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# Nitrite Toxicity to Fathead Minnows: Effect of Fish Weight

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Nitrite is an intermediate product of nitrification that sometimes reaches toxic levels in aquatic ecosystems and aquaculture environments. The toxicity of nitrite in vertebrates is related to its ability to oxidize hemoglobin to methemoglobin, a form not capable of carrying oxygen. Toxic levels of nitrite to several species of fishes have been reported (see review by RUSSO & THURSTON 1977, TOMASSO et al. 1981).

Some investigators have suggested that smaller fish and sac fry are more tolerant to nitrite than larger fish of the same species (SMITH & WILLIAMS 1974, RUSSO et al. 1974, PERRONE & MEADE 1977). However, no apparent differences were observed in rainbow trout (Salmo gairdneri) ranging in weight from 2 to 387 g (RUSSO 1980). Recent observations of fathead minnows (Pimephales promelas) in our laboratory suggested that smaller fathead minnows were more tolerant to nitrite than larger fish. The purpose of this study was to determine if nitrite toxicity is related to fish weight in the fathead minnow.

## MATERIALS AND METHODS

Fish were obtained from culture ponds at the San Marcos (Texas) National Fish Hatchery and Development Center. They were then held in large fiberglass tanks for at least 4 weeks prior to use. Each holding tank received a constant supply of well water (7 turnovers/hour; temperature = 23°C; hardness = 310 mg/L; dissolved oxygen > 6.0 mg/L; total alkalinity = 316 mg/L; chloride = 22 mg/L; pH = 7.2). Fish were fed a commercial fish food (>40% protein) daily. Feeding was suspended 24 h prior to moving fish to experimental aquaria.

Fish were allowed to acclimatize to experimental aquaria for 24 h prior to nitrite exposure. Desired nitrite concentrations were obtained by the addition of reagent grade sodium nitrite.

All tests were carried out in glass aquaria containing 30 L of well water. Dissolved oxygen levels were maintained near saturation by constant aeration. Water quality was determined in randomly selected tanks after 0, 24, 48, 72 and 96 h of exposure. Dissolved oxygen did not fall below 7.8 mg/L (dissolved oxygen/ temperature meter). The pH ranged from 7.7-8.2 (expanded scale pH meter). Total alkalinity averaged 250 $\pm$ 4 mg/L (mean $\pm$ S.E.) at the beginning of the test and gradually decreased to 153 $\pm$ 4 mg/L after 96 h (HACH CHEMICAL COMPANY 1973). Total hardness averaged 268 $\pm$ 3 mg/L at time 0 and gradually decreased to 191 $\pm$ 4 mg/L after 96 h (HACH CHEMICAL COMPANY 1973). Temperature remained constant at 23$^{\circ}$C (dissolved oxygen/temperature meter). Measured nitrite concentrations averaged 101 $\pm$ 1% of nominal for all sampling times (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 1974).

Fish were exposed in groups of 10 for 96 h to geometrically increasing nitrite concentrations of 75-492 mg/L. Median lethal concentrations (LC-50) for 24, 48, 72 and 96 h of exposure were calculated according to THOMPSON (1947). Dead fish were removed and weighed (0.1g) every 24 h. Thirteen LC-50 tests were conducted. Mean fish weights for each test were as follows: 0.3 $\pm$ .02, 0.4 $\pm$ .02, 0.4 $\pm$ .02, 0.6 $\pm$ .02, 0.8 $\pm$ .02, 0.9 $\pm$ .03, 1.1 $\pm$ .03, 1.2 $\pm$ .02, 1.5 $\pm$ .03, 2.0 $\pm$ .03, 2.8 $\pm$ .07, 3.2 $\pm$ .12 and 3.3 $\pm$ .11 g. A two-tailed *t* test was used where appropriate for further analysis of data.

## RESULTS AND DISCUSSION

The results were extremely variable after 24 h of exposure; LC-50's ranged from greater than 1000 mg/L for 0.3 g fish to 152 mg/L for 1.1 g fish. By 48 h the variability of responses had decreased considerably, and it was evident to the investigators that fish weighing 0.3 to 0.8g were more tolerant to nitrite than fish weighing 0.9 to 3.3 g. Means and variability were then calculated for each of the two groups at 24, 48, 72 and 96 h (Fig. 1). A *t* test demonstrated that a significant difference ($p < 0.01$) existed between smaller and larger fish at 48, 72 and 96 h. The *t* test was not used to test the 24 h groups due to the unequal variability of the groups. No control fish died at any time during the test.

