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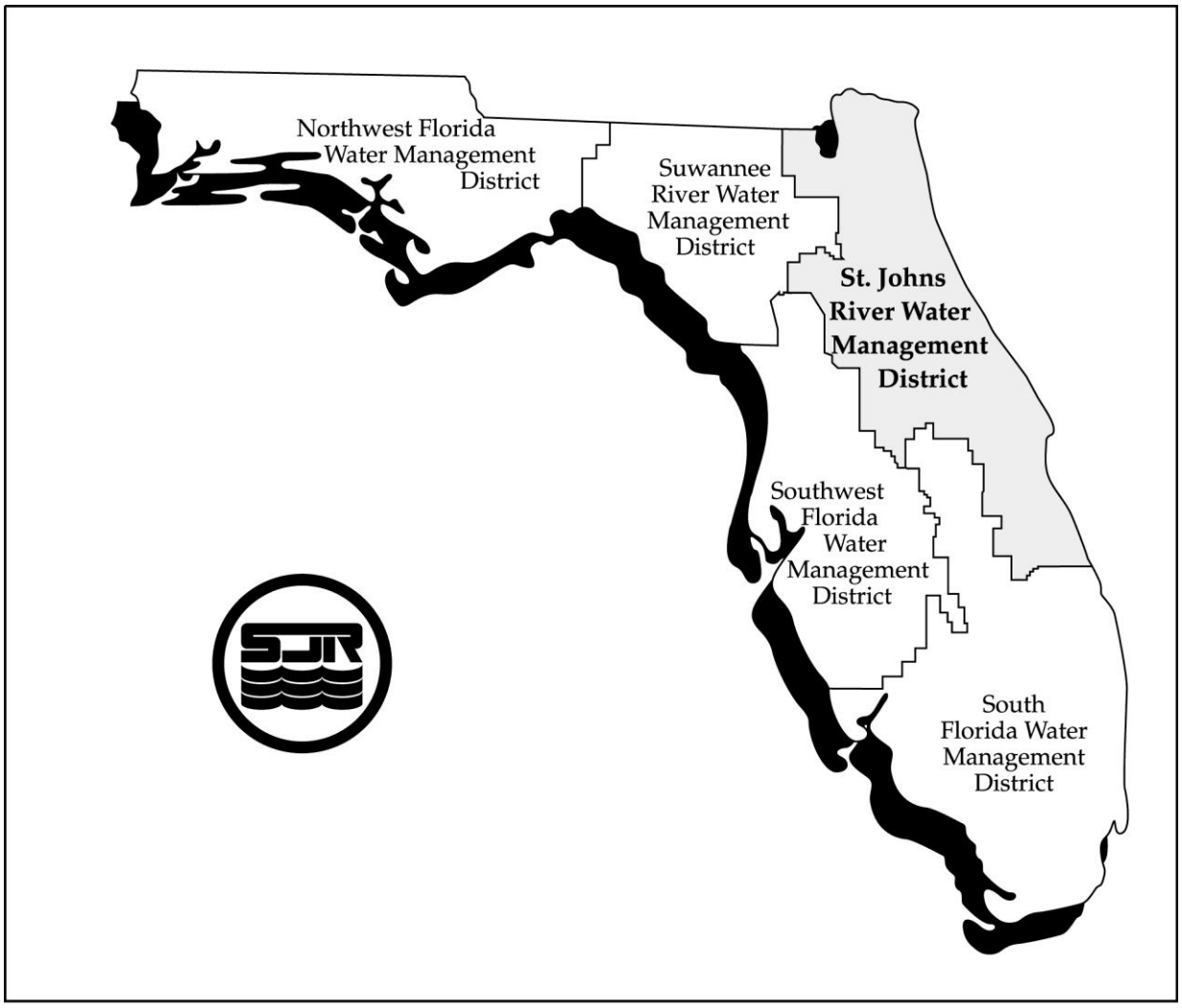

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ABSTRACT

The global nitrogen cycle has been altered substantially by human activity over the last century. This alteration has resulted in increased loads of nitrogen to surface- and groundwater. Considerable research attention has been given to the ecological effects of nitrogen enrichment on primary producers and the concurrent effects on aquatic food webs. An emerging body of research data suggests that, in addition, there are toxic effects of nitrogen enrichment on aquatic fauna, not only from ammonia and nitrite (which have been well-established), but also from nitrate. Here, the current literature of nitrate toxicity is reviewed and the implications for Florida's springs are discussed. Nitrate toxicity thresholds down to 0.23 mg/ L NO_3-N were reported for invertebrates, down to 1.1 mg/ L NO_3-N were reported for fishes, and down to 5 mg/ L NO_3-N were reported for amphibians. A number of springs in Florida display nitrate concentration levels above these thresholds, which suggests there may be toxic effects on springs' fauna.

INTRODUCTION

The global nitrogen cycle has been substantially altered by human activities over the last century (Vitousek et al. 1997). Industrial fixation of nitrogen to produce fertilizer has resulted in dramatically increased use and application of fertilizer to the landscape. This, along with increases in intensive animal husbandry, increased nitrogen deposition from air pollution, and increased generation of wastewater from human settlements, has roughly doubled the total mass of nitrogen introduced annually into the earth's biosphere (Vitousek et al. 1997). While the benefits of this have been profound in terms of increased food production and better overall health of the earth's human population, the ecological consequences of this additional nitrogen input have been equally profound; the cultural eutrophication of inland and coastal aquatic ecosystems, due in part to this increased nitrogen input, has changed their ecological structure and function in myriad ways (Carpenter et al. 1998; Bricker et al. 1999; Howarth et al. 2000; Rabalais 2002).

In Florida, the issue of nitrogen enrichment has focused on the state's springs and spring-run streams (stream systems entirely or largely fed by spring inflow). Many springs in Florida exhibit elevated nitrate concentrations compared to background or historic conditions (Scott et al. 2004; Florida Springs Task Force 2006). This parallels increased concentrations of nitrate in the Floridan aquifer, the primary groundwater resource that feeds most Florida springs (Katz et al. 1999; Toth and Fortich 2002; Phelps 2004). Much of the focus on the effects of this nitrate enrichment has centered on primary productivity and how

increased plant production alters water quality and other ecosystem characteristics. Not considered as much has been the potential effects of nitrate as an agent of toxicity. The purpose of this paper is to summarize the existing literature on nitrate toxicity and evaluate its relevance to the problem of nitrate enrichment of Florida springs and spring-run streams (collectively termed "spring systems" in this review).

Nitrogen Chemistry in the Aquatic Environment

In natural waters, nitrate (NO_3^-) coexists with nitrite (NO_2^-) as the two major forms of oxidized nitrogen in aqueous solution. Nitrite is produced from the breakdown and degradation of various forms of organic nitrogen and ammonia, mediated by nitrifying bacteria as part of the nitrogen cycle in aquatic environments (Wetzel 1975; Lewis and Morris 1986). In aerobic conditions, most nitrite is converted to the more stable nitrate and nitrite is typically present at very low concentrations (Guillette and Edwards 2005). Nitrate is highly soluble in water, and any nitrate not taken up by plants or converted back to nitrogen gas by denitrification is transported to surface- or groundwaters.

Current analytical methods typically used to measure levels of nitrate in surface- and groundwaters actually measure both NO_3^- and NO_2^-, and levels are expressed as mg/ L nitrate/ nitrite-nitrogen (NO_3/ NO_2-N or "NOx-N") (Edwards et al. 2004). In this review, "NOx-N" will be referred to as NO_3-N. In the older literature, nitrate may be reported as mg/ L NO_3, which is roughly four times higher than NO_3-N (Bruning-Fann and Kaneene 1993). Nitrate concentrations are typically expressed as parts per million, or mg/ L, or they may be expressed as molarity (Armstrong 1979; Bruning-Fann and Kaneene 1993). Bruning-Fann and Kaneene (1993) provide a useful table for conversion between the various units and chemical forms of nitrate.

Nitrate Toxicity Considerations and Its Mechanisms

The toxicity of various nitrogen compounds in aqueous solution (ammonia, nitrite, and nitrate) has been known for many years (Russo 1985), primarily due to their harmful effects in closed circulating systems such as aquaria and fish hatcheries (Armstrong 1979; Shimura et al. 2002). In general, the degree of toxicity is ammonia > nitrite > nitrate (Armstrong 1979; Russo 1985). Nitrate toxicity is also well known in agricultural and veterinary practice (Bruning-Fann and Kaneene 1993; *http://www.vetmed.ucdavis. edu/vetext/INF-BE_cca/INF-BE_cca01/INF-BE_cca0111.html*), due to nitrogen enrichment of livestock drinking water by urine/ manure or fertilizer runoff.

