatus*) fry, calculated 24, 48, 72 and 96 h LC_{50} values of 267, 219, 199 and 191 mg NO_3-N/l (Table 3). Tomasso and Carmichael (1986) reported that the 96 h LC_{50} value of nitrate for the Guadalupe bass *Micropterus treculi* was 1261 mg NO_3-N/l (Table 3). Tilak et al. (2002), using static and continuous flow through systems, determined 24 h LC_{50} values of 1565 and 1484 mg NO_3-N/l for the Indian major carp *Catla catla* (Table 3).

Scott and Crunkilton (2000), after conducting laboratory experiments to examine the acute toxicity of $NaNO_3$ to larvae (<8 day old) of the fathead minnow *Pimephales promelas*, found that the 96 h LC_{50} value fell within the range of 1010–1607 mg NO_3-N/l (average LC_{50} value of 1341 mg NO_3-N/l; Table 3). Scott and Crunkilton (2000) also reported that the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC), for the growth of newly hatched larvae (<24 h old) of *P. promelas* after an exposure of 7 days, were 358 and 717 mg NO_3-N/l (Table 3). These larvae were lethargic and exhibited bent spines before death at a nitrate concentration of 717 mg NO_3-N/l.

With regard to marine fishes, Brownell (1980) reported 24 h LC_{50} values (mg NO_3-N/l) in 34‰ seawater of 3450 for *Lithognathus mormyrus*, 3560 for *Diplodus saegus*, and 5050 for *Heteromycteris capensis* (Table 3). Pierce et al. (1993) estimated 96 h LC_{50} values (mg NO_3-N/l) in 32‰ seawater of 573 for the planehead filefish *Monocanthus hispidus*, >960 for the clearnose skate *Raja eglanteria*, 1000 for the Florida pompano *Trachinotus carolinus*, 2400 for the black sea bass *Centropristis striata*, and >3000 for the beaugregory *Pomacentrus leucostritus* (Table 3).

5. Toxicity to amphibians

Current field data suggest that nitrogen fertilizers, such as ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$), may be contributing (with pesticides) to the decline of amphibian populations in agricultural areas (Wederkinch, 1988; Berger, 1989; Hecnar, 1995; Oldham et al., 1997; Birge et al., 2000). Laboratory studies have shown that the toxicity of nitrate compounds to amphibians increases with increasing nitrate concentrations and exposure times (Baker and Waights, 1993, 1994; Hecnar, 1995; Xu and Oldham, 1997; Marco et al., 1999; Schuytema and Nebeker, 1999a,b,c). The tolerance of amphibians to nitrogen fertilizers may however increase with increasing body size (Schuytema and Nebeker, 1999a,b) and environmental adaptation (Johansson et al., 2001).

Baker and Waights (1993), studying the toxicity of $NaNO_3$ to tadpoles of the common toad *Bufo bufo*, found that these animals exhibited reduced feeding activity, weight loss and decreased survival (84.6% mortality) when exposed for 13 days to a nitrate concentration of 9.1 mg NO_3-N/l (Table 4). Similarly, Baker and Waights (1994), examining the toxicity of $NaNO_3$ to tadpoles of the treefrog *Litoria caerulea*, found that these animals exhibited reduced feeding activity, weight loss and decreased survival (58.0% mortality) when exposed for 16 days to a nitrate concentration of 22.7 mg NO_3-N/l (Table 4).

