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Review article

Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment

Julio A. Camargo [a,*], Álvaro Alonso [b]

[a] Departamento de Ecología, Edificio de Ciencias, Universidad de Alcalá, 28871 Alcalá de Henares (Madrid), Spain
[b] Laboratorio de Ecotoxicología, Departamento de Medio Ambiente, INIA, 28040 Madrid, Spain

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Abstract

We provide a global assessment, with detailed multi-scale data, of the ecological and toxicological effects generated by inorganic nitrogen pollution in aquatic ecosystems. Our synthesis of the published scientific literature shows three major environmental problems: (1) it can increase the concentration of hydrogen ions in freshwater ecosystems without much acid-neutralizing capacity, resulting in acidification of those systems; (2) it can stimulate or enhance the development, maintenance and proliferation of primary producers, resulting in eutrophication of aquatic ecosystems; (3) it can reach toxic levels that impair the ability of aquatic animals to survive, grow and reproduce. Inorganic nitrogen pollution of ground and surface waters can also induce adverse effects on human health and economy.

Because reductions in SO_2 emissions have reduced the atmospheric deposition of H_2SO_4 across large portions of North America and Europe, while emissions of NO_x have gone unchecked, HNO_3 is now playing an increasing role in the acidification of freshwater ecosystems. This acidification process has caused several adverse effects on primary and secondary producers, with significant biotic impoverishments, particularly concerning invertebrates and fishes, in many atmospherically acidified lakes and streams. The cultural eutrophication of freshwater, estuarine, and coastal marine ecosystems can cause ecological and toxicological effects that are either directly or indirectly related to the proliferation of primary producers. Extensive kills of both invertebrates and fishes are probably the most dramatic manifestation of hypoxia (or anoxia) in eutrophic and hypereutrophic aquatic ecosystems with low water turnover rates. The decline in dissolved oxygen concentrations can also promote the formation of reduced compounds, such as hydrogen sulphide, resulting in higher adverse (toxic) effects on aquatic animals. Additionally, the occurrence of toxic algae can significantly contribute to the extensive kills of aquatic animals. Cyanobacteria, dinoflagellates and diatoms appear to be major responsible that may be stimulated by inorganic nitrogen pollution. Among the different inorganic nitrogenous compounds (NH_4^+, NH_3, NO_2^-, HNO_2, NO_3^-) that aquatic animals can take up directly from the ambient water, unionized ammonia is the most toxic, while ammonium and nitrate ions are the least toxic. In general, seawater animals seem to be more tolerant to the toxicity of inorganic nitrogenous compounds than freshwater animals, probably because of the ameliorating effect of water salinity (sodium, chloride, calcium and other ions) on the tolerance of aquatic animals.

Ingested nitrites and nitrates from polluted drinking waters can induce methemoglobinemia in humans, particularly in young infants, by blocking the oxygen-carrying capacity of hemoglobin. Ingested nitrites and nitrates also have a potential role in developing cancers of the digestive tract through their contribution to the formation of nitrosamines. In addition, some scientific evidences suggest that ingested nitrites and nitrates might result in mutagenicity, teratogenicity and birth defects, contribute to the risks of non-Hodgkin's lymphoma and bladder and ovarian cancers, play a role in the etiology of insulin-dependent diabetes mellitus and in the development of thyroid hypertrophy, or cause spontaneous abortions and respiratory tract infections. Indirect health hazards can occur as a consequence of algal toxins, causing nausea, vomiting, diarrhoea, pneumonia, gastroenteritis, hepatoenteritis, muscular cramps, and several poisoning syndromes (paralytic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning). Other indirect health hazards can also come from the potential relationship between inorganic nitrogen pollution and human infectious diseases (malaria, cholera). Human sickness and death, extensive kills of aquatic animals, and other negative effects, can have elevated costs on human economy, with the recreation and tourism industry suffering the most important economic impacts, at least locally.

It is concluded that levels of total nitrogen lower than 0.5–1.0 mg TN/L could prevent aquatic ecosystems (excluding those ecosystems with naturally high N levels) from developing acidification and eutrophication, at least by inorganic nitrogen pollution. Those relatively low TN levels could also protect aquatic animals against the toxicity of inorganic nitrogenous compounds since, in the absence of eutrophication, surface waters

* Corresponding author. Fax: +34 91 885 49 29.
E-mail address: julio.camargo@uah.es (J.A. Camargo).

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usually present relatively high concentrations of dissolved oxygen, most inorganic reactive nitrogen being in the form of nitrate. Additionally, human health and economy would be safer from the adverse effects of inorganic nitrogen pollution.

Keywords: Inorganic nitrogen pollution; Aquatic ecosystems; Ecological and toxicological effects; Human risk; Global assessment

Contents

1. Introduction

Nitrogen is the most abundant chemical element of the Earth's atmosphere (almost 80%), and also one of the essential components of many key biomolecules (e.g., amino acids, nucleotides). It ranks fourth behind carbon, oxygen and hydrogen as the commonest chemical element in living tissues (Campbell, 1990). An increase in the environmental availability of inorganic nitrogen usually boosts life production, firstly increasing the abundance of primary producers. However, high levels of inorganic nitrogen that cannot be assimilated by the functioning of ecological systems (i.e., N saturated ecosystems) can cause adverse effects on the least tolerant organisms.

Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are the most common ionic (reactive) forms of dissolved inorganic nitrogen in aquatic ecosystems (Kinne, 1984; Howarth, 1988; Day et al., 1989; Wetzel, 2001; Rabalais, 2002). These ions can be present naturally as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen-rich geological deposits, N_2 fixation by certain prokaryotes (cyanobacteria with heterocysts, in particular), and biological degradation of organic matter (Kinne, 1984; Howarth, 1988; Day et al., 1989; 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) (Sharma and Ahlert, 1977; Wetzel, 2001). The nitrification process can even occur if levels of dissolved oxygen decline to a value as low as 1.0 mg O_2/L (Stumm and Morgan, 1996; Wetzel, 2001). NH_4^+, NO_2^- and NO_3^- may however be removed from water by macrophytes, algae and bacteria which assimilate them as sources of nitrogen (Howarth, 1988; Harper, 1992; Paerl, 1997; Wetzel, 2001; Dodds et al., 2002; Smith, 2003). Furthermore, in anaerobic waters and anoxic sediments, facultative anaerobic bacteria (e.g., *Achromobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas*) can utilize nitrite and nitrate as terminal acceptors of electrons, resulting in the ultimate formation of N_2O and N_2 (Austin, 1988; Stumm and Morgan, 1996; Wetzel, 2001; Paerl et al., 2002).

During the past two centuries, and especially over the last five decades, humans have substantially altered the global nitrogen cycle (as well as the global cycles of other chemical elements), increasing both the availability and the mobility of nitrogen over large regions of Earth (Vitousek et al., 1997; Carpenter et al., 1998; Howarth et al., 2000; Galloway and Cowling, 2002). Consequently, in addition to natural sources, inorganic nitrogen can enter aquatic ecosystems via point and nonpoint sources derived from human activities (Table 1). Nonpoint sources generally are of greater relevance than point sources since they are larger and more difficult to control (Howarth et al., 2000; National Research Council, 2000). Moreover, anthropogenic inputs of particulate nitrogen and organic nitrogen to the environment can also result in inorganic nitrogen pollution (National Research Council, 2000; Smil, 2001). Concentrations of inorganic nitrogenous compounds (NH_4^+, NO_2^-, NO_3^-) in ground and surface waters are hence increasing around the world, causing significant effects on many aquatic organisms and, ultimately, contributing to the degradation of freshwater, estuarine, and coastal marine ecosystems (Neilson and Cronin, 1981; Russo, 1985; Meybeck et al., 1989; Camargo, 1992; Gleick, 1993; Nixon, 1995; Paerl, 1997; Smith et al., 1999; Howarth et al., 2000; National Research Council, 2000; Smil, 2001; Anderson et al., 2002; Philips et al., 2002; Rabalais and

Table 1
Major anthropogenic sources of inorganic nitrogen in aquatic ecosystems (Vitousek et al., 1997; Carpenter et al., 1998; Howarth et al., 2000; National Research Council, 2000; Smil, 2001; Galloway and Cowling, 2002)

Point sources
– Wastewaters from livestock (cattle, pigs, chickens) farming
– N releases from aquaculture (fish, prawns, shrimps) operations
– Municipal sewage effluents (including effluents from sewage treatment plants that are not performing tertiary treatments)
– Industrial wastewater effluents
– Runoff and infiltration from waste disposal sites
– Runoff from operational mines, oil fields, and unsewered industrial sites
– Overflows of combined storm and sanitary sewers
Nonpoint sources
– Widespread cultivation of N_2-fixing crop species, and the subsequent N mobilization among terrestrial, aquatic and atmospheric realms
– Use of animal manure and inorganic N fertilizers, and the subsequent runoff from agriculture
– Runoff from burned forests and grasslands
– Runoff from N saturated forests and grasslands
– Urban runoff from unsewered and sewered areas
– Septic leachate and runoff from failed septic systems
– Runoff from construction sites and abandoned mines
– N loadings to ground waters and, subsequently, to receiving surface waters (rivers, lakes, estuaries, coastal zones)
– Emissions to the atmosphere of reduced (from volatilization of manure and fertilizers) and oxidized (from combustion of fossil fuels) N compounds, and the subsequent atmospheric (wet and dry) deposition over surface waters
– Other activities that can mobilize nitrogen (from long-term storage pools) such as biomass burning, land clearing and conversion, and wetland drainage

Nixon, 2002; Constable et al., 2003; Jensen, 2003; Smith, 2003; Camargo et al., 2005a).

Nevertheless, in spite of the current worldwide environmental concern, no study has provided a global assessment, with detailed multi-scale data, of the ecological and toxicological effects generated by inorganic nitrogen pollution in aquatic ecosystems. We have performed such a study, and our synthesis of the published scientific literature shows three major environmental problems: (1) inorganic nitrogen pollution can increase the concentration of hydrogen ions in freshwater ecosystems without much acid-neutralizing capacity, resulting in acidification of those ecological systems; (2) inorganic nitrogen pollution can stimulate or enhance the development, maintenance and proliferation of primary producers, resulting in eutrophication of freshwater, estuarine, and coastal marine ecosystems. In some cases, inorganic nitrogen pollution can also induce the occurrence of toxic algae; (3) inorganic nitrogen pollution can impair the ability of aquatic animals to survive, grow and reproduce as a result of direct toxicity of inorganic nitrogenous compounds. In addition, inorganic nitrogen pollution of ground and surface waters can induce adverse effects on human health and economy.

