Chemosphere xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Influence of low levels of water salinity on toxicity of nitrite to anuran larvae

C. Shinn [a,*], A. Marco [a], L. Serrano [b]

[a] Estación Biológica de Doñana, CSIC, C/Americo Vespucio, s/n, 41092 Isla de la Cartuja, Sevilla, Spain
[b] Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, PO Box 1095, 41080 Sevilla, Spain

HIGHLIGHTS

- ▶ Presence of Cl^- increased survival, activity and growth of NO_2^- exposed anuran larvae.
- ▶ Mountain populations were more sensitive to NO_2^- and Cl^- than coastal conspecifics.
- ▶ In general, Cl^- attenuated the toxicity of NO_2^- to developing amphibians.

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form 10 January 2013
Accepted 22 January 2013
Available online xxxx

Keywords:
Nitrite
Salinity
Amphibian larvae
Sub-lethal effects

ABSTRACT

Reactive nitrogen compounds such as nitrite (NO_2^-) are highly toxic to aquatic animals and are partly responsible for the global decline of amphibians. On some fish and Caudata amphibian species low levels of sodium chloride significantly reduce the toxicity of nitrite. However, the nitrite–salinity interaction has not been properly studied in anuran amphibians. To verify if chloride (Cl^-) attenuates NO_2^- toxicity, eggs and larvae of three anuran species were subjected to a series of NO_2^- solutions combined with three salt concentrations (0, 0.4 and 2 or 0, 0.052 and 0.2 g L^{-1} NaCl). One of the species tested originated from two different populations inhabiting highly contrasted nutrient richness environments: lowland Doñana Natural Park and Sierra de Gredos Mountain. In general, the presence of Cl^- increased survival and growth of lowland *Pelophylax perezi* and activity of mountain *P. perezi* larvae exposed to NO_2^-, thus attenuating the toxicity of NO_2^- to developing amphibians. Mountain amphibian populations appeared to be much more sensitive to the concentrations of NO_2^- and Cl^- used in this experiment than coastal conspecifics, suggesting possible adaptation of populations to local conditions. Nitrogen pollution in coastal wetlands poses a serious threat to aquatic organisms, causing direct toxicity or indirect effects via ecosystem eutrophication. The presence of low to medium levels of salinity that would be common in coastal wetlands may attenuate the direct effects of increasing concentrations of nitrogenous compounds in water bodies. Furthermore, treating cultures of endangered anurans with small amounts of NaCl may provide an additional protective measure.

1. Introduction

Nitrogen-based agricultural fertilizers are one of many anthropogenic contaminants having serious effects on natural ecosystems, especially surface freshwaters. Of the total nitrogen applied to agricultural land, only a small portion is required by plants to grow; the excess accumulates in the soil, leaches into adjacent water bodies, or enters groundwaters and the atmosphere (Vitousek et al., 1997). Eutrophic conditions in surface waters can arise due to a small excess of nitrogen, resulting in a decrease in dissolved oxygen. Eutrophication-induced anoxia or hypoxia has been reported to cause biodiversity losses, outbreaks of nuisance species and alteration of food chain structures (Vitousek et al., 1997; Smith et al., 1999). The degradation of water resources can also cause the loss of ecosystem services, leading to economic effects (Carpenter et al., 1997).

Several studies have reported the toxic effects of nitrogenous compounds on various aquatic organisms including fish, invertebrates and amphibians (Marco et al., 1999; Randall and Tsui, 2002; Alonso and Camargo, 2004). Of all intervening compounds of the nitrogen cycle, nitrite (NO_2^-) is one of the most toxic when present in an already unbalanced environment (Wetzel, 2001; Marco and Ortiz-Santaliestra, 2009). Once absorbed into an organism, NO_2^- reacts with haemoglobin and oxidizes ferrous iron to

* Corresponding author. Present address: IMAR-Instituto do Mar, c/o Dep. Ciências da Vida, Univ. Coimbra, 3004-517 Coimbra, Portugal. Tel.: +351 239836386; fax: +351 239823603.