Specific mechanisms of nitrate toxicity vary depending upon a number of factors, both *in situ* and *in vivo*. Nitrate and/ or nitrite enter an animal either through permeable membranes, such as the gills of fish or tadpoles or the skin of adult amphibians, or via ingestion (mammals and birds). It appears that nitrite is actively taken up by the chloride transport mechanism across gill membranes (Jensen 1996; Lewis and Morris 1986). Within body fluids, nitrate can be converted to nitrite or nitrate can accumulate due to hepatic detoxification of nitrite (Edwards et al. 2004). Bruning-Fann and Kaneene (1993) note that toxicity of nitrate to higher vertebrates (birds and mammals) is actually attributable to metabolites of nitrate.

In a review of toxic effects of nitrate, Edwards et al. (2004) noted that nitrate can interfere with steroid hormone synthesis, can affect sperm motility and viability, can affect fecundity, or can be toxic to embryos.

Hrubec et al. (2002) indicate that nitrate may decrease immune response and induce hematological and biochemical changes. Nitrate interferes with hemoglobin in the bloodstream, reducing its ability to carry oxygen and causing tissue hypoxia and cyanosis. In vertebrate animals, nitrate toxicity is manifested as methemoglobinemia (Lewis and Morris 1986; Bruning-Fann and Kaneene 1993; Marco and Blaustein 1999), a disorder that involves oxidation of the iron on the hemoglobin molecule by nitrate. This disrupts the ability of hemoglobin to bind oxygen reversibly, reducing its oxygen-carrying capacity. An emerging body of data also indicates that nitrate may act as an endocrine disruptor in fishes and reptiles (Edwards 2005; Guillette and Edwards 2005), affecting various metabolic processes through disruption of hormone synthesis.

An increasing literature is developing on the toxicity of nitrate in the environment at concentrations typically measured in aquatic ecosystems. Much attention has been focused on amphibians due to the global decline in amphibian populations and biodiversity (Stuart et al. 2004; Whiles et al. 2006). Nitrogen enrichment has been implicated in some of these declines (Berger 1989; Rouse et al. 1999). Recent work has also focused on nitrate toxicity in Florida spring systems (Edwards et al. 2004).

Conventional toxicology typically deals with highly controlled laboratory studies on single species which derive the concentrations of toxicant that kill test organisms, usually expressed as an "LC_{50}" —the concentration that kills 50% of the test organisms over some defined period of time. This is termed acute toxicity. The standard in Florida law is the "96-hour LC_{50}"; the concentration that kills 50% of the test organisms over a 4-day period (Chapter 62-302.200, *Florida Administrative Code* [*F.A.C.*]). Chronic toxicity, concentrations of toxicant that don't necessarily kill the test organisms but result in other, sublethal detrimental effects, is also a subject of conventional toxicity studies, but the length of time needed to conduct these types of lab experiments generally precludes their being done, and various methods have been developed to attempt to extrapolate the results of acute testing to estimate chronic toxicity (Lee et al. 1995).

In evaluating the effects of toxicity from an ecological perspective, a distinction needs to be made between the toxicological effects of nitrate on individual organisms and its ecotoxicological effects on individual species and the populations, communities, and ecosystems of which they are a part. Ecotoxicology is concerned with acute and chronic toxicity effects on individual organisms within the context of how these in turn affect the structure and function of ecosystems (Relyea and Hoverman 2006). In particular, toxicants can have "density-mediated effects" on ecosystems through acute toxicity (killing organisms directly), or can have "trait-mediated effects" through chronic, sublethal effects on organism behavior, physiology, reproduction and development, or morphology (Releya and Hoverman 2006). Both density- and trait-mediated effects can have ecological ramifications, not only to the individual organism but also to the ecosystem of which it is a part, largely through food web interactions (Releya and Hoverman 2006; Whiles et al. 2006). It is the trait-mediated effects of nitrate toxicity on aquatic fauna that deserve careful attention, since these are the effects most likely to be seen at existing ambient levels of nitrate in spring systems.

SUMMARY OF LITERATURE ON NITRATE TOXICITY IN AQUATIC ECOSYSTEMS

Literature searches were conducted using several search engines available on the Internet. The Web of Science/ Science Citation Index, the U.S. Environmental Protection Agency on-line library database, AGRICOLA (U.S. Department of Agriculture National Agriculture Library), INFOMINE internet resource search, the BioOne database, and the contents of the libraries of the University of Florida and the St. Johns River Water Management District, as well as the cited sections of other papers listed in this report, were searched for this review. Searches used the terms "nitrate" and "toxicity." Additional searches were conducted with these two terms paired with "fish" or "fishes," "amphibians," "invertebrates," "birds" and "mammals." This literature review focused specifically on papers dealing with toxicity of nitrate on aquatic animals.

Seventy-nine papers and reports were found dealing with toxicity of nitrate and/ or nitrite compounds to aquatic life. All sources are listed in the literature citation and bibliography section herein. This review included nitrite since this species of dissolved nitrogen is closely related to nitrate and could cause toxicity if water quality conditions allow for its accumulation. Of the papers reviewed, 64 reported actual toxicity concentrations from laboratory studies or review of the literature. The other 15 publications dealt with a range of subjects, including physiological effects of nitrogen compounds (Bruning-Fann and Kaneene 1993; Crow et al. 2001), statistical methodology to estimate chronic toxicity from acute toxicity data (Mayer et al. 1992; Lee et al. 1995), and general overviews of the effects of fertilizers and other nitrogen compounds in the landscape (Berger 1989; Rouse et al. 1999).

Of the papers and reports that provided actual toxicity numbers, the bulk of these (76%) dealt with vertebrates (Figure 1), primarily fishes (37% of the papers) and amphibians (38%). Only one paper dealt with reptiles; none addressed nitrate toxicity to aquatic birds or mammals. The remainder of the studies examined aquatic invertebrates, including crustaceans, insects, and mollusks. Summaries of the toxicity data are presented within tables in the Appendix. These show nitrate toxicity values only (not nitrite).

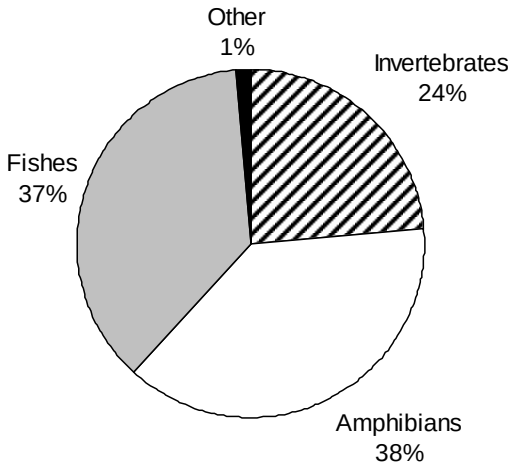

Figure 1. Proportions of taxa studies/reported in the literature providing actual nitrogen/nitrate or nitrite toxicity values

RESULTS OF LITERATURE REVIEW

Invertebrate Toxicity Studies

Scott and Crunkilton (2000) examined toxicity of nitrate to two planktonic crustaceans, the cladocerans *Ceriodaphnia dubia* and *Daphnia magna*. Acute toxicity was evaluated in test concentrations of 150 to 2,500 mg/ L NO_3-N. Median LC_{50} values for *C. dubia* were 374 mg/ L NO_3-N and 323-611 mg/ L NO_3-N for *D. magna*. Chronic toxicity was evaluated by measuring neonate

production in these two cladocerans. The "no observed effect concentration" (NOEC) ranged from 7.1 to 56.5 mg/ L NO_3-N for *C. dubia* and 358 mg/ L for *D. magna*. The "lowest observed effect concentration" (LOEC) ranged from 14.1 to 113 mg/ L for *C. dubia* and was 717 mg/ L for *D. magna*. In a study using amphipod crustaceans, Camargo et al. (2005) reported on the results of toxicity tests with *Eulimnogammarus toletanus* and *Echinogammarus echinosetosus* and determined short-term safe levels (expressed as 120-hr $LC_{0.01}$) of 4.4 mg/ L NO_3-N and 2.8 mg/ L NO_3-N, respectively. Camargo et al. (2005) suggested a maximum level of 2 mg/ L NO_3-N to protect the most sensitive freshwater species.