Hecnar (1995), examining the acute toxicity of NH_4NO_3 to tadpoles of the American toad *Bufo americanus*, the chorus frog *Pseudacris triseriata*, the leopard frog *Rana pipiens* and the green frog *Rana clamitans*, reported 96 h LC_{50} values within the range 13.6–39.3 mg NO_3-N/l (Table 4). Hecnar (1995) also examined the chronic (100 days) toxicity of NH_4NO_3 to these amphibian species, and found that tadpoles of chorus frog and leopard frog exhibited lower survivorship at a nitrate concentration of 10.0 mg NO_3-N/l (Table 4). Signs of anormal behavior and development were similar in acute and chronic experiments: tadpoles swam and fed less vigorously, exhibited swelled and transparent bodies, developed head and digestive-system deformities, and suffered edemas and paralysis before death. Although Hecnar (1995) only considered nitrate toxicity when using ammonium nitrate, the toxicity of H_4NO_3 could be due not only to nitrate but also to ammonia (the unionized form of NH_4^+). Because laboratory conditions during toxicity tests were 7.6 for pH and 20 °C for temperature (Hecnar, 1995), it may be estimated that maximum ammonia levels in acute and chronic exposures were 1.0 and 0.20 mg NH_3/l, respectively. These NH_3 levels are higher than the established safe levels of ammonia for aquatic animals (Alabaster and Lloyd, 1982; US Environmental Protection Agency, 1986)

Table 4
Comparative toxicity of nitrate-nitrogen (NO_3-N) to amphibians

Species	Developmental stage	Toxicological parameter (mg NO_3-N/l)	References
Bufo bufo	Tadpoles	9.1 (84.6% mortality 13 d)[a]	Baker and Waights (1993)
	Tadpoles	384.8 (96 h LC_{50})[c]	Xu and Oldham (1997)
	Tadpoles	369.6 (168 h LC_{50})[c]	Xu and Oldham (1997)
	Tadpoles	22.6 (30 d LOEC)[c]	Xu and Oldham (1997)
Litoria caerulea	Tadpoles	22.7 (58% mortality 16 d)[a]	Baker and Waights (1994)
Bufo americanus	Tadpoles (from Ojibway)	13.6 (96 h LC_{50})[c]	Hecnar (1995)
	Tadpoles (from Harrow)	39.3 (96 h LC_{50})[c]	Hecnar (1995)
	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Pseudacris triseriata	Tadpoles	17 (96 h LC_{50})[c]	Hecnar (1995)
	Tadpoles	10.0 (100 d LOEC)[c]	Hecnar (1995)
Rana pipiens	Tadpoles	22.6 (96 h LC_{50})[c]	Hecnar (1995)
	Tadpoles	10.0 (100 d LOEC)[c]	Hecnar (1995)
	Larvae	30.0 (NOAEL)[a]	Allran and Karasov (2000)
Rana clamitans	Tadpoles	32.4 (96 h LC_{50})[c]	Hecnar (1995)
Rana sylvatica	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Rana pretiosa	Newly hatched larvae	16.45 (15 d LC_{50})[b]	Marco et al. (1999)
Ambystoma jeffersonianum	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Ambystoma maculatum	Fertilized eggs	9.0 (NOAEL)[a]	Laposata and Dunson (1998)
Ambystoma gracile	Newly hatched larvae	23.39 (15 d LC_{50})[b]	Marco et al. (1999)
Pseudacris regilla	Embryos	643 (96 h LC_{50})[a]	Schuytema and Nebeker (1999a)
	Embryos	578 (240 h LC_{50})[a]	Schuytema and Nebeker (1999a)
	Embryos	56.7 (10 d NOAEL)[a]	Schuytema and Nebeker (1999a)
	Tadpoles	1749.8 (96 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	266.2 (240 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	30.1 (10 d NOAEL)[a]	Schuytema and Nebeker (1999b)
Xenopus laevis	Embryos	438.4 (120 h LC_{50})[a]	Schuytema and Nebeker (1999a)
	Embryos	24.8 (5 d NOAEL)[a]	Schuytema and Nebeker (1999a)
	Tadpoles	1655.8 (96 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	1236.2 (240 h LC_{50})[a]	Schuytema and Nebeker (1999b)
	Tadpoles	65.6 (10 d NOAEL)[a]	Schuytema and Nebeker (1999b)
	Tadpoles	66.0 (40 d NOAEL)[a]	Sullivan and Spence (2003)
Rana aurora	Embryos	636.3 (16 d LC_{50})[a]	Schuytema and Nebeker (1999c)
	Embryos	29.0 (16 d NOAEL)[a]	Schuytema and Nebeker (1999c)
Rana temporaria	Larvae (northern Scandinavia)	5.0 (8 w LOEC)[a]	Johansson et al. (2001)
	Larvae (southern Scandinavia)	5.0 (10 w NOEC)[a]	Johansson et al. (2001)

Values of toxicological parameters (LC_{50}, NOAEL, NOEC, LOEC) at different exposure times for several species of amphibians.
[a] Animals were exposed to sodium nitrate ($NaNO_3$).
[b] Animals were exposed to potassium nitrate (KNO_3).
[c] Animals were exposed to ammonium nitrate (NH_4NO_3).

and, consequently, we can assume that, in addition to nitrate toxicity, some toxicity might have been caused by NH_3.

