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Nitrite Exposure and Respiration Rates in Fathead Minnows

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Nitrite in aquatic systems is potentially toxic to many organisms, and has recently become the subject of much research. Aquacultural systems or waters receiving nitrogenous effluent may accumulate relatively high nitrite concentrations due to imbalances in the nitrification pathway of the nitrogen cycle (Sawyer 1960; Westin 1974).

Nitrite toxicity is relatively variable both within and between fish species: reported 96-h LC50 values range from less than 1 to about 70 mg NO_2-N/L (Freeman et al. 1983; Palachek and Tomasso 1984). Increases in pH, chloride concentration, or hardness have been shown to decrease nitrite toxicity in various freshwater fish species (Perrone and Meade 1977; Tomasso et al. 1979; Russo et al. 1981). Demonstrated mechanisms of acute nitrite toxicity in vertebrates include methemoglobin formation, inhibition of enzymes with exposed amine and sulfhydryl groups, and oxidation of cellular membrane lipids (Huey et al. 1980; Mensi et al. 1982; Margiocco et al. 1983).

In addition to serving as a sublethal bioassay tool, respirometry may provide indirect insight to the mode of toxicity of a chemical (Hughes 1981). Several researchers have utilized various measures of metabolic rate to ascertain sublethal stress in fish. Suspended solids, crude oil, zinc, copper and cadmium directly or indirectly affect metabolic rates of some fish (Neumann et al. 1982; Thomas and Rice 1975; Skidmore 1970; Sellers et al. 1974; Thurberg and Dawson 1974). The conversion of hemoglobin to methemoglobin in nitrite-exposed fish may reduce their oxygen uptake capability, and thereby depress their metabolic rate. This study attempted to determine if nitrite influences the routine weight specific oxygen consumption of fathead minnows, a species found to be particularly tolerant of nitrite exposure.

MATERIALS AND METHODS

Fathead minnows (*Pimephales promelas*) were obtained from a local fish hatchery and maintained in a 450 L recirculating "living stream" (Frigid Units, Inc.). Holding water was aerated and charcoal filtered continuously, and consisted of deionized water

reconstituted into hard water as per US EPA (1975). Fish were held at 20 ± 1°C ($\bar{X}$ ± SD) for at least 3 weeks prior to experimentation and experienced a 16L:8D photoperiod cycle throughout holding and testing. At no time did the holding water contain greater than 0.1 mg NO_2-N/L. Fish were fed ground catfish chow at 1% body weight per day until 3 days prior to experimentation.

Acute (24 h), static exposures of fathead minnows to nitrite were conducted in aerated, reconstituted hard water at 19 ± 1°C ($\bar{X}$ ± SD) in 30-L all glass aquaria (US EPA 1975). Palachek and Tomasso (1984) found that 24 h nitrite lethality to fathead minnows is highly variable and inversely related to weight: 24-h LC50's ranged from 46 mg NO_2-N/L for 1.1 g fish to over 300 mg/L for 0.3 g fatheads. Minnows used in this study weighed 0.60 ± 0.15 g ($\bar{X}$ ± SD), and nominal doses of 0, 9, 18, and 27 mg NO_2-N/L were employed. All exposures were replicated, with 15 to 20 fish per replicate. Actual nitrite concentrations of water samples collected at the beginning and end of 24 h exposures were measured (± 0.1 mg NO_2-N/L) by the azo-dye colorimetric technique (APHA 1975)

Routine respiration rates were determined from consumption of oxygen by individual fish in 300 ml BOD bottles closed for 90 min at a temperature of 19.2 ± 0.2°C ($\bar{X}$ ± SD) (Anderson et al. 1980). BOD bottles were filled with water from the fish's exposure aquarium. In 90 min the typical test fish depleted dissolved oxygen in a bottle to an easily measurable but unstressful level (Doudoroff and Shumway 1970). Dissolved oxygen determinations were made to ± 0.1 mg O_2/L with a Yellow Springs Instruments dissolved oxygen meter equipped with a stirring probe. The D.O. meter was calibrated by comparison to the azide modification of Winkler's iodometric method (APHA 1975). Respirometry trials occurred in a quiet, dimly lit setting. Bottles without fish were treated similarly to quantify meter drift and/or microbial oxygen consumption. Since oxygen concentration of these bottles decreased an average of 0.05 mg O_2/1 (± SD of 0.05), this amount was subtracted from the measured oxygen consumption of each fish. The weight of each fish was measured after respirometry to the nearest 0.01 g, and weight-specific routine respiration rates were calculated.

Since deviations from normality were observed in distribution of respiration rates of fish from two of the exposure groups (Shapiro and Wilk's test, $p<0.01$), Kruskal-Wallis (nonparametric ANOVA) and Dunnett's (multiple range test on ranks, $\alpha = 0.05$) tests were employed to compare mean weight specific oxygen consumption rates of minnows from the four exposure groups (Zar 1984). Statistical Analysis System procedures conducted all tests (SAS 1982).

