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Article *in* Journal of Aquatic Animal Health · March 1999

DOI: 10.1577/1548-8667(1999)011<0076:CAIONU>2.0.CO;2

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ISSN: 0899-7659 (Print) 1548-8667 (Online) Journal homepage: http://www.tandfonline.com/loi/uahh20

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To cite this article: Q. C. Fontenot , J. J. Isely & J. R. Tomasso (1999) Characterization and Inhibition of Nitrite Uptake in Shortnose Sturgeon Fingerlings, Journal of Aquatic Animal Health, 11:1, 76-80, DOI: 10.1577/1548-8667(1999)011<0076:CAIONU>2.0.CO;2

To link to this article: http://dx.doi.org/10.1577/1548-8667(1999)011<0076:CAIONU>2.0.CO;2

Journal of Aquatic Animal Health 11:76–80, 1999

Characterization and Inhibition of Nitrite Uptake in Shortnose Sturgeon Fingerlings

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Abstract.—Efforts are underway to culture the endangered shortnose sturgeon *Acipenser brevirostrum* for possible reintroduction. As part of a larger project to develop culture techniques for this species, the uptake of nitrite was evaluated in fingerlings (16.5 ± 4.85 g; mean ± SD). Plasma nitrite concentrations increased significantly with exposure time (0–5 d) and dose (0–4 mg nitrite-N/L). Shortnose sturgeon fingerlings were able to concentrate nitrite in their plasma to more than 63 times the environmental concentration. Chloride, as either sodium chloride or calcium chloride, partially inhibited nitrite uptake. However, calcium chloride was a better inhibitor. After previous exposure (2 d at 2.13 ± 0.080 mg nitrite-N/L) plasma nitrite-N decreased from 165.5 to 36.7 mg/L during a 3-d simultaneous exposure to 2.13 ± 0.080 mg nitrite-N/L and treatment with 40 mg chloride/L as calcium chloride. The addition of calcium chloride to the water appeared to be an effective means of preventing nitrite uptake and treating nitrite toxicity in hatchery-reared shortnose sturgeon fingerlings.

The shortnose sturgeon *Acipenser brevirostrum* is listed as an endangered species in the United States under the Endangered Species Act and faces localized extinction (Kynard 1997). Shortnose sturgeon are believed to remain in their natal rivers for life; therefore, recolonization will likely be slow in rivers with extirpated populations (Kynard 1997). Efforts are currently underway to develop techniques to culture shortnose sturgeon for possible reintroduction. Salinity and dissolved oxygen tolerances of early life stages of shortnose sturgeon fingerlings have been determined (Jenkins et al. 1995) as have the 96-h median-lethal concentrations of ammonia and nitrite (Fontenot et al. 1998). The dynamics of uptake of nitrite, however, have not been determined. Nitrite can reach lethal concentrations for shortnose sturgeon fingerlings under routine culture conditions (Fontenot et al. 1998) due to an imbalance in the nitrification processes that oxidize ammonia to nitrate (Tomasso 1994). Exposure to nitrite has been shown to decrease growth and increase mortality for several fishes (Colt and Tchobanoglous 1976; Tomasso 1994).

The toxicity of nitrite to fishes depends on the ability of the fish to concentrate nitrite in the plasma (Palachek and Tomasso 1984; Tomasso 1986). Nitrite is actively transported into many freshwater fishes via the same mechanism as chloride, and nitrite uptake can be competitively inhibited by environmental chloride (Williams and Eddy 1986; Tomasso 1994; Jensen 1995). Fish that have been exposed to environmental nitrite can be treated by immersion in nitrite-free water or by adding chloride to the contaminated water (Tomasso et al. 1979; Eddy et al. 1983). The objectives of this study were to characterize nitrite uptake rates, to determine the ability of chloride to inhibit the uptake of nitrite, and to evaluate the effectiveness of chloride as a treatment for shortnose sturgeon fingerlings exposed to high levels of environmental nitrite.