Xu and Oldham (1997) examined lethal and sublethal effects of NH_4NO_3 on tadpoles of the common toad *Bufo bufo*. They reported 96 and 168 h LC_{50} values of 384.8 and 369.6 mg NO_3-N/l (Table 4). Tadpoles exhibited certain unusual behavior (either undirected swimming movements or twisting laterally), remaining static unless disturbed. In a subchronic exposure (30 days) at a nitrate concentration of 22.6 mg NO_3-N/l, there was 21% mortality and a further 17% failed to resorb their tails at metamorphosis (Table 4). As in the case of Hecnar (1995), Xu and Oldham (1997) only considered

nitrate toxicity when using ammonium nitrate. Because laboratory conditions during toxicity tests were 7.4 for pH and 27.5 °C for temperature (Xu and Oldham, 1997), it may be estimated that maximum ammonia levels in acute and subchronic exposures were 9.2 and 0.51 mg NH_3/l, respectively. In consequence, we can assume that, in addition to nitrate toxicity, some toxicity might have been caused by NH_3.

Laposata and Dunson (1998) exposed fertilized eggs of the wood frog *Rana sylvatica*, the American toad *Bufo americanus*, the Jefferson salamander *Ambystoma jeffersonianum* and the spotted salamander *A. maculatum* to a nitrate concentration of 9.0 mg NO_3-N/l (from $NaNO_3$). They found that there was no significant difference in the hatching success with regard to control eggs in any of the four amphibian species (Table 4).

Marco et al. (1999), studying the effects of KNO_3 on several amphibian species indigenous of the Pacific Northwest (USA), found that newly hatched larvae of *Rana pretiosa* and *Ambystoma gracile*, exposed for 15 days to nitrate concentrations within the range 0.78–25.0 mg NO_3-N/l, reduced feeding activity, swam less vigorously, suffered edemas and paralysis, and eventually died. They calculated 15 d LC_{50} values of 16.45 mg NO_3-N/l for *R. pretiosa* and 23.39 mg NO_3-N/l for *A. gracile* (Table 4).

Schuytema and Nebeker (1999a,b,c) examined the toxic effects of $NaNO_3$ on embryos and tadpoles of the Pacific treefrog *Pseudacris regilla*, the African clawed frog *Xenopus laevis* and the red-legged frog *Rana aurora*. Schuytema and Nebeker (1999a) calculated 96 and 240 h LC_{50} values (mg NO_3-N/l) of 643 and 578 for embryos of *P. regilla*, and a 120 h LC_{50} value of 438.4 mg NO_3-N/l for embryos of *X. laevis* (Table 4). NOAEL (no observed adverse effect level) values, based on reduced growth (wet weight) of embryos, were 56.7 mg NO_3-N/l for *P. regilla* and 24.8 mg NO_3-N/l for *X. laevis* (Table 4). Schuytema and Nebeker (1999b) calculated 96 and 240 h LC_{50} values (mg NO_3-N/l) of 1749.8 and 266.2 for tadpoles of *P. regilla*, and 1655.8 and 1236.2 for tadpoles of *X. laevis* (Table 4). NOAEL values, based on reduced growth (wet weight) of tadpoles, were 30.1 mg NO_3-N/l for *P. regilla* and 65.6 mg NO_3-N/l for *X. laevis* (Table 4). Lastly, Schuytema and Nebeker (1999c) reported, for embryos of *R. aurora*, a 16 d LC_{50} value of 636.3 mg NO_3-N/l and a NOAEL value (based on length) of 29 mg NO_3-N/l (Table 4).