Armstrong (1979) summarized his work and the literature on toxicity of ammonia, nitrite, and nitrate to a variety of crustaceans, including cladocerans, shrimp, and lobsters. Armstrong notes that nitrate is the least toxic of the three nitrogen compounds. Lethal (acute toxicity) concentrations of nitrate ranged from 160 to 3,400 mg/ L NO_3-N for juvenile and larval shrimp (*Macrobrachium*, *Penaeus*, and *Palaemonetes* spp.). Sublethal (chronic) effect concentrations of nitrate (affecting growth rates or time of larval development) were 174–450 mg/ L NO_3-N for these taxa. Armstrong notes that nitrate toxicity more closely approximates the toxicity of nitrite and ammonia when expressed as molarity.

Jensen (1996) found that the crayfish *Astacus astacus* actively accumulates nitrite by way of the same mechanism used in chloride uptake. Nitrate, however, passively accumulates via simple diffusion across permeable membranes. Meade and Watts (1995) found no toxic effects of nitrate on the crayfish *Cherax quadricarinatus*. Within crayfish, blood nitrite appears to be detoxified to nitrate (Jensen 1996). Jensen (1996) believed the main physiological effects of nitrate/ nitrite on crayfish would be on osmoregulation and ionic balance. However, "methemoglobinemia-like" symptoms may also occur. Oxygen transport in crustacean blood is based on haemocyanin. Meade and Watts (1995) indicated that effects of nitrite on oxygen consumption by the crayfish *C. quadricarinatus* were due to formation of methemocyanin. Tahon et al. (1988) showed that nitrite converted haemocyanin to deoxyheamocyanin. Cheng and Chen (1999) showed that oxygen affinity of hemocyanin may decrease with nitrite exposure, especially at low pH. The effect of nitrite on hemocyanin appears to be less than the effect on hemoglobin. The hemocyanin condition would not technically be called methemoglobinemia, but it is the haemocyanin equivalent, indicating that oxygen transport can be affected in animals with hemocyanin.

In a study using aquatic insects, Camargo and Ward (1992) conducted short-term acute toxicity tests of sodium nitrate on early and last instar larvae of the caddisflies *Cheumatopsyche pettiti* and *Hydropsyche occidentalis*. Animals were exposed for 5 days and LC_{50} values reported for 72-, 96-, and 120 hours. They used conductivity controls (with sodium chloride) to demonstrate that mortality was fundamentally due to the nitrate ions, not conductivity. The 120-hour LC_{50} values for early and last instar larvae of *C. pettiti* were 106.5 mg/ L and 119.0 mg/ L, respectively. For *H. occidentalis* early and last instar larvae had LC_{50} values of 65.5 and 77.2 mg/ L, respectively. LC_{50} values for 72- and 96 hours are also reported in the paper; these values are higher than for the 120 hours. Larvae of *H. occidentalis* appear significantly ($P > 0.05$) more sensitive to nitrate toxicity than larvae of *C. pettiti*. In a separate study, Camargo et al. (2005) report a "safe level" (defined as the 120-hour $LC_{0.01}$)

of 11.9 mg/ L NO_3-N for the caddisfly *Hydropsyche exocellata*.

In a subsequent study, Camargo and Ward (1995) used a multifactor probit analysis of the results of their prior study (Camargo and Ward 1992) to evaluate long-term effects of nitrate (over the course of a year). They estimated "safe concentrations" (SCs) of nitrate for larvae of the caddisflies *C. pettiti* and *H. occidentalis*. They noted that SC is similar to the "no observed effect concentration" (NOEC) of traditional toxicity tests. Early instar larvae were more sensitive to nitrate than last instar stages, in that they exhibited higher mortality in response to nitrate. Mean SCs (expressed as 8760-hour [1-year] $LC_{0.01}$) were 2.4 mg/ L for early instar *C. pettiti* and 1.4 mg/ L for *H. occidentalis*. Mean SCs for last instar larvae of these two insects were 3.5 and 2.2 mg/ L NO_3-N. From their analyses, Camargo and Ward concluded that benthic invertebrates may be more sensitive to nitrate toxicity than fish.

Beketov (2004) compared the toxic effects of nitrogen compounds on six species of mayflies found in Siberian streams using a mixture of ammonia, nitrite, and nitrate. Beketov found that the species differ significantly in their tolerance to nitrogen compounds. Furthermore, species with higher tolerance levels were found in a larger number of water bodies and their habitat included comparatively polluted ones. Beketov (2004) believed his data to suggest that the mixture of the three compounds was additively more toxic to the mayfly *Ephemerella lenoki* than any one of them individually; however, he quotes an earlier study which found antagonistic as well as additive effects of different nitrogen compounds.

Alonso and Camargo (2003) determined a 96-hour $LC_{0.01}$ value of 195 mg/ L NO_3-N for the aquatic hydrobiid snail *Potamopyrgus antipodarum*, indicating that this species is relatively tolerant to nitrate toxicity. Armstrong (1979) stated that mollusks appeared to be least sensitive to toxicity from ammonia (compared to crustaceans and fish), but that there were few data to evaluate relative toxicity to nitrate. That situation appears to be the same to date.

Younger life stages of invertebrates appear to be more sensitive to nitrate toxicity effects. As noted above, Camargo and Ward (1995) showed that early instar caddisfly larvae were more sensitive to the toxic effects of nitrate than late instar larvae. Armstrong (1979) found that larvae of the shrimp *Macrobrachium rosenbergii* were three times more sensitive to nitrite than juveniles in terms of acute toxicity, and the sensitivity of larvae was even greater (eight times) when sublethal effects were considered. This may also apply to nitrate toxicity. Muir et al. (1991) found that protozoea of the shrimp *Penaeus monodon* exhibited "significant mortality" at 1 mg/ L NO_3. This equates to a concentration of 0.23 mg/ L NO_3-N. Increased salinity appears to reduce the toxic effects of nitrite and possibly nitrate to invertebrates (Chen and Lin 1991)

Summary of Invertebrate Toxicity

A wide range of nitrate toxicity values (acute or chronic) is reported for invertebrates, from a low of 0.23 mg/ L to a high of 3,400 mg/ L NO_3-N. Lowest toxicity numbers for adult invertebrates were levels of 2.8–4.4 mg/ L NO_3-N for two species of amphipods (Camargo et al. 2005); these were for chronic toxicity effects. In general, freshwater invertebrates appear to be more sensitive to the toxic effects of nitrate than marine invertebrates (Camargo et al. 2005). Many of the reported values in the lower range have greater effect on larval phases, including 0.23 mg/ L NO_3-N for shrimp larvae (Muir et al. 1991) and 1.4–2.4 mg/ L NO_3-N for early instar caddisfly larvae

(Camargo and Ward 1995). From this information, it can be surmised that nitrate toxicity may have its most severe ecotoxicological effect on invertebrate growth and development rates, due to greater effects on larval phases.

Fish Toxicity Studies

Armstrong (1979), Russo (1985), Edwards, et al. (2004) and Camargo et al. (2005) reviewed literature on nitrate toxicity effects on fish. Several studies experimentally evaluated nitrate toxicity on various fishes, using test values ranging from 50 mg/ L up to over 1,000 mg/ L NO_3-N.

Lewis and Morris (1986) and Russo and Thurston (1977) reviewed aspects of the toxicity of nitrite to fish. Under conditions of low dissolved oxygen (DO) and/ or high pH, higher levels of nitrite may occur than found under normal, more aerobic conditions. Nitrate may also be converted to nitrite in the body of fish after absorption (Armstrong 1979; Edwards et al. 2004). Lewis and Morris (1986) regarded channel catfish (*Ictalurus punctatus*) as a "sensitive" species to nitrite (NO_2) toxicity, although black bullhead (another catfish) was not as sensitive. Centrarchids, such as largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*), were considered by them to be "insensitive taxa." The range of values for bluegill, however, was reported to be as low as 2.4 mg/ L NO_2-N. It is not known if nitrate toxicity is proportional to nitrite toxicity (i.e., species more sensitive to nitrite are also more sensitive to nitrate), but this would be worth investigation.

