

Nitrite influence on fish: a review

H. KROUPOVA¹, J. MACHOVA¹, Z. SVOBODOVA^{1,2}

¹University of South Bohemia, Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic

²University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: Nitrite is an intermediate in the oxidation of ammonium to nitrate. An elevated ambient nitrite concentration is a potential problem for freshwater fish since nitrite is actively taken up across the gills in competition with chloride. Nitrite is a well-known toxicant for fish as well as a disrupter of multiple physiological functions including ion regulatory, respiratory, cardiovascular, endocrine and excretory processes. One critical consequence of nitrite accumulation is the oxidation of haemoglobin to methaemoglobin, compromising blood oxygen transport. Nitrite toxicity to fish varies considerably and depends on a large number of external and internal factors. Among the most important ones are water quality (e.g. pH, temperature, cation, anion and oxygen concentration), length of exposure, fish species, fish size and age, and individual fish susceptibility. Chloride concentration in water is considered one of the most important factors influencing nitrite toxicity to fish. The importance of individual factors is assessed and re-evaluated continuously.

Keywords: nitrite; fishes; toxicity mechanism; aquatic environment; physiological disturbances; water quality; age; length of exposure

Contents

- | | |
|--|---|
| <ul style="list-style-type: none"> 1. Introduction 2. Nitrite in aquatic environment 3. Nitrite uptake and toxicity mechanisms <ul style="list-style-type: none"> 3.1. Blood methaemoglobin content 3.2. Mechanism of detoxification 3.3. Physiological disturbances induced by nitrite 4. Factors affecting nitrite toxicity <ul style="list-style-type: none"> 4.1. Length of nitrite exposure | <ul style="list-style-type: none"> 4.2. Water quality <ul style="list-style-type: none"> 4.2.1. Chloride 4.2.2. Other anions 4.2.3. Cations 4.2.4. Ammonia 4.2.5. pH 4.2.6. Oxygen and temperature 4.3. Fish size and age 4.4. Intraspecific differences 4.5. Fish species 5. Conclusion 6. References |
|--|---|

1. Introduction

Nitrite is a natural component of the nitrogen cycle in ecosystems, and its presence in the environment is a potential problem due to its well-documented toxicity to animals (e.g. Lewis and Morris, 1986; Jensen, 2003).

Aquatic animals are at higher risk of nitrite intoxication than terrestrial animals. Since nitrite in the ambient water can be actively taken up across the gill epithelium and can accumulate to very high concentrations in the body fluids. Studies on fish and crustaceans revealed that nitrite induced a large variety of physiological disturbances,

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 6007665809) and Internal Grant Agency of the University of South Bohemia (Grant No. 24/2004/P-VURH).

many of which contribute to toxicity (Jensen, 1995, 2003).

The purpose of this paper is to survey the present knowledge of nitrite toxicity to fish.

2. Nitrite in the aquatic environment

Nitrite is an intermediate and important product in bacterial nitrification and denitrification processes in the nitrogen cycle. The concentration of nitrite in natural water is typically low in the μM range. Elevated concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments or in effluents from industries producing metals, dyes and celluloid (Pitter, 1999).

Elevated nitrite concentrations cause great problems in intensive culture of commercial fish species and ornamental fish (Dvorak, 2004; Svobodova et al., 2005a). Intensive rearing methods are commonly used today. These methods mostly rely on recirculating water systems that remove waste ammonia from water, which leads to the hazard of possible incomplete oxidation of ammonia accompanied by accumulation of nitrite in the system. Upon the initiation of nitrification process in biological filters, or during imbalance in the process, concentrations of nitrite can reach 1 mM (50 mg/l) or more (Avnimelech et al., 1986; Kamstra et al., 1996). This may result in mass fish mortality (Svobodova and Kolarova, 2004; Svobodova et al., 2005a). Factors that affect the nitrification process include pH, temperature, concentration of dissolved oxygen, number of nitrifying bacteria, and the presence of inhibiting compounds, e.g. nitrous acid, NH_3 , methylene blue, antibiotics, and some organic compounds (aniline, dodecylamine, p-nitrobenzaldehyde) (as reviewed by Russo and Thurston, 1991).

3. Nitrite uptake and toxicity mechanisms

Freshwater fish and crustaceans are hyperosmotic to their environment and require an active uptake of ions across the gills to compensate for ions lost with urine and via passive efflux across the gills. Fish gain ions through their diet, and most fish are also able to accumulate ions through active uptake mechanisms associated with the chloride cells of gills (Maetz, 1971). In fresh water, these cells can

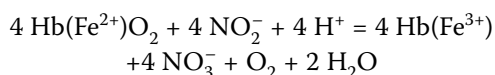
give off ammonium or hydrogen ions in exchange for an equal number of sodium ions and can give off bicarbonate ions in exchange for an equivalent number of chloride ions (Love, 1980).

Problems with nitrite in freshwater animals stem from the fact that NO_2^- has an affinity for the branchial Cl^- uptake mechanism, presumably the $\text{Cl}^-/\text{HCO}_3^-$ exchanger; thus, whenever nitrite is present in the ambient water, a part of the Cl^- uptake will be shifted to NO_2^- uptake (Jensen, 2003). A shared uptake route for nitrite and chloride is also supported by the fact that fish with high branchial Cl^- uptake rates (e.g. rainbow trout, perch, pike) are more sensitive to nitrite than species with low uptake rates (eel, carp, tench) (Williams and Eddy, 1986).

It has been considered that some nitrite may enter fish via diffusion of HNO_2 , but this route seems insignificant in most cases (Jensen, 2003). Only a minute fraction of nitrite will be present as HNO_2 at natural pH values (pKa for nitrous acid is ~ 3.35). This means that NO_2^- highly prevails over HNO_2 at pH > 5 , and both forms appear in a molar ratio of 1 : 1 at pH 3.35 (Pitter, 1999). Additionally, diffusion of HNO_2 does not explain the well-documented protective effect of environmental chloride (Jensen, 2003).