Methods

Fingerling shortnose sturgeon were obtained from the Bear's Bluff National Fish Hatchery (U.S. Fish and Wildlife Service), South Carolina, transported by truck to the Aquatic Animal Research Laboratory at Clemson University, and stocked in 800-L flow-through water tanks. Tanks received 1.0 L/min dechlorinated tap water (18 ± 0.8°C, mean ± SD; pH 7.1 ± 0.11) and were continuously aerated. Mean un-ionized ammonia concentrations were less than 0.01 mg/L, and mean nitrite levels were 0.03 ± 0.092 mg/L. Fish were fed live black worms *Lumbriculus variegatus* (Cherokee Trout Farms, Cherokee, North Carolina) two or three

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Received March 6, 1998 Accepted July 20, 1998

times daily. Feeding was discontinued 24 h before trials began.

All experiments were conducted in glass aquaria, each containing one shortnose sturgeon fingerling (16.5 ± 4.85 g; 174.5 ± 12.15 mm, TL) and 30 L of continuously aerated test water (18.0 ± 0.13°C). Test water contained 1.8 mg Ca/L and less than 1.0 mg Cl/L. Fish were placed in aquaria 1 d before the experiments began. Fish were not offered feed during any experiment, and dead fish were removed daily. Nitrite was added to the experimental aquaria as sodium nitrite. Nitrite concentrations for each aquarium were determined by the azo-dye method (USEPA 1974) at the beginning and end of each experiment. An Accumet model 915 pH meter (Fisher Scientific Co., Pittsburgh, Pennsylvania) was used to measure pH, which ranged from 6.7 to 7.6 (7.3 ± 0.15). Unionized ammonia-N levels in all experimental aquaria were 0.0046 mg/L or less, calculated from the tables in Piper et al. (1982). No control fish died in any study.

To determine the rate of nitrite uptake, 24 fish were exposed to nitrite-N at 2.17 ± 0.156 mg/L for 0, 1, 2, 3, 4, or 5 d. Four fish were sampled each day. One fish died after 4 d of exposure and was excluded from the study.

In a second experiment, 24 fish were exposed to 0, 1, 2, or 4 mg nitrite-N/L for 2 d ($N = 6$ per exposure concentration) to determine the dose–response of shortnose sturgeon fingerlings. Measured nitrite-N concentration in all test aquaria was 101.4 ± 5.85% of the expected dose. Due to inaccurate dosing of tanks, two fish were excluded from this experiment. No fish died during this experiment; therefore, five or six independent observations for each treatment were used for analysis.

To evaluate the ability of chloride to inhibit nitrite-N uptake, 16 fish were simultaneously exposed to 2.05 ± 0.090 mg nitrite-N/L and 10 mg chloride/L as either sodium chloride or calcium chloride for 2 d (8 fish/treatment). Eight other fish were exposed for 2 d to nitrite-N (2.05 ± 0.090 mg/L) without a chloride treatment and served as positive controls.

To determine the effect of increasing concentration of chloride on uptake of nitrite, 24 fish were exposed to 2.23 ± 0.089 mg nitrite-N/L and either 0, 5, 10, or 20 mg/L additional chloride as calcium chloride for 2 d. Six fish were exposed in each treatment group.

Treatment of hypernitritemia was studied first by exposing 24 fish to 2.13 ± 0.080 mg nitrite-N/L for 2 d. Six fish were then bled and plasma nitrite concentrations were determined; 40 mg of chloride/L (as calcium chloride) was added to the test solution of six other fish; six fish were gently moved to aquaria containing freshwater only (nitrite-N = 0.02 ± 0.008 mg/L); and six fish remained in the original test water. After 24 h all remaining fish were bled. Three fish died during this experiment, thus each mean is based on four to six independent observations.

The time course of treatment of hypernitritemia with calcium chloride was studied by exposing 24 shortnose sturgeon fingerlings to 2.14 ± 0.069 mg nitrite-N/L for 2 d at which time six were bled. Forty mg chloride/L (as calcium chloride) was then added to the test water of each of the remaining 18 fish. These 18 fish were divided into three groups. A group of six fish each was bled after 1, 2, and 3 d.

Plasma nitrite concentrations were determined with a modification of the azo-dye method (USEPA 1974; Palachek and Tomasso 1984). After each fish had been independently exposed to a specific treatment, blood was collected in sodium heparin-coated capillary tubes from the hemal arch of the severed caudal peduncle and immediately centrifuged for 180 s. After centrifugation, the capillary tubes were broken at the plasma–cell interface. Each plasma sample (10 μL) was transferred to a test tube containing 6 mL of deionized water and 50 μL of azo-dye reagent. After 10 min, the solution was transferred to a spectrophotometer tube and the absorbance at 540 nm was determined. Because of the low plasma nitrite-N concentration in control and low-exposure groups, 20 μL plasma, 20 μL azo-dye, and 3 mL deionized water were used to determine plasma nitrite-N concentrations for those groups. Recoveries of standard additions of nitrite-N to fingerling shortnose sturgeon plasma samples were 107.5 ± 26.64% of the expected value. Data were subjected to analysis of variance (ANOVA) followed by a Tukey range test ($P \leq 0.05$).