E-mail addresses: candida.shinn@gmail.com (C. Shinn), amarco@ebd.csic.es (A. Marco), serrano@us.es (L. Serrano).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.01.079

ferric iron, thus producing methaemoglobin, which in turn cannot bind or transport oxygen, thereby causing tissue hypoxia (US EPA, 1986).

Ponds and lakes adjacent to agricultural fields are particularly vulnerable environments as toxic compounds such as fertilizers and pesticides can reach the aquatic system directly via runoff. Toxic and even lethal concentrations of NO_2^- can occur in this type of closed aquatic systems (Bogardi et al., 1991). However, studies on the impact of NO_2^- exposure to fish species have resulted in contradictory results. Fish tolerance to nitrite exposure may increase in environments with greater salinity (Crawford and Allen, 1977; Lewis and Morris, 1986; Sampaio et al., 2002). The key factor to such results is the attenuating effect of monovalent ions, such as chloride (Cl^-), on the toxicity of NO_2^- to organisms (Perrone and Meade, 1977). Membrane flux studies have revealed that NO_2^- is a competitive inhibitor of Cl^- uptake, and vice-versa (Williams and Eddy, 1986). For instance, fish species with greater Cl^- uptake rates are more sensitive to NO_2^- than species with lower Cl^- uptake rates (Williams and Eddy, 1986). Therefore, ambient Cl^- can ameliorate NO_2^- toxicity through competitive inhibition (Perrone and Meade, 1977; Bath and Eddy, 1980; Alonso and Camargo, 2008). In fact, adding calculated quantities of chlorine to aquaculture water systems is a way of protecting freshwater fish from NO_2^- contamination (Francis-Floyd, 1995). In the whole, as the concentration of Cl^- in the water increases, the capacity of the NO_2^- ions to enter the blood stream decreases (Lewis and Morris, 1986; Alonso and Camargo, 2008).

Nitrogen pollution has been appointed as one of the major causes for the ongoing global amphibian population decline (Blaustein et al., 2003). Several studies have demonstrated the toxic effects of NO_2^- on amphibian larval stages (Huey and Beitinger, 1980a, 1980b; Marco and Blaustein, 1999; Marco et al., 1999; Griffis-Kyle, 2005). However, there is also a strong variability in sensitivity to NO_2^- among species and even among different studies conducted on the same species (Marco and Ortiz-Santaliestra, 2009). Genetic, maternal or ontogenetic effects may explain part of this variability (Gomez-Mestre and Tejedo, 2003; Ortiz-Santaliestra et al., 2006; Shinn et al., 2008). As occurs in fishes, environmental factors such as the presence of small amounts of Cl^- in some freshwater bodies may also explain the low toxicity of NO_2^- to amphibians in some studies. Huey and Beitinger (1980a) found that relatively low test concentrations of Cl^- protected *Ambystoma texanum* larvae from the toxic effects of NO_2^-. This species suffered a 0% mortality rate at 3 mg L^{-1} N–NO_2^- in the presence of high test concentrations of Cl^- (300 mg L^{-1}). *A. texanum* exposed to $NaNO_2$ at low concentrations of Cl^- had a 96 h LC50 (Lethal Concentration to half of tested individuals) of 0.33 mg L^{-1} N–NO_2^-, and a 100% mortality rate when larvae were exposed to 0.76 mg L^{-1} N–NO_2^- (Huey and Beitinger, 1980b). Conversely, a recent study of Ortiz-Santaliestra et al. (2010a) revealed synergistic lethal effect of salinity and ammonium nitrate in a *Pelophylax perezi* population that was not naturally exposed to high salinity levels. This was not observed in *P. perezi* individuals collected from ponds with higher salinity, suggesting population-level adaptation to salinity.

The present study has experimentally assessed the effects of low levels of salinity over NO_2^- toxicity in larval stages of three anuran species, testing the interaction between Cl^- and NO_2^-. Amphibians do not tolerate high levels of salinity (over 5 g L^{-1}) but can develop in waters with low levels of salt. When comparing different amphibian habitats, salt concentrations usually vary within 0 and 5 mg L^{-1} (Gomez-Mestre and Tejedo, 2003). Low concentrations of sodium chloride (NaCl), often found in amphibian habitats, were used to verify whether low levels of salt can reduce the toxicity of NO_2^- to anuran larvae.