Nitrite concentrations in the blood plasma may be more than 60 times higher than the concentrations in the surrounding medium (Fontenot and Isely, 1999). However, nitrite also accumulates in other tissues such as gills, liver, brain and muscle (Margiocco et al., 1983). Nitrite concentrations in all the examined organs do not reach the same levels as in the blood although in the liver and in the brain of torpid and overturning animals the levels about 30 times higher than the environmental concentration can be detected (Margiocco et al., 1983). Nevertheless, the blood appears to be the primary target of nitrite action. From the blood plasma, nitrite diffuses into red blood cells, where it oxidises iron in haemoglobin (Hb) to the +3 oxidation state. Haemoglobin that is changed in this way is called methaemoglobin or ferrihaemoglobin (Kiese, 1974), which lacks the capacity to bind oxygen reversibly (Bodansky, 1951). The methaemoglobin reduces the total oxygen-carrying capacity of the blood (Cameron, 1971). It gives whole blood a brownish colour and has an optical spectrum with maximum absorption at about 635 nm. So a visible symptom of high methaemoglobin levels is the brown colour of blood and gills. The brown colour of blood was

observed in Nile tilapia (*Oreochromis niloticus*) as soon as methaemoglobin levels reached about 20% of total haemoglobin (Svobodova et al., 2005a). The reaction of haem group proceeds via a series of intermediate reactions mediated by the radical NO_2^\cdot , overall stoichiometry of the reaction being given by Kosaka and Tyuma (1987):



Thus NO_2^\cdot turnover by haemoglobin is followed by the production of NO_3^- , whereas methaemoglobin is eventually reduced to haemoglobin by methaemoglobin reductase. Other haem proteins of cells may react with nitrite in a similar way (Doblender and Lackner, 1997).

Inactive fish have a very low oxygen demand and may not be immediately threatened by severe methaemoglobinaemia (Crawford and Allen, 1977). However, if a fish with methaemoglobinaemia is frightened or otherwise forced to become active, it may die of anoxia (Huey et al., 1980). Individuals in better physical condition may have higher stores of glycogen in the liver and can derive a greater portion of their energy requirement from anaerobic glycolysis. Lowering their requirement for oxygen, they may cope with elevated levels of methaemoglobin for longer periods (Perrone and Meade, 1977).

The amount of methaemoglobin necessary to kill, to reduce the growth of, or to prevent the normal behaviour of fish varies with species and with environmental conditions. As a rough rule of thumb, methaemoglobin concentrations in excess of 50% are considered threatening to fish (Bowser et al., 1983). Channel catfish (*Ictalurus punctatus*) with 100% methaemoglobin survived for 2 days in warm water (25°C) although the fish were inactive (Tomasso et al., 1979). When methaemoglobin concentrations are below 50%, there is usually no mortality (Lewis and Morris, 1986).

3.1. Blood methaemoglobin content

Methaemoglobin forms spontaneously, although slowly, in the absence of nitrite. Thus fish blood typically contains a measurable amount of methaemoglobin even in the absence of nitrite. Auto-oxidation of haemoglobin, i.e. the oxidation of haemoglobin by molecular oxygen to methaemoglobin, causes the presence of a small portion of methaemoglobin in normal red blood cells. The

rate of haemoglobin auto-oxidation is much higher at pH 5.7 than at pH 7 and maximal at a low oxygen pressure when nearly half the haemoglobin is deoxygenated (Kiese, 1974). The increase in methaemoglobin content may also occur as a response to unspecified and unrecognised stress conditions. Specifically, the state of energy metabolism of the red blood cells may be affected by overall nutrition, hypoxia and exercise, resulting in disturbance of the normal methaemoglobin-haemoglobin equilibrium (Cameron, 1971). Reported values are 0.9 to 3.6% for rainbow trout (*Oncorhynchus mykiss*) (Cameron, 1971; Brown and McLeay, 1975) and 10.9% for prespawning pink salmon (*Oncorhynchus gorbuscha*) (Cameron, 1971). It is evident that the presence of methaemoglobin in the blood, even somewhat above 10%, should not be viewed as exceptional among fish. Fish differ in this aspect from mammals whose methaemoglobin levels seldom exceed 1% (Beutler, 1968).

3.2. Mechanism of detoxification

The red blood cells of fish contain methaemoglobin reductase reconverting methaemoglobin to haemoglobin (Cameron, 1971; Huey and Beitinger, 1982). This occurs steadily and restores the normal proportion of haemoglobin within 24–72 hours if a fish is transferred to water that lacks nitrite (Huey et al., 1980; Knudsen and Jensen, 1997). When nitrite is present, the ultimate level of methaemoglobin in the blood is a result of the balance between methaemoglobin formation and conversion to haemoglobin by methaemoglobin reductase (Lewis and Morris, 1986). As fish are poikilotherms, the efficiency of the reductase enzyme may vary with seasonal temperatures (Perrone and Meade, 1977).

Fish are also capable of detoxifying nitrite by oxidising it to low-toxic nitrate (Doblender and Lackner, 1996). Several systems are known to produce nitrate from nitrite in animals, the most commonly known being haemoglobin, catalase and cytochrome oxidase. A part of detoxification takes place in the liver, as revealed by the ability of trout hepatocytes to oxidise nitrite to nitrate (Doblender and Lackner, 1996). Attention was also paid to the capacity of erythrocytes to convert nitrite to nitrate (Doblender and Lackner, 1997). Oxygenated trout erythrocytes detoxify nitrite to nitrate whereas anoxic ones do not (Doblender and Lackner, 1997). The mechanism is via the oxida-

tion of haemoglobin to methaemoglobin depicted in the equation described before. This reaction can therefore be considered both a toxic mechanism (by converting haemoglobin to methaemoglobin) and a detoxification mechanism (by converting nitrite to nitrate) (Doblender and Lackner, 1997). The reaction between haemoglobin and nitrite may serve as a model reaction between iron-containing protein and nitrite. The intensity of biotransformation may be governed by the availability of haem proteins (ferrous and/or ferric ones) and by the presence of antioxidants. There are several low molecular weight redox substances which reduce nitrite toxicity: methylene blue, ascorbic acid, uric acid, methyl uric acid, violuric acid, ribosyl uric acid and glutathione (as reviewed by Doblender and Lackner, 1996). Nitrate, the final product of the detoxification mechanism, is possibly excreted via urine and bile.