2. Acidification of freshwater ecosystems

Sulphur dioxide (SO_2), nitrogen dioxide (NO_2) and nitrogen oxide (NO) have been traditionally recognized as the major acidifying pollutants in lakes and streams (Schindler, 1988; Irwin, 1989; Mason, 1989; Baker et al., 1991). Once emitted into the atmosphere, these gaseous pollutants can undergo complex chemical reactions (see, for example, Mason, 1989), resulting in the formation of sulfuric acid (H_2SO_4) and nitric acid (HNO_3). The subsequent atmospheric (wet and dry) deposition of these acid compounds may increase not only the concentrations of SO_4^{2-} and NO_3^- in surface waters, but also the concentration of H^+ in freshwater environments without much acid-neutralizing capacity (i.e., with moderate or low alkalinity) (Schindler, 1988; Irwin, 1989; Mason, 1989; Baker et al., 1991). Moreover, a decrease in the pH value of water can increase the concentration of dissolved aluminium (Al^{3+}) and other trace metals (e.g., Cd, Cu, Pb, Zn) owing to enhanced metal mobilization and/or decreased metal sedimentation (Bache, 1986; Borg et al., 1989; Nelson and Campbell, 1991). Part of the dissolved aluminium can subsequently settle in the sediments of atmospherically acidified lakes and reservoirs, reducing orthophosphate availability and disrupting the P cycling in those water bodies (Ulrich and Pöthig, 2000; Kopáèek et al., 2001).

Reductions in SO_2 emissions throughout the 1980s and 1990s have reduced the atmospheric deposition of H_2SO_4 across large portions of North America and Europe, while emissions of NO_x have gone unchecked (Vitousek et al., 1997; Driscoll et al., 1998; Stoddard et al., 1999; Skjelkvale et al., 2001). In consequence, HNO_3 is now playing an increasing role in the acidification of freshwater ecosystems (Vitousek et al., 1997; Driscoll et al., 1998; Stoddard et al., 1999; Skjelkvale et al., 2001). Significant inputs of NH_4^+ can also contribute to the acidification process since ammonium nitrification produces hydrogen ions (Schuurkes and Mosello, 1988; Vitousek et al., 1997; Wetzel, 2001). Currently, eastern North America and northern and central Europe are major acidified regions on Earth that present lakes and streams with pH values usually ranging from 4.5 to 5.8 (Driscoll et al., 1998; Stoddard et al., 1999; Skjelkvale et al., 2001; Doka et al., 2003).

The anthropogenic acidification of lakes and streams can cause several adverse effects on primary and secondary producers (Table 2). Biotic impoverishments, particularly concerning invertebrates (crustaceans, gastropods, leeches) and fish (salmonids), have indeed been observed in many atmospherically acidified lakes and streams of North America and Europe during the last four decades (Krause-Dellin and Steinberg, 1986; Huckabee et al., 1989; Morris et al., 1989; Schindler et al., 1989; Fjellheim and Raddum, 1990; Meriläinen and Hynynen, 1990; Ormerod et al., 1990; Cummins, 1994; Allan, 1995). A pH range of 5.5–6.0 seems to be an important threshold below which damage to sensitive aquatic biota will remain a major local and regional environmental problem (Doka et al., 2003). The damage of anthropogenic acidification to sensitive aquatic biota may however be mitigated by high concentrations of calcium, sodium, potassium and chloride in the aquatic environment (Morris et al., 1989; Ormerod et al., 1990; Baker et al., 1991; Camargo and Ward, 1993; Cummins, 1994; Allan, 1995).

Key microbial processes for nutrient cycling and ecosystem functioning can also be inhibited or altered as a consequence of decreased pH values. NH_4^+ nitrification ceased at pH values below 5.6 in experimentally acidified Canadian lakes, with aquatic denitrification being stimulated (Rudd et al., 1988, 1990). N_2

Table 2
Adverse effects of anthropogenic acidification on freshwater plants and animals

Adverse effects	References
Depression of net photosynthesis in planktonic and attached algae	Schindler, 1988; Huckabee et al., 1989
Reduction of net productivity inplanktonic and attached algae	Schindler, 1988; Huckabee et al., 1989
Increased bioaccumulation of aluminium and other trace metals in aquatic (especially submerged) macrophytes	Sprenger and McIntosh, 1989
Increased abundance of filamentous green algae no longer attached to the substratum (metaphyton)	Schindler, 1988; Huckabee et al., 1989; Allan, 1995
Declined species diversity in phytoplankton and periphyton communities, with the loss of sensitive species	Schindler, 1988; Huckabee et al., 1989; Allan, 1995
Disruption of ionic regulation, especially loss of body sodium and failure to obtain sufficient calcium, in molluscs, insects, crustaceans, fish, and amphibians	Alabaster and Lloyd, 1982; Morris et al., 1989; Cummins, 1994
Respiratory and metabolic disturbances in molluscs, insects, crustaceans, fish, and amphibians	Alabaster and Lloyd, 1982; Morris et al., 1989; Cummins, 1994
Increased bioaccumulation and toxicity of aluminium and other trace metals in insects, crustaceans, fish, and amphibians	Morris et al., 1989; Spry and Wiener, 1991; Cummins, 1994
Arrested development of fish and amphibian embryos, presenting in some cases skeletal deformities	Alabaster and Lloyd, 1982; Morris et al., 1989; Cummins, 1994
Hatching delay of fish and amphibian eggs	Morris et al., 1989; Cummins, 1994
Disruption of molting and emergence in insects and crustaceans	Morris et al., 1989; Cummins, 1994
Reduced growth rates in cladocerans, fish, and amphibians	Morris et al., 1989; Cummins, 1994
Reduced efficiency or activity by grazing zooplankton (cladocerans), producing ramifying effects on the phytoplankton community	Locke, 1991; Cummins, 1994
Reduced efficiency or activity of prey capture by copepods, planarians, and fish, producing ramifying effects on prey populations and on populations of other predators	Morris et al., 1989; Locke, 1991; Camargo and Ward, 1992a; Cummins, 1994
Increased migration of aquatic insects (caddisfly larvae) from their retreat and capture nets	Camargo and Ward, 1993
Increased drift behaviour of benthic invertebrates to be transported downstream	Camargo and Ward, 1993; Cummins, 1994
Avoidance of acid spawning sites by insects, fish, and amphibians	Cummins, 1994; Doka et al., 2003
Declined species diversity in zooplankton, macrobenthic, fish, and amphibian communities	Schindler, 1988; Morris et al., 1989; Cummins, 1994; Doka et al., 2003

fixation ceased at pH values below 5.0 in the experimentally acidified Little Rock Lake (Canada), but no effect on aquatic nitrification was observed (Schindler et al., 1991). Cessation of ammonium nitrification in the atmospherically acidified Plesne Lake (Central Europe) led to an atypical situation in which the lake became a net source of NH_4^+ (Kopáèek et al., 2004). Leaf-litter decomposition decreased in atmospherically acidified woodland streams because of the negative effects on microbial decomposers (bacteria and fungi), with aquatic invertebrates (detritivore species, mainly) being affected due to changes in food quality and in food availability (Hildrew et al., 1984; Chamier, 1987; Groom and Hildrew, 1989; Griffith and Perry, 1994; Dangles et al., 2004). The rate of microbial decomposition could however be increased or even recovered, and the abundance of detritivores could also increase, after liming (Cummins, 1994; Allan, 1995; Dangles et al., 2004).

3. Eutrophication of aquatic ecosystems

Limitation of inorganic nitrogen characterizes large portions of the world´s coastal and estuarine environments, net primary production being mainly controlled by N inputs (Neilson and Cronin, 1981; Kinne, 1984; Howarth, 1988; Day et al., 1989; Nixon, 1995; Paerl, 1997; Howarth et al., 2000; Rabalais and Nixon, 2002). However, in estuaries and coastal marine ecosystems that are receiving high N inputs, phosphorus can become relatively more limiting as the N:P loading ratio tends to increase (Fisher et al., 1992; Vollenweider et al., 1992; Anderson et al., 2002; Turner, 2002). In freshwater environments, phosphorus has often been identified as the foremost limiting nutrient for algal growth (Schindler, 1977; Margalef, 1983; Harper, 1992; Reynolds, 1998; Biggs, 2000; Downing et al., 2001; Smith, 2003; Camargo et al., 2005b), though in many instances nitrogen can also play an important role in net primary production, especially in lakes and streams with low N:P loading ratios (Elser et al., 1990; Harper, 1992; Allan, 1995; Borchardt, 1996; Francouer, 2001; Wetzel, 2001; Dodds et al., 2002; Smith, 2003). Other nutrients, such as silicon and iron, also can significantly influence the growth and abundance of algae (e.g., diatoms) but, in general, to a lesser extent than N and P (Wetzel, 2001; Anderson et al., 2002; Smith, 2003). Freshwater and marine ecosystems might however move towards a higher incidence of Si limitation as a result of increased N and P loadings (Turner et al., 1998, 2003a,b).

Elevated concentrations of NH_4^+, NO_2^- and NO_3^-, derived from human activities, can therefore stimulate or enhance the development, maintenance and proliferation of primary producers (phytoplankton, benthic algae, macrophytes), contributing to the widespread phenomenon of the cultural (man-made) eutrophication of aquatic ecosystems (Howarth, 1988; Harper, 1992; Allan, 1995; Borchardt, 1996; Paerl, 1997; National Research Council, 2000; Wetzel, 2001; Anderson et al., 2002; Dodds et al., 2002; Rabalais and Nixon, 2002; Turner, 2002; Smith, 2003). In fact, over the last four decades, human activities are estimated to have increased N fluxes (mainly as nitrate) into the coastal waters of the northeastern USA by 6- to 8-fold, into the coastal waters of the Gulf of Mexico by 4- to 5-fold, and into the European rivers draining to the North Sea region by 6- to 20-fold, linking these increased N fluxes to increased numbers of algal blooms (Vitousek et al., 1997; Howarth et al., 2000; Rabalais et al., 2002; Smith, 2003).