Results and Discussion

Plasma nitrite concentrations of shortnose sturgeon fingerlings increased with time of exposure to nitrite (Figure 1). Nitrite concentrations in plasma were elevated, more than 63 times the environmental concentration, after 5 d of exposure. Other fish shown to concentrate nitrite in plasma to levels greater than environmental concentrations are channel catfish *Ictalurus punctatus* (Tomasso et al. 1979; Palachek and Tomasso 1984; Tomasso 1994), blue tilapia *Tilapia aurea* (Palachek and Tomasso 1984), and rainbow trout *Oncorhynchus mykiss* (Eddy et al. 1983). Shortnose

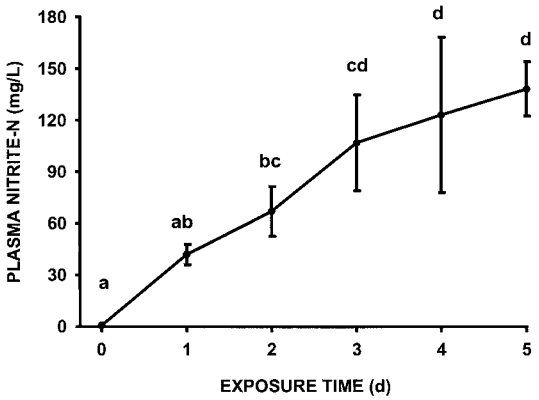

FIGURE 1.—Plasma nitrite concentrations (mean ± SD; $N = 3$ or 4 per treatment) in shortnose sturgeon fingerlings for each day exposed to nitrite-N at 2.2 ± 0.16 mg/L. Means with letters in common are not statistically different.

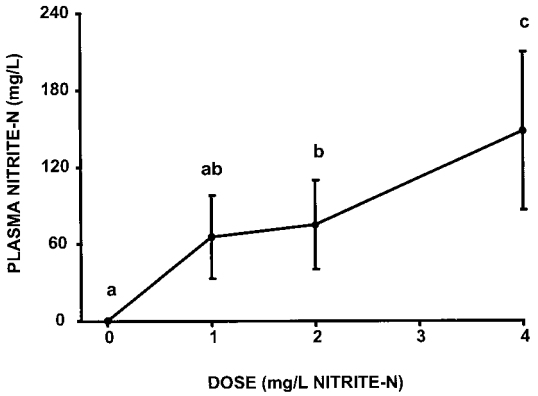

FIGURE 2.—Plasma nitrite concentrations (mean ± SD; $N = 5$ or 6 per treatment) in shortnose sturgeon fingerlings exposed to different doses of nitrite-N for 2 d. Means with letters in common are not statistically different.

sturgeon fingerlings are strong concentrators of nitrite compared with other warmwater fishes (Tomasso 1986).

Plasma nitrite concentrations of shortnose sturgeon also increased with exposure to increasing environmental nitrite concentrations (Figure 2). Nitrite concentrations were significantly higher in those fish exposed for 48 h to 4 mg nitrite-N/L than those exposed to 1 or 2 mg/L. Palachek and Tomasso (1984) found similar results with channel catfish and tilapia but not largemouth bass *Micropterus salmoides.*

Chloride was more effective at inhibiting the uptake of environmental nitrite when added as calcium chloride than as sodium chloride (Figure 3). Chloride is effective at reducing the uptake of nitrite in several fishes, especially in the form of calcium chloride (Tomasso 1994). Nitrite is approximately 55 times more toxic to milkfish *Chanos chanos* reared in freshwater than in 16‰ brackish water (Almendras 1987). Calcium chloride is more than twice as effective as sodium chloride at reducing nitrite toxicity in striped bass *Morone saxatilis* (Mazik et al. 1991) or sunshine bass (hybrid of female white bass *Morone chrysops* × male striped bass; Weirich et al. 1993). The toxicity of nitrite to juvenile steelhead (anadromous rainbow trout) was reduced by a factor of up to three by treatment with sodium chloride and by a factor of up to 50 with calcium chloride (Wedemeyer and Yasutake 1978).