3.3. Physiological disturbances induced by nitrite

Nitrite has multiple physiological effects (e.g. Jensen, 2003). One critical consequence of nitrite accumulation is the oxidation of haemoglobin to methaemoglobin (as mentioned above). However, mortality in nitrite-exposed fish can be associated with both high and low methaemoglobin levels (Margiocco et al., 1983), suggesting that the toxicity mechanism of nitrite can be connected with different effects – independent or synergistic – and that methaemoglobinaemia is only one of these effects.

As a consequence, the hypoxic conditions caused by methaemoglobinaemia could damage various organs such as liver (Ariillo et al., 1984) or retina (Hofer and Gatumu, 1994) to a different extent depending on their characteristic of anatomy and blood flow conditions. Tissue hypoxia and anaerobic energy production can be reflected in elevated plasma lactate concentrations (Jensen et al., 1987; Stormer et al., 1996). Moreover, nitrite also induces lysosomal and liver mitochondria alterations in fish resulting in a remarkable increase in the lability of these organelles (Mensi et al., 1982; Ariillo et al., 1984). Nitrite concentrations are comparable in the liver and in the brain after nitrite intoxication. However, the high methaemoglobin values neither cause a cerebral hypoxic state nor alter the mitochondrial function in this organ; thus, anaerobic

hypoxia damages the brain less than the liver (Ariillo et al., 1984).

The general oxygen starvation eventually leads to the gasping behaviour frequently seen in nitrite-intoxicated fish. Nitrite exposure induces significant hyperventilation (Jensen et al., 1987; Aggergaard and Jensen, 2001).

Histopathological studies of fish exposed to pollutants revealed that fish organs were efficient indicators of water quality (Cardoso et al., 1996; Barlas, 1999; Cengiz et al., 2001). The gills are important organs in fish for respiration, osmotic regulation, acid base balance and nitrogenous waste excretion (Heath, 1987). Hyperplasia, vacuolisation and elevated numbers of chloride cells were the main histological lesions that occurred in the gills of nitrite treated carp (*Cyprinus carpio*) (Svobodova et al., 2005b). Michael et al. (1987) observed hyperplasia and hypertrophy in the gills of *Clarias lazera* chronically exposed to nitrite.

Nitrite critically influences the potassium balance. It was originally observed that nitrite exposure significantly elevated extracellular K^+ in carp (Jensen et al., 1987). The rise in extracellular K^+ results from the loss of K^+ from red blood cells and skeletal muscle, as revealed by studies on trout (Stormer et al., 1996) and carp (Jensen, 1990; Knudsen and Jensen, 1997). The rise of extracellular K^+ is unfavourable for the heart and other excitable tissues because it causes depolarisation that can potentially lead to heart failure and nerve malfunction. The decline in intracellular K^+ content is also critical due to its possible influence on the muscular metabolism and function (Jensen, 2003). The efflux of K^+ from red blood cells seems to result from the activation of a K^+/Cl^- cotransporter that is normally involved in cell volume regulation (Jensen, 1990, 1992). The activation of K^+/Cl^- efflux draws osmotically obligated water out of the cells and hence induces erythrocyte shrinkage (Jensen, 1990, 1992). Red blood cell shrinkage is usually followed by the loss of haemoglobin solubility, resulting in haemoglobin crystals and structural damage to erythrocytes (Jensen et al., 1987). Furthermore, the high activity of the methaemoglobin-reductase system to convert methaemoglobin to haemoglobin during nitrite exposure results in a high metabolic cost to the red blood cells, shortening the normal life span of these cells (Scarano et al., 1984; Jensen et al., 1987). These above-mentioned disturbances are followed by an increase in haematocrit, red blood cell counts and in the haemoglobin concentration

(Brown and McLeay, 1975; Jensen, 1990; Avilez et al., 2004). The erythrocytes of nitrite treated carp showed a significantly higher number of elongated erythrocytes with the nucleus located in one cell pole and all erythrocytes had remarkably pale cytoplasm compared to the control group (Svobodova et al., 2005b).

Nitrite exposure can potentially affect cardiovascular functions. For example, a rapid and persistent increase in the heart rate developed in rainbow trout exposed to 1mM ambient nitrite (Aggergaard and Jensen, 2001). This suggests nitrite-induced vasodilation possibly via nitric oxide generated from nitrite (Gladwin et al., 2000) that is countered by increased cardiac pumping to re-establish the blood pressure. The heart rate variability (i.e. variability in the time elapsed between consecutive heartbeats) also decreased in nitrite-exposed trout (Aggergaard and Jensen, 2001). The reduced heart rate variability may be due to a critical change in the automatic control of the heart, and it seems to reflect the physiological deterioration (Jensen, 2003).

Nitrite may have some general effects on nitrogen metabolism and excretion whose manifestation probably depends on the degree of nitrite intoxication (Jensen, 2003). In rainbow trout, small elevations in ammonia excretion across the gills and via urine were observed (Zachariasen, 2001). Changes in nitrogenous excretion were also observed in marine decapod crustaceans. Under the stress of nitrite Kumura shrimp increased their haemolymph urea with a concomitant decrease in haemolymph ammonia and increases in ammonia-N excretion, urea-N excretion and organic-N excretion indicating both ammoniogenesis and ureogenesis take place (Cheng and Chen, 2001). Further studies are needed to better understand the interaction between nitrite and the nitrogen metabolism of fish.

The physiological messenger molecule of nitric oxide can be produced from nitrite, this process being promoted by low pH, hypoxia and by a high nitrite concentration (Benjamin et al., 1994; Zweier et al., 1999). So it is possible that nitrite may interfere with a number of processes that are regulated by this local hormone, including blood pressure, vascular tone, neural signalling and immunological functions. Besides that, NO is proved to be a potent inhibitor of steroid hormone synthesis (Ahsan et al., 1997; Cymeryng et al., 1998). Nitrite was reported to decrease steroid

hormone synthesis in Leydig tumour cells in mice *in vitro* and in rats *in vivo* (Panesar and Chan, 2000). Rats given distilled water containing 50 mg/l NaNO_2 for 4 weeks daily drank significantly less. At the end, their blood corticosterone and testosterone levels were significantly decreased (Panesar and Chan, 2000). The mechanism presumably involves conversion of nitrite to NO, which inhibits cytochrome P450_{scc} (cholesterol side chain cleavage enzyme), the initial and rate limiting step in the pathway leading from cholesterol to steroid hormones (Panesar and Chan, 2000). The impact of environmental nitrites on steroid hormone synthesis in aquatic animals should also be addressed.

