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# Acute and chronic nitrite toxicity in juvenile pike-perch (*Sander lucioperca*) and its compensation by chloride

S. Wuertz [a,b,*], S.G.E. Schulze [b], U. Eberhardt [a], C. Schulz [b,c], J.P. Schroeder [b,c]

[a] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany
[b] Gesellschaft für Marine Aquakultur mbH, Hafentörn 3, 25761 Büsum, Germany
[c] Institut für Tierzucht und Tierhaltung, Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40, 24098 Kiel, Germany

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ABSTRACT

Pike-perch *Sander lucioperca* is currently considered as one of the most promising candidates for production in freshwater recirculation aquaculture systems (RAS). Here, due to the lack of studies on nitrite ($NO_2^-$) toxicity in pike-perch, a flow-through exposure at 0, 0.44, 0.88, 1.75, 3.5, 7, 14 and 28 mg/L $NO_2^-$–N was carried out to determine the acute and chronic toxicity over a period of 32 days. In juvenile pike-perch, 120 h $LC_{50}$ was 6.1 mg/L $NO_2^-$–N and at ≥14 mg/L $NO_2^-$–N all fish had died within 24 h. Chronic exposure revealed a significant build up of $NO_2^-$ in the plasma as well as in the muscles at ≥0.44 mg/L $NO_2^-$–N peaking in fish exposed to the highest concentration of 3.5 mg/L $NO_2^-$–N after 32 days. Still, due to high individual variation methemoglobin (MetHb) was only significantly increased ($p<0.01$) at 3.5 mg/L $NO_2^-$–N. No adverse effects on red blood cells (RBC) and hematocrit were observed in any of the treatments. In a second experiment, compensation of $NO_2^-$ toxicity at increasing chloride concentrations (40 (freshwater), 65, 90, 140, 240, 440 mg/L $Cl^-$) was observed at a constant exposure of 10 mg/L $NO_2^-$–N for 42 days. At ≥240 mg/L $Cl^-$, $NO_2^-$ build-up in blood plasma and muscle was completely inhibited. At lower $Cl^-$ concentrations (≤140 mg/L), $NO_2^-$ was significantly increased in plasma, but only insignificantly elevated in muscle due to high individual variation. MetHb was increased significantly difference only at 40 mg/L $Cl^-$ (freshwater control) compared to the control. Again, high individual variations were observed. As a conclusion, *S. lucioperca* is moderately sensitive towards $NO_2^-$ and acceptable levels in RAS should hence not exceed 1.75 mg/L $NO_2^-$–N to avoid MetHb formation. However, based on the 120 h $LC_{50}$ and a factor of 0.01 according to Sprague (1971), a $NO_2^-$ concentration of ≤0.061 mg/L $NO_2^-$–N is considered as "safe." Thereby, no $NO_2^-$ should accumulate in the plasma or muscle tissue during chronic exposure. For 10 mg/L $NO_2^-$–N, ≥240 mg/L chloride compensates for $NO_2^-$ uptake in plasma and muscle.



## 1. Introduction

Currently, fish and seafood products account for at least 15% of the animal protein consumed which, given the stagnation of global fisheries landings, can only be met by aquaculture production. This is reflected by a doubling of the global aquaculture production every decade over the past 40 years (FAO, 2011). Thereby, aquaculture undoubtedly depicts the fastest growth in the agriculture sector (FAO, 2011). In contrast to the global trend, central European aquaculture is rather static since production costs as well as stringent environmental regulations clash with the actual price levels of the common species such as trout and carp grown in ponds. In Germany, a small domestic production is overwhelmingly overshadowed by a total of approximately 1070000 t imported aquaculture products. Traditional culture systems such as ponds and raceways as well as species farmed (carp and trout) do not show promising growth potential (FEAP 2008), while new production methods and new species are on the rise. In particular, aquaculture production of new species with a high market value is gaining more and more relevance.

Defined by a daily water renewal of <10% (FAO, 2006), such recirculation aquaculture systems (RAS) recirculate process water after water treatment, comprising biological filter units that allow for a conversion of toxic ammonia derived from fish, faeces or uneaten food. Typically, in intensive production, more than 50% of the protein fed is not retained in the fish and most of the nitrogen is actually directed to the biofilter, either as ammonia or organic nitrogen in food and faeces (Handy and Poxton, 1993; Avnimelech, 2006). In the course of microbial nitrification, ammonia is converted in a two step process into less toxic $NO_2^-$ and nitrate, but imbalances of this process may lead to a build up of nitrite to 50 mg/L $NO_2^-$ or more in the recirculating process water and impact on fish health or even mass mortalities are regularly observed in commercial facilities (Svobodova and Kolarova, 2004; Svobodova et al., 2005). Consequently, to safeguard animal

* Corresponding author. Tel.: +49 30 641 81 683; fax: +49 30 641 81 614.
*E-mail address:* wuertz@igb-berlin.de (S. Wuertz).

