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Sensitivity of Brown Trout Alevins (*Salmo trutta* L.) to Nitrite at Different Chloride Concentrations

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It has been known for decades that nitrite can be very toxic for salmonid fishes. Nitrite is a blood poison which oxidizes the iron of the heme group, causing the formation of methemoglobin, which cannot efficiently bind oxygen (Brown and McLeay 1975). Although low amounts of nitrite occur naturally in running waters as part of the oxidizing process of ammonia to nitrate, it can reach high concentrations through the introduction of nitrogen-rich agricultural and industrial waste water (Borchardt 1992). Due to its importance for fish hatcheries, nitrite toxicity has been studied extensively on salmonids, which are especially sensitive to poor water quality (Smith and Williams 1974). Other studies have revealed that the toxicity of nitrite to fish is dependent on other water parameters, such as the pH-value and the chloride, bicarbonate, calcium, phosphate and sulfate concentrations (Bath and Eddy 1980; Russo et al. 1981). Of these factors, chloride seems to have the most deciding influence. It is believed that nitrite is actively taken up by fish mainly through the chloride cells of their gills. This would explain the moderating effect that higher chloride concentrations have on nitrite toxicity; chloride competes with nitrite for the carriers on the gill epithelia (Bath and Eddy 1980; Huey et al. 1982; Gaino et al. 1984). Due to the uptake being an active process, nitrite can be accumulated in the blood to concentrations many times higher than in the surrounding water (Margiocco et al. 1983).

Although the toxicity of nitrite to adult salmonids has been well studied, little is known about the tolerance of salmonid eggs and larvae to nitrite. Due to the life cycle of salmonid fishes, the eggs and larvae of these species can be exposed to considerable concentrations of nitrite (up to 1,5 mg/L NO_2-N); their chance of survival in the interstitial water can be endangered by decreasing oxygen concentrations directly (Ingendahl and Neumann 1996) and probably also indirectly by nitrite generated anaerobically through the denitrification of nitrate contained in the downwelling surface water (Borchardt 1992).

Brown and rainbow trout eggs seem to be impervious even to high concentrations of nitrite (at least 3.1 mg/L NO_2-N, unpublished experiments), meaning that larvae are first exposed to nitrite during hatching. The object of this study was to examine the susceptibility of alevins (after the end of the yolk-sac stage) to concentrations of nitrite known to be toxic to adult trout. At the same time, the influence of different chloride concentrations on nitrite toxicity was tested. As a comparison, adult brown trout were also tested to check for agreement with data given in the literature. Using this comparison, it was possible to establish that the alevins were actually less sensitive to nitrite than the adult fish. The consequences on the evaluation of limiting chemical conditions for salmonids will be discussed.

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MATERIALS AND METHODS

Brown trout eggs (*S. trutta* f. *fario*) were obtained from the North Rhine Westphalian agency for ecology, land and forestry (fisheries divsion, LÖBF) in Kirschhundem/Albaum, NRW, Germany. The eggs were collected from several 3+ generation adult brown trout which had not bred previously. The eggs were reared in the same water used later for experimentation, which had the parameters given in table 1.

Table 1. Physiochemical parameters of the water used for rearing and experimentation of all test animals (in mg/L when not otherwise noted).

parameter	mean +/- std	parameter	mean +/- std
temperature (°C)	9.0 +/- 0.6	calcium	4.88 +/- 0.13
pH	7.1 +/- 0.1	magnesium	0.51 +/- 0.01
conductance (µS/cm)	51.5 +/- 3.1	potassium	0.41 +/- 0.02
oxygen	10.8 +/- 0.7	iron	< 0.1
chloride	3.1 +/- 0.3	copper	< 0.3
ammonia-N (µg/L)	48.9 +/- 3.1	phosphate	< 0.1
nitrite-N (µg/L)	5.5+/- 0.6	light:dark (h)	9:15
nitrate-N	1.4 +/- 0.3		

After hatching, which occurred after 330-380 degree-days, the eleutheroembryos (sac fry) were collected and distributed into small (2 L) flow-through tanks at two different chloride concentrations: 3 mg/L and 10 mg/L, whereby the latter concentration was achieved by the addition of sodium chloride. The embryos were left to adjust to the different chloride concentrations until after the yolk had been fully assimilated, which occured after 21 days (wet weight of larvae ca. 75 mg). Nitrite was then added in the form of sodium nitrite to several of the tanks, resulting in the five experimental conditions given in figure 1 (next page).