Acute toxicity tests with fathead minnow found a median 96-hr LC_{50} of 1,341 mg/ L NO_3-N (Scott and Crunkilton 2000). Reported LC_{50} values for several fish species were in the 1,000–2,000 mg/ L range. Colt and Tchobanoglous (1976) report a 96-h LC_{50} for channel catfish fingerlings as 1,400 mg/ L NO_3-N. Knepp and Arkin (1973) observed a nitrate tolerance of channel catfish and largemouth bass of 400 mg/ L after 160 days of exposure. Tomasso and Carmichael (1986) studied nitrate toxicity of Guadalupe bass (*Micropterus treculi)* fingerlings and reported a 96-h LC_{50} value of 1,261 mg/ L NO_3-N. Westin (1974) reports 96-h LC_{50} values for chinook salmon (*Oncorhynchus tshatscha)* and rainbow trout (*Ocorhynchus mykiss)* fingerlings as 1,310 mg/ L NO_3-N and 1,355 mg/ L NO_3-N, respectively. Most of these studies used fingerlings in the toxicity assays and earlier developmental stages could potentially be more sensitive to nitrate toxicity (see below).

Edwards, et al. (2004) and Edwards (2005) examined reproductive characteristics of male and female mosquito fish (*Gambusia holbrooki*) that were collected from eight north Florida springs having nitrate concentrations of 0.23–4.52 mg/ L NO_3-N.

Reproductive characteristics examined were

Males: body size, gonadosomatic index (GSI), hepatosomatic index (HSI), tissue androgen levels, sperm counts and viability, gonopodium length, and testicular histology

Females: body size, GSI, HSI, tissue estradiol levels, fecundity (number of embryos/ female), mean embryo weight

In springs with nitrate levels $\geq$ 4 mg/ L NO_3-N, adult male mosquito fish exhibited testicular hypertrophy, reduced levels of one androgen (11-ketotestosterone), and reduced total sperm counts per sperm packet. Similarly, among females, nitrate exposure (>4 mg/ L NO_3-N) was related to low dry weight of developing *Gambusia*

embryos and decreased rate of reproductive activity among mature females. This indicated what is termed "reproductive disturbance" among mosquito fish when nitrate levels exceed a 4 mg/ L NO_3-N threshold.

Hrubec et al. (2002) found that prolonged exposure of hybrid striped bass to nitrate levels of 200 mg/ L NO_3-N resulted in decreased immune response, hematologic and biochemical changes, and increased mortality. By using different salts of nitrate (sodium, calcium, and potassium nitrate) and sodium bicarbonate as a conductivity control, Hrubec et al. believed their results suggested the responses observed were due to a pathological response to nitrate, not osmotic or other stress. In general, more studies examining sublethal physiological responses, such as those conducted by Hrubec et al. (2002) and Edwards et al. (2004), are needed to evaluate chronic nitrate toxicity to fishes.

Edwards et al. (2004) noted that one study of salmonids found larval mortality at nitrate concentrations of 2.3–7.6 mg/ L, and their studies of mosquito fish in Florida springs found adverse effects on embryos within this range. Kincheloe et al. (1979) examined toxicity of nitrate to the eggs and fry of several taxa of salmonids. They used sodium nitrate concentrations of 0–20 mg/ L, which equated to effective NO_3-N concentrations of 0–4.5 mg/ L. Statistically significant increased egg mortality was seen at a threshold NO_3-N concentration of 1.1 mg/ L, depending upon the species of fish. McGurk et al. (2006) found that NO_3-N concentrations of 1.6 mg/ L NO_3-N resulted in sublethal effects (delayed hatching and development and reduced wet weight) on swim-up fry of lake trout (*Salvelinus namaycush*); McGurk et al. believed this constituted a NOEC for this fish taxon.

Summary of Fish Toxicity

A wide range of nitrate toxicity values for fishes is reported in the literature. High values range up to >1,000 mg/ L NO_3-N. Lowest values (for sublethal effects) are around 4 mg/ L NO_3-N (Edwards et al. 2004). Although Armstrong (1979) indicates that fish may be more sensitive to nitrate toxicity than invertebrates, the greater number of invertebrate studies with lower acute or chronic toxicity thresholds would suggest that fish are less sensitive. As seen with invertebrates, serious consideration needs to be given to potential nitrate toxicity impacts on more sensitive, younger life stages such as eggs, embryos and fry. Although the study by Kincheloe et al. (1979) was excluded from the data set used by Canada to develop nitrate water quality guidelines (McGurk et al. 2006), their results, along with the others reported here, indicate that impacts on eggs, embryos, and/ or fry are a concern and need additional investigation. Increased salinity may buffer the toxic effects of nitrate and nitrite on fish (Tomasso 1994; Russo et al. 1981).

Amphibian Toxicity Studies

Amphibians are often promoted as excellent "sentinel" species to examine the ecological effects of toxicants (Murphy et al., 2000). Studies have been conducted on amphibians for two main reasons: the physiological importance of their skin in respiration and hydration and their complex life history, encompassing both aquatic and terrestrial habitats. Edwards et al. (2004) reviewed a number of studies reported in the literature. Negative effects included behavioral abnormalities (decreased activity, reduced feeding, disequilibrium), deformities, mortality, and altered timing of metamorphosis. Edwards et al. (2004) concluded from their literature survey and their own work that nitrate

must be considered a possible cause of amphibian deformities and has the potential to be an endocrine disrupter. Many of the field studies reviewed cite confounding factors such as habitat destruction and introduced species that complicate isolation of nitrate toxicity impacts to amphibian populations in the field. Nonetheless, nitrate toxicity is a factor that should be considered in amphibian conservation.

In addition to literature surveys, Edwards et al. (2004) conducted laboratory studies on effects of nitrate in filtered water and unfiltered spring water on development and metamorphosis of the Southern toad (*Bufo terrestris*). Egg clutches were obtained and raised to metamorphosis (emergence of forelimbs). Two sets of this experiment were conducted: one using filtered water treated by reverse osmosis (RO) and another in unfiltered spring water. Nitrate levels of 0–30 mg/ L NO_3-N were added to these two water types. Tadpoles raised at nitrate levels of 30 mg/ L took less time to reach metamorphosis in the filtered (RO) water, but more time (about 6 days longer than controls) in spring water with nitrate added. Edwards et al. (2004) believed this indicated that the source of water in laboratory experiments needs to be a consideration, with "natural" water from the ecosystem being tested and possibly being preferred. Tadpoles exposed to concentrations of nitrate that fluctuated between 0 and 30 mg/ L NO_3-N had reduced levels of the hormone thyroxine, which governs the timing of metamorphosis.

Langan (2003) also examined the effects of nitrate on growth and development of *B. terrestris* tadpoles. Langan used solutions of sodium nitrate from 5 to 30 mg/ L, which equaled effective NO_3-N concentrations of 0.824–4.944 mg/ L. No statistically significant effects on growth were found, but tadpoles raised at the highest nitrate level exhibited significantly shorter time to metamorphosis. Langan surmised that this might be due to stress effects at the higher nitrate concentration, with the tadpoles developing faster in order to escape from the nitrate-enriched water. Vaala et al. (2004) found no significant acute toxicity effects of nitrate on tadpoles of gray treefrog (*Hyla versicolor*).

Laboratory studies showed that significantly fewer *Litoria aurea* tadpoles survived to metamorphosis in 10 and 15 mg/ L ammonium nitrate during 150-, 91-, and 21-day experiments, while there were no effects on the survivorship of *Crinia signifera* and *Limnodynastes peronii* (Hamer et al. 2004). Smith et al. (2003) studied the effects of nitrate concentrations between 0–20 mg/ L NO_3-N on the tadpoles of *Rana catesbeiana* and *R. clamitans* during a 15-day laboratory experiment. Survival of both species was negatively affected at the highest NO_3-N concentration (20 mg/ L NO_3-N), with *R. clamitans* being more strongly affected. Nitrate concentrations also had an effect on the weight of *R. catesbeiana*, with tadpoles exposed to intermediate concentrations (5 mg/ L) being the heaviest. In this study, lethal effects of nitrate first appeared after 8 days of exposure in both species, which suggests that lethal effects might require chronic exposure at levels of 0-20 mg/ L NO_3-N. Based on this observation, the commonly used 96-hour tests may underestimate the lethal effects of nitrate.