It is well known that nitrite strikingly reacts with NH_2 and SH groups through its derivatives, thus being able to inhibit several enzymes and to generate mutagenic or carcinogenic compounds such as nitrosamide-like compounds (De Flora and Arillo, 1983).

The presence of sublethal concentrations of noxious chemicals in freshwater environments can promote the emergence and development of infectious diseases in fish (Carballo and Munoz, 1991; Carballo et al., 1995). Rainbow trout exposed for 24 hours to 0.24 mg/l NO_2^- (corresponding to 50% of lethal concentration 96h LC_{50}) were challenged after toxin exposure with *Saprolegnia parasitica* causing mycotic dermal infection. The acute stress response provoked by the toxin exposure accounts for the main contribution to the increase in saprolegniosis susceptibility, representing approximately a 100% increase in the percentage of infected fish when compared with the control group (Carballo et al., 1995).

4. Factors affecting nitrite toxicity

4.1. Length of nitrite exposure

Generally, a 24–48 hour exposure is required for maximum accumulation of nitrite in fish (Huey et al., 1980; Eddy et al., 1983; Aggergaard and Jensen, 2001). As expected, the lethal concentration LC_{50} declines after 24 hours. The rate of decline is very low by the time the exposure has reached 96 hours. Thus the relevant duration for short-term toxicity testing is probably 24 to 96 hours as is the case for many toxicants (Lewis and Morris, 1986).

The literature dealing with long-term toxicity of sublethal nitrite concentrations corresponding to

10% of 96hLC₅₀ suggests that such a concentration should not be detrimental to freshwater fish. Neither growth suppression nor tissue damage was observed (Wedemeyer and Yasutake, 1978; Colt et al., 1981).

The results of an experiment conducted on channel catfish clearly indicated that these fish acclimated to nitrite (Tucker and Schwedler, 1983). The fish that were not previously exposed to > 0.01 mg/l N-NO₂⁻ (NO₂⁻/Cl⁻ molar ratio < 0.003) developed higher levels of methaemoglobin than the fish with immediate past history of exposure to relatively high NO₂⁻/Cl⁻ molar ratios. These fish were exposed to > 3.0 mg/l N-NO₂⁻ for the preceding 2 weeks (NO₂⁻/Cl⁻ molar ratio approximately 0.26). Doblender and Lackner (1997) and Machova et al. (2004) reported similar results.

4.2. Water quality

4.2.1. Chloride

Since 1977 nitrite toxicity has been known to depend greatly on the salinity of the water in which the nitrite exposure took place (Crawford and Allen, 1977). Mortality in seawater occurred at nitrite concentrations 50 to 100 times higher than in fresh water (Crawford and Allen, 1977). The effect of chloride on the toxicity of nitrite is now known to be so great that experiments in which chloride concentrations are not documented are of very low value because they cannot be meaningfully compared with the results of other studies. According to the EIFAC (1984) recommendation, it is very important to monitor the Cl⁻/N-NO₂⁻ ratio in aquaculture. The ratio of 17 and 8 is recommended for salmon and rough fish, respectively. According to our observations, in the case of death of wels (*Silurus glanis*) and tench (*Tinca tinca*) the Cl⁻ to N-NO₂⁻ weight ratios ranged between 13 and 28 and between 11 and 19, respectively (Svobodova et al., 2005a). In the case of Nile tilapia (*Oreochromis niloticus*) health impairment without symptoms of toxicity, the ratios ranged between 50 and 150 (Svobodova et al., 2005a). The experiments conducted on rainbow trout and fathead minnow (*Pimephales promelas*) showed that the relationship between nitrite toxicity and chloride concentration was linear (Russo and Thurston, 1977; Palachek and Tomasso, 1984b; McConnell, 1985). Machova et al.

(2004) also proved the linear relationship between lethal concentration and chloride concentration in water for ornamental fish *Poecilia reticulata*. The 96hLC₅₀ values of NaNO₂ ranged between 39 and 436 mg/l depending on the chloride concentration (10–190 mg/l). The effect of chloride on LC₅₀ for nitrite appears to be inversely related to the sensitivity of a fish species to nitrite. The most sensitive species benefit from chloride addition to the least extent although the benefit is large even for sensitive fish (Lewis and Morris, 1986).

4.2.2. Other anions

Other anions were found to inhibit nitrite toxicity to a different extent. Bromide, which is chemically similar to chloride, was studied by Eddy et al. (1983), who found that 1mM of sodium bromide (80 mg/l) was enough to offset the presence of 0.7mM nitrite (32 mg/l nitrite-N) almost completely for Atlantic salmon (*Salmo salar*) in fresh water. It was documented in literature that bicarbonate and nitrate had detectable effects but they were not so effective as those of chloride and bromide. Divalent and trivalent anions (such as sulphate, phosphate and borate) had very low effects on nitrite toxicity (Lewis and Morris, 1986).

4.2.3. Cations

Calcium, magnesium, sodium and potassium are typically present in considerable quantities in fresh waters. The effect these ions might have on nitrite toxicity is therefore of interest. However, the mechanism of any effects would obviously be different from that of chloride.

Calcium chloride was more effective than sodium chloride at reducing nitrite toxicity in striped bass (*Morone saxatilis*) (Mazik et al., 1991) or short-nose sturgeon fingerlings (*Acipenser brevirostrum*) (Fontenot and Isely, 1999). On the other hand, Bowser et al. (1983) found that sodium chloride and calcium chloride provided equivalent protection against nitrite toxicity for channel catfish, suggesting that the identity of the metal cation was of small importance. Krous et al. (1982) pointed out that high concentrations of calcium generally reduced the loss of chloride through the gills. This in turn diminished the requirement for nitrite uptake. Thus there are theoretical reasons to expect that calcium ions will reduce ni-

trite toxicity although experimental work that has been done so far proves that the effect is a weak one (Lewis and Morris, 1986).