Starting after the yolk sac had been fully absorbed, the fish were fed *ad libitum* on a mixed diet of *Bosmina, Chironomus* larvae, and *Daphnia.* For each of the five experimental conditions, 124-126 alevins were divided among two flow tanks. The water in each of the tank pairs was pumped from the same reservoir tank, at the rate of 3.5-4.0 L/d. The experiment ran for thirty days, during which time all mortalities as well as morphological and behavioral differences were noted.

As a comparison, adult brown trout (1+ generation) were tested under conditions given by experiment no. 3 in fig. 1, i.e. 3 mg/L Cl^- and 4 mg/L NO_2-N. The twenty trout, weighing 10-36 g (9-14 cm in length) were also obtained from the aforementioned agency and were kept for twenty days under natural lighting at 9 °C and 3 mg/L chloride before the start of the experiments (i.e. the addition of nitrite), in order to allow them to adapt to the tank and water. Ten fish were used as control animals, the other ten as test animals. Each of the two tanks contained 300 L of aerated water (of the same composition as by the alevins), circulation was provided by large pumps. 15% of the water was changed every other day.

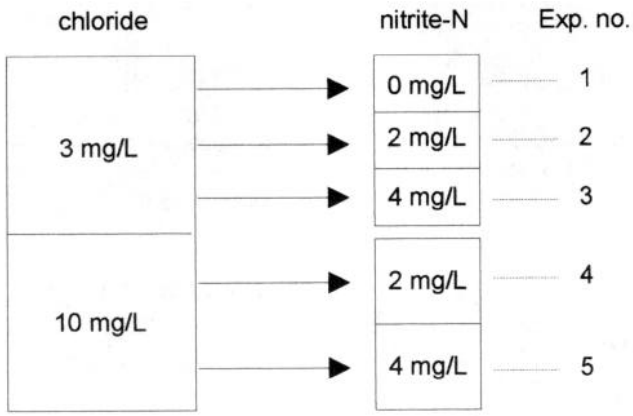

Figure 1. Experimental combinations of chloride and nitrite concentrations.

RESULTS AND DISCUSSION

As shown in fig. 2, the rate of alevin mortality was strongly influenced by the nitrite concentration. At a concentration of 4 mg/L NO_2N (in connection with 3 mg/L of chloride), 5% and 50% mortality were reached after ca. 15 and 22 days, respectively; 95% mortality was reached on the 27th day. In comparison, at a concentration of 2 mg/L NO_2-N (at the same chloride concentration), it took 20 and 27 days to reach 5% and 50% mortality, respectively; by the end of the experiment (day 30) there were still 39 of 125 (31%) alevins alive. Very few control animals (i.e. without nitrite) died during the experiment; by the 30th day only two of 124 (1.6%) alevins had died.

The nitrite-induced mortality was dramatically reduced with increasing chloride concentration, as depicted in fig. 3. In this test series the water contained 10 mg/L of chloride instead of 3 mg/L. At the same two nitrite concentrations used above (2 and 4 mg/L NO_2-N), alevin mortality started considerably later. At 4 mg/L NO_2-N, 5% mortality was reached on the 24th day (a delay of nine days from the observation by 3 mg/L Cl^-). At 2 mg/L NO_2-N, the 5% mortality mark was not reached until the 29th day (in comparison to the 20th day in connection with 3 mg/L Cl^-, also a delay of nine days).

This reduction of nitrite toxicity at higher chloride concentrations has been well documented in adult trout (e.g. Gaino et al. 1984; Rodriguez-Moreno and Tarazona 1994; Williams and Eddy 1986). Most experiments concerning nitrite toxicity to fish have been LC-50 (96 hr) or other short-term, high concentration tests. Because this experiment lasted 30 days, it was possible to observe that higher chloride concentrations only slow down the nitrite poisoning. Nitrite seems to be still taken up by the chloride cells but at a reduced rate. For example, 3 mg/L of chloride were able to prevent alevin mortality at 4 mg/L NO_2-N only until about the 15th day (5% mortality) after which time the mortality increased at a fairly constant rate (fig. 2). A similar effect was seen in the other test series shown in figs. 2 and 3.