Although the monitoring and assessment of cultural eutrophication has been traditionally based on phosphorus and chlorophyll-

Table 3
Ecological and toxicological effects of cultural eutrophication in aquatic ecosystems

Ecological and toxicological effects	References
Reductions in water column transparency and light availability	Wetzel, 2001; Rabalais and Nixon, 2002
Increased sedimentation of organic matter	Wetzel, 2001; Rabalais and Nixon, 2002
Decreased concentrations of dissolved oxygen (hypoxic or anoxic conditions) in bottom waters and sediments	Diaz and Rosenberg, 1995; Wetzel, 2001; Rabalais and Nixon, 2002
Formation of reduced (toxic) chemical compounds (e.g., H_2S) in bottom waters and sediments	Diaz and Rosenberg, 1995; Wetzel, 2001; Rabalais and Nixon, 2002
P releases from sediments that can reinforce eutrophication	Wetzel, 2001; Rabalais and Nixon, 2002
Increased biomass and productivity of phytoplankton	Wetzel, 2001; Rabalais and Nixon, 2002
Shifts in phytoplankton composition to bloom-forming species, some of which may be toxic (e.g., *Microcystis* cyanobacteria in fresh waters and *Alexandrium* dinoflagellates in coastal waters)	Paerl, 1997; Van Dolah, 2000; Wetzel, 2001; Anderson et al., 2002; Smith, 2003
Increased biomass, and changes in productivity and species composition, of freshwater periphyton, being usually favoured filamentous species (e.g., *Cladophora*) at the expense of other attached microalgae	Allan, 1995; Wetzel, 2001; Dodds et al., 2002; Camargo et al., 2005b
Increased biomass, and changes in productivity and species composition, of freshwater macrophytes, often with proliferation of duckweeds	Allan, 1995; Wetzel, 2001; Cirujano and Medina, 2002
Increased biomass and productivity, and shifts in species composition, of marine macroalgae, being usually favoured fast-growing seaweeds at the expense of sensitive seagrasses	Rabalais and Nixon, 2002; Smith, 2003
Losses of species diversity in phytoplankton, periphyton, macrophyte and macroalgae communities	Harper, 1992; Wetzel, 2001; Rabalais and Nixon, 2002; Smith, 2003
Increased biomass, and changes in productivity and species composition, of zooplankton, being usually favoured invertebrate grazers (e.g., *Daphnia*) at the expense of other trophic groups	Harper, 1992; Wetzel, 2001; Rabalais and Nixon, 2002; Smith, 2003
Changes in biomass, productivity and species composition of benthic invertebrates and fish, often with mass mortality events in sensitive populations and reductions in the area of suitable habitat for reproduction. Tolerant grazers (e.g., *Lymnaea*) may proliferate at the expense of other trophic groups	Day et al., 1989; Diaz and Rosenberg, 1995; Wetzel, 2001; Rabalais and Nixon, 2002; Smith, 2003; Camargo et al., 2005b
Losses of species diversity in zooplankton, benthic invertebrates and fish communities	Diaz and Rosenberg, 1995; Wetzel, 2001; Rabalais and Nixon, 2002; Smith, 2003
Reductions in the health and size of marine coral populations, often with large increases in cover and biomass of fleshy macroalgae (e.g., *Lobophora*)	Koop et al., 2001; Rabalais and Nixon, 2002
Losses of species diversity in marine coral communities	Koop et al., 2001; Rabalais and Nixon, 2002
Alterations in the food web structure of freshwater, estuarine, and coastal marine ecosystems, with ramifying effects on every trophic level	Day et al., 1989; Wetzel, 2001; Rabalais and Nixon, 2002; Camargo et al., 2005b

a concentrations (see, for example, OECD, 1982), recent nutrient criteria to prevent and manage eutrophication of aquatic ecosystems include both phosphorus and nitrogen, owing to the increasing link between nitrogen pollution and cultural eutrophication. Dodds et al. (1998) have suggested upper limits of total nitrogen and total phosphorus for eutrophic temperate lakes (1260 μg TN/L and 71 μg TP/L) and eutrophic temperate streams (1500 μg TN/L and 75 μg TP/L). The Swedish Environmental Protection Agency (2000) has considered that levels higher than 440 μg TN/L and 30 μg TP/L can result in eutrophication of coastal marine ecosystems. The US Environmental Protection Agency (2002) has recently published nutrient-criteria technical guidance manuals for freshwater and coastal marine ecosystems, taking into account two causal variables (TN and TP) and two response variables (algal biomass and water clarity). Those nutrient criteria however could ultimately depend on the TN:TP loading ratios, being able to induce multiple effects on the biomass and composition of phytoplankton (Turner, 2002; Smith, 2003).

Cultural eutrophication of freshwater, estuarine, and coastal marine ecosystems can cause ecological and toxicological effects that are either directly or indirectly related to the proliferation of primary producers (Table 3). This proliferation and the subsequent decomposition of organic matter usually lead to low dissolved oxygen concentrations in bottom waters and sediments of eutrophic and hypereutrophic aquatic ecosystems with low water turnover rates. Hypoxic (or anoxic) conditions indeed occur throughout the world in stratified rivers, lakes, reservoirs, estuaries and coastal waters that receive high loadings of nutrients from anthropogenic sources, being able to affect large areas as in the well-known cases of the Chesapeake Bay, the Baltic and Black Seas, and the northern Gulf of Mexico (Neilson and Cronin, 1981; Margalef, 1983; Day et al., 1989; Harper, 1992; Vollenweider et al., 1992; Diaz and Rosenberg, 1995; Wetzel, 2001; Anderson et al., 2002; Paerl et al., 2002; Rabalais et al., 2002; Smith, 2003).

Extensive kills of both invertebrates and fishes (sensitive benthic species, particularly) are probably the most dramatic manifestation of hypoxia (or anoxia) in eutrophic and hypereutrophic aquatic ecosystems, with significant reductions in the area of suitable habitat for food, growth and reproduction of these aquatic organisms (Diaz and Rosenberg, 1995; Wetzel, 2001; Anderson et al., 2002; Breitburg, 2002). The decline in dissolved oxygen concentrations can also promote the formation of reduced compounds, such as hydrogen sulphide (H_2S), resulting in higher adverse effects on aquatic animals (Diaz and Rosenberg, 1995; Wetzel, 2001; Breitburg, 2002). Hydrogen sulphide is a very toxic compound that can cause acute mortalities in aquatic animals at relatively low concentrations by affecting the nervous system (Ortiz et al., 1993). Additionally, mass occurrences of toxic algae,

currently a global phenomenon that appears to be favoured by nutrient pollution (Carmichael, 1997; Paerl and Whitall, 1999; Hitzfeld et al., 2000; Van Dolah, 2000; Chorus, 2001; Anderson et al., 2002; Landsberg, 2002; Smith, 2003), can significantly contribute to the extensive kills of aquatic animals.

4. Occurrence of toxic algae

Algae can cause toxicity to aquatic (and terrestrial) animals because of the synthesis of certain toxins (harmful metabolites). These toxins can remain inside algal cells (intracellular toxins), or they may be released into the surrounding water (extracellular toxins) during active algal growth or when algal cells lyse (Chorus, 2001; Landsberg, 2002). In consequence, animals may be directly exposed to toxins by absorbing toxins from water, drinking water with toxins, or ingesting algal cells via feeding activity (Hallegraeff, 1993; Carmichael, 1997; Hitzfeld et al., 2000; Van Dolah, 2000; Chorus, 2001; Landsberg, 2002). Because algal toxins can be bioaccumulated, biotransferred and biomagnified through food chains and food webs, aquatic and terrestrial animals (e.g., carnivorous fishes, dolphins, seals, birds, humans) may also be indirectly exposed to toxins when they consume other animals containing toxins (Hallegraeff, 1993; Carmichael, 1997; Hitzfeld et al., 2000; Van Dolah, 2000; Chorus, 2001; Landsberg, 2002).

Among the different taxonomic groups contributing to the occurrence of toxic algae, cyanobacteria, dinoflagellates and diatoms appear to predominate and may be stimulated by inorganic nitrogen pollution (Hallegraeff, 1993; Anderson, 1994; Carmichael, 1997; Paerl and Whitall, 1999; Hitzfeld et al., 2000; Van Dolah, 2000; Chorus, 2001; Wetzel, 2001; Anderson et al., 2002; Landsberg, 2002; Rabalais and Nixon, 2002; Smith, 2003). In the case of other groups, such as raphidophytes (with brevetoxins) and prymnesiophytes (with hemolysins), the link between inorganic N pollution and the increased occurrence of toxic algae is much less evident (Paerl and Whitall, 1999; Van Dolah, 2000; Anderson et al., 2002; Landsberg, 2002).

4.1. Toxic cyanobacteria and toxins

The prokaryotic cyanobacteria (or blue-green algae) are generally more abundant in freshwater environments than in estuarine and coastal marine ecosystems (Fogg et al., 1973; Sellner, 1997; Wetzel, 2001). Environmental conditions promoting the formation of cyanobacterial blooms seem to be ample sunlight, relatively elevated water temperature, and high concentrations of inorganic nutrients (phosphate, ammonium, nitrate) (Carmichael, 1997; Smith et al., 1999; Hitzfeld et al., 2000; Chorus, 2001; Wetzel, 2001; Smith, 2003). Because cyanobacteria can fix molecular nitrogen, many limnologists have traditionally considered that elevated P concentrations is the primary mechanism for cyanobacterial dominance in eutrophic and hypereutrophic aquatic ecosystems (see, for example, Schindler, 1977; Margalef, 1983; Harper, 1992; Reynolds, 1998; Downing et al., 2001).