4.2.4. Ammonia

An urgent problem associated with the recirculation of water on a fish farm is potential accumulation of both ammonia and nitrite, making it pertinent to study possible interactive effects of these pollutants. In a study on channel catfish, no synergistic or additive effects on plasma corticosteroid levels were observed when the fish were exposed to a combination of ammonia and nitrite (Tomasso et al., 1981). In this study, however, high amounts of NH_4Cl were added together with NaNO_2 . Since the elevation of ambient chloride concentrations protects against nitrite toxicity, the added chloride might have affected the results obtained. In a study on rainbow trout (Vedel et al., 1998), when the desired nitrite and ammonia concentrations were achieved by adding dissolved NaNO_2 and NH_4NO_3 , the combined nitrite and ammonia exposure resulted in high mortality at the highest exposure concentrations ($600\mu\text{M NO}_2^-$ and $18\mu\text{M NH}_3$). This was not explained by synergistic effects of ammonia and nitrite on any of the measured parameters (plasma osmolality, amino acid and ion concentrations, blood respiratory parameters, muscle potassium concentrations and water content, plasma transaminase activity, brain glutamate and glutamine concentrations).

4.2.5. pH

The effect of the hydrogen ion concentration on toxicity of nitrite is still uncertain. Contributions to the literature on this subject are not frequently definitive because they fail to separate possible effects of anions from those of acidity, or they use pH ranges outside the normal adaptive range of fish (as reviewed by Lewis and Morris, 1986). The effect of pH on nitrite toxicity within the natural pH range appears to be minute.

4.2.6. Oxygen and temperature

Oxygen can affect nitrite toxicity because nitrite reduces the oxygen-carrying capacity of blood. A

reduction in oxygen supply in the external medium will exacerbate the oxygen supply problem in fish. Bowser et al. (1983) showed that an oxygen concentration of 5 mg/l, in the presence of nitrite, was not sufficient for channel catfish even though channel catfish normally tolerate oxygen concentrations below this value.

Temperature, which influences tissue oxygen demand, could also be expected to affect nitrite toxicity. Over a relatively small range (22–30°C), Colt and Tchobanoglous (1976) showed no significant relationship between nitrite toxicity and temperature. In the study of Huey et al. (1984), channel catfish kept at 30°C in the presence of 0.91 mg/l nitrite-N over a period 24 hours developed methaemoglobin concentrations almost twice as high as those of fish held at 10°C. Huey et al. (1984) also found that the fish kept at 30°C showed a more rapid return to background haemoglobin levels in the absence of nitrite.

A higher amount of oxygen in water at lower temperatures and lower metabolic rates of fish at lower temperatures might render nitrite a less potent toxin at lower temperatures. However, it is also assumed that lower temperatures reduce the efficiency of detoxification mechanisms (Lewis and Morris, 1986). General conclusions should be approached with caution.

4.3. Fish size and age

The study by Perrone and Meade (1977) indicated that coho salmon (*Oncorhynchus kisutch*) fry had a higher tolerance to nitrite than coho yearlings. Russo et al. (1974) and Bartlett and Neumann (1998) also observed this phenomenon in other species of salmonids. There may be a variation in the activity of the methaemoglobin reductase system between young and adult individuals (Kiese, 1974). Spicer and Reynolds (1949) reported that under certain conditions red cells from weanling rabbits reduced methaemoglobin more rapidly than cells from their adult counterparts. Another explanation might be found in different respiratory physiology of larvae and adults. Because scales first start to form toward the end of the alevin phase of development (Balon, 1975), cutaneous oxygen uptake is higher for fry than for adults. The surface area to total mass ratio is considerably higher for younger fish, allowing a higher rate of oxygen diffusion (Rombough and Moroz, 1990). Due to these factors, oxygen taken

up through the skin permeates to the internal tissues far better in an alevin than in an older fish. When one takes into consideration that nitrite is a blood poison, it seems logical that fry could survive for a longer time when the oxygen-carrying capacity of blood is reduced than would an adult who depends on oxygen delivered by the blood stream for survival (Bartlett and Neumann, 1998). In a lethal experiment conducted on Nile tilapia (*Oreochromis niloticus*) Atwood et al. (2001) found out that tolerance to nitrite was significantly affected by the fish size (the difference in age and sexual development between both groups was not mentioned). The 96hLC₅₀ for smaller fish (average weight 4.4 ± 1.50 g) was 81 mg/l N-NO₂⁻ and larger fish (90.7 ± 16.43 g) demonstrated a 96hLC₅₀ of 8 mg/l N-NO₂⁻.

4.4. Intraspecific differences

Some fish species show intraspecific variability in nitrite uptake and in nitrite susceptibility. These differences between individuals were often observed in rainbow trout (Margiocco et al., 1983; Arrilo et al., 1984; Aggergaard and Jensen, 2001). According to Aggergaard and Jensen (2001), rainbow trout exposed to nitrite (1mM) could be divided into two distinct groups based on the rate of nitrite accumulation and time of mortality. Group 1 accumulated nitrite in the plasma to 2.9mM after 24 hours and died within 48 hours. Group 2 showed a slower accumulation of nitrite, plasma nitrite reached only 1.8mM after 24 hours and the fish survived for 96–144 hours. The differences in nitrite accumulation could be due to a variable number and surface area of branchial chloride cells (Perry and Goss, 1992). Another explanation of the intraspecific differences would be a variable nitrite detoxification and elimination mechanism (Aggergaard and Jensen, 2001). Future studies are needed to evaluate the cause of the intraspecific difference in nitrite susceptibility.

4.5. Fish species

Data from acute toxicity tests for a variety of fishes reveal a very wide range of results, not just between different families of fish, but within families as well.