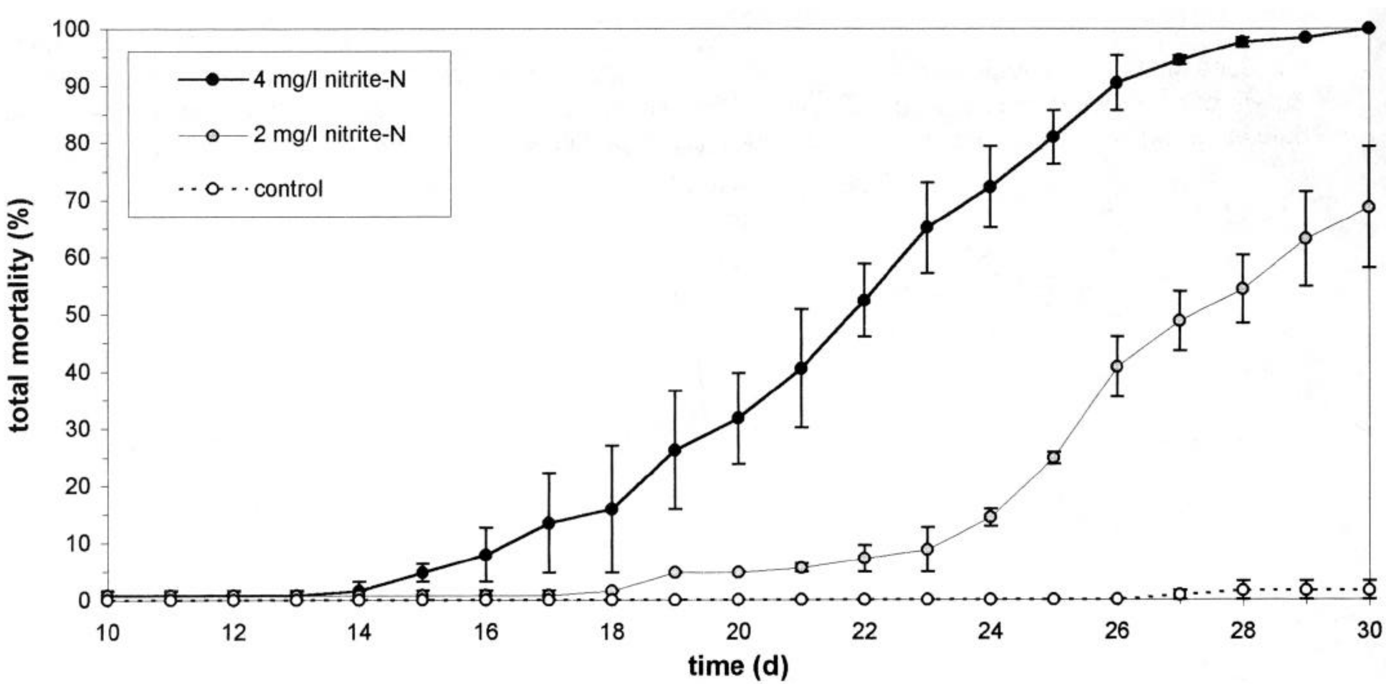

Figure 2. Mortality of brown trout alevins exposed to different concentrations of nitrite in water containing 3 mg/l chloride. Nitrite was added in the form of sodium nitrite at time = 0 d. For each point, n = 125 +/- 1; points represent mean from two seperate flow tanks with n = 62-63 each, bars above and below each point represent standard deviation.