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health and welfare, species-specific, maximum exposure limits based on acute and chronic toxicity data need to be determined, particularly since $NO_2^-$ toxicity in fish varies over a wide range (Williams and Eddy, 1986; Tomasso and Grosell, 2005).

$NO_2^-$ competes for the branchial $Cl^-$ uptake mechanism, presumably the $Cl^-/HCO_3^-$ exchanger, shifting $Cl^-$ to $NO_2^-$ uptake (Jensen, 2003). The subsequent $NO_2^-$ accumulation does not only cause acute toxicity and subsequent fish mortalities, but may impact fish chronically even at lower concentrations if exposed over prolonged periods. Surprisingly, data on chronic $NO_2^-$ toxicity are restricted to a few species (Siikavuopio and Saether, 2006; Kroupova et al., 2008). In the past, sublethal effects have been reported at concentrations down to 3–12% of lethal toxicity (Tomasso, 1994). Both lethal and sublethal toxicity are assumed to be mainly caused by the uptake of $NO_2^-$ and the subsequent oxidation of hemoglobin to methemoglobin (MetHb), impairing oxygen binding due to the oxidation of $Fe^{2+}$ to $Fe^{3+}$, which results in hypoxia (Tomasso, 1994; Jensen, 2003). Still, other modes of action have been documented in the past, including anaerobic substrate oxidation as a result of energy drains for detoxification processes (de Lima et al., 2011), ion imbalances ($Cl^-$ uptake inhibition or efflux of $K^+$ from skeletal muscle) as well as inhibition of steroidogenesis resulting in hormone imbalances (for review see Jensen, 2003).

From a European perspective, pike-perch (*Sander lucioperca*; Linnaeus, 1758) is among the most promising candidates for a production in RAS (Rennert et al., 2005; Fontaine, 2009). Its delicate meat characterised by a mild taste and good texture achieves prices above 8€/kg (Zienert et al., 2005; Dill, 2008). Currently, 75% of pike-perch consumed in Germany is actually imported (1600 t), mainly originating from wild fisheries in Eastern Europe. Most importantly for RAS-based production, rearing temperature can be used to control production, shifting energy from gonad development to somatic growth at temperatures around 23 °C (Zakes and Demska-Zakes, 2009; Hermelink et al., 2011). The emerging commercial interest in RAS-based production is often impeded by limited data on the requirements of the species (Zienert et al., 2005). Although considered a typical freshwater species, pike-perch is also locally abundant in coastal habitats throughout the Baltic Sea and tolerates salinities of up to 12 psu (Hilge and Steffens, 1996; Koed et al., 2000; Brown et al., 2001). With regard to this salt tolerance, elevation of chloride concentration in aquaculture process water thus represents a potential countermeasure to compensate for high $NO_2^-$ toxicity. Currently, no data are available on either acute or chronic $NO_2^-$ toxicity in pike-perch.

In the present study, acute and chronic $NO_2^-$ toxicity in juvenile pike-perch *S. lucioperca* were studied to determine safe concentrations in freshwater and to investigate the potential of chloride addition as a preventive measure to reduce $NO_2^-$ uptake in the blood of the fish exposed. Therefore, two experimental exposures (exp 1: freshwater/exp 2: various salinities) were carried out assessing acute toxicity by 120 h $LC_{50}$ and - over a prolonged exposure time – $NO_2^-$ accumulation in the fish (plasma and muscle), hematologic parameters characterising respiratory function (methemoglobin MetHb, red blood cell count RBC, hemoglobin Hb, hematocrit Hcrit) as well as histological alterations of the gills.

## 2. Materials and methods

### 2.1. Experimental setup

Two experimental $NO_2^-$ exposures, built upon one another, were carried out with juvenile pike-perch (*Sander lucioperca*) (exp. 1: 33.8 ± 5.3 g/exp. 2: 47.3 ± 15.4 g) in a flow-through exposure system.

