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Evaluation of the effect of water type on the toxicity of nitrate to aquatic organisms

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HIGHLIGHTS

- Acute and chronic NO_3 toxicity to aquatic organisms assessed.
- *Hyalella azteca* and *Ceriodaphnia dubia* most sensitive to effects of nitrate.
- Strong influence of ionic strength on nitrate toxicity.
- 2–10-fold reductions in chronic NO_3 toxicity as water changed from soft to hard.

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ABSTRACT

A suite of acute and chronic toxicity tests were conducted to evaluate the sensitivity of freshwater organisms to nitrate (as sodium nitrate). Acute exposures with rainbow trout (*Onchorhynchus mykiss*) and amphipods (*Hyalella azteca*), as well as chronic exposures with *H. azteca* (14-d survival and growth), midges (*Chironomus dilutus*; 10-d survival and growth), daphnids (*Ceriodaphnia dubia*; 7-d survival and reproduction), and fathead minnows (*Pimephales promelas*; 7-d survival and growth) were used to determine sublethal and lethal effect concentrations. Modification of nitrate toxicity was investigated across a range of ionic strengths, created through the use of very soft water, and standard preparations of synthetic soft, moderately-hard and hard dilution waters. The most sensitive species tested were *C. dubia* and *H. azteca*, in soft water, with reproduction and growth IC25 values of 13.8 and 12.2 mg/L NO_3-N, respectively. All of the organisms exposed to nitrate demonstrated significantly reduced effects with increasing ionic strength associated with changes in water type. Possible mechanisms responsible for the modifying effect of increasing major ion concentrations on nitrate toxicity are discussed.

1. Introduction

Nitrogen is an essential element for all organisms, being a major component of amino acids, nucleic acids, and other biological materials. In aquatic environments, biologically-available nitrogen occurs primarily in the chemical forms of ammonia, nitrite and nitrate, with the relative concentrations of these materials determined by biological and chemical processes associated with the nitrogen cycle. Nitrate is the most common aqueous form of nitrogen, and is produced primarily from the oxidation of plant and animal debris (Camargo and Alonso, 2006). Anthropogenic sources of nitrogen have increased concentrations of nitrate in many aquatic environments (Puckett, 1995), exceeding 25 and 100 mg/L NO_3-N in contaminated surface and ground waters, respectively (Camargo and Alonso, 2006). Mining activities often discharge nitrate into receiving environments, primarily as a result of use of nitrogen-containing blasting agents, such as ammonium nitrate/fuel oil or AN/FO (Camargo et al., 2005; Zaitsev et al., 2008).

Significant research has been conducted on the toxicity of ammonia and nitrite, however, less information is available regarding the concentrations of nitrate which cause adverse effects to aquatic organisms. Direct nitrate toxicity has previously been considered to be negligible, and concern was primarily associated with the potential for nitrate to stimulate eutrophication (Camargo and Alonso, 2006). However, studies have demonstrated that environmentally-relevant concentrations of nitrate can lead to

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direct toxicity to aquatic organisms (Camargo et al., 2005).

Inhibition of the oxygen-carrying capacity of hemoglobin has been implicated as the cause of reduced performance of aquatic organisms in the presence of elevated concentrations of nitrate (Grabda et al., 1974). This toxic action is similar to that of nitrite (Lewis Jr and Morris, 1986), likely due to reduction of nitrate to nitrite in the blood (Guillette and Edwards, 2005) resulting in a similar metabolic pathway involving production of nitric oxide (Hannas et al., 2010). Effects on osmoregulation resulting from nitrate exposures have also been identified (Gulyassy et al., 1962; Hrubec et al., 1997).

Research related to the toxicity of ammonia and nitrite has identified key toxicity-modifying factors for these compounds (*i.e.,* pH and chloride, respectively); these factors have been incorporated into water quality benchmarks for these constituents (British Columbia Ministry of Environment, 2009; Canadian Council of Ministers of the Environment, 2010; U.S. Environmental Protection Agency, 2013). Due to a limited focus on evaluating toxicity of nitrate, regulatory agencies have developed guidelines based on limited datasets (British Columbia Ministry of Environment, 2009; Canadian Council of Ministers of the Environment, 2012; Environment Australia, 2000) and the role of toxicity-modifying factors for nitrate has not been comprehensively evaluated, although the effect of chloride on toxicity of nitrate to amphipods has been reported (Soucek and Dickinson, 2016).

The aquatic toxicity of sulphate and chloride has been reported to be reduced with increasing water hardness (Davies and Hall, 2007; Elphick et al., 2011a, 2011b; Lasier and Hardin, 2010; Soucek and Kennedy, 2005), although the mechanism associated with this effect has not yet been clearly defined. The effect of water hardness on the toxicity of nitrate has not previously been evaluated, although the potential for this interaction has been hypothesized (Camargo et al., 2005; Scott and Crunkilton, 2000).