Salmonids are among the most sensitive of the taxa that have been studied, and show very small

differences between species. There is a considerable variation among the warmwater fish taxa: channel catfish are as sensitive as salmonids, and logperch (*Percina caprodes*), brook stickleback (*Culaea inconstans*) and blue tilapia (*Tilapia aurea*) seem to be similarly sensitive or only slightly less sensitive. The cyprinids, catostomids, mottled sculpin (*Cottus bairdi*) and black bullhead (*Ictalurus melas*) are considerably less sensitive. The centrarchids are especially insensitive to nitrite toxicity (Lewis and Morris, 1986). Critical concentrations for the largemouth bass (*Micropterus salmoides*) are quite high because the largemouth bass, unlike other fishes whose blood nitrite concentrations have been studied, does not concentrate nitrite in the blood plasma, and thus appears to discriminate nitrite from chloride (Palachek and Tomasso, 1984a).

5. Conclusion

In recent years the harmful effect of nitrite on fish has attracted a lot of attention. In particular in aquacultural facilities with water re-use systems, high levels of nitrite have been found to cause severe physiological disturbances or they have resulted in mass fish mortalities.

The above-mentioned data document that nitrite toxicity to fish depends on a large number of external and internal factors. The importance of individual factors is continuously assessed and re-evaluated. Different authors often arrive at contradictory conclusions, and no final explanation of the combined effect of individual internal and external factors on nitrite toxicity to fish has been put forward. Future research is needed to be able to better understand the nitrite toxicity mechanism.

6. REFERENCES

- Aggergaard S., Jensen F.B. (2001): Cardiovascular changes and physiological response during nitrite exposure in rainbow trout. *Journal of Fish Biology*, 59, 13–27.
- Ahsan S., Lacey M., Whitehead S.A. (1997): Interactions between interleukin-1 beta, nitric oxide and prostaglandin E2 in the rat ovary: Effects on steroidogenesis. *European Journal of Endocrinology*, 137, 293–300.
- Arrilo A., Gaino E., Margiocco C., Mensi P., Schenone G. (1984): Biochemical and ultrastructural effects of nitrite in rainbow trout: Liver hypoxia as the root of the

- acute toxicity mechanism. *Environmental Research*, 34, 135–154.
- Atwood H.L., Fontenot Q.C., Tomasso J.R., Isely J.J. (2001): Toxicity of nitrite to Nile tilapia: effect of fish size and environmental chloride. *North American Journal of Aquaculture*, 63, 49–51.
- Avilez I.M., Altran A.E., Aguiar L.H., Moraes G. (2004): Haematological responses of the neotropical teleost matrinxã (*Brycon cephalus*) to environmental nitrite. *Comparative Biochemistry and Physiology, Part C*, 139, 135–139.
- Avnimelech Y., Weber B., Hefner B., Milstein A., Zorn M. (1986): Studies in circulated fish ponds: organic matter recycling and nitrogen transformation. *Aquaculture and Fisheries Management*, 17, 231–242.
- Balon E.K. (1975): Terminology of intervals in fish development. *Journal of the Fisheries Research Board of Canada*, 32, 1663–1670.
- Barlas N. (1999): Histopathological examination of gill, liver and kidney tissues of carp (*Cyprinus carpio* L., 1758) fish in the upper Sakarya River Basin. *Turkish Journal of Veterinary & Animal Sciences* 23, 277–284.
- Bartlett F., Neumann D. (1998): Sensitivity of brown trout alevins (*Salmo trutta* L.) to nitrite at different chloride concentrations. *Bulletin of Environmental Contamination and Toxicology*, 60, 340–346.
- Benjamin N., O'Driscoll F., Dougall H., Duncan C., Smith L., Golden M., McKenzie H. (1994): Stomach NO synthesis. *Nature*, 368, 502.
- Beutler E. (1968): *Hereditary Disorders of Erythrocyte Metabolism*. Grune and Stratton, New York. 185 pp.
- Bodansky O. (1951): Methaemoglobinaemia and methaemoglobin-producing compounds. *Pharmacological Review*, 3, 144–196.
- Bowser P.R., Falls W.W., VanZandt J., Collier N., Phillips J.D. (1983): Methaemoglobinaemia in channel catfish: methods of prevention. *Progressive Fish-Culturist*, 45, 154–158.
- Brown D.A., McLeay D.J. (1975): Effect of nitrite on methaemoglobin and total haemoglobin of juvenile rainbow trout. *Progressive Fish-Culturist*, 37, 36–43.
- Cameron J.N. (1971): Methaemoglobin in erythrocytes of rainbow trout. *Comparative Biochemistry and Physiology A, Comparative Physiology*, 40, 743–749.
- Carballo M., Munoz M.J. (1991): The effect of sublethal concentrations of four chemicals on susceptibility of juvenile rainbow trout (*Oncorhynchus mykiss*) to saprolegniosis. *Applied and Environmental Microbiology*, 57, 1813–1816.
- Carballo, M., Munoz, M.J., Cuellar, M., Tarazona, J.V. (1995): Effect of waterborne copper, cyanide, ammonia, and nitrite on stress parameters and changes in susceptibility to saprolegniosis in rainbow trout (*Oncorhynchus mykiss*). *Applied and Environmental Microbiology*, 61, 2108–2112.
- Cardoso E.L., Chiarini-Garcia H., Ferreira R.M.A., Poli C.R. (1996): Morphological changes in the gills of *Lophiosilurus alexandri* exposed to unionized ammonia. *Journal of Fish Biology*, 49, 778–787.
- Cengiz E.I., Unlu E., Balci, K. (2001): The histopathological effects of Thiodan® on the liver and gut of mosquitofish, *Gambusia affinis*. *Journal of Environmental Science and Health B*, 36, 75–85.
- Cheng S.Y., Chen J.C. (2001): The time-course change of nitrogenous excretion in the Kuruma shrimp *Penaeus japonicus* following nitrite exposure. *Aquatic Toxicology*, 51, 443–454.
- Colt J., Tchobanoglous G. (1976): Evaluation of short-term toxicity of nitrogenous compounds in the channel catfish, *Ictalurus punctatus*. *Aquaculture*, 8, 209–224.
- Colt J., Ludwig R., Tchobanoglous G., Cech J.J. (1981): The effect of nitrite on the short-term growth and survival of channel catfish, *Ictalurus punctatus*. *Aquaculture*, 24, 111–122.
- Crawford R.E., Allen G.H. (1977): Seawater inhibition of nitrite toxicity to chinook salmon. *Transactions of the American Fisheries Society*, 106, 105–109.
- Cymeryng C.B., Dada L.A., Podesta E.J. (1998): Effect of nitric oxide on rat adrenal zona fasciculata steroidogenesis. *Journal of Endocrinology*, 158, 197–203.
- De Flora S., Arillo A. (1983): Mutagenic and DNA damaging activity in muscle of trout exposed *in vivo* to nitrite. *Cancer Letters*, 20, 147–155.
- Doblender C., Lackner R. (1996): Metabolism and detoxification of nitrite by trout hepatocytes. *Biochimica et Biophysica Acta*, 1289, 270–274.
- Doblender C., Lackner R. (1997): Oxidation of nitrite to nitrate in isolated erythrocytes: a possible mechanism for adaptation to environmental nitrite. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 157–161.
- Dvorak P. (2004): Selected specificity of aquarium fish disease (in Czech). *Bulletin VURH Vodnany*, 40, 101–108.
- Eddy F.B., Kunzlik P.A., Bath R.N. (1983): Uptake and loss of nitrite from the blood of rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L. in fresh water and in dilute sea water. *Journal of Fish Biology*, 23, 105–116.
- EIFAC (1984): Water quality criteria for European freshwater fish: Report on nitrite and freshwater fish. EIFAC Technical Paper, 46, 19 pp.
- Fontenot Q.C., Isely J.J. (1999): Characterization and inhibition of nitrite uptake in shortnose sturgeon fingerlings. *Journal of Aquatic Animal Health*, 11, 76–80.