Schuytema and Nebeker (1999a) studied the effects of ammonium and nitrate compounds on embryos of *Pseudacris regilla* (Pacific treefrog) and *Xenopus laevis* (African clawed frog). The sodium nitrate LC_{50} for *P. regilla* exposed for 10 days was 578.0 mg/ L NO_3-N and for *X. laevis* the LC_{50} after 5 days of exposure was 438.4 mg/ L. The lowest observed adverse effect level (LOAEL) and no observed adverse effect level (NOAEL)

for *P. regilla* were 111.0 and 56.7 mg/ L NO_3-N, respectively. LOAEL for *X. laevis* ranged from 56.7– >470.4 mg/ L NO_3-N and NOAEL ranged from 24.8– >470.4 mg/ L NO_3-N.

In a separate study, Schuytema and Nebeker (1999b) investigated the effects of ammonium nitrate, sodium nitrate, and urea on *Rana aurora* (Red-legged frogs), *Pseudacris regilla* (Pacific treefrog) and *Xenopus laevis* (African clawed frog). Study results indicated that ammonium ion is more toxic and the primary cause of mortality in ammonium nitrate exposure. For toxicity of sodium nitrate, the LOAEL and NOAEL for *R. aurora* were <29.1 mg/ L NO_3-N for length, and 235.0 and 116.8 mg/ L NO_3-N, respectively, for weight. The exposure time in this experiment was 16 days.

Hecnar (1995) examined the effects of nitrate (from ammonium nitrate) at concentrations of 2.5 to 10 mg/ L on growth and development of tadpoles of *Bufo americanus*, *Pseudacris triseriata*, *Rana pipiens* and *R. clamitans* from the Ontario region. Statistically significant effects on metamorphosis and survivorship were seen in the 5 mg/ L and 10 mg/ L treatments (reduced survivorship and lower rate of metamorphosis). Behavioral differences (reduced swimming and feeding activity) were noted in all treatment concentrations down to 2.5 mg/ L. Decreased swimming activity, paralysis, and deformities (bent tails) were noted with increasing time of exposure at most concentrations. In contrast to what Schuytema and Nebeker (1999a) stated about the results, Hecnar believed the effects he observed were due to the nitrate ion, not ammonium.

Summary of Amphibian Toxicity

Amphibian species vary in their sensitivity towards nitrate toxicity and, as with fishes, a wide range of toxicity values is evident in the literature. The form of nitrogen compound used may be important in assessing toxicity effects, since results of studies using ammonium nitrate may be due in part to ammonia toxicity as well as toxic effects of nitrate. Chronic, sublethal effects may not be detectable until past the 96-hour time limit used in standard acute toxicity assays.

Other Aquatic Fauna Toxicity Studies

This literature review found only one study of nitrate toxicity on another group of aquatic animal, reptiles (Guillette and Edwards 2005), and no nitrate toxicity studies on aquatic birds or mammals. Toxicity to birds and mammals occurs through ingestion of contaminated water, and the literature generally indicates that effects on mammals are not observed until NO_3-N levels of 20–40 mg/ L are reached. Guillette and Edwards (2005) evaluated effects of nitrate levels in juvenile alligators from seven central Florida lakes. They found that plasma testosterone levels in both male and female juvenile alligators were negatively correlated with lake Total N and nitrate-N levels. In female juveniles, a significant positive correlation was found between plasma E2 (estradiol) and lake nitrate concentrations between 0.01–0.04 mg/ L NO_3-N. (Guillette and Edwards, 2005). This analysis was conducted retrospectively by using data collected from a different study that had objectives other than determining nitrate toxicity. These findings should be regarded as preliminary until more definitive cause-and-effect studies are done.

Nitrate Toxicity and Interactions With Other Toxicants

An additional consideration is the interaction of nitrate with other compounds, including other dissolved

nitrogen compounds (ammonia and nitrite) and other toxicants (pesticides). Most studies conducted on this phenomenon indicate that nitrate may be more toxic in combination with other toxicants. As noted above, Beketov (2004) believed nitrate may be more toxic to mayfly nymphs in combination with nitrite and ammonia than the toxicity of nitrate alone. Orton et al. (2006) found that nitrate in combination with the herbicide atrazine had greater chronic toxicity (altered sex ratios and histological changes) on tadpoles of *R. pipiens* than either compound alone. In contrast, Allran and Karasov (2000) found no synergistic or additive interactions of atrazine and nitrate on tadpoles of this frog. Sullivan and Spence (2003) found that atrazine interacted synergistically with nitrate at 37 mg/ L NO_3 (8.32 mg/ L NO_3-N) for tadpoles of the African clawed frog to result in reduced tadpole size at metamorphosis (based on lower snout-vent length than expected in controls). Boone et al. (2005) found that nitrate in combination with the insecticide carbaryl had negative effects (reductions in mass and development time) on tadpoles of *R. clamitans*., compared to positive effects (higher mass and development time) by each compound individually. Allran and Karasov (2000) believed some of these effects were exerted through negative effects on cladoceran grazers of the algae, also fed upon by tadpoles, in combination with behavioral and physiological effects on the tadpoles. Hatch and Blaustein (2003) found that nitrate in combination with UV-B radiation together reduced survival or mass of tadpoles of *Hyla regilla*, while Blaustein et al. (2001) suggested that UV-B in combination with nitrate fertilizers may negatively affect development of the salamander *Ambystoma macrodactylum*. In summary, the preponderance of literature reviewed indicates that nitrate toxicity may be increased in combination with exposure to other stressors, such as other toxicants, UV radiation, or potentially parasites.

Boone et al. (2005) and Schuytema and Nebeker (1999a) believed that toxic responses to ammonium nitrate in amphibians were largely due to ammonium ion, rather than nitrate ion. Total ammonia is typically reported from ambient water quality sampling (Russo 1985), which consists of both ammonium (NH_4^+) and unionized ammonia (NH_3). The balance between these two is dependent upon temperature, pH, conductivity, and DO levels, with higher temperature and pH and lower DO generally favoring formation of more unionized ammonia, which is the form of ammonia toxic to aquatic life (Armstrong 1979; Russo 1985). Of the literature reviewed, none were found to indicate that ammonium had toxic properties. Hecnar (1995) believed that the acute and chronic effects of ammonium nitrate observed on amphibians were due to the nitrate ion, not the ammonia ion. However, Camargo et al. (2005) and Schuytema and Nebeker (1999a) believed that some of the effects they observed were due to unionized ammonia in test tanks, with this based on the reported experimental conditions (temperature and pH). Hrubec et al. (2002) used an assortment of nitrate salts and strongly suggested that the chronic toxicity effects seen in fish were due to the effects of the nitrate ion, not ammonia or other salts. Camargo and Ward (1992) used sodium nitrate and sodium chloride conductivity controls to show that the toxic effects observed in caddisfly larvae were due to the nitrate ion, not to conductivity impacts.

Nitrate appears to manifest toxicity through effects on the oxygen-carrying capacity of the blood, in both invertebrates and vertebrates (Camargo et al. 2005; Edwards et al. 2004; Cheng and Chen 1999; Tahon et al. 1988), although other physiological

effects may also be active (Hrubec et al. 2002; Guillette and Edwards 2005). The literature appears to clearly establish that nitrate is toxic to aquatic invertebrates, fish, and amphibians when they are exposed to concentrations high enough and/ or for a long enough time period (Camargo et al. 2005). Some of the literature reviewed herein suggests that nitrate toxicity may be manifest at the ambient concentrations seen in Florida spring systems; this is the important issue and question that needs to be addressed.

DISCUSSION AND IMPLICATIONS

Background nitrate levels in the Floridan aquifer and Florida springs have been reported to be as low as 0.05 mg/ L NO_3-N (Maddox et al. 1992; Scott et al. 2004) and, similarly, springs not impacted by nitrate enrichment from land use in their contributing areas exhibit nitrate levels $\leq$0.1 mg/ L NO_3-N (Wetland Solutions 2004; 2005; Mattson et al. 2006). Levels above 0.2 mg/ L NO_3-N are generally regarded as "enriched" due to human activities (Madison and Brunett 1985; Osburn et al. 2002; Toth and Fortich 2002). Numerous studies in Florida have shown nitrate enrichment in the groundwater of the Floridan aquifer in association with human development of the landscape (Toth et al. 1989; Katz et al. 1999; Phelps 2004) and that the enrichment is having an impact on Florida springs (Jones et al. 1996; Toth and Fortich 2002; Phelps 2004). This is compelling evidence that current nitrate levels in many Florida springs are elevated due to human activities. A number of efforts are now under way to evaluate how this nitrate is affecting springs and spring run ecosystems. Any efforts should consider and incorporate nitrate toxicity effects.