Nevertheless, the process of N_2 fixation in the open (fresh and marine) waters has been basically related to the presence of cyanobacteria with heterocysts (e.g., *Anabaena*, *Aphanizomenon*, *Nodularia*), with non-heterocystous cyanobacteria (e.g., *Microcystis*, *Planktothrix*) apparently lacking the ability to fix N_2 (non-heterocystous *Trichodesmium* cyanobacteria are a remarkable exception) (Fogg et al., 1973; Riddolls, 1985; Sellner, 1997; Wetzel, 2001). Furthermore, the process of N_2 fixation is decreased or even inhibited when the N availability in the aquatic environment is elevated, probably because it requires significant amounts of reducing power and adenosine triphosphate (Fogg et al., 1973; Riddolls, 1985; Sellner, 1997; Wetzel, 2001). In consequence, the availability of ammonium or nitrate may hence be an important factor in determining which cyanobacterial species can become dominant in eutrophic and hypereutrophic aquatic ecosystems.

The genera most often implicated in toxicity of cyanobacterial blooms are *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia* and *Planktothrix* (syn. *Oscillatoria*) (Carmichael, 1997; Sellner, 1997; Hitzfeld et al., 2000; Chorus, 2001; Landsberg, 2002). Wild populations of the most common bloom-forming non-heterocystous genus, *Microcystis*, are almost always toxic, while species of other genera usually comprise toxic and non-toxic strains (Carmichael, 1997; Sellner, 1997; Hitzfeld et al., 2000; Chorus, 2001; Landsberg, 2002). Laboratory and field studies have shown that toxicity of a strain ultimately depends both on whether or not it contains the gene for toxin production and on environmental factors promoting the gene expression (Dittman et al., 1996; Chorus, 2001; Landsberg, 2002). However, environmental conditions leading to the dominance of toxic strains over non-toxic strains in cyanobacterial blooms are not well understood (Sellner, 1997; Chorus, 2001; Landsberg, 2002).

Major groups of toxins found in species/strains of cyanobacteria are anatoxins, microcystins, nodularins and saxitoxins (Table 4). Comparatively, neurotoxins can kill faster than hepatotoxins, but hepatotoxic microcystins are more common and ubiquitous (Carmichael, 1997; Hitzfeld et al., 2000; Chorus, 2001; Landsberg, 2002). Although the relationship between microcystin concentrations and environmental factors seems to be complex, recent field studies have shown that levels of microcystins can strongly (but nonlinearly) correlate with total nitrogen, maximal microcystin concentrations occurring between 1.5 and 4.0 mg TN/L (Graham et al., 2004). Other cyanotoxins, such as neosaxitoxin, homoanatoxin-a, mueggelone and cylindrospermopsin, have been isolated from certain cyanobacteria, but field reports on their toxicity to aquatic animals are much more scarce (Chorus, 2001; Landsberg, 2002).

Toxic cyanobacteria have been implicated in acute mortalities of freshwater fishes such as bluegill, bream, carp, catfish, eel, perch, pike, pikeperch, roach, sucker and trout (Persson et al., 1984; Toranzo et al., 1990; Rodger et al., 1994; Reguera et al., 1998; Giovannardi et al., 1999; Chorus, 2001; Landsberg, 2002). Toxic cyanobacteria have also caused sublethal or chronic effects (reduced feeding and fecundity) on mussels, cladocerans and copepods (Gilbert, 1990; De Mott et al., 1991; Negri and Jones, 1995; Sellner et al., 1996; Landsberg, 2002). Zooplankton species can differ markedly in their sensitivity to cyanotoxins: 48-hour LC50 values (mg/L) of purified hepatotoxic microcystins were 0.45–1.0 for the copepod *Diaptomus birget*, 9.6 for the cladoceran *Daphnia pulex*, 11.6 for the

Table 4
Major groups of toxins in cyanobacteria, dinoflagellates and diatoms (Hallegraeff, 1993; Carmichael, 1997; Hitzfeld et al., 2000; Van Dolah, 2000; Chorus, 2001; Landsberg, 2002)

Toxins	Chemical structure	Site and mode of action	Characteristic species
Anatoxin-a	Secondary amine alkaloid	Nervous system by mimicking the action of acetylcholine, and overstimulating muscle cells	*Anabaena circinalis Anabaena flos-aquae Anabaena planctonica Aphanizomenon flos-aquae*
Anatoxin-a (s)	Organophosphate	Nervous system by inhibiting acetyl-cholinesterase, and overstimulating muscle cells	*Anabaena flos-aquae Anabaena lemmermannii*
Brevetoxins	Polycyclic ethers	Nervous system by binding to sodium channels (site 5), and disrupting nerve conduction	*Karenia brevis*
Domoic acid	Tricarboxylic amino acid	Nervous system by binding to kainate glutamate receptors, and causing depolarization of the neurons, with a subsequent increase in intracellular Ca^{2+}, neuronal swelling, and cell death	*Pseudo-nitzschia australis Pseudo-nitzschia delicatissima Pseudo-nitzschia multiseries Pseudo-nitzschia seriata*
Hemolysins	Fatty acids	Target cells by altering membrane function, and causing cell lysis	*Alexandrium monilatum Gymnodinium aureolum Karenia mikimotoi*
Microcystins	Cyclic heptapeptides	Liver, hepatopancreas by shrinking the cytoskeleton, distorting cells and causing hepatic haemorrhages, and by inhibiting protein phosphatases, and causing tumor promotion	*Anabaena flos-aquae Microcystis aeruginosa Microcystis viridis Planktothrix agardhii Planktothrix rubescens*
Nodularins	Cyclic pentapeptides	Liver, hepatopancreas by shrinking the cytoskeleton, distorting cells and causing hepatic haemorrhages, and by inhibiting protein phosphatases, and causing tumor promotion	*Nodularia spumigena*
Saxitoxins	Carbamate alkaloids	Nervous system by blocking sodium channels (site 1), and disrupting nerve conduction	*Anabaena circinalis Aphanizomenon flos-aquae Alexandrium catenella Alexandrium tamarense Gymnodinium catenatum*

cladoceran *Daphnia hyalina*, and 21.4 for the cladoceran *Dapnia pulicaria* (De Mott et al., 1991; Landsberg, 2002).

4.2. Toxic dinoflagellates and toxins

In contrast with cyanobacteria, the eukaryotic dinoflagellates (or red-brown algae) are generally more abundant in marine environments than in freshwater ecosystems (Spector, 1984; Anderson et al., 1985). Environmental conditions promoting blooms of dinoflagellates (red tides) are not completely clear, and depend in many instances on the species involved. However, the relationship between red tides and inorganic nitrogen pollution is increasing worldwide (Hallegraeff, 1993; Anderson, 1994; Richardson, 1997b; Burkholder, 1998; Reigman, 1998; Paerl and Whitall, 1999; Van Dolah, 2000; Anderson et al., 2002; Landsberg, 2002; Rabalais and Nixon, 2002; Smith, 2003). Moreover, toxic red tides have apparently caused more mortality events in populations of aquatic animals than cyanobacterial blooms (Van Dolah, 2000; Landsberg, 2002).

The genera most often implicated in toxicity of red tides are *Alexandrium*, *Gymnodinium* and *Karenia* (Hallegraeff, 1993; Van Dolah, 2000; Landsberg, 2002). In terms of their frequency and the range of species affected, *Karenia* blooms have been more devastating than blooms of other dinoflagellates (Hallegraeff, 1993; Landsberg, 2002). Major groups of toxins found in species/strains of these genera are brevetoxins, hemolysins and saxitoxins (Table 4). Although brevetoxins have been found in the marine dinoflagellate *Karenia brevis* (= *Gymnodinium breve*), this group of neurotoxins is better known from marine raphidophytes (Landsberg, 2002). Other toxins, such as ciguatoxins, hemolysins, yessotoxins, tetrodotoxins, pectenotoxins, gymnodimine, azaspiracid, okadaic acid and dinophysistoxins, have been isolated from certain dinoglagellates, but field reports on their toxicity to aquatic animals are more limited and, in some cases, refer to benthic dinoflagellates (Van Dolah, 2000; Landsberg, 2002).

Toxic dinoflagellates of the genera *Alexandrium*, *Gymnodinium* and *Karenia* have been implicated in acute mortalities of marine animals such as clams, mussels, oysters, crabs, prawns, shrimps, fishes, birds, otters, seals, dolphins, manatees and whales (Geraci et al., 1989; Granéli et al., 1990; Horstman et al., 1991; Hallegraeff, 1993; Smayda and Shimizu, 1993; Lush and Hallegraeff, 1996; Van Dolah, 2000; Landsberg, 2002). Toxic dinoflagellates have also caused sublethal or chronic effects (reduced clearance rate, inhibition of shell valve activity, decreased development, lethargy and paralysis) on clams, mussels, oysters and copepods (Huntley et al., 1987; Lesser and Shumway, 1993; Turner et al., 1996; Lassus et al., 1999).

On the other hand, fish kills have also been associated with the occurrence of generalist predatory dinoflagellates of the genus *Pfiesteria* in eutrophic estuaries and coastal waters of the mid-Atlantic and south-eastern USA (Burkholder et al., 1992; Glasgow et al., 1995; Noga et al., 1996; Van Dolah, 2000; Landsberg, 2002). In addition to fish kills, these dinoflagellates have been linked to mortalities of other marine animals such as shellfish, blue crabs, oysters, scallops and quahogs (Landsberg, 2002).

Pfiesteria dinoflagellates are capable of consuming a diverse range of prey (e.g., finfish, shellfish, micro-fauna, algae, bacteria), and are described as having a complex life cycle involving free-swimming flagellates, resting cysts and amoeboid forms, with toxins being produced mostly by flagellated stages (Burkholder and Glasgow, 1997; Glasgow et al., 2001; Landsberg, 2002; Burkholder et al., 2005). *P. piscicida* and *P. shumwayae* are toxic representative species that can present different strains ranging from non-toxic to highly toxic (Landsberg, 2002; Burkholder et al., 2005). The expression of toxicity by toxic strains depends greatly on culture (i.e., environmental) conditions (Burkholder et al.,

2005). A hydrophilic *Pfiesteria* toxin, isolated in 1997 and consisting in a metallated organic complex, has been observed to cause neurological effects and inflammatory responses in the brain of fish and mammals (Landsberg, 2002; Levin et al., 2003; Burkholder et al., 2005).