Table 1. Survival and weight specific oxygen consumption of fathead minnows from various 24 h nitrite exposures.

mg NO_2-N/L		Exposure Survival	Respirometry Survival	Weight (g)		$\dot{V}O_2$ (mg $O_2 g^{-1} h^{-1}$)		Dunnett's Grouping
$\bar{X}$	SD			$\bar{X}$	SD	$\bar{X}$	SD	
0.0	0.0	30/30 (100%)	30/30 (100%)	0.64	0.16	0.32	0.08	A
9.3	0.1	30/31 (96.8%)	27/30 (90.0%)	0.60	0.12	0.31	0.05	A
18.3	0.1	31/35 (88.6%)	26/29 (89.7%)	0.57	0.15	0.24	0.06	B
27.7	0.3	29/35 (82.9%)	22/28 (78.6%)	0.59	0.16	0.25	0.06	B

RESULTS AND DISCUSSION

Some fathead minnows died both during exposure to nitrite and during subsequent respirometry at all nitrite concentrations (Table 1). Lethality was significantly correlated ($p<0.05$) with nitrite concentration during both nitrite exposure and respirometry (Pearson's $r = 0.987$ and 0.955, respectively). Some fish exhibited stress during respirometry. Observed symptoms considered indicators of stress included darkened coloration, rapid operculation rate, and loss of equilibrium. Control fish did not display these symptoms, and only two minnows from the lowest nitrite dose appeared stressed. Cursory observation of minnows during respirometry revealed minimal activity in most cases, and severely stressed fish were somewhat less active. Overall, dissolved oxygen in BOD bottles containing fish decreased from 8.6 ± 0.1 mg O_2/L to 7.7 ± 0.4 ($\bar{X} \pm SD$). The lowest terminal D.O. measurement observed was 6.6 mg O_2/L.

Mean weights of minnows from the various nominal concentrations were not significantly different (ANOVA: $F = 1.26$, $p>0.29$). Kruskal-Wallis test found differences between mean weight specific oxygen consumption of fathead minnows from the various groups to be highly significant ($X^2 = 22.41$, $p<0.001$). Although mean routine respiration rates of minnows from the lowest nitrite exposure was not significantly lower than that of the controls, rates of the two highest nitrite exposures were significantly lower than controls (Dunnett's test, $\alpha=0.05$; Table 1). The effect of any chemical on oxygen consumption of an organism may occur at the site of oxygen uptake, in the circulatory system, or in the internal tissues. Zinc is known to decrease oxygen uptake of fish by disruption of the gill epithelia, and nickel and cadmium operate in a similar fashion (Skidmore 1970; Sellers et al. 1974; Hughes 1981; Thurberg and Dawson 1974). Organic compounds, particularly naphthalene, are often metabolized within an organism, and so increase energetic costs and oxygen consumption (Lee et al. 1972; Thomas and Rice 1975).

Fathead minnows are extremely tolerant of nitrite; their LC50's are the highest yet reported for any aquatic organism (Palachek and Tomasso 1984). The reduction in weight-specific oxygen consumption of fathead minnows exposed to 18.3 and 27.7 mg NO_2-N/L may represent saturation of whatever mechanism(s) *P. promelas* possess to prevent or ameliorate nitrite intoxication. Chronic sublethal nitrite exposure has resulted in minor gill damage in steelhead trout (*Salmo gairdneri*), and several possible modes of nitrite toxicity exist in vertebrates. The acute toxic effects of nitrite on respiration rate, however, are probably founded in the circulatory system, where nitrite renders hemoglobin useless as an oxygen-carrying pigment by oxidizing it to methemoglobin (Wedemeyer and Yasutake 1978; Margiocco et al. 1983; Bodansky 1951). Significant reduction of blood oxygen-carrying capacity could concomitantly reduce O_2 uptake at the gills of nitrite-exposed minnows.

Most vertebrates, including many freshwater fish, possess enzymatic systems to reduce methemoglobin back to hemoglobin (Huey and Beitinger 1982; Freeman et al. 1983). Although methemoglobin-reductase activity has not been studied in *P. promelas*, a highly active reductase system and/or other biochemical mechanisms to ameliorate primary and secondary toxic effects of nitrite could explain their relative resistance to its toxicity. Alternatively, fathead minnows may not take up nitrite as readily as other more susceptible species, or may depurate this chemical more rapidly.

This study demonstrated that acute exposure of fathead minnows to nitrite reduced their weight specific oxygen consumption by about 22%. Although routine respiration rate determinations detected a sublethal effect of nitrite on *P. promelas*, such determinations are usually insensitive indicators of changes in metabolic rate induced by chemicals because activity is unregulated (Anderson et al. 1980). Effects of chemicals on metabolic rate of organisms are better evaluated with standard or active weight specific oxygen consumption when possible.

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