Nitrate Toxicity Relative to Existing Levels in Florida Spring Systems

Mattson et al. (2005) presented a summary of the lower nitrate toxicity thresholds reported in the literature for aquatic insects, amphibians, and fish, relative to ambient nitrate levels (as nitrate/ nitrite nitrogen or NO_3-N) over the past 22 years in the Wekiva River and Rock Springs Run in central Florida (Figure 2). Chronic toxicity levels for the lower caddisfly (aquatic insect) threshold were equaled or exceeded in both spring run streams over this period. Rock Springs Run exhibited the higher amount of exceedance, with 38% of the data exceeding the lower caddisfly chronic toxicity threshold (1.4 mg/ L NO_3-N). In the Wekiva River, 3% of the data exceeded this threshold.

Statewide, about 15% of Florida springs exceed an average nitrate level of 1.5 mg/ L NO_3-N (Wetland Solutions 2005). The median average statewide is 0.48 mg/ L NO_3-N, so well over half the springs in the state exceed a nitrate concentration (0.23 mg/ L NO_3-N) that was shown to be toxic to the larvae of shrimp (*Penaeus monodon*, Muir et al. 1991). A review summary of some of the lower toxicity thresholds is displayed in Figure 3 and includes recent (fall 2005) nitrate levels measured in selected major Florida springs of the Florida Geological Survey monitoring program.

Ecotoxicological Implications of Nitrate Toxicity in Florida Springs

If nitrate is having acute or chronic toxicological effects on the invertebrates, fish, and/ or amphibians living in Florida spring systems, this gives cause to wonder "What are the broader implications of this? How could this be affecting the ecological structure and function of these aquatic ecosystems?" Relyea and Hoverman (2006) emphasize that toxicological effects on

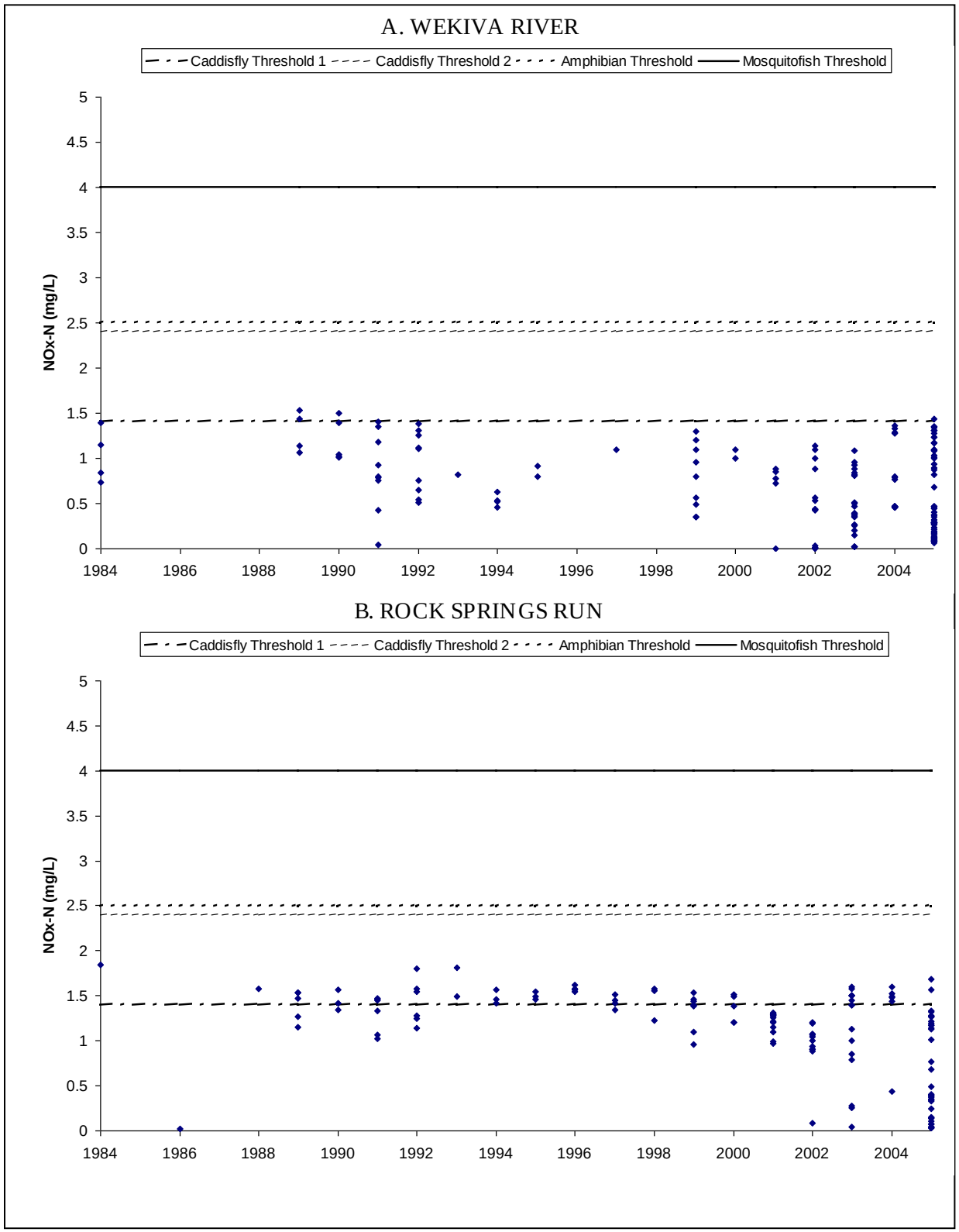

Figure 2. Nitrate levels over the past 22 years in the Wekiva River (A) and Rock Springs Run (B) and nitrate toxicity thresholds for aquatic insects, amphibians, and fish