Although *Pfiesteria* dinoflagellates are non-photosynthetic, heterotrophic organisms, there is compelling evidence that their occurrence at toxic levels coincides with the eutrophication of coastal waters, where nutrient (nitrate, mainly) enrichment enhances the growth of autotrophic algae that in turn support the proliferation of *Pfiesteria* dinoflagellates (Paerl, 1997; Burkholder, 1998; Van Dolah, 2000). These organisms may however represent a minor component of the plankton community during fish kill events, and are not typically associated with the production of discoloured waters so common to other pigmented species (Burkholder, 1998; Landsberg, 2002).

4.3. Toxic diatoms and toxins

The eukaryotic diatoms (or siliceous algae) are abundant in freshwater and marine environments (Wetzel, 2001; Rabalais and Nixon, 2002). Although some diatoms (e.g., *Chaetoceros convolutus*, *Skeletonema costatum*, *Thalassiosira aestivalis*) have caused gill lesions and mortality events in invertebrates and fish as a consequence of mechanical damage, other diatoms (*Pseudo-nitzschia* spp.) have been implicated in intoxications to marine animals by the synthesis of domoic acid (Table 4). This food-web-transferred neurotoxin has been detected in different marine animals such as molluscs, crustaceans, cephalopods, fishes, birds and mammals, in some cases causing significant mortality events in cormorants, pelicans and sea lions (Work et al., 1992; Sierra Beltran et al., 1997; Scholin et al., 2000; Pan et al., 2001; Gulland et al., 2002; Landsberg, 2002; Lefebvre et al., 2002; Costa et al., 2004; Busse et al., 2006). In addition, certain laboratory studies have demonstrated that domoic acid can cause direct toxicity to copepods and anchovies at very low concentrations and, consequently, these marine animals might be adversely affected by domoic acid during toxic diatom blooms (Shaw et al., 1997; Lefebvre et al., 2001; Landsberg, 2002).

Nine species of the ubiquitous marine genus *Pseudo-nitzschia* have now been identified to produce domoic acid (*P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. multiseries*, *P. multistriata*, *P. pseudodelicatissima*, *P. pungens*, *P. seriata* and *P. turgidula*) (Pan et al., 2001; Landsberg, 2002; Costa et al., 2004; Busse et al., 2006). Furthermore, the abundance of toxic *Pseudo-nitzschia* diatoms has apparently increased in several areas of the world (the northern Gulf of Mexico, the coasts of Washington and British Columbia, the Irish and Scottish waters) (Dortch et al., 1997; Adams et al., 2000; Cusack et al., 2002; Parsons et al., 2002; Fehling et al., 2004b; Marchetti et al., 2004). This increased abundance has been related to nutrient enrichment on continental shelves, with inorganic nitrogen pollution (nitrate, mainly) being an important causative factor (Dortch et al., 1997; Adams et al., 2000; Cusack et al., 2002; Parsons et al., 2002; Fehling et al., 2004b; Marchetti et al., 2004). Certain field and laboratory studies also suggest that inorganic nitrogen pollution might induce a higher production of domoic acid in toxic diatoms as a result of decreased Si:N loading ratios (i.e., Si-limiting conditions) (Turner et al., 1998; Turner, 2002; Fehling et al., 2004a,b).

5. Toxicity of inorganic nitrogenous compounds

Aquatic animals are, in general, better adapted to relatively low levels of inorganic nitrogen since natural (unpolluted) ecosystems often are not N saturated and natural concentrations of inorganic nitrogenous compounds usually are not elevated (Wetzel, 2001; Constable et al., 2003; Jensen, 2003; Camargo et al., 2005a). Therefore, high levels of ammonia, nitrite and nitrate, derived from human activities, can impair the ability of aquatic animals to survive, grow and reproduce, resulting in direct (acute or chronic) toxicity of these inorganic nitrogenous compounds (Russo, 1985; Lewis and Morris, 1986; Eddy and Williams, 1987; Adams and Bealing, 1994; Richardson, 1997a; Philips et al., 2002; Constable et al., 2003; Jensen, 2003; Camargo et al., 2005a).

5.1. Ammonia toxicity

The ionized ammonia (NH_4^+) and unionized ammonia (NH_3) are interrelated through the chemical equilibrium $NH_4^+ - OH^- \leftrightarrow NH_3 \cdot H_2O \leftrightarrow NH_3 + H_2O$ (Emerson et al., 1975; Russo, 1985). The relative concentrations of NH_4^+ and NH_3 are basically dependent on the pH and temperature of the water: as values of pH and temperature tend to increase, the concentration of NH_3 also increases but the concentration of NH_4^+ decreases. The concentration of total ammonia is the sum of NH_4^+ and NH_3 concentrations, and it is total ammonia that is analytically measured in water samples. To estimate the relative concentrations of NH_4^+ and NH_3 from total ammonia, Emerson et al.'s (1975) formulas are recommended (Alabaster and Lloyd, 1982; Russo, 1985; Adams and Bealing, 1994; Richardson, 1997a).

Unionized ammonia is very toxic to aquatic animals, particularly to fish, whereas ionized ammonia is nontoxic or appreciably less toxic (Russo, 1985; Adams and Bealing, 1994; Richardson, 1997a; Environment Canada, 2001; Constable et al., 2003). Moreover, unionized ammonia can cause toxicity to *Nitrosomonas* and *Nitrobacter* bacteria, inhibiting the nitrification process (Anthonisen et al., 1976; Russo, 1985). This inhibition can also result in increased accumulation of NH_4^+ (plus NH_3) in the aquatic environment, intensifying the toxicity to bacteria and aquatic animals (Russo, 1985).

The toxic action of unionized ammonia on aquatic animals, particularly on fish, may be due to one or more of the following causes (Tomasso et al., 1980; Alabaster and Lloyd, 1982; Russo, 1985; Adams and Bealing, 1994; Richardson, 1997a; Environment Canada, 2001; Augspurger et al., 2003): (1) damage to the gill epithelium causing asphyxiation; (2) stimulation of glycolisis and suppression of Krebs cycle causing progressive acidosis and reduction in blood oxygen-carrying capacity; (3) uncoupling oxidative phosphorylation causing inhibition of ATP production and depletion of ATP in the basilar region of the brain; (4) disruption of blood vessels and osmoregulatory activity upsetting the liver and kidneys; (5) repression of immune system increasing the susceptibility to bacterial and parasitic diseases. In addition, ammonium ions can contribute to ammonia toxicity by reducing internal Na^+ to

possibly fatally low levels (Russo, 1985; Adams and Bealing, 1994; Environment Canada, 2001; Augspurger et al., 2003). These negative physiological effects can result in reduced feeding activity, fecundity and survivorship, decreasing populations sizes of aquatic animals (Environment Canada, 2001; Constable et al., 2003; Alonso and Camargo, 2004; Alonso, 2005).

Several environmental factors can affect ammonia toxicity to aquatic animals. In the case of fish, the most important factors are pH, temperature, dissolved oxygen, salinity and calcium (Tomasso et al., 1980; Alabaster and Lloyd, 1982; Russo, 1985; Adams and Bealing, 1994; Richardson, 1997a; Environment Canada, 2001; Augspurger et al., 2003). An increase in the pH value of the water at the gill surface induces an increase in the concentration of unionized ammonia that may be absorbed through the gill epithelium. Similarly, an increase in the water temperature will increase the concentration of unionized ammonia at the gill surface. A reduction in the level of dissolved oxygen (O_2) in the water can increase the susceptibility of fish to ammonia toxicity. In contrast, increases in salinity and calcium (Ca^{2+}) concentration in the water can reduce the susceptibility of fish to ammonia toxicity. The susceptibility of fish can also decrease due to acclimation to high environmental levels of ammonia (Russo, 1985; Adams and Bealing, 1994; Environment Canada, 2001; Augspurger et al., 2003). On the other hand, mixtures of ammonia and other chemical pollutants, such as copper, cyanide, phenol, zinc and chlorine (with the formation of inorganic chloramines), may result in additive toxicity or even cause synergistic effects (Alabaster and Lloyd, 1982; Russo, 1985; Adams and Bealing, 1994; Environment Canada, 2001).

Although numerous field observations have recorded fish kills following anthropogenic discharges containing high levels of total ammonia (Adams and Bealing, 1994; Environment Canada, 2001; Constable et al., 2003), concentrations of unionized ammonia causing direct toxicity to aquatic animals have been examined by laboratory studies (Tomasso et al., 1980; Alabaster and Lloyd, 1982; Russo, 1985; Adams and Bealing, 1994; Richardson, 1997a; Environment Canada, 2001; Augspurger et al., 2003; Alonso and Camargo, 2004; Alonso, 2005). Among the different taxonomic groups of aquatic animals that have been exposed to ammonia toxicity, certain freshwater invertebrates (molluscs, planarians) and fishes (salmonids) seem to be the most sensitive (Table 5), exhibiting acute toxicities (96-hour LC_{50}) lower than 0.6 mg NH_3–N/L and chronic toxicities (30–60-day LOEC and 72-day LC_{50}) of 0.05 mg NH_3–N/L.

On the basis of acute and chronic toxicity data, water quality criteria, ranging 0.05–0.35 mg NH_3–N/L for short-term exposures and 0.01–0.02 mg NH_3–N/L for long-term exposures, have been estimated and recommended to protect sensitive aquatic animals (US Environmental Protection Agency, 1986, 1999; Environment Canada, 2001; Constable et al., 2003; Alonso, 2005).

5.2. Nitrite toxicity

The nitrite ion (NO_2^-) and unionized nitrous acid (HNO_2) are interrelated through the chemical equilibrium $NO_2^- + H^+ \leftrightarrow HNO_2$ (Russo et al., 1981; Russo, 1985). The relative concentrations of NO_2^- and HNO_2 are basically dependent on the pH of the water: as the value of pH tends to increase, the concentration of NO_2^- can also increase but the concentration of HNO_2 decreases. The HNO_2 concentration is 4–5 orders of magnitude less than the NO_2^- concentration within the pH range 7.5–8.5 (Russo et al., 1981; Russo, 1985). To estimate the concentration of unionized nitrous acid from the analytically measured nitrite concentration, Russo et al.'s (1981) equation may be used (Russo, 1985).