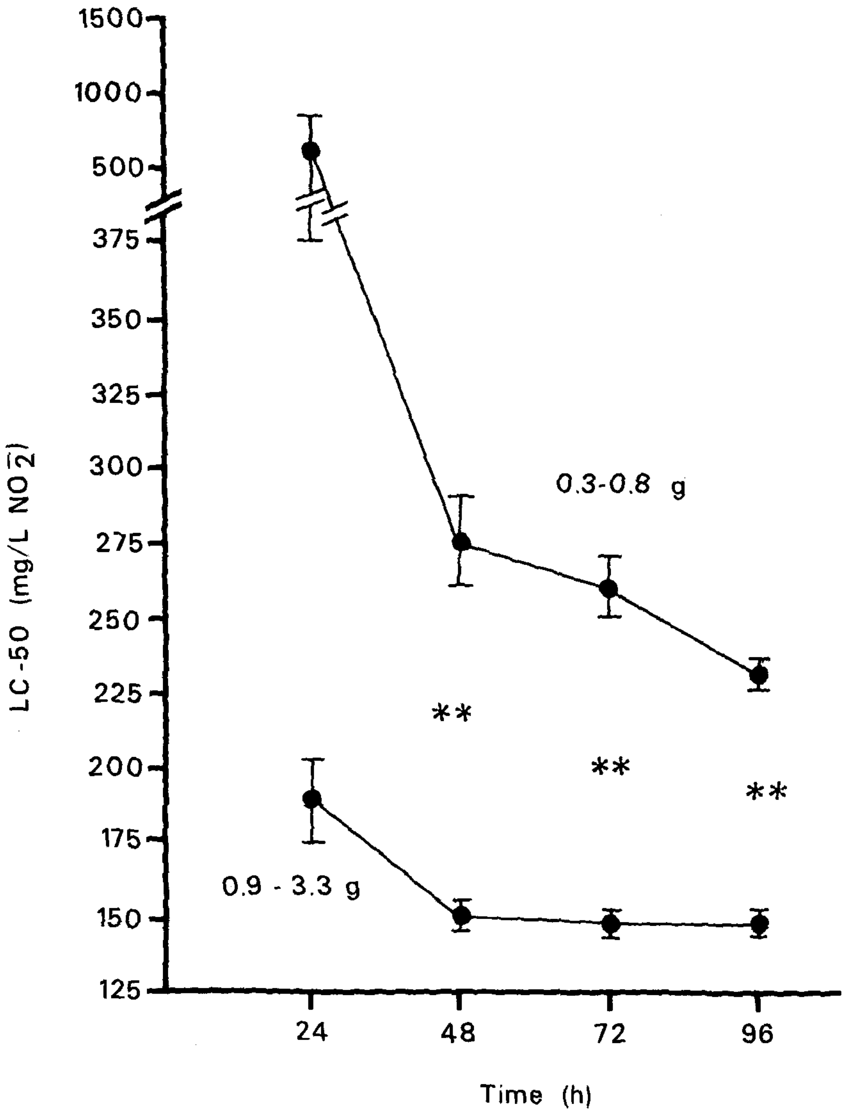


Fig. 1. Median lethal concentration (LC-50) of nitrite to fathead minnows of differing weight classes. Dots with vertical lines represent mean ± S.E. Double asterisks represent a significant difference (<.01) between means of different size classes at same sampling time.

The biological basis for this difference in tolerance is not totally understood. PERRONE & MEADE (1977), citing KIESE (1974), suggested that young individuals might have a more active methemoglobin reductase system which would counteract the methemoglobin forming tendencies of nitrite allowing fry and smaller fish to withstand increased concentrations for a longer period of time.

Two other observations should be considered. First, the data tended to fall into 2 groups with no graded relationship between weight and tolerance. Second, by 72 h the slopes of the toxicity curves of the larger fish were flat but the curves for the smaller fish were generally still falling. It is possible that if this study had been extended to 120 or 144 h, the differences between groups would no longer be significant.

This study points out that fish weight is an important consideration when evaluating nitrite toxicity data, and that although no weight-tolerance interaction was found in rainbow trout (RUSSO 1980), it may exist in other as yet untested species.

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