Bull. Environ. Contam. Toxicol. (1986) 37:802–807

Environmental Contamination and Toxicology

Resistance of Nitrite-Exposed Channel Catfish, *Ictalurus punctatus*, to Hypoxia

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In ponds, aquatic recirculating culture systems, laboratory holding systems, sewage plant receiving waters and natural systems where biomass is high, nitrite can reach lethal or limiting concentrations particularly if imbalances occur in the relative abundances of bacteria, Nitrosomonas and nitrobacter which oxidize ammonia and nitrite respectively. Nitrite is toxic to many aquatic animals including invertebrates (Beitinger and Huey 1981), amphibians (Huey and Beitinger 1980a,b) and fish (see review E.I.F.A. 1984). Reported median lethal concentrations of NO_2-N in 96-h tests range from 0.3 to more than 100 mg/L and are strongly influenced by water chemistry. Acute nitrite toxicity appears to be inversely related to pH, chloride concentration and water hardness (e.g., Tomasso et al. 1979; Huey et al. 1982). Among its apparent multiple modes of toxic action (Arillo et al. 1984), nitrite autocatalytically oxidizes hemoglobin to methemoglobin, a form incapable of binding oxygen. Methemoglobinemia has been well documented in fish. Channel catfish, Ictalurus punctatus, exposed to water-borne nitrite rapidly develop methemoglobinemia with concentrations approaching 70 % methemoglobin. Not surprisingly, vertebrates afflicted with methemoglobinemia have reduced oxygen carrying capacity.

Previous research in our laboratory has documented that exposure of channel catfish to sublethal concentrations of water-borne nitrite reduced both upper temperature tolerance (Watenpaugh et al. 1985) and swimming performance (Watenpaugh and Beitinger 1985). In both cases, effects were highly significantly correlated ($p < 0.001$) with nitrite concentrations. Since we found that nitrite negatively affected the ability of channel catfish to tolerate high temperature, we conducted research to determine if oxygen tolerance would be similarly influenced. As a major abiotic environmental limiting factor in aquatic environments, oxygen influences the physiology, biochemistry and behavior of fish and hence the productivity and economic returns from intensive aquaculture operations (Smart 1981). We hypothesized that nitrite exposure and resultant methemoglobinemia would compromise the ability of channel catfish to resist a low constant oxygen concentration.

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MATERIALS AND METHODS

Channel catfish, Ictalurus punctatus, wet wt. = 9.3 ± 2.1 g ($\bar{X}$ ± SD) obtained from a local fish hatchery were placed in a 450-L aquarium and held at 30 ± 0.2 C for a minimum of four weeks. Fish were fed catfish chow daily until two days before experimentation. Holding and testing water was reconstituted from deionized water following a recipe for hard water by USEPA (1975). Water was aerated and continuously filtered through charcoal. Five g/L chloride as NaCl was added to prevent nitrite toxicity and inhibit disease in fish during holding. Nitrite-N levels did not exceed 0.1 mg NO_2-N/L during holding.

Channel catfish were exposed to nitrite in all glass aquaria containing 30 L of aerated, reconstituted hard water. Aquaria were partially immersed in a water bath to maintain temperature at 30 ± 0.1 C ($\bar{X}$ ± SD). Nominal exposure concentrations of 0.0, 0.5, 1.0, and 1.5 mg NO_2-N/L were chosen to produce a graded yet sublethal increase in methemoglobin concentration of channel catfish blood following Huey et al. (1980). Nitrite was measured with the azo-dye method (APHA 1975). Nominal nitrite concentrations were maintained throughout the 24-h exposure period by addition of reagent grade sodium nitrite or deionized water, as necessary. Five fish were used in each exposure, and all exposures were duplicated.

Hypoxic resistance times of channel catfish were determined in hard water at 30 C, deoxygenated by nitrogen gas stripping to levels less than 1 mg O_2/L. Removal of oxygen from reconstituted water occurred in a 170-L aquarium with a counter-current stripper. Actual exposures of catfish to hypoxia occurred in a 60-L aquarium containing 35 L of water siphoned from the 170-L stripping tank. Fish were segregated in five 10-cm by 10-cm plastic mesh enclosures during hypoxic resistance trials, and were denied access to the surface by a 6.2-mm thick piece of plexiglass. A circulating thermoregulator maintained water temperature at 30 ± 0.1 C ($\bar{X}$ ± SD) in the resistance aquarium. The test endpoint criterion was cessation of opercular movements. At that point, a fish was rapidly removed from its enclosure, and blood collected from its severed caudal peduncle was analyzed for hemoglobin and methemoglobin concentrations (see Huey and Beitinger 1980a). Dissolved oxygen was measured before and after hypoxic resistance trials with the azide modification of Winkler's iodometric method (APHA 1975).

Relationships between nitrite exposure, hypoxic resistance time and percentage of methemoglobin (% MHb) were described with simple linear regression and the effects of nitrite on resistance time and % MHb were were further assessed by analysis of variance (ANOVA) and Duncan's multiple range test (α = 0.05). The robustness of these parametric procedures tolerates moderate deviations from normality which occurred in hypoxic resistance times.

RESULTS AND DISCUSSION

During hypoxic resistance trials, dissolved oxygen concentration decreased from 0.85 ± 0.1 to 0.65 ± 0.14 mg O_2 /L ($\bar{X}$ ± SD). After introduction to hypoxic water catfish generally settled to the bottom and became motionless, except for an unquantified but seemingly large increase in both opercular rate and stroke volume relative to those noted prior to hypoxic exposure. Although a few fish exhibited brief yet increasingly frequent bouts of activity during hypoxic exposure, more often fish remained largely immobile during the majority of a trial. Most fish progressed from immobility to loss of equilibrium followed by a steady decline in amplitude of opercular movements, occasional coughing, erratic locomotor activity and finally cessation of operculation.