Both chemical species, nitrite ion and unionized nitrous acid, may contribute to the total toxicity of nitrite to aquatic animals (Russo et al., 1981; Russo, 1985). Furthermore, as in the case of unionized ammonia, HNO_2 can cause toxicity to *Nitrosomonas* and *Nitrobacter* bacteria, inhibiting the nitrification process (Anthonisen et al., 1976; Russo, 1985). This inhibition can also result in increased accumulation of NO_2^- (plus HNO_2) in the aquatic environment, intensifying the toxicity to bacteria and aquatic animals (Russo, 1985). Nevertheless, because in aquatic ecosystems the NO_2^- concentration usually is much higher than the HNO_2 concentration, nitrite ions are considered to be major responsible for nitrite toxicity to aquatic animals (Russo, 1985; Lewis and Morris, 1986; Eddy and Williams, 1987; Tahon et al., 1988; Chen and Chen, 1992; Jensen, 2003).

The main toxic action of nitrite on aquatic animals, particularly on fish and crayfish, is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, causing hypoxia and ultimately death. In fish, entry of nitrite into the red blood cells is associated with the oxidation of iron atoms ($Fe^{2+} \rightarrow Fe^{3+}$), functional hemoglobin being converted into methemoglobin that is unable to release oxygen to body tissues because of its high dissociation constant (Russo, 1985; Lewis and Morris, 1986; Eddy and Williams, 1987; Jensen, 2003). Similarly, in crayfish, entry of nitrite into the blood plasma is associated with the oxidation of copper atoms ($Cu^{1+} \rightarrow Cu^{2+}$), whereby functional hemocyanin is converted into methemocyanin that cannot bind reversibly to molecular oxygen (Tahon et al., 1988; Chen and Chen, 1992; Jensen, 2003). In addition, the following toxic effects of nitrite on fish and crayfish have been found (Lewis and Morris, 1986; Eddy and Williams, 1987; Harris and Coley, 1991; Jensen, 2003): (1) depletion of extracellular and intracellular Cl^- levels causing severe electrolyte imbalance; (2) depletion of intracellular K^+ and elevation of extracellular K^+ levels affecting membrane potentials, neurotransmission, skeletal muscle contractions, and heart function; (3) formation of *N*-nitroso compounds that are mutagenic and carcinogenic; (4) damage to mitochondria in liver cells causing tissue O_2 shortage; (5) repression of immune system decreasing the tolerance to bacterial and parasitic diseases.

Among the different environmental factors that can affect nitrite toxicity to aquatic animals, the water chloride concentration seems to be the most important. Because, in the gills of fish and crayfish, nitrite ions enter via the same route as chloride ions by being competitive inhibitors of the active branchial chloride uptake mechanism, elevated Cl^- concentrations in the ambient water may inhibit the NO_2^- uptake and thereby protect fish and crayfish against nitrite toxicity (Tomasso et al., 1979; Gutzmer and Tomasso, 1985; Eddy and Williams, 1987; Harris and Coley, 1991; Jensen, 2003). Calcium (Ca^{2+}) and seawater (probably because of the high concentration of chloride and other ions) also can significantly reduce nitrite toxicity to fish and crayfish (Russo, 1985; Lewis and Morris, 1986; Jensen, 2003). Moreover,

Table 5
Ammonia toxicity to sensitive freshwater invertebrates and fishes

Species	Toxicological parameters	References
Villosa iris (mollusc; juveniles)	0.11 (96 h LC_{50})	Mummert et al., 2003
Lampsilis cardium (mollusc; juveniles)	0.15 (96 h LC_{50})	Newton et al., 2003
Lampsilis fasciola (mollusc; juveniles)	0.26 (96 h LC_{50})	Mummert et al., 2003
Polycelis felina (planarian; adults)	0.39 (96 h LC_{50}) 0.05 (30 d LOEC)	Alonso, 2005
Sphaerium novaezelandiae (mollusc; juveniles)	0.49 (96 h LC_{50}) 0.05 (60 d LOEC)	Hickey and Vickers, 1994; Hickey and Martin, 1999
Polycelis tenuis (planarian; adults)	0.58 (96 h LC_{50})	Williams et al., 1986
Eulimnogammarus toletanus (amphipod; adults)	0.65 (96 h LC_{50}) 0.09 (96 h $LC_{0.01}$)	Alonso and Camargo, 2004
Oncorhynchus gorbuscha (salmonid; fry)	0.08 (96 h LC_{50})	Rice and Bailey, 1980
Oncorhynchus mykiss (salmonid; alevins)	0.16–0.37 (96 h LC_{50}) 0.05 (72 d LC_{50})	Calamari et al., 1977
Salmo salar (salmonid; fry)	0.23 (24 h LC_{50})	Herbert and Shurben, 1965
Perca fluviatilis (percoid; fry)	0.29 (96 h LC_{50})	Ball, 1967
Rutilus rutilus (cyprinid; fry)	0.35 (96 h LC_{50})	Ball, 1967

Values of toxicological parameters (LC_{50}, $LC_{0.01}$, LOEC), at different exposure times (hours or days), are expressed in mg NH_3–N/L.

several studies on the physiological effects of nitrite in teleosts and crustaceans suggest that the susceptibility to nitrite toxicity can decrease because of acclimation to high environmental levels of nitrite (Lewis and Morris, 1986; Jensen, 2003).

Anthropogenic discharges containing elevated nitrite concentrations have been associated with fish kills in aquatic ecosystems (Lewis and Morris, 1986; Philips et al., 2002). However, as in the case of ammonia, nitrite concentrations causing direct toxicity to aquatic animals have been examined by laboratory studies (Tomasso et al., 1979; Russo et al., 1981; Russo, 1985; Lewis and Morris, 1986; Eddy and Williams, 1987; Tahon et al., 1988; Chen and Chen, 1992; Jensen, 2003; Alonso, 2005). These studies have shown that seawater animals are more tolerant to nitrite toxicity than freshwater animals, probably because of the ameliorating effect of chloride ions on the tolerance of aquatic animals (Russo, 1985; Lewis and Morris, 1986; Jensen, 2003; Alonso, 2005). Among the different taxonomic groups of freshwater invertebrates and fish that have been exposed to nitrite toxicity, certain crustaceans (decapods, amphipods), insects (ephemeropterans), and fishes (salmonids, cyprinids) seem to be the most sensitive (Table 6), exhibiting acute toxicities (96-hour LC_{50}) lower than 3 mg NO_2–N/L and short-term safe levels (96-hour $LC_{0.01}$) lower than 0.25 mg NO_2–N/L.

On the basis of acute toxicity data, Alonso (2005) has recently estimated water quality criteria, ranging 0.08–0.35 mg NO_2–N/L, that may be adequate to protect sensitive aquatic animals, at least during short-term exposures.

5.3. Nitrate toxicity

The nitrate ion (NO_3^-) does not form an unionized species in the aquatic environment (i.e., HNO_3 is completely dissociated to H^+ and NO_3^-), and consequently nitrate toxicity to aquatic animals is due to nitrate ions (Russo, 1985; Camargo et al., 2005a).

As in the case of nitrite, the main toxic action of nitrate on aquatic animals, particularly on fish and crayfish, seems to be the conversion of oxygen-carrying pigments (hemoglobin, hemocyanin) to forms that are incapable of carrying oxygen (methemoglobin, methemocyanin) (Jensen, 1996; Scott and Crunkilton, 2000; Cheng et al., 2002). In fact, before it becomes toxic, nitrate must be converted into nitrite under internal body conditions (Cheng and Chen, 2002). Nevertheless, owing to the low branchial permeability to nitrate ions, the NO_3^- uptake in aquatic animals is more limited than the NO_2^- uptake, which contributes to the relatively low toxicity of nitrate (Jensen, 1996; Scott and Crunkilton, 2000; Cheng and Chen, 2002; Alonso and Camargo, 2003; Camargo et al., 2005a).

The toxicity of nitrate ions in aquatic ecosystems has been traditionally considered to be irrelevant (Russo, 1985; Camargo et al., 2005a), despite the fact that elevated nitrate concentrations can 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 (Gleick, 1993; Ministry of Agriculture, Fisheries and Food, 1993; Steinheimer et al., 1998). Furthermore, several laboratory studies have shown that 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, sensitive aquatic animals (Canadian Council of Ministers of the Environment, 2003; Camargo et al., 2005a).

Freshwater animals appear to be more sensitive to nitrate toxicity than seawater animals, owing to the likely ameliorating effect of water salinity (Camargo et al., 2005a). However, early developmental stages of some marine invertebrates, naturally well adapted to low nitrate concentrations, may be so sensitive to nitrate ions as freshwater animals despite the ameliorating effect of water salinity (Muir et al., 1991). Among the different taxonomic groups of freshwater invertebrates and fish that have been exposed to nitrate toxicity, certain caddisflies, amphipods, and salmonid fishes seem to be the most sensitive (Table 7), exhibiting short-term safe levels (120-hour $LC_{0.01}$) and no-observed effect concentrations (30-day NOEC) lower than 5 mg NO_3–N/L.

The sensitivity of certain amphibians to nitrate toxicity may be high and similar to that of sensitive freshwater invertebrates and fishes (Table 7), though their tolerance can increase with increasing body size and environmental adaptation (Schuytema and Nebeker, 1999; Johansson et al., 2001). Current field data suggest that nitrogen-based fertilizers, such as ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$), may be contributing to the decline of amphibian

Table 6
Nitrite toxicity to sensitive freshwater invertebrates and fishes

Species	Toxicological parameters	References
Cherax quadricarinatus (decapod; adults)	1.03 (96 h LC_{50})	Rouse et al., 1995
Hexagenia sp. (ephemeropteran; larvae)	1.40 (96 h LC_{50})	Kelso et al., 1999
Eulimnogammarus toletanus (amphipod; adults)	2.09 (96 h LC_{50}) 0.18 (96 h $LC_{0.01}$)	Alonso, 2005
Ephemerella sp. (ephemeropteran; larvae)	2.50 (96 h LC_{50})	Kelso et al., 1999
Echinogammarus echinosetosus (amphipod; adults)	2.59 (96 h LC_{50}) 0.21 (96 h $LC_{0.01}$)	Alonso, 2005
Gammarus fasciatus (amphipod; adults)	5.89 (96 h LC_{50})	Ewell et al., 1986
Procambarus clarkii (decapod; adults)	8.91 (96 h LC_{50})	Gutzmer and Tomasso, 1985
Helisoma trivolvis (mollusc; adults)	10.9 (96 h LC_{50})	Ewell et al., 1986
Oncorhynchus mykiss (salmonid; fry)	0.1–0.4 (96 h LC_{50})	Russo et al., 1981
Salmo clarki (salmonid; fry)	0.5–0.6 (96 h LC_{50})	Thurston et al., 1978
Oncorhynchus tshawytscha(salmonid; fry)	0.9 (96 h LC_{50})	Westin, 1974
Pimephales promelas (cyprinid; fry)	2.3–3.0 (96 h LC_{50})	Russo and Thurston, 1977

Values of toxicological parameters (LC_{50}, $LC_{0.01}$), at an exposure time of 96 h, are expressed in mg NO_2–N/L.

populations in agricultural areas because of impaired swimming ability, decreased body size, and reduced fecundity and survivorship (Hecnar, 1995; Birge et al., 2000).