Allran and Karasov (2000), studying $NaNO_3$ toxicity to larvae of the leopard frog *Rana pipiens* exposed from first-feeding stage through metamorphosis, found that a nominal nitrate concentration of 30 mg NO_3-N/l had no significant effect on development rate, percent metamorphosis, time to metamorphosis, percent survival, mass at metamorphosis, or hematocrit. Although the growth of larvae was slowed, this growth inhibition was not biologically important when compared with natural variation in the environment.

Johansson et al. (2001), after conducting a comparison of nitrate tolerance between different populations of the common frog *Rana temporaria*, reported that a nitrate concentration of 5.0 mg NO_3-N/l might reduce the growth rate and metamorphic size in larvae (stage 25) from the northern parts of Scandinavia (less well adapted to cope with high environmental nitrate levels), but not in larvae from the southern parts of Scandinavia (better adapted to cope with high environmental nitrate levels) (Table 4). They concluded that increased anthropogenic nitrate pollution could impact more the northern than the southern Swedish common frog populations.

Sullivan and Spence (2003), examining $NaNO_3$ toxicity to tadpoles of the African clawed frog *Xenopus laevis*, found that a nominal nitrate concentration of 66 mg NO_3-N/l had no significant effect on the survival and metamorphosis of these animals during a exposure of 40 days (Table 4).

6. Concluding remarks

It should be evident, from data presented in this review, that nitrate discharges from anthropogenic sources may result in a serious ecological risk for certain aquatic animals. Indeed, as a consequence of nitrogen pollution, nitrate concentrations in surface waters can actually exceed values of 25 mg NO_3-N/l (Bogardi et al., 1991; Gleick, 1993; Ministry of Agriculture, Fisheries and Food, 1993). Because a nitrate concentration of 10 mg NO_3-N/l (USA federal maximum level for drinking water) can adversely affect, at least during long-term exposures, freshwater invertebrates (*Eulimnogammarus toletanus*, *Echinogammarus echinosetosus*, *Cheumatopsyche pettiti*, *Hydropsyche occidentalis*), fishes (*Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, *Salmo clarki*), and amphibians (*Pseudacris triseriata*, *Rana pipiens*, *Rana temporaria*, *Bufo bufo*) (Tables 1–4), safe levels below this nitrate concentration are therefore recommended to protect these sensitive freshwater animals from nitrate pollution. Furthermore, following Kincheloe et al.'s (1979) recommendation, we consider that a maximum level of 2.0 mg NO_3-N/l would be appropriate for protecting the most sensitive freshwater species. In the case of marine invertebrates and fishes, we consider that the proposed maximum level of 20 mg NO_3-N/l for culturing seawater animals (Spotte, 1979) may in general be acceptable. However, early developmental stages of some marine invertebrates (Muir et al., 1991), that are well adapted to low nitrate concentrations, may be so susceptible to nitrate as sensitive freshwater invertebrates (Tables 1 and 2).

In spite of this proposal of preliminary safe levels of nitrate for aquatic animals, further studies, especially

long-term studies, are required to check and improve the recommended safe levels. Additional studies must also examine the influence of water hardness, salinity, pH, temperature, dissolved oxygen and other chemical compounds on nitrate toxicity to aquatic animals. Lastly, because aquatic organisms are subjected to biotic interactions (e.g., competition, predation, parasitism) and diseases, field and laboratory studies should be carried out to assess the effects of elevated nitrate concentrations on these ecological and evolutionary agents of natural selection.

Acknowledgement

Funds for this research came from Madrid Community (research project 07M/0065/2001) and Ministry of Science and Technology (research project REN2001-1008/HID) in Spain. The University of Alcala provided logistical support. Comments by two anonymous reviewers are gratefully acknowledged. A. Alonso was supported by a pre-doctoral grant from the Council of Castilla-La Mancha. Part of this work was presented at the 5th Iberian and 2nd Iberoamerican Congress on Environmental Contamination and Toxicology, held in Porto (Portugal) from 22 to 24 September of 2003.

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