2. Materials and methods

2.1. Study areas

In all studies, amphibians were handled in accordance with national and international guidelines for the protection of animal welfare (Directive 86/609/EEC; European Union, 1986; in force at the time the studies were conducted). Amphibian eggs and larvae were collected from ponds located in the lowland, coastal marshland area of the Doñana Natural Park (DNP) and in the inland Sierra de Gredos mountain (GM). Authorization for the collection from the wild and use of the organisms in experiments was obtained from the Consejaría de Medio Ambiente de Andalucía, the Consejaría de Medio Ambiente de Castilla y León, and from the head offices of the Parque Nacional de Doñana and of the Parque Regional de la Sierra de Gredos.

The Doñana region is located on the Atlantic coast of southwestern Spain. This region includes an extraordinary variety of aquatic systems and some of them exhibit the highest degree of environmental protection in Spain. Outside the protected areas, watersheds are severely altered by human activities. The lower valley of the Guadalquivir River is devoted to agriculture (traditional cultivars of olive trees, irrigated crops, and rice fields) as well as farming of cattle and horses. The water chemical composition of the Doñana wetlands is mainly influenced by rainfall, evaporative concentration, groundwater discharge, biogeochemical interactions at the sediment–water interface, and the water quality of their watersheds (Serrano et al., 2006). The Doñana marshland is a silty-clay floodplain which is seasonally fed by rainfall and river outflow, and to a much lesser extent, tidal water. High dissolved nutrient concentrations (maximum NO_2^- concentrations can reach 0.47 mg L^{-1} maximum) is the main cause of poor water quality in the various river basins, while high inorganic suspended matter is the main cause of pollution in floodplains fed by the tidal water from the estuary of the Guadalquivir River (Serrano et al., 2006). Both the solubilization of salts from the dry sediment and the concentrative effect of evaporation ensure a relatively high mineralization level, though salinity drops markedly during heavy floods and hence conductivity has been reported to vary from 2 to >30 mS cm^{-1} across the Doñana marshland in the same year (Serrano et al., 2006). Frogs in the DNP breed in late winter or early spring in shallow warm, eutrophic water.

The Sierra de Gredos Mountain is located within the Iberian Peninsula Central Range. The main anthropogenic stressors on GM are cattle and intense tourism, both causing eutrophication of lake and pond waters due to terrain erosion and deposition of biological waste. Wastewater discharge into a lake due to faulty wastewater treatment installations of a mountain refuge has been reported in the area. The GM is covered by snow for 5–7 months of the year, with water levels in lakes responding rapidly to temperature increase in the spring. At the altitude of the study (around 1900 m.a.s.l.) the habitat is of humid meadows with ponds and rapid streams that are fed principally by heavy rain events. The mean water conductivity is very low in GM, varying between 4 and 15 μS cm^{-1} in the various lakes. Water pH is slightly acid (ranging between 6.2 and 6.8 in lakes and going as low as 5.6 in streams), contributing to a very low buffering capacity in surface waters. Nitrate concentration is low (<60 μg L^{-1} N–NO_3), ammonium concentration is usually under 90 μg L^{-1} N–NH_4, and total phosphorus concentrations do not exceed 25 μg L^{-1}. Pond water temperatures during the amphibian breeding season – mid-April to mid-June – vary from 6 °C to 20 °C and ponds occasionally dry-up in the summer (Lizana and Pedraza, 1998). Thus, GM frog populations breed in colder and more oligotrophic conditions, and with lower risk of NO_2^- pollution, than DNP populations.

Please cite this article in press as: Shinn, C., et al. Influence of low levels of water salinity on toxicity of nitrite to anuran larvae. Chemosphere (2013), http://dx.doi.org/10.1016/j.chemosphere.2013.01.079