Bull. Environ. Contam. Toxicol. (1994) 52:74–79

Environmental Contamination and Toxicology

Effect of Nitrite on the Survival of Grass Carp, *Ctenopharyngodon idella* (Val.), with Relation to Chloride

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The accumulation of nitrite in the environment is one of the principal problems in freshwater fish culture. Nitrite is highly toxic to freshwater fishes, but its toxic action is as yet poorly understood. It is well-established for several fish species that nitrite can cause methemoglobinemia. Methemoglobin is a form incapable of binding oxygen (Lewis and Morris 1986). Besides, other alterations caused by nitrite have been reported, like inhibition of growth (Colt et al. 1981), depression of thermal tolerance (Watenpaugh and Beitinger 1985), pathological changes in branchial and hepatic tissue (Michael et al. 1987), enzymatic alterations (Gaino et al. 1984) and hematic modified patterns (Hilmy et al. 1987). Nitrite rapidly moves from the water through the gills to inside the organism, reaching high concentrations in blood and other tissues (Bath and Eddy 1980; Margiocco et al. 1983).

A number of studies have reported the use of chloride to inhibit nitrite inducing methemoglobinemia in Oncorhynchus kisutch (Perrone and Meade 1977). This has been confirmed in Salmo gairdneri (Bath and Eddy 1980), in Ictalurus punctatus (Tomasso et al 1979). Perrone and Meade (1977) reported that there is an inverse relationship between the nitrite lethal concentration and the environmental chloride concentration. Nitrite acts as a competitive inhibitor of chloride uptake at the gills (Williams and Eddy 1986); so, when chloride concentration in environment increases, the chloride uptake is little modified in presence of nitrite. Our purpose was to determine the median lethal concentration (96-hr LC 50) of nitrite in the presence of chloride in the herbivorous carp Ctenopharyngodon idella.

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MATERIALS AND METHODS

Juveniles of grass carp Ctenopharyngodon idella (0.17 to 0.75 g wet weight) were obtained from the Centro de Producción Piscícola at Tezontepec, Estado de Hidalgo, México. The fish were held 15 d in 60-L aquaria with tap water at 24 °C, pH: 7 (Watmann pH meter; + 0.01), DO: 6.8 mg O/L (YSY oxymeter; + 0.1 mg O2/L), alkalinity: 44 mg CaCO3/L and 125 mg Cl^-/L (APHA, 1985). Photoperiod was established at 12/12 h light/dark. Fish were fed with lucern (25%) and commercial food until 1 d before starting the experiments. Once the period to acclimation was over, the fish were divided in six groups with soft water, which had a pH of 6.7-6.9, an alkalinity of 44 mg CaCO3/L, 6.1-6.4 mg O2/L, 3 mg Cl^-/L and 24 °C. The fish were kept for 24 hr under these conditions.

Toxicity test was conducted as static dose-response test (Buikema et al. 1982). One group of 10 fishes was considered the control; this group was kept in soft water without nitrite. The other five groups of ten fish each were exposed to 1, 2, 4, 8 and 10 mg $N\text{-}NO_2^-$/L. Glass aquaria (20 L) were used with mild and constant aeration at a temperature of 24 °C. Nitrite was used in the form of NaNO2 (Merck, 99.9%). Nitrite concentration was quantified every 24 hr by the azo dye colorimetric method (APHA 1985). Dead fish were removed from aquaria daily. The absence of response to prodding by a glass road was considered as criteria for death. Median lethal concentration (96-hr LC 50), for the fish exposed for 48, 72, 96 and 120 hr to nitrite was calculated using the DORES program from Ramírez (1989).

The effect of nitrite and chloride concentration on the mortality of juvenile of C. idella was investigated by exposing carps to 1.7, 2.7 and 4.5 mg $N\text{-}NO_2^-$/L in combination to 5, 6 and 6.5 mg Cl^-/L added as NaCl (Merk 99.9%). Control group was kept in soft water (3 mg Cl^-/L) without nitrite. Dead carp were removed from aquaria every 24 hr. Mortality percentage was registered in both groups after 96 hr.

In order to establish the effect of nitrite-chloride concentration on the mortality (%) of C. idella, a second-order polynomial model in two variables was fitted and the two-dimensional response surface was obtained (Montgomery and Peck 1982). All analysis were conducted with STATGRAPHICS procedures (Statist. Graph. Syst. Co. 1985).

RESULTS AND DISCUSSION

Static bioassay was conducted for a maximum of 120 hr. The median lethal concentrations (LC 50) of nitrite in C. idella for 48, 72, 96 and 120 hr are presented in Table 1. Mortality was not registered in the control group. As expected, mortality increased as nitrite concentration and expure-time increased.

Table 1. Lethal median concentration of nitrite for C. idella at different time of exposure. R^2 is the determination coefficient; df = degree of freedom and SE = Standard Error.

TIME (hr)	R^2	df	96-h LC50 + SE
48	0.87	2	4.45 + 0.57
72	0.91	2	3.04 + 0.55
96	0.89	2	1.71 + 0.21
120	0.86	2	1.50 + 0.22

There are few studies that describe the effect of nitrite in cyprinids because these organisms are considered less sensitive to nitrite than other fish (Williams and Eddy 1986). Lewis and Morris (1986) reported that 96-h LC 50 of nitrogen-nitrite for Cyprinus carpio is greater than 32 mg $N\text{-}NO_2^-$/L. However, it is not possible to make direct comparison between this concentration and the LC 50 obtained in this study because of the different water quality used in both studies. Although methemoglobin was not determined here, the fish showed methemoglobinemia signs, e.g., external paleness and brown-colored blood (Hilmy et al. 1987; Tucker et al. 1989).

The effect of chloride on the toxicity of nitrite in C. idella was estimated through the mortality of fish, which increased in the groups exposed to the nitrite LC 50 of 1.71 mg $N\text{-}NO_2^-$/L and 5, 6 and 6.5 mg Cl^-/L but in fish exposed to 2.7 and 4.5 mg $N\text{-}NO_2^-$/L mortality decreased as chloride environmental concentration increased from 5 to 6.5 mg Cl^-/L (Table 2).

The mortality values (%) were codified by means of an angular transformation (