This study was designed to expand upon the available data on the toxicity of nitrate using additional freshwater species, while exploring the relationship between nitrate toxicity and the ionic characteristics of water (*e.g.*, hardness). The relationships between nitrate and water quality characteristics are potentially significant in understanding mechanisms of nitrate toxicity and in establishing whether toxicity modifying factors should be incorporated into water quality benchmarks for nitrate.

2. Methods

Toxicity tests were conducted at Nautilus Environmental (Burnaby, BC, Canada) in walk-in environmental chambers with temperature (±1 °C) and photoperiod (16:8 light:dark) control. Water quality parameters, including dissolved oxygen, pH and temperature, were recorded daily throughout the exposures.

Test waters were prepared by addition of reagent-grade salts to achieve the target water hardness types, with the exception of very soft water (*i.e.,* 10–15 mg/L as $CaCO_3$), which was dechlorinated Metro Vancouver municipal tap water. Salt additions followed the ratios of salts specified by USEPA (U.S. Environmental Protection Agency, 2002), with the exception of tests using *Hyalella azteca* and *Chironomus dilutus*, which employed a recipe containing a higher concentration of chloride, as described by Environment Canada (Environment Canada, 1997a).

Tests using rainbow trout (*Onchorhynchus mykiss*) and fathead minnows (*Pimephales promelas*) were conducted at four hardnesses: very soft water (VSW, 10–15 mg/L as $CaCO_3$); soft water (SW, 40–55 mg/L as $CaCO_3$); moderately-hard water (MHW, 80–100 mg/L as $CaCO_3$); and hard water (HW, 160–180 mg/L as $CaCO_3$). The invertebrate species (*i.e., Ceriodaphnia dubia*, *C. dilutus* and *H. azteca*) were tested at the three higher hardnesses (Table 2). These water types are commonly used in evaluations of water hardness as a toxicity modifying factor; however, in addition to ions contributing to hardness (*i.e.*, Ca and Mg) other major ions (*i.e.*, Na, K, HCO_3, Cl and SO_4) also co-vary in these water types (Table 1). Hardness and alkalinity were measured on waters at test initiation using titration techniques.

Table 1
Water chemistry for dilution waters used in nitrate exposures. Nominal concentrations based on salt additions (mg/L).

Constituent (mg/L)	*P. promelas/O. mykiss*				*C. dubia*			*H. azteca/C. dilutus*		
	VSW	SW	MHW	HW	SW	MHW	HW	SW	MHW	HW
Na	1.8	15.0	28.1	54.4	13.1	26.3	52.5	15.0	28.1	54.4
Mg	0.1	6.2	12.3	24.4	6.1	12.7	24.2	3.2	6.2	12.3
Ca	3.9	10.9	17.9	31.8	7.0	14.7	27.9	18.7	33.5	63.2
K	0.2	1.2	2.3	4.4	1.0	2.1	4.2	1.2	2.3	4.4
HCO_3	11.0	45.9	80.8	150.5	34.9	69.7	139.5	45.9	80.8	150.5
Cl	2.0	2.9	3.9	5.8	1.0	1.9	3.8	18.8	36.0	69.4
SO_4	2.7	43.4	84.0	165.4	40.7	85.4	162.7	28.6	54.5	106.4
pH	6.9–7.3	7.3–7.7	7.7–8.0	7.9–8.3	7.6–7.9	8.1	8.2–8.4	7.4–7.7	7.7–8.0	8.0–8.2
Hardness (as $CaCO_3$)	10–15	40–55	80–100	160–180	40–55	80–100	160–180	40–55	80–100	160–180

Very soft water: 10–15 mg/L as $CaCO_3$; soft water: 40–55 mg/L as $CaCO_3$; moderately-hard water: 80–100 mg/L as $CaCO_3$; and, hard water: 160–180 mg/L as $CaCO_3$.

Table 2
Summary of toxicity test endpoints and test concentrations.

Test species	Test duration	Test endpoint(s)	Nominal concentrations tested (mg/L NO_3-N)
O. mykiss	96-hr	Survival	312, 625, 1250, 2500, 5000
H. azteca	96-hr	Survival	103, 206, 412, 824, 1647
	14-day	Survival, growth	10[a], 20, 40, 80, 160, 320, 640[b]
C. dilutus	10-day	Survival, growth	10[a], 20, 40, 80, 160, 320, 640[b]
C. dubia	7 ± 1 day	Survival, reproduction	5[c], 10, 20, 40, 80, 160, 320, 640[d]
P. promelas	7-day	Survival, growth	50, 100, 200, 400, 800, 1600

[a] Tested in soft water only.
[b] Tested in moderately hard and hard water only.
[c] Tested in moderately hard water.
[d] Tested in soft water and hard water.