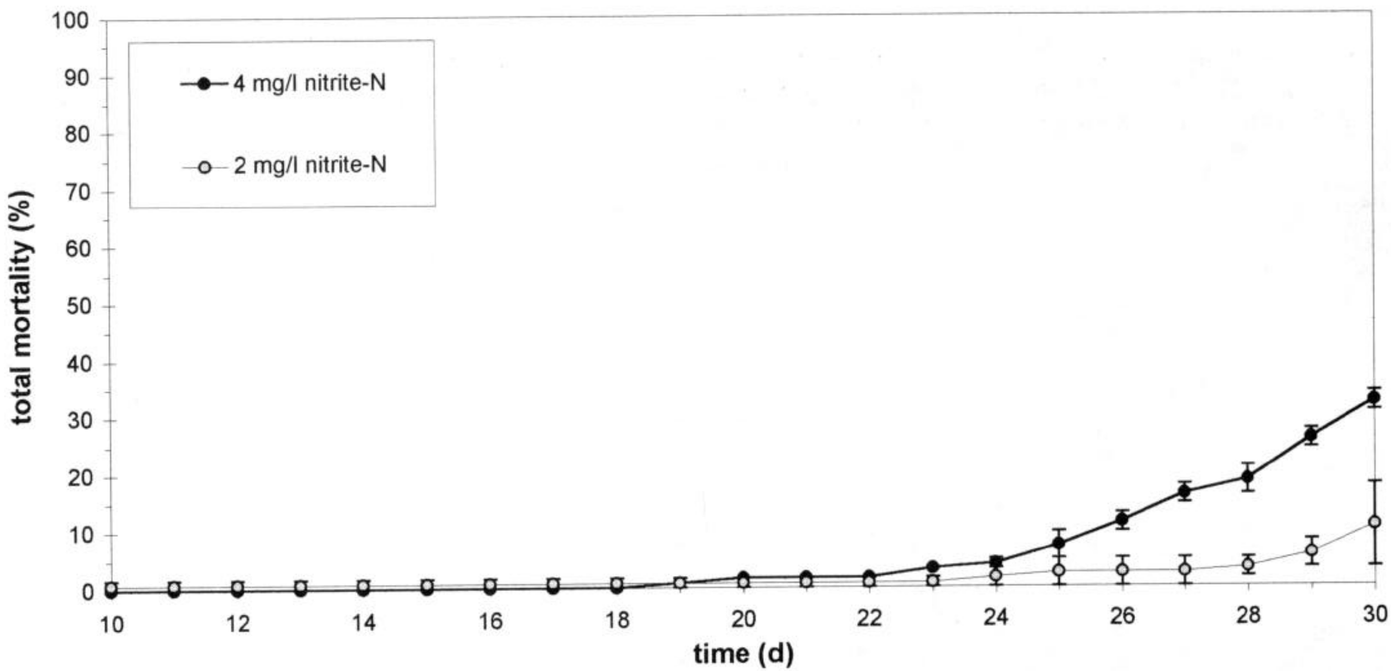

Figure 3. Mortality of brown trout alevins exposed to different concentrations of nitrite in water containing 10 mg/l chloride. Nitrite was added in the form of sodium nitrite at time = 0 d. For each point, n = 125 +/- 1; points represent mean from two seperate flow tanks with n = 62-63 each, bars above and below each point represent standard deviation.

It was also noted that prior to death, the alevins exposed to toxic nitrite concentrations were more strongly pigmented and usually lay motionless on the floor of the tank, breathing heavily. This lack of activity can probably be attributed to the blood poisoning (leading to decreased oxygen concentrations in the blood) caused by nitrite. The increased pigmentation was probably a stress response well known in aquarium fish.

When the ten adult brown trout kept at a concentration of 3 mg/L chloride were exposed to 4 mg/L of NO_2-N (the same water composition by which 50% mortality of the alevins occured after 22 days), all ten test fish died within 24 hrs. There were no mortalities among the ten control animals. These results are in agreement with the known literature on nitrite toxicity to adult trout; according to calculations by Lewis and Morris (1986), the LC-50 (96 hr) nitrite concentration for adult rainbow trout kept in water containing 3 mg/L of chloride is about 1,4 mg/L NO_2-N. Because our experiments on trout alevins and adult trout were both conducted using test water from the same source, we can be certain that observed differences in the tolerance to nitrite between the different age groups are not based on possible other differences in water chemistry.

Although further tests are necessary to determine the cause of the high tolerance of trout alevins to nitrite, one explanation might be found in the differing respiratory physiology of larvae and adults. Because scales first start to form near the end of the alevin phase of development (Balon 1975) cutaneous oxygen uptake is higher for trout fry than for adults. In addition, cutaneous surfaces account for about 96% of the total respiratory surface area in chinook salmon sac fry (45 mg wet weight, 3.7 d post-hatch); the branchial area of the developing gills does not exceed the cutaneous surface area until the young salmon weigh 2.5 - 4.0 g (Rombough and Moroz 1990). Although the mass-specific basal metabolism is higher per mass unit for smaller fish (i.e. small fish need more O_2 per gram body mass), the surface area to total mass ratio is considerably higher for younger trout, allowing a higher rate of oxygen diffusion.
Due to these factors, oxygen taken up through the skin is able to permeate to the internal tissues far better in an alevin than in an older trout. When one takes into consideration that nitrite is a blood poison, it seems logical that trout fry would be able to survive for longer under reduced oxygen carrying capacity of the blood than would an adult, which depends on the oxygen delivered by the blood stream for survival. Although further tests are necessary in order to determine the effect of low oxygen levels on the tolerance of salmonid larvae to nitrite, it seems unlikely that high nitrite concentrations in connection with normal oxygen levels would endanger trout alevins in nature (or in fish farms) over short periods of time

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