In a first experiment (exp. 1), both acute and chronic $NO_2^-$ toxicity were investigated in freshwater (40 mg/L $Cl^-$) by a 32 d exposure to 0, 0.44, 0.88, 1.75, 3.5, 7, 14, 28 mg/L $NO_2^-$–N (corresponding to 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1 and 2 mM). In a second experiment (exp. 2), considering the results of exp. 1, the compensatory effect of chloride-ions on chronic $NO_2^-$ toxicity was studied in a 42 d exposure experiment, assessing $NO_2^-$ accumulation and chronic effects on blood parameters at 40 (concentration in exp. 1, "freshwater" control), 65, 90, 140, 240, 440 mg/L $Cl^-$ and a constant $NO_2^-$ concentration of 10 mg/L $NO_2^-$–N (0.7 mM) compared to a control at 440 mg/L $Cl^-$ and 0 mg/L $NO_2^-$–N (detection limit <0.002 mg/L $NO_2^-$–N).

Both exposure experiments were performed in a flow-through exposure system (flow-through approx. 225 L/d) of 55 L aquaria equipped with adjusted aeration, providing duplicates per treatment (fish per tank exp. 1: 15/exp. 2: 10). The aquaria of each treatment were supplied from a 450 L reservoir; each reservoir was refilled twice daily from a second reservoir, where $NO_2^-$ concentration was adjusted with a 5 M $NaNO_2$ stock solution. Water exchanged approximately 5–7 times per day. The entire experimental unit was shielded and moderately darkened by a screen to minimize stress during the experiment.

Fish were fed a commercial diet (Aller Metabolica XS, 3 mm, Aller Aqua, Christiansfeld, Denmark) at 0.7% of total body weight and faeces were removed once a day to reduce ammonia formation. Animal care and layout of the experiments were performed according to the animal experiment approval V 313-72241 J23-34 (57–5110) of the Ministry for Agriculture, the Environment and Rural Areas Schleswig-Holstein.

### 2.2. Water analysis

Routinely, $NO_2^-$ was determined in the reservoir and in each aquarium with a DR/2800 Spectrophotometer (Hach Lange GmbH, Germany) using the Diazotization Method (HACH 8507 NitriVer 3). Actual $NO_2^-$ concentrations are given in Table 1. Salinity, pH, $O_2$ and temperature were determined once a day (multi 350i, WTW), total ammonia (TAN) every second day (Salicylate Method, HACH 8155) revealing comparable conditions between treatments in both experiments (exp. 1/exp. 2: 1.32 ± 0.10/0.54 ± 0.36 mg/L TAN, 8.7 ± 0.1/8.9 ± 0.4 mg/L $O_2$, pH 8.1 ± 0.2/8.3 ± 0.1, 22.0 ± 0.6/20.3 ± 1.4 °C) within the range recommended for pike-perch (Zienert et al., 2005).

### 2.3. Acute toxicity

Acute toxicity was assessed in accordance with the OECD Guidelines for Testing of Chemicals 203 (Fish Acute Toxicity Test, OECD, 1992) and 204 (Fish Prolonged Toxicity Test (14-day Study), OECD, 1984) by detecting dose-mortality relationships. Death was considered as cessation of respiration and failure to respond to tactile stimuli. Dead fish were immediately removed from the tanks and cumulative mortalities were recorded every 24 h. Toxicity data were statistically analysed by calculating standard $LC_{50}$ values and their 95% confidence limits after 96 and 120 h according to the Spearman–Karber method (Hamilton et al., 1977) using TSK v.1.5 software (EPA, USA) by setting the trim to 0%. The 24, 48 and 72 h $LC_{50}$ values had to be determined graphically by linear interpolation as no partial mortalities occurred in the respective time frame.

A "safe" concentration level for rearing juvenile pike-perch was calculated by multiplying the 120 h $LC_{50}$ value by 0.01 as

**Table 1**
Nominal and actual nitrite concentrations in the exposure experiment (exp 1). SD, standard deviation.

| Nominal [mM] | 0 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 |
|---|---|---|---|---|---|---|---|---|
| Nominal [mg/L $NO_2^-$–N] | 0 | 0.4375 | 0.875 | 1.75 | 3,5 | 7 | 14 | 28 |
| Real [mg/L $NO_2^-$–N] | 0.01 | 0.25 | 0.56 | 1.3 | 3.1 | 6.18 | 13.82 | 29.92 |
| ± SD | 0.04 | 0.08 | 0.07 | 0.2 | 0.25 | 0.66 | 0.82 | 2.18 |