The ability of calcium chloride to prevent the uptake of environmental nitrite increased with exposure to increasing concentrations of calcium chloride (Figure 4). After a 2-d exposure, blood plasma nitrite-N concentrations were significantly lower in those fish treated with 20 mg chloride/L than in those treated with 5 mg chloride/L as calcium chloride. An increase in plasma nitrite concentration is directly correlated with an increase in methemoglobin levels (Tomasso 1986). Although a molecular ratio of 9 chloride ions to 1 nitrite ion did not completely inhibit nitrite-N uptake in shortnose sturgeon fingerlings, a ratio of 16:1 was sufficient to completely inhibit an increase in methemoglobin levels in channel catfish (Tomasso et al. 1979).

Plasma nitrite concentrations did not continue to

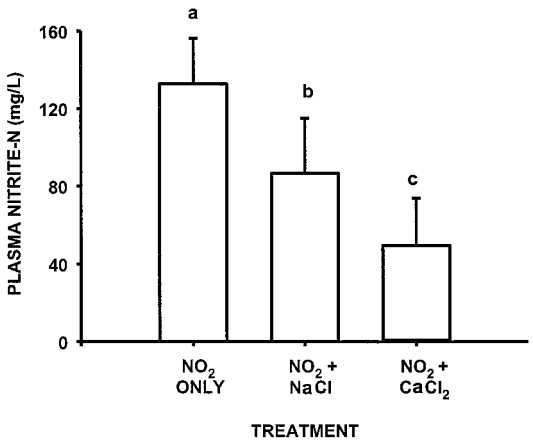

FIGURE 3.—Plasma nitrite concentrations (mean ± SD; $N = 8$ per treatment) in shortnose sturgeon fingerlings exposed to 2.05 ± 0.092 mg nitrite-N/L for 2 d with either no chloride or 10 mg chloride/L as either sodium chloride or calcium chloride. Means with letters in common are not statistically different.

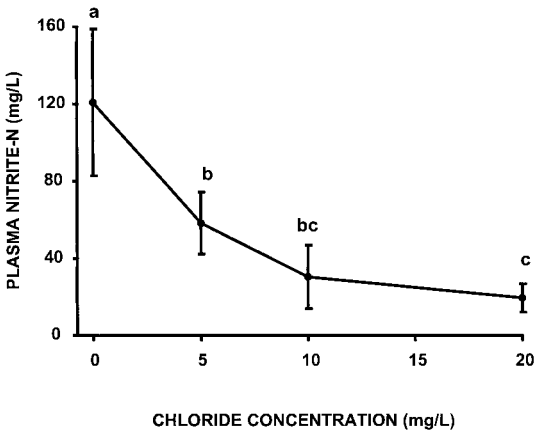

FIGURE 4.—Plasma nitrite concentrations (mean ± SD; $N = 6$ per treatment) in shortnose sturgeon fingerlings exposed to different concentrations of chloride as calcium chloride and 2.23 ± 0.089 mg nitrite-N/L for 2 d. Means with letters in common are not statistically different.

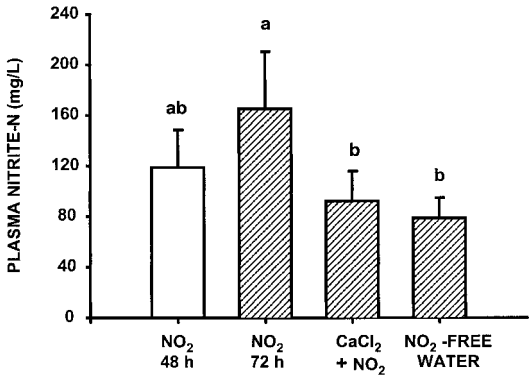

FIGURE 5.—Plasma nitrite concentrations (mean + SD; $N = 4$–6) in shortnose sturgeon fingerlings exposed to nitrite-N (2.13 ± 0.080 mg/L) for 48 h (open bar) and then, for 24 h (cross-hatched bars), either held in test water containing nitrite, exposed simultaneously to 2.13 ± 0.080 mg nitrite-N/L and 40 mg chloride/L (as calcium chloride), or moved to nitrite-free water. Means with letters in common are not statistically different.