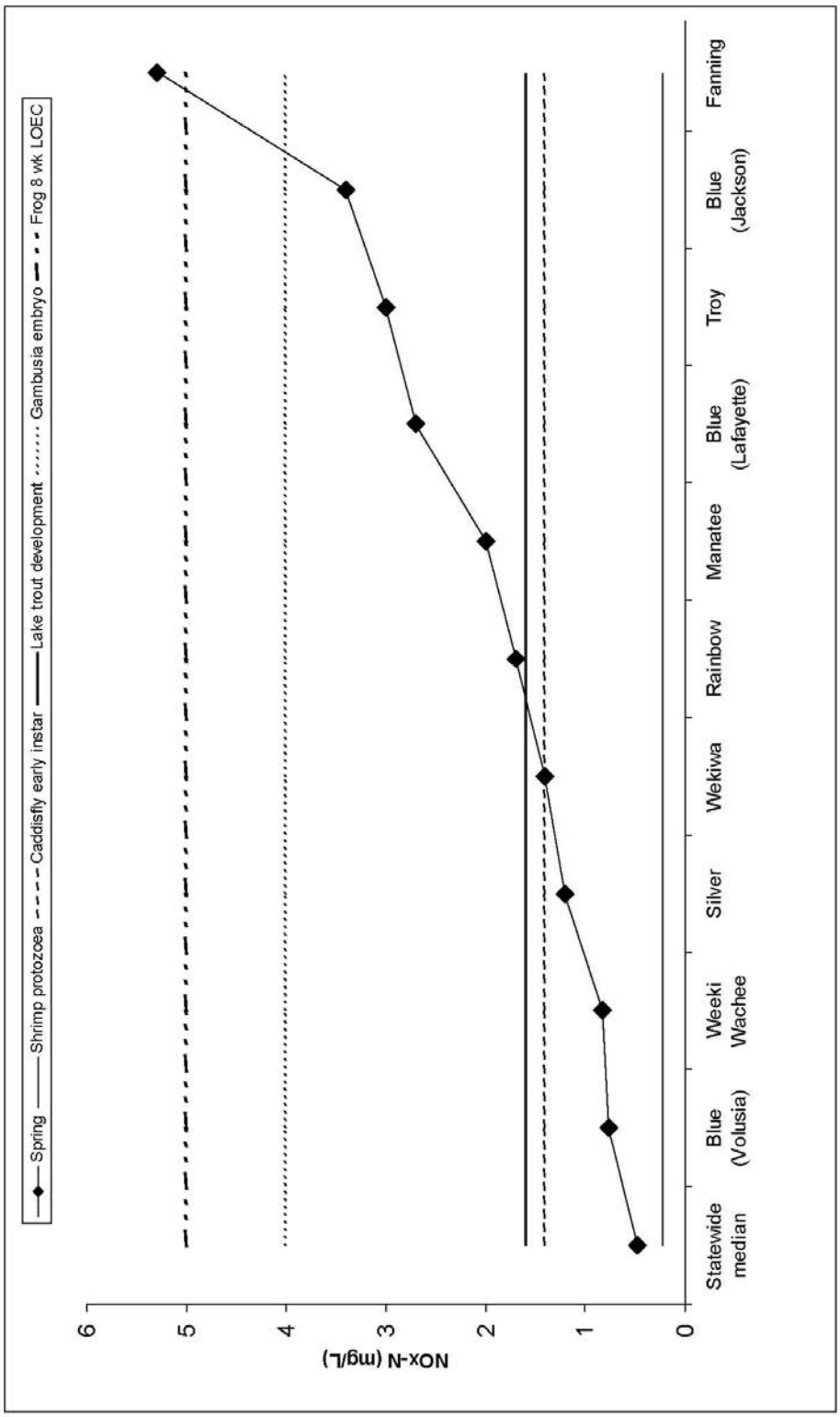

Figure 3. Selected nitrate thresholds from this literature review relative to the median for sampled springs in Florida (from Wetland Solutions 2005) and from fall 2005 levels measured in selected major Florida springs (Source: Florida Department of Environmental Protection data)

individual animals need to be ecologically interpreted in terms of impacts on food webs and species interactions. Nitrate toxicity in agricultural landscapes has been implicated in the decline of amphibian biodiversity in several areas (Berger 1989; Rouse et al. 1999). Hamer et al. (2004) found evidence that the range of *Litoria aurea* diminished in response to increased fertilizer loading into aquatic habitats, whereas *Crinia signifera* and *Limnodynastes peronii* showed no decline. Loss of one amphibian species is essentially equivalent (ecologically) to the loss of two species in the overall landscape (Whiles et al. 2006) because of their complex, biphasic life cycle. The aquatic (tadpole) phase is an important algal herbivore in aquatic food webs and prey for fish and invertebrate predators (Whiles et al. 2006), while the terrestrial adult is an important insect predator and prey for snakes and birds. Thus, loss of an amphibian species in the landscape could negatively impact both aquatic and terrestrial ecosystems through disruption of food web dynamics.

Nitrate levels as low as 2.8 mg/ L were chronically toxic to the gammarid amphipod *Echinogammarus echinosetosus* (Camargo et al. 2005). In Florida springs, the talitrid amphipod *Hyalella azteca* is an abundant, sometimes dominant, member of the benthic invertebrate community. This crustacean is an important food item in the diets of many fish and probably invertebrate predators (odonate nymphs, chironomids), and reductions in its abundance may affect food webs. Lobinske (1995) showed that larval biomass of chironomids in Rock Springs Run (with higher nitrate levels) was higher than biomass in Blackwater Creek (with lower nitrate levels); however, adult biomass (as emergence of adults) was similar in both streams. Lobinske speculated this difference might be due to higher larval mortality in the spring-fed Rock Springs Run. Since Camargo and Ward (1992; 1995) demonstrated that nitrate at levels seen in Rock Springs run may be chronically toxic to larval caddisflies (Figure 2), high nitrate in this stream may be a factor in chironomid larval mortality, reducing the emergence of adult chironomids, which are important food sources (both as larvae and adults) for many insectivorous animals. Thus, nitrate toxicity to invertebrates may have broader effects in spring system food webs.

Other food web-related effects in springs may result from interactions between grazers and primary producers, specifically periphytic and filamentous algae. A chief focus of the research on increased nitrate in Florida springs has to do with increased algal growth. This could be the result of either a “bottom up” effect (higher nutrients promoting algal growth), or a “top down” effect (reduced grazer abundance or activity allowing for more algal growth and biomass accumulation). Studies on some amphibians have shown reduced feeding rates (Edwards et al. 2004, Hecnar 1995), which could result in increased periphytic algal abundance on submersed aquatic vegetation. Increased mortality of invertebrate grazers could result in the same effect, although this could have positive effects on amphibians. As noted earlier, Boone et al. (2005) found positive effects of the insecticide carbaryl and nitrate separately on Green frog tadpoles (increased growth and mass) and negative effects on cladocerans in experimental mesocosms. Positive effects on tadpoles were attributed to the availability of higher periphyton biomass as food; however, Boone et al. could not separate the effects of increased nitrate from grazer effects. Watt and Oldham (1995) found that ammonium nitrate fertilizer (which was also used by Boone et al.) resulted in negative behavioral effects on *Daphnia* sp. (increased time spent at the water surface), which suggests that the results of Boone et al. (2005) could be

due to negative effects on cladoceran algal grazers. Separating bottom-up and top-down effects such as these would have to be done under controlled experiment conditions.

The literature appears to indicate that nitrate toxicity is increased in the younger, more sensitive life stages of many animal taxa. This has a variety of implications for systemwide ecological effects because of disruptions in life cycles (reproduction and development). Low concentrations of NO_3-N (0.23 mg/ L) were found to be acutely toxic to larvae of the shrimp *Penaeus monodon*. Shrimps of closely related genera are a primary target of the commercial shrimp fishery in Florida. This could have implications for the state's fishery, since shrimp use the low-salinity areas of estuaries as nursery habitat (Comp and Seaman 1985), where toxicity effects could be more likely (Chen and Lin 1991). Armstrong (1979) showed that larvae of the shrimp *Macrobrachium* were more sensitive to the toxic effects of nitrite; the same may be true for nitrate toxicity.

SUMMARY

1. Camargo et al. (2005) conducted a comprehensive review of much of the same literature reviewed here for nitrate toxicity to aquatic animals and compared this to their own work with aquatic invertebrates. In general, they note that nitrate toxicity increases with greater concentration and/ or time of exposure. Based on the work of Camargo et al. with invertebrates, freshwater animals appear to be more susceptible to nitrate toxicity than marine animals, and salinity (mainly the chloride ion) appears to ameliorate the toxic effects of nitrate (Chen and Lin 1991; Tomasso 1994). See the Appendix for tables summarizing the toxicity levels of nitrate on invertebrates, fish, and amphibians. These were primarily adapted from Camargo et al. (2005), with additions provided from this paper's review of the literature.

2. Nitrate appears to manifest its toxic effects by affecting the oxygen-carrying capacity of the blood after transformation to nitrite. It may also alter blood chemistry, affect immune response, and act as an endocrine disruptor, either as the nitrate anion or after transformation to another form of nitrogen.

3. Nitrate appears to be more toxic to younger stages (eggs, embryos, and fry), resulting in implications for the disruption in life cycles of aquatic animals.

4. Invertebrates appear to be more sensitive to nitrate toxicity effects, although this has not been as well studied as for fish and amphibians.

5. Over half the sampled springs in Florida exceed a nitrate level shown to be acutely toxic to protozoea larvae of shrimp (*Penaeus monodon)*. Many large (first magnitude) Florida springs have nitrate concentrations exceeding one or more of the lower chronic toxicity thresholds described in this review.

6. Nitrate toxicity that results in the loss of amphibian species may have a twofold impact on food webs due to their biphasic life cycle—aquatic larvae and terrestrial adults, thus affecting both aquatic and terrestrial ecosystems.

7. Nitrate toxicity may be amplified through interactions with other toxicants (pesticides) or through

interaction with other dissolved nitrogen compounds.

8. Nitrate toxicity could be a factor in the increased biomass of algae seen in nitrate-enriched springs due to negative effects on algal grazers in addition to the effects of nitrate in promoting increased algal growth and biomass accumulation.

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APPENDIX

Summary Tables of Nitrate Toxicity Concentrations on Aquatic Fauna

Table 1. Invertebrates
Table 2. Fish
Table 3. Amphibians

All tables are adapted from Camargo et al. (2005) and other sources, as defined within tables.