On the basis of toxicity data, the Canadian Council of Ministers of the Environment (2003) has recommended water quality criteria, ranging 2.9–3.6 mg NO_3–N/L, to protect freshwater and marine life, and Camargo et al. (2005a) have recently proposed a maximum level of 2 mg NO_3–N/L for the protection of sensitive aquatic animals.

6. Adverse effects on human health and economy

There is no doubt that the increased use of inorganic fertilizers and fossil fuels around the world has brought enormous health and economic benefits to humans, dramatically increasing food production and human population. Nevertheless, because human society is greatly dependent on surface and ground water resources, as well as on fish and shellfish harvesting, it should be evident that the excessive nitrogen pollution of aquatic ecosystems can cause adverse effects on human health and economy.

6.1. Effects on human health

Ingested nitrates and nitrites, from polluted drinking water, can induce methemoglobinemia in humans by the reduction of nitrates to nitrites, under anaerobic conditions in the digestive tract, and the subsequent blockade of the oxygen-carrying capacity of hemoglobin, resulting in methemoglobin (Craun et al., 1981; Nash, 1993; Ayebo et al., 1997; Knobeloch et al., 2000; Fwetrell, 2004; Greer and Shannon, 2005). Normal levels of methemoglobin in human blood can range from 1 to 3%, and reduced oxygen transport is clinically noted when methemoglobin levels reach 10% or more (Knobeloch et al., 2000; Fwetrell, 2004). Typical symptoms of methemoglobinemia are cyanosis, headache, stupor, fatigue, tachycardia, coma, convulsions, asphyxia and ultimately death (Craun et al., 1981; Nash, 1993; Ayebo et al., 1997; Knobeloch et al., 2000; Fwetrell, 2004; Greer and Shannon, 2005). Infants less than 4 months of age seem to be particularly susceptible because of their greater fluid intake relative to body weight, their higher proportion of readily oxidizable fetal hemoglobin, their lower methemoglobin reductase activity (a red blood cell enzyme that converts methemoglobin back to hemoglobin), their higher gastric pH (which allows greater invasion of bacteria and enhances conversion of ingested nitrates to nitrites), and their increased susceptibility to gastroenteritis (Craun et al., 1981; Ayebo et al., 1997; Knobeloch et al., 2000; Greer and Shannon, 2005).

Since 1945 more than 3000 cases of methemoglobinemia have been reported worldwide, most of which were associated with private wells with high nitrate concentrations (>10 mg NO_3–N/L)

Table 7
Nitrate toxicity to sensitive freshwater invertebrates, fishes, and amphibians

Species	Toxicological parameters	References
Echinogammarus echinosetosus (amphipod; adults)	62.5 (96 h LC_{50}) 2.8 (120 h $LC_{0.01}$)	Camargo et al., 2005a
Eulimnogammarus toletanus (amphipod; adults)	85.0 (96 h LC_{50}) 4.4 (120 h $LC_{0.01}$)	Camargo et al., 2005a
Hydropsyche occidentalis (caddisfly; larvae)	97.3 (96 h LC_{50}) 4.5 (120 h $LC_{0.01}$)	Camargo and Ward, 1992b; Camargo and Ward, 1995
Cheumatopsyche pettiti (caddisfly; larvae)	113.5 (96 h LC_{50}) 6.7 (120 h $LC_{0.01}$)	Camargo and Ward, 1992b; Camargo and Ward, 1995
Hydropsyche exocellata (caddisfly; larvae)	269.5 (96 h LC_{50}) 11.9 (120 h $LC_{0.01}$)	Camargo et al., 2005a
Ceriodaphnia dubia (cladoceran; neonates)	374 (48 h LC_{50}) 7.1–56.5 (7 d NOEC)	Scott and Crunkilton, 2000
Oncorhynchus mykiss (salmonid; fry)	2.3 (30 d LOEC) 1.1 (30 d NOEC)	Kincheloe et al., 1979
Oncorhynchus tshawytscha (salmonid; fry)	4.5 (30 d LOEC) 2.3 (30 d NOEC)	Kincheloe et al., 1979
Salmo clarki (salmonid; fry)	7.6 (30 d LOEC) 4.5 (30 d NOEC)	Kincheloe et al., 1979
Pseudacris triseriata (anuran; tadpoles)	17.0 (96 h LC_{50}) 10.0 (100 d LOEC)	Hecnar, 1995
Rana pipiens (anuran; tadpoles)	22.6 (96 h LC_{50}) 10.0 (100 d LOEC)	Hecnar, 1995
Rana temporaria (anuran; larvae)	5.0 (56 d LOEC)	Johansson et al., 2001

Values of toxicological parameters (LC_{50}, $LC_{0.01}$, LOEC, NOEC), at different exposure times (hours or days), are expressed in mg NO_3–N/L.

(Nash, 1993; World Health Organization, 1996; Ayebo et al., 1997; Knobeloch et al., 2000; Wolfe and Patz, 2002). On the basis of case data, and because nitrates are endogenously reduced to nitrites at an average percentage of 5 to 10%, maximum contaminant levels of 10 mg NO_3–N/L and 1 mg NO_2–N/L for drinking water have been recommended to prevent methemoglobinemia in humans (US Environmental Protection Agency, 1986; World Health Organization, 1996; Wolfe and Patz, 2002). Nevertheless, Fwetrell (2004) has recently indicated that nitrate (and nitrite) may be one of a number of co-factors that play a sometimes complex role in causing methemoglobinemia and, consequently, it would be inappropriate to attempt to link illness rates with drinking-water nitrate levels. Greer and Shannon (2005) have also reported that breastfeeding infants are not at risk of methemoglobinemia even when mothers ingest water with very high nitrate concentrations (100 mg NO_3–N/L).

Ingested nitrates and nitrites also have a potential role in developing cancers of the digestive tract through their contribution to the bacterial formation of *N*-nitroso compounds (i.e., nitrosamines), which are among the most potent of the known carcinogens in mammals (Nash, 1993; Knobeloch et al., 2000; Wolfe and Patz, 2002; Fwetrell, 2004). A likely and common means of synthesizing nitrosamines in the digestive tract, particularly in the stomach, seems to be the low-pH reaction of a secondary amine and nitrite, resulting in the formation of dimethylnitrosamines (Manahan, 1992). Carcinogenicity by dimethylnitrosamines (*N*-nitrosodimethylamines) may result from short-term exposure to a single large dose or from chronic exposure to relatively small doses, with DNA alkylation as the main carcinogenic mechanism (Manahan, 1992; Wolfe and Patz, 2002). In some cases, long-term consumption of drinking water with nitrate concentrations even below the maximum contaminant level of 10 mg NO_3–N/L may stimulate the endogenous formation of nitrosamines (Van Maanen et al., 1996; Townsend et al., 2003).

On the other hand, some scientific evidences suggest that ingested nitrates and nitrites might result in mutagenicity, teratogenicity and birth defects (Dorsch et al., 1984; Luca et al., 1987), contribute to the risks of non-Hodgkin's lymphoma (Ward et al., 1996), coronary heart disease (Cerhan et al., 2001), and bladder and ovarian cancers (Weyer et al., 2001), play a role in the etiology of insulin-dependent diabetes mellitus (Virtanen et al., 1994; Parslow et al., 1997; Van Maanen et al., 2000) and in the development of thyroid hypertrophy (Van Maanen et al., 1994), or cause spontaneous abortions (Centers for Disease Control and Prevention, 1996) and respiratory tract infections (Gupta et al., 2000). Long-term consumption of drinking water with nitrate concentrations even below the maximum contaminant level of 10 mg NO_3–N/L has been linked to higher risks for non-Hodgkin's lymphoma (Ward et al., 1996) and for bladder and ovarian cancers (Weyer et al., 2001).

Indirect health hazards can occur as a consequence of algal toxins. Several laboratory studies have indeed shown that toxins from cyanobacteria and dinoflagellates may be very toxic to mammals: LD_{50} values (μg/kg body weight) in mouse for intraperitoneal administration of purified toxins have been estimated to be 10–30 for neurotoxic saxitoxins (Sasner et al., 1984; Chorus, 2001), 30–50 for hepatotoxic nodularins (Main et al., 1977; Eriksson et al., 1988), 20–200 for neurotoxic anatoxins (Devlin et al., 1977; Matsunaga et al., 1989; Carmichael, 1997), 50–300 for hepatotoxic microcystins (Botes et al., 1982; Dawson, 1998; Chorus, 2001), and 500 for neurotoxic brevetoxins (Baden, 1989; Landsberg, 2002). Toxicity of domoic acid from *Pseudo-nitzschia* diatoms seems to be lower: in rats, the LD_{50} value for intraperitoneal administration of purified toxin was estimated to be 4.000 μg/kg body weight (Iverson et al., 1989; Van Dolah, 2000).

Blooms of toxic cyanobacteria (*Anabaena*, *Microcystis* and *Planktothrix*, mainly) in water storage reservoirs, lakes and rivers, leading to adverse health effects (eye irritation, skin rash, fever, vomiting, diarrhoea, pneumonia, gastroenteritis, hepatoenteritis, muscular cramps) following consumption of contaminated drinking water or after recreational exposure, have been reported from Australia, Brazil, Canada, China, Sweden, United Kingdom, USA and other countries (Hitzfeld et al., 2000; Chorus, 2001). In some instances, human exposures to cyanotoxins were lethal, with children being the most affected (Teixera et al., 1993; Hitzfeld et al., 2000). The consumption of dietary supplements, made from blue-green algae, may also be another important route of exposure to cyanobacterial toxins, particularly to microcystins (Gilroy et al., 2000).

