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# Effect of pH and Chloride on Nitrite-induced Lethality in Bluegill (*Lepomis macrochirus*)

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Nitrite ($NO_2^-$) toxicity is a problem frequently encountered in intensive, biofiltered fish culture systems (HUEY et al. 1980), fish culture ponds from denitrification in anaerobic sediments (BOYD & HOLLERMAN 1980), and streams receiving sewage treatment effluents (RUSSO et al. 1981).

Nitrite toxicity has been studied in several fish species (eg. KLINGER 1959; SMITH & WILLIAMS 1974; WESTIN 1974; RUSSO & THURSTON 1977), nonfish aquatic vertebrates (SULLIVAN & RIGGS 1964; HUEY & BEITINGER 1980a, b), and crayfish (BEITINGER & HUEY in press). The major toxic action of nitrite is oxidation of hemoglobin which produces methemoglobin, a derivative incapable of binding oxygen (BODANSKY 1952). Toxicity of nitrite both among and within species varies and is highly dependent upon water quality. Reported lethal concentration as 96 h LC-50s range from 27 $mgL^{-1}$ for channel catfish, Ictalurus punctatus, (KONIKOFF 1975) to 0.7 $mgL^{-1}$ for rainbow trout, Salmo gairdneri (RUSSO et al. 1974). Various anions influence the toxicity of nitrite (PERRONE & MEADE 1977; WEDEMEYER & YASUTAKE 1978; HUEY et al. 1980; HUEY & BEITINGER 1980a, b; RUSSO et al. 1981). TOMASSO et al. (1979) reported that a molar ratio of 18:1 $Cl^-:NO_2^-$ eliminated $NO_2^-$ lethality in channel catfish. In contrast enhanced toxicity of nitrite has been observed in channel catfish, rainbow trout and crayfish (Procambarus simulans) at increased hydrogen ion concentrations (HUEY et al. 1980; RUSSO et al. 1981; BEITINGER & HUEY, in press).

We conducted experiments to evaluate the combined effects of hydrogen ion and chloride concentrations on nitrite toxicity in bluegill (Lepomis macrochirus). Our working hypotheses proposed (1) no chloride amelioration of nitrite toxicity at low pH, (2) significant toxicity reduction at high pH and (3) increased nitrite toxicity in all groups at low pH relative to neutral pH.

## MATERIALS AND METHODS

Bluegill (17.3-7.4 g) obtained from a pond in Denton County, Texas were held for at least 5 days in 200-L tanks containing dechlorinated, continuously filtered tapwater at 29-30°C. Holding water was analyzed daily for ammonia and nitrite concentrations using an Orion specific ion probe and an azo-dye method

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(STANDARD METHODS 1975), respectively. Standard methodology for static toxicity testing (E.P.A. 1975) was employed and all fish were post-absorptive four days prior to testing. Trials were conducted in 30°C, $O_2$ saturated, soft (48 $mgL^{-1}$ total hardness) water. Toxicity tests were conducted under four environmental conditions with pH and chloride ($Cl^-$) as variables. Composition of test water varied as follows: (1) pH 4.0 and 18:1 $Cl^-:NO_2^-$ molar ratio (2) pH 4.0 and 5.0 $mgL^{-1}$ $Cl^-$ (3) pH 7.2 and 18:1 $Cl^-:NO_2^-$ molar ratio (4) pH 7.0 and 5.0 $mgL^{-1}$ $Cl^-$. A 0.02 M phosphate buffer was used to maintain pH at 7.2 and a potassium hydrogen phthalate buffer to maintain a pH of 4.0. Chloride was added as sodium chloride, nitrite as sodium nitrite and all chemicals were mixed with a mechanical stirrer. Chloride ion concentrations were quantified using a mercuric nitrate titration and nitrites determined by an azo-dye method (STANDARD METHODS 1975). During trials nitrites and hydrogen ion concentrations were monitored at 6 h intervals and adjusted as necessary. Chlorides were determined at the beginning of the experiment. Seven to ten trials with various constant nitrite concentrations were conducted at each of the four test conditions. Eleven bluegill were exposed to each test concentration. Lethal concentration, as 48 h LC-50, was calculated using the Statistical Analysis System probit package (FINNEY 1971) for each of the four test conditions.