The 96-hour LC_{50} value is shown because that is the basis for the state standards for acute and chronic toxicity (Chapter 62-302.200, *Florida Administrative Code*,

[*F.A.C.*]). Acute toxicity is defined as one-third of the lowest 96-hr LC_{50} test result; chronic toxicity is defined as one-twentieth of the lowest 96-hr LC_{50} test result.

Other toxicity thresholds shown provide additional guidance on potential nitrate concentration criteria to consider for water quality management.

Appendix Table 1. Summary of acute and chronic nitrate toxicity thresholds for freshwater and selected marine** invertebrates. Most data from Camargo et al. (2005) and references therein. Other data from Armstrong (1979); Beketov (2004); and Watt and Oldham (1995).

Species	Life Stage	96-hour LC_{50} (mg/L NO_3-N)	Other Toxicity Threshold (mg/L NO_3-N)	Notes on "Other" Threshold
EPHEMEROPTERA				
Baetis vernus	Nymph		0.73	NO_3–N concentration in mixture of nitrate, nitrite, and ammonia
Potamanthus luteus	Nymph		0.70	NO_3–N concentration in mixture of nitrate, nitrite, and ammonia
Baetis fuscatus	Nymph		0.74	NO_3–N concentration in mixture of nitrate, nitrite, and ammonia
Cloeon bifidum	Nymph		0.72	NO_3–N concentration in mixture of nitrate, nitrite, and ammonia
Ephemerella lenoki	Nymph		0.74	NO_3–N concentration in mixture of nitrate, nitrite, and ammonia
Heptagenia sulphurea	Nymph		0.70	NO_3–N concentration in mixture of nitrate, nitrite, and ammonia
TRICHOPTERA				
Cheumatopsyche pettiti	Early instar larvae	113.5	2.4	"Safe Concentration" (defined as 8760 hr $LC_{0.01}$)
	Last instar larvae	165.5	3.5	"Safe Concentration" (defined as 8760 hr $LC_{0.01}$)
Hydropsyche exocellata	Last instar larvae	269.5	11.9	120 hr $LC_{0.01}$
H. occidentalis	Early instar larvae	97.3	1.4	"Safe Concentration" (defined as 8760 hr $LC_{0.01}$)
	Last instar larvae	109	2.2	"Safe Concentration" (defined as 8760 hr $LC_{0.01}$)
CLADOCERA				
Ceriodaphnia dubia	Neonates		7.1 - 56.5	Chronic toxicity NOEC for neonate production
Daphnia magna	Neonates		358	Chronic toxicity NOEC for neonate production
Daphnia sp.	Adults		34.8 - 87.1	Behavioral effects
AMPHIPODA				
Eulimnogammarus toletanus	Adults	85.0	4.4	120 hr $LC_{0.01}$
E. echinosetosus	Adults	62.5	2.8	120 hr $LC_{0.01}$
DECAPODA				
Cherax quadricarinatus	Juveniles		1,000	5 day NOAEL
Astacus astacus	Juveniles		14	7 day NOAEL
*Penaeus monodon***	Protozoea		0.23	Saltwater taxon that uses low-salinity estuarine areas: 31-37% mortality/40 hr exposure
Macrobrachium rosenbergii	Juveniles		160	21 day LC_{50}
	Juveniles		175	Growth reduced 50% over 3-5 weeks
GASTROPODA				
Potamopyrgus antipodarum	Adults	1,042	195	96-hr $LC_{0.01}$

Appendix Table 2. Summary of acute and chronic nitrate toxicity thresholds for freshwater fish. Most data from Camargo et al. (2005) and references therein. Other data from Edwards et al. (2004); Hrubec et al. (2002); McGurk et al. (2006); and Shimura et al. (2002).

Species	Life Stage	96-hour LC_{50} (mg/L NO_3-N)	Other Toxicity Threshold (mg/L NO_3-N)	Notes on "Other" Threshold
SALMONIDAE				
Oncorhynchus mykiss	Eggs		1.1	30 day LOEC and NOEC
	Fry		1.1 - 4.5	30 day NOEC
	Fry		2.3	30 day LOEC
	Fingerlings	1,355	14	8 day NOAEL
O. kisutch	Eggs		4.5	30 day NOEC
	Fry		4.5	30 day NOEC
O. tshawytscha	Eggs		4.5	30 day NOEC
	Fry		2.3 & 4.5	30 day NOEC and LOEC
	Fingerlings	1,310		
O. clarki	Eggs		2.3 & 4.5	30 day NOEC and LOEC
	Fry		4.5 & 7.6	30 day NOEC and LOEC
Salvelinus namaycush	Swim-up fry	1,121.4		
	Embryo		100	NOEC for survival to fry from embryo stage
	Alevin	2,342.5	400	NOEC for survival to alevin from embryo stage
	Embryo to Swim-up fry		1.6	Significantly delayed hatching and development (NOEC)
Coregonus clupeaformis	Swim-up fry	1,902.7		
	Embryo		25	NOEC for survival to fry from embryo stage
	Alevin	2,185.7	25	NOEC for survival to alevin from embryo stage
	Embryo to Swim-up fry		6.25	Significantly delayed development and reduced fry wet weight (NOEC)
MORONIDAE				
Morone sp. hybrid	Unknown Life stage (juveniles?)		200	Fish had changes in blood serum chemistry; altered immune responses, blindness after 3 wks; death after 6 wks
CENTRARCHIDAE				
Lepomis macrochirus	Fingerlings	1,975	761	24 hour LC_{50}
Micropterus treculi	Fingerlings	1,261		
ICTALURIDAE				
Ictalurus punctatus		1,355 - 1,423	90	164 day NOAEL
CYPRINIDAE				
Pimephales promelas	Larvae	1,010 - 1,607	358	7 day NOEC
Catla catla	Fry		1,484	24 hr LC_{50} in flow-through system

Appendix Table 2—*continued*

Species	Life Stage	96-hour LC_{50} (mg/L NO_3-N)	Other Toxicity Threshold (mg/L NO_3-N)	Notes on "Other" Threshold
POECILIDAE				
Gambusia affinis	Embryos		4	Reduced dry weight
	Adults		4	Alteration in endocrine and reproductive characteristics
Poecilia reticulatus	Fry	191		
ADRIANICHTHYIDAE				
Oryzias latipes	Spawning		30	Retardation of spawning and reduced egg production
	Juveniles		50	Reduced body weight

Appendix Table 3. Summary of acute and chronic nitrate toxicity thresholds for amphibians. Most data from Camargo et al. (2005) and references therein. Other data from Edwards et al. (2004); Langan (2003); Vaala et al. (2004); and Watt and Oldham (1995).

Species	Life Stage	96-hour LC_{50} (mg/L NO_3-N)	Other Toxicity Threshold (mg/L NO_3-N)	Notes on "Other" Threshold
FROGS & TOADS				
Bufonidae				
Bufo bufo	Tadpoles	384.8	22.6	30 day LOEC
B. americanus	Tadpoles	13.6 - 39.3		
	Fertilized eggs		9.0	NOAEL
B. terrestris	Tadpoles		30.0	Delayed time to metamorphosis in spring water
	Tadpoles		4.9	Shorter development time
Xenopus laevis	Embryos		24.8	5 day NOAEL
	Tadpoles	1,655.8	65.6	10 day NOAEL
Hylidae				
Hyla versicolor			20	No acute or chronic effects at highest level of exposure to sodium nitrate
Litoria caerulea	Tadpoles		22.7	58% mortality in 16 days of exposure
Pseudacris triseriata	Tadpoles	17	10.0	100 day LOEC and NOAEL
P. regilla	Embryos	643	56.7	10 day NOAEL
	Tadpoles	1,749.8	30.1	10 day NOAEL
Ranidae				
Rana pipiens	Tadpoles	22.96	10.0 & 30.0	100 day LOEC and NOAEL
R. clamitans	Tadpoles	32.4		
R. sylvatica	Fertilized eggs		9.0	NOAEL
R. pretiosa	Newly-hatched larvae		16.5	15 day LC_{50}
R. temporaria	Larvae		5	8 week LOEC/10 week NOEC
R. aurora	Embryos		29.0	16 day NOAEL
SALAMANDERS				
Ambystoma maculatum	Fertilized eggs		9.0	NOAEL
A. jeffersonianum	Fertilized eggs		9.0	NOAEL
A gracile	Newly-hatched larvae		23.4	15 day LC_{50}
Triturus vulgaris	Larvae		17.4	Reduced size at metamorphosis with at least 24 hr of exposure