increase when exposed fish were treated with calcium chloride or moved to freshwater (Figure 5). Plasma nitrite levels in fish that remained in nitrite solutions without chloride protection were significantly higher than those in fish that received either calcium chloride or were transferred to freshwater. Also, no difference was found between plasma nitrite-N concentrations in fish treated with calcium chloride or freshwater. The addition of calcium chloride to water with an elevated concentration of nitrite is similar to moving exposed shortnose sturgeon fingerlings to nitrite-free water. Plasma nitrite concentrations in rainbow trout exposed to 24-h LC50 (concentrations lethal to 50% of fish) of nitrite-N for 20 h were significantly reduced after fish were transferred to either freshwater or salt water (16‰) for 28 h (Eddy et al. 1983).

Plasma nitrite concentrations in shortnose sturgeon fingerlings were negatively correlated with number of days of exposure to 40 mg chloride/L as calcium chloride added to the test water (Figure 6; $r^2 = 0.8598$, $P = 0.05$). Although plasma nitrite concentrations did not reach control levels (0.40 ± 0.440 mg nitrite-N/L) after 3 d exposure to the calcium chloride treatment, the following linear model,

$$\text{plasma nitrite-N} = 137.225 - 33.96\ (\text{days of exposure}),$$

predicts that plasma nitrite-N concentrations should reach zero in about 4 d for shortnose sturgeon fingerlings exposed to 2.14 ± 0.069 mg nitrite-N/L for 48 h (a molecular ratio of 18.7 chloride ions to 1 nitrite ion). The plasma nitrite concentration in rainbow trout decreased from about 6.8 to about 1.1 mmol nitrite-N after 28 h exposure to 16‰ seawater (Eddy et al. 1983).

Nitrite is taken up from the environment and concentrated in the plasma of shortnose sturgeon fingerlings to many times the environmental concentration, presumably by the chloride uptake mechanism (Perrone and Meade 1977; Palachek and Tomasso 1984). Although several warmwater

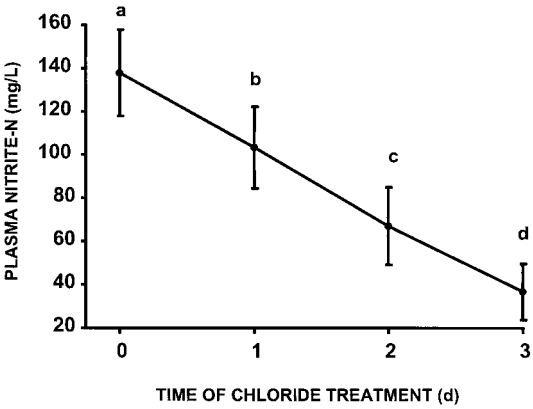

FIGURE 6.—Plasma nitrite concentrations (mean ± SD; $N = 4$ or 6) in shortnose sturgeon fingerlings exposed to 2.14 ± 0.069 mg nitrite-N/L for 48 h and then simultaneously exposed to 2.14 ± 0.069 mg nitrite-N/L and 40 mg chloride/L (as calcium chloride) for exposure times of 1, 2, or 3 d. Means with letters in common are not statistically different.

fishes concentrate nitrite in their plasma above environmental levels (Tomasso 1986, 1994), shortnose sturgeon fingerlings tend to concentrate nitrite in their plasma to greater levels, similar to that demonstrated in salmonids (Margiocco et al. 1983). Environmental nitrite can be competitively excluded by chloride, and calcium chloride is more effective than sodium chloride. The addition of calcium chloride to the environment appears to be an effective means of preventing nitrite uptake and treating nitrite toxicity in shortnose sturgeon fingerlings reared in high-density fish culture systems.

Acknowledgments

Funding for this project was provided by the U.S. Fish and Wildlife Service and the Aquaculture Research Initiative of the South Carolina Agricultural Experiment Station. Chris Kempton, Kent Ware, and Win Taylor assisted with the project. Cooperating agencies for the South Carolina Cooperative Fish and Wildlife Research Unit are the U.S. Geological Survey Biological Resources Division, Clemson University, the South Carolina Department of Natural Resources, and the Wildlife Management Institute.

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