- Gladwin M.T., Shelhamer J.H., Schechter A.N., Pease-Fye M.E., Waclawiw M.A., Panza J.A., Ognibene F.P., Cannon R.O. (2000): Role of circulating nitrite and S-nitrosohaemoglobin in the regulation of regional blood flow in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 11482–11487.
- Heath A.G. (1987): *Water Pollution and Fish Physiology*. CRC Press, Inc., Florida. 272 pp.
- Hofer R., Gatumu E. (1994): Necrosis of trout retina (*Oncorhynchus mykiss*) after sublethal exposure to nitrite. *Archives of Environmental Contamination and Toxicology*, 26, 119–123.
- Huey D.W., Beitinger T.L. (1982): A haemoglobin reductase system in channel catfish *Ictalurus punctatus*. *Canadian Journal of Zoology*, 60, 1511–1513.
- Huey D.W., Simco B.A., Criswell D.W. (1980): Nitrite-induced methaemoglobin formation in channel catfish. *Transactions of the American Fisheries Society*, 109, 558–562.
- Huey D.W., Beitinger T.L., Wooten M.C. (1984): Nitrite-induced methaemoglobin formation and recovery in channel catfish (*Ictalurus punctatus*) at three acclimation temperatures. *Bulletin of Environmental Contamination and Toxicology*, 32, 674–681.
- Jensen F.B. (1990): Nitrite and red cell function in carp: control factors for nitrite entry, membrane potassium ion permeation, oxygen affinity and methaemoglobin formation. *Journal of Experimental Biology*, 152, 149–166.
- Jensen F.B. (1992): Influence of haemoglobin conformation, nitrite and eicosanoids on K⁺ transport across the carp red blood cell membrane. *Journal of Experimental Biology*, 171, 349–371.
- Jensen F.B. (1995). Uptake and effects of nitrite and nitrate in animals. In: Walsh P.J., Wright P. (eds.): *Nitrogen Metabolism and Excretion*. CRC Press, Boca Raton. 289–303.
- Jensen F.B. (2003): Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology, Part A*, 135, 9–24.
- Jensen F.B., Andersen N.A., Heisler N. (1987): Effects of nitrite exposure on blood respiratory properties, acid-base and electrolyte regulation in the carp (*Cyprinus carpio*). *Journal of Comparative Physiology B*, 157, 533–541.
- Kamstra A., Span J.A., Van Weerd J.H. (1996): The acute toxicity and sublethal effects of nitrite on growth and feed utilization of European eel, *Anguilla anguilla* (L.). *Aquaculture Research*, 27, 903–911.
- Kiese M. (1974): *Methaemoglobinaemia: A Comprehensive Treatise*. CRC Press, Cleveland, OH.
- Knudsen P.K., Jensen F.B. (1997): Recovery from nitrite-induced methaemoglobinaemia and potassium balance disturbances in carp. *Fish Physiology and Biochemistry*, 16, 1–10.
- Kosaka H., Tyuma I. (1987): Mechanism of autocatalytic oxidation of oxyhaemoglobin by nitrite. *Environmental Health Perspectives*, 73, 147–151.
- Krous S.R., Blazer V.S., Meade T.L. (1982): Effects of acclimation time on nitrite movement across the gill epithelia of rainbow trout: the role of “chloride cells”. *Progressive Fish-Culturist*, 44, 126–130.
- Lewis W.M., Morris D.P. (1986): Toxicity of nitrite to fish: A Review. *Transactions of the American Fisheries Society*, 115, 183–195.
- Love M.R. (1980): *The chemical biology of fishes*. Academic Press, New York.
- Maetz J. (1971): Fish gills: mechanism of salt transfer in fresh water and sea water. *Philosophical Transactions of the Royal Society of London Series B Biological Sciences*, 262, 209–249.
- Machova J., Kroupova H., Piackova V., Hamackova J., Valentova O. (2004): Acute toxicity of nitrite for fish in relation to chloride concentration in water. In: *Ochrona zdrawia ryb-aktualne problemy*. 275 pp.
- Margiocco C., Arillo A., Mensi P., Shenone G. (1983): Nitrite bioaccumulation in *Salmo gairdneri* Rich. and haematological consequences. *Aquatic Toxicology*, 3, 261–270.
- Mazik P.M., Hinman M.L., Winkelmann D.A., Klaine S.J., Simco B.A. (1991): Influence of nitrite and chloride concentrations on survival and haematological profiles of striped bass. *Transactions of the American Fisheries Society*, 120, 247–254.
- McConnell R. (1985): Toxicity of nitrite to the fathead minnow. Colorado Department of Health File Report, Denver.
- Mensi P., Arillo A., Margiocco C., Schenone G. (1982): Lysosomal damage under nitrite intoxication in rainbow trout (*Salmo gairdneri* Rich.). *Comparative Biochemistry and Physiology*, 73, 161–165.
- Michael M.I., Hilmy A.M., el-Domiatiy N.A., Wershana K. (1987): Serum transaminases activity and histopathological changes in *Clarias lazera* chronically exposed to nitrite. *Comparative Biochemistry and Physiology C*, 86, 255–262.
- Palachek R.M., Tomasso J.R. (1984a): Toxicity of nitrite to channel catfish (*Ictalurus punctatus*), tilapia (*Tilapia aurea*), and largemouth bass (*Micropterus salmoides*): evidence for a nitrite exclusion mechanism. *Canadian Journal of Fisheries and Aquatic Sciences*, 41, 1739–1744.
- Palachek R.M., Tomasso J.R. (1984b): Nitrite toxicity to fathead minnows: effect of fish weight. *Bulletin of Environmental Contamination and Toxicology*, 32, 238–242.