Hypoxic resistance times of the ten control catfish were variable ranging from 92 to 362 minutes. Mean resistance time was the highest in the controls (173.3 min) and decreased with increasing nitrite concentration: 117.6, 52.3, and 37.8 min respectively (Table 1).

Table 1. Percentage methemoglobin (%) and resistance times (min) of nitrite exposed channel catfish placed in hypoxic water.

NO -N mg/L	Percent Methemoglobin X	SD	Grouping*	Resistance Times-minutes X	SD	Grouping*
0.0	13.0	4.3	A	173.3	86.5	A
0.5	43.6	8.1	B	117.6	69.9	A
1.0	65.3	8.5	C	52.3	42.3	B
1.5	78.5	14.3	D	37.8	43.7	B

*Column labelled "Grouping" identify significantly different percent methemoglobin and resistance time groups by Duncan's multiple range test ($\alpha = 0.05$). For each group n = 10.

Resistance times and nitrite concentrations were significantly related: time (min) = 166 - 94 (mg NO_2 -N/L), $p < 0.001$ and $r = -0.65$. ANOVA substantiated this conclusion ($F = 9.75$, $p < 0.001$) and mean hypoxic resistance times of fish from the two higher nitrite exposure groups were distinguished from the control and 0.5 mg NO_2-N/L group means by Duncan's test (Table 1).

Each nominal exposure group contained one exceptionally tolerant fish: a catfish exposed to 0.5 mg NO_2-N/L survived 293 min in hypoxic water, and one fish from each of the two higher exposures lasted 157 min. A fish from the 1.5 mg NO_2 -N/L exposure had the shortest time to death, 9 min.

Upon completion of resistance measurements, mean % MHb concentrations of catfish from the control and three nitrite exposure groups were different (ANOVA $F = 90.69$, $p < 0.001$) and

partitioned by Duncan's multiple range test (α=0.05) into four statistically distinct means (Table 1). Regression analysis yielded a best-fit model of MHb = 17.0 + 43.8 (mg NO_2-N/L). This model is highly statistically significant ($p < 0.001$) and has a $r = 0.927$. As expected, hypoxic resistance times of individual catfish were highly significantly related to methemoglobin percentage: Time (min) = 194 - 2 (% MHb), $p = 0.001$ and $r = -0.64$.

Channel catfish behavior during hypoxic exposure in this study was similar to that observed by Caillouet (1968), and supports Scott and Rodgers' (1981) suggestion that channel catfish are oxygen conformers in that physiologically and biochemically they accommodate environmental hypoxia by decreasing oxygen consumption rates by minimizing activity, i.e., a "wait it out" approach.

Nitrite-induced methemoglobin formation has been previously observed in salmonids, channel catfish and other species; our nitrite methemoglobin dose-respose relationship compares well with other reports (Tomasso et al. 1979; Huey et al, 1980). Methemoglobinemia decreases the oxygen carrying capacity of blood by making less hemoglobin available for oxygen transport and by shifting the oxygen dissociation curve to the left. The latter effect may be confounded in hypoxic catfish:decreased blood pH and elevated pCO_2 both shift blood oxygen dissociation curves right (Bohr shift). Nevertheless, reduced delivery of oxygen in an organism will accelerate tissue depletion of oxygen and anaerobic production of lactate. The depression of hypoxic resistance of channel catfish exposed to nitrite; therefore, may be explained at least partially by the hematologic effects of nitrite. The general variability observed in hypoxic resistance of both control and nitrite exposed channel catfish may be due to individual differences in aerobic and anaerobic capacity. Scott and Rogers (1981) noted highly variable plasma lactate levels after exposure of catfish to 1.5 mr O_2/L for 24 h, and Caillouet (1968) reported similar results for lethal exposures. Although their experimental design was considerably different, our results qualitatively corroborate those of Bowser et al. (1983). In 120-h trials at 23 to 25°C, the lethality of catfish subjected to progressively decreasing oxygen was directly related to environmental nitrite and blood methemoglobin concentrations. The mean methemoglobin level of the 38 catfish that died was 82.1 %. Controls exposed to nitrite-free water exhibited essentially no mortality even though oxygen concentrations at the conclusion of the trial approached 1.5 mg O_2/L.

Dissolved oxygen is a primary limiting factor in aquaculture of channel catfish (Lovell 1979). Maintenance of dissolved oxygen above 3 mg/L allows maximal food conversion efficiency in catfish, yet high feeding rates, overstocking of culture ponds, high temperature and/or cloudy weather can lower dissolved oxygen to 1 mg/L or less (Smart 1981, Tucker et al. 1979; Romaire and Boyd 1979). Nitrite-N in aquatic systems can increase before,

during or after periods of oxygen depletion (Sawyer 1960). This laboratory research demonstrated that acute sublethal exposure of channel catfish to nitrite reduces their ability to survive subsequent low oxygen exposure, and by inference that the toxic effects of nitrite on catfish could increase mortality or aggravate stress associated with environmental hypoxic conditions.

Acknowledgments. We sincerely thank Dub Roland for providing fish. A North Texas State University Faculty Research Grant to TLB funded this research.

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Received 27 January 1986; accepted 14 April 1986