## RESULTS AND DISCUSSION

The 48 h LC-50s for bluegill, Lepomis macrochirus, in pH 4.0 water were 4.4 $mgL^{-1}$ $NO_2^-$ in high chloride water and 4.6 $mgL^{-1}$ $NO_2^-$ in low chloride water. At pH 7.0 LC-50s equalled 211.3 $mgL^{-1}$ $NO_2^-$ in high chloride water and 281.9 $mgL^{-1}$ in low chloride water. (Table 1).

TABLE 1. Lethal concentration 50%, LC-50 (48 h) for bluegill, L. macrochirus, exposed to nitrite under four environmental conditions. Eleven fish were used in each trial.

| Group | Number of trials | pH | Chlorides | 48 h LC-50 $NO_2^-$ $mgL^{-1}$ | 95% Fiducial Limits $NO_2^-$ $mgL^{-1}$ | |
|---|---|---|---|---|---|---|
| I | 7 | 4.0 | + | 4.4 | 3.0 | 12.9 |
| II | 7 | 4.0 | - | 4.6 | 3.9 | 5.8 |
| III | 8 | 7.2 | + | 211.3 | 210.5 | 212.3 |
| IV | 10 | 7.2 | - | 281.9 | 251.0 | 345.7 |

Chlorides (+) indicates an 18:1 $Cl^-:NO_2$ molar ratio was used in each dose. (varied - high).
Chlorides (-) indicates 5.0 $mgL^{-1}$ $Cl^-$ was used for each dose. (fixed-low).

Fish exposed in low pH water exhibited immediate stress at concentrations of 6.9 $mgL^{-1}$ $NO_2^-$ and higher. These fish gulped at

the surface, produced excess slime and none survived the 48 h test period. At pH 7.2 all fish survived 48 h exposures at nitrite concentrations 13 fold higher than those that killed all fish at pH 4.0. Although pH had a dramatic effect on nitrite toxicity, chloride concentration had little or no effect.

Enhanced nitrite toxicity at high hydrogen ion concentrations was expected and corroborates results obtained by HUEY et al. (1980), MEADE & PERRONE (1980), RUSSO et al. (1981) and BEITINGER & HUEY (in press). This toxicity increase is due to the permeability of the uncharged nitrous acid form of nitrite predominant at low pH (HUEY et al. 1980, BEITINGER & HUEY in press). We suggest that the $HNO_2$ molecule passes rapidly across the gills causing profound methemoglobinemia and death by anoxia. KLINGER (1959) describes nitrite as a slow-acting fish poison, which appears to be correct for pH levels of 7.0 and above, where $NO_2^-$ is the predominant form of nitrite. Both forms of nitrite ($HNO_2$ and $NO_2^-$) are known to be toxic (RUSSO et al. 1981); however, we suggest that the $HNO_2$ uptake is much more rapid and the sudden nitrite load converts a majority of the fish's hemoglobin to methemoglobin resulting in death. At pH levels where $NO_2^-$ is predominant, uptake occurs by means of gill anion gates and is much slower (HUEY et al. 1980). A low rate of toxicant uptake allows the methemoglobin reductase system time to function before lethal methemoglobin concentrations occur. Although this system cannot supply enough reducing power in prolonged high nitrite exposure, it would significantly increase short term resistance to nitrite toxicity. In contrast, $HNO_2$ floods across the gill membranes causing a lethal concentration of methemoglobin and the methemoglobin reductase system functions too slowly to prevent rapid lethality.

At low pHs no decrease in nitrite toxicity was observed in high chloride exposures. This was expected because chloride competitively interferes with $NO_2^-$ at ionic uptake sites on the gills; however, chloride cannot interfere with uptake of $HNO_2$, the predominant nitrite form at pH 4.0 (BEITINGER & HUEY in press). At pH 7.2 we expected results similar to those obtained by CRAWFORD & ALLEN (1977), PERRONE & MEADE (1977), TOMASSO et al. (1979), and HUEY et al. (1980); however, 48 h LC-50s were similar in high and low chloride tests. It is possible that interaction between chloride, nitrite, phosphate buffers and high temperature ($30^oC$) caused the chloride dosed groups to suffer lethality that could not be explained by nitrite toxicity alone.

Our data support both the hypotheses that nitrite is more toxic at low ph and that chloride protection is lost at low pH.

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