- Panesar N.S., Chan K.W. (2000): Decreased steroid hormone synthesis from inorganic nitrite and nitrate: studies *in vitro* and *in vivo*. *Toxicology and Applied Pharmacology*, 169, 222–230.
- Perrone S.J., Meade T.L. (1977): Protective effect of chloride on nitrite toxicity to coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Research Board of Canada*, 34, 486–492.
- Perry S.F., Goss G.G. (1992): The interrelationships between gill chloride cell morphology and ionic uptake in four freshwater teleosts. *Canadian Journal of Zoology*, 70, 1775–1786.
- Pitter P. (1999): *Hydrochemistry (in Czech)*. Editor VSCHT, Prague. 568 pp
- Rombough P.J., Moroz B.M. (1990): The scaling and potential importance of cutaneous and branchial surfaces in young chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Experimental Biology*, 154, 1–12.
- Russo R.C., Thurston R.V. (1977): The acute toxicity of nitrite to fishes. In: Tubb R.A. (ed.): *Recent Advances in Fish Toxicity*. EPA-600/3-77-085. US Environmental protection Agency, Corvallis, OR. 118–131.
- Russo R.C., Thurston R.V. (1991): Toxicity of ammonia, nitrite and nitrate to fishes. In: Brune D.E., Tomasso J.R. (eds.): *Aquaculture and Water Quality*. World Aquaculture society, Baton Rouge, Louisiana. 58–89.
- Russo R.C., Smith C.E., Thurston R.V. (1974): Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, 31, 1653–1655.
- Scarano G., Saroglia M.G., Gray R.H., Tibali E. (1984): Haematological responses of sea bass *Dicentrarchus labrax* to sublethal nitrite exposures. *Transactions of the American Fisheries Society*, 113, 360–364.
- Spicer S.S., Reynolds H. (1949): Individual and age variation in methaemoglobin formation and reduction in rabbit erythrocytes. *The American Journal of Physiology*, 159, 47–56.
- Stormer J., Jensen F.B., Rankin J.C. (1996): Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: effects on ionic balance. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 1943–1950.
- Svobodova Z., Kolarova J. (2004): A review of the diseases and contaminant related mortalities of tench (*Tinca tinca* L.). *Veterinarni Medicina*, 49, 19–34. <http://www.vri.cz/docs/vetmed/49-1-19.pdf>
- Svobodova Z., Machova J., Poleszczuk G., Huda J., Hamackova J., Kroupova H. (2005a): Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems: three case studies. *Acta Veterinaria Brno*, 74, 129–137.
- Svobodova Z., Machova J., Drastichova J., Groch L., Luskova V., Poleszczuk G., Velisek J., Kroupova H. (2005b): Haematological and biochemical profile of carp blood following nitrite exposure at different concentration of chloride. *Aquaculture Research*, 36, 1177–1184.
- Tomasso J.R., Simco B.A., Davis K.B. (1979): Chloride inhibition of nitrite-induced methaemoglobinaemia in Channel catfish (*Ictalurus punctatus*). *Journal of the Fisheries Research Board of Canada*, 36, 1141–1144.
- Tomasso J.R., Davis K.B., Simco B.A. (1981): Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrite. *Canadian Journal of Fisheries and Aquatic Sciences*, 38, 1106–1112.
- Tucker C.S., Schwedler T.E. (1983): Acclimation of channel catfish (*Ictalurus punctatus*) to nitrite. *Bulletin of Environmental Contamination and Toxicology*, 30, 516–521.
- Vedel N.E., Korsgaard B., Jensen F.B. (1998): Isolated and combined exposure to ammonia and nitrite in rainbow trout (*Oncorhynchus mykiss*): effect on electrolyte status, blood respiratory properties and brain glutamine/glutamate concentrations. *Aquatic Toxicology*, 41, 325–342.
- Wedemeyer G.A., Yasutake W.T. (1978): Prevention and treatment of nitrite toxicity in juvenile steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, 35, 822–827.
- Williams E.M., Eddy F.B. (1986): Chloride uptake in freshwater teleosts and its relationship to nitrite uptake and toxicity. *Journal of Comparative Physiology B*, 156, 867–872.
- Zachariasen F. (2001): Intraspecific differences in nitrite tolerance of rainbow trout. The role of gill and kidney. [Cand. Scient. Thesis.] Institute of Biology, University of Southern Denmark, Odense.
- Zweier J.L., Samouilov A., Kuppasamy P. (1999): Non-enzymatic nitric oxide synthesis in biological systems. *Biochimica et Biophysica Acta*, 1411, 250–262.

Received: 05–05–03

Accepted: 05–11–14

Corresponding Author

Hana Kroupova, University of South Bohemia, Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Zatisi 728/II, 389 01 Vodnany, Czech Republic
Tel. +420 383 382 402, fax +420 383 382 396, e-mail: kroupova@vurh.jcu.cz