Blooms of toxic dinoflagellates in estuaries and coastal waters, leading to several poisoning syndromes following consumption of contaminated seafood or after water or aerosol exposure, have been reported from Australia, Europe, Japan, North America, Southeast Asia and other parts of the world (Van Dolah, 2000; Van Dolah et al., 2001). The paralytic shellfish poisoning (PSP) and the neurotoxic shellfish poisoning (NSP) are syndromes often associated with red tides (Van Dolah, 2000): PSP is caused by the consumption of molluscan shellfish contaminated with saxitoxins (from *Alexandrium* and *Gymnodinium*, mainly). Clinical symptoms of PSP are tingling and numbness of the perioral area and extremities, loss of motor control, drowsiness, incoherence, and respiratory paralysis; NSP generally results from the consumption of molluscan shellfish contaminated with brevetoxins (from *Karenia brevis*). Clinical symptoms of NSP include nausea, tingling and numbness of the perioral area and extremities, loss of motor control, and severe muscular ache. On a global basis, more than 2000 cases of human intoxications by PSP and NSP are reported per year, but only for PSP some intoxications have been fatal (Van Dolah, 2000; Van Dolah et al., 2001).

Aerosol exposure to toxic *Pfiesteria* dinoflagellates was linked to a human intoxication syndrome, the possible estuary associated syndrome (PEAS), during the 1990s large-scale mortality events among fish and other aquatic organisms in eutrophic estuaries and coastal waters on the eastern seaboard of North America (Glasgow et al., 1995; Van Dolah, 2000; Hudnell, 2005). PEAS is characterized by multiple-system symptoms including fatigue, headache, respiratory irritation, skin lesions or burning sensations on contact, disorientation, deficits in cognitive function, memory loss, and rapid and severe decrements in visual contrast sensitivity (Glasgow et al., 1995; Van Dolah, 2000; Hudnell, 2005). Currently, there is no evidence that toxicity of *Pfiesteria* dinoflagellates may be transferred through seafood (Van Dolah, 2000; Hudnell, 2005).

Domoic acid was not suspected as a health hazard for humans before 1987 (Wright et al., 1989; Todd, 1993). On that year, more than 100 people became ill and 3 died after consuming contaminated blue mussels in Prince Edward Island, Canada (Todd, 1993; Van Dolah, 2000; Landsberg, 2002). The source of domoic acid was the diatom *Pseudo-nitzschia multiseries*, causing the amnesic shellfish poisoning (ASP) syndrome (Wright et al., 1989; Todd, 1993). Clinical symptoms of ASP are nausea, vomiting, diarrhoea, abdominal cramps, reduced consciousness, confusion, disorientation, lethargy, seizures, and permanent loss of short-term memory (Todd, 1993; Van Dolah, 2000; Landsberg, 2002). Individuals found most susceptible were the elderly and those with impaired renal function resulting in poor toxin clearance (Todd, 1993; Van Dolah, 2000). However, no human outbreaks of ASP have apparently occurred since the original 1987 incident in Canada (Van Dolah, 2000; Thessen et al., 2005; Busse et al., 2006).

Other indirect health hazards can also come from the potential relationship between inorganic nitrogen pollution and human infectious diseases since increasing nutrient availability may often favour opportunistic, disease-causing organisms (Townsend et al., 2003). Several studies have shown positive correlations between concentrations of inorganic nutrients in surface waters and larval abundances for *Anopheles*, *Culex* and *Aedes* mosquitoes which may be carriers of pathogenic micro-organisms causing malaria, La Crosse encephalitis, Japanese encephalitis, and West Nile virus (Rejmankova et al., 1991; Walker et al., 1991; Teng et al., 1998; Toth and Melton, 2000; Townsend et al., 2003). However, because not all such correlations are positive, concurrent increases in mosquitoes and eutrophic conditions are probably species, site, and season specific (Townsend et al., 2003). Finally, high nutrient conditions favouring coastal algal blooms have been associated with some cholera outbreaks (Epstein, 1993, Colwell and Huq, 2001; Townsend et al., 2003).

6.2. Effects on human economy

Human sickness and death, resulting directly (e.g., ingested nitrates and nitrites from polluted drinking water) or indirectly (e.g., aerosol exposure to algal toxins, consumption of contaminated seafood causing poisoning syndromes) from inorganic nitrogen pollution, can have elevated economic costs because of lost wages and work days, and because of medical treatment and investigation (Van Dolah et al., 2001; Hoagland et al., 2002; Van den Bergh et al., 2002; Wolfe and Patz, 2002). For example, the annual average estimate of the public health costs of illnesses due to shellfish poisoning in USA could range from $400 thousand to $2 million (Todd, 1989; Hoagland et al., 2002). Moreover, individuals who become sick and experience strong pain and suffering could develop further psychological disorders with long-term medical treatments.

Extensive kills of both invertebrates and fishes (wild or cultured stocks) may result in much higher economic costs because of reduced yields of desirable fish and shellfish species, and because of the subsequent contraction of consumer demand usually associated with closing of commercially important fisheries (Van Dolah et al., 2001; Hoagland et al., 2002; Philips et al., 2002; Smith, 2003). For example, Tester et al. (1991) estimated total commercial harvest losses of clams, oysters, scallops and finfish to be about $8 million during a November 1987 to February 1988 *K. brevis* bloom in North Carolina (USA), and Hoagland et al. (2002) estimated total economic effects of $18 million arising from the deaths of farmed Atlantic salmon killed by phytoplankton blooms during 1987, 1989 and 1990 in Washington (USA).

Other collateral effects can also have elevated costs on human economy (Hitzfeld et al., 2000; Van Dolah et al., 2001; Hoagland et al., 2002; Philips et al., 2002; Van den Bergh et al., 2002; Doka et al., 2003; Smith, 2003): (1) taste and odor problems in drinking water supplies; (2) additional improvements in water treatment procedures (e.g., activated carbon adsorption, chemical coagulation, liming, ozonation, ultrafiltration); (3) increased expenditures in monitoring and management of water pollution, including cleanup and restoration procedures; (4) decreases in the perceived aesthetic value of water bodies; (5) reduced use of degraded aquatic ecosystems for recreation and tourism.

Although the economic costs in the last category are highly uncertain and, consequently, very difficult to estimate, many experts consider that negative effects on the recreation and tourism industry may be responsible for the most important economic impacts, at least locally (Van Dolah et al., 2001; Hoagland et al., 2002; Smith, 2003).

7. Concluding remarks

This global assessment, with detailed multi-scale data, has clearly shown that inorganic nitrogen pollution of aquatic ecosystems may result in three major environmental problems: water acidification, cultural eutrophication (including occurrence of toxic algae), and direct toxicity of inorganic nitrogenous compounds (ammonia, nitrite and nitrate). Water acidification adversely affects freshwater ecosystems without much acid-neutralizing capacity (Table 2). Cultural eutrophication and toxicity of ammonia, nitrite and nitrate can however affect many aquatic ecosystems. In general, freshwater animals seem to be more sensitive to the toxicity of inorganic nitrogenous compounds than seawater animals, with nitrate being less toxic than ammonia and nitrite in any case (Tables 5, 6 and 7).

Extensive kills of invertebrates and fishes, particularly sensitive benthic species, are probably the most dramatic manifestation of hypoxia (or anoxia) in eutrophic and hypereutrophic aquatic ecosystems with low water turnover rates (Table 3). The decline in dissolved oxygen concentrations can also promote the formation of reduced compounds, such as hydrogen sulphide, resulting in higher adverse (toxic) effects on aquatic animals. Moreover, occurrence of toxic algae can significantly contribute to the extensive kills of aquatic animals. Cyanobacteria, dinoflagellates and diatoms appear to be major responsible that may be stimulated by inorganic nitrogen pollution (Table 4): in freshwater ecosystems, the most common bloom-forming non-heterocystous cyanobacterial genus, *Microcystis*, and its hepatotoxic microcystins; in estuarine and coastal marine ecosystems, dinoflagellates of the genera *Alexandrium*, *Gymnodinium* and *Karenia*, with neurotoxic saxitoxins and brevetoxins,

and domoic acid-producing diatoms of the ubiquitous marine genus *Pseudo-nitzschia*.

Because anthropogenic inputs of particulate nitrogen and organic nitrogen can also contribute to the inorganic nitrogen pollution of aquatic ecosystems (National Research Council, 2000; Smil, 2001), and because several studies indicate that total nitrogen (TN) may be related more strongly with algal biomass and toxin production than is dissolved inorganic nitrogen (DIN) (Dodds et al., 1997, 1998; Swedish Environmental Protection Agency, 2000; Turner, 2002; Turner et al., 2003b; Fehling et al., 2004a,b; Graham et al., 2004), we consider that TN criteria are more appropriate than DIN criteria for preventing impacts by inorganic nitrogen pollution in aquatic ecosystems. In consequence, and taking into account the whole information exposed above, we can conclude that TN levels lower than the range 0.5–1.0 mg/L might prevent aquatic ecosystems (excluding those ecosystems with naturally high N levels) from developing acidification and eutrophication, at least by inorganic nitrogen pollution. These relatively low levels of total nitrogen could also protect aquatic animals against the toxicity of inorganic nitrogenous compounds since, in the absence of eutrophication, surface waters usually present relatively high concentrations of dissolved oxygen, most of the dissolved inorganic nitrogen being in the form of nitrate. In addition, human health and economy would be safer from the adverse effects of inorganic nitrogen pollution.

It should be evident that humans must drastically reduce anthropogenic N inputs to the environment. Otherwise, ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems may become worse, especially under expected changes in global climatic conditions (Mann et al., 1998). Increased warming and decreased rainfall in some continental areas (e.g., in southern Spain) might lead to a higher occurrence of toxicity processes in freshwater ecosystems because of elevated concentrations of inorganic nitrogen compounds (resulting from reduced dilution), and because of increased metabolism in ectothermic aquatic animals. Additionally, increases in water temperature might stimulate or enhance the development, maintenance and proliferation of primary producers, resulting in a higher occurrence of eutrophication processes and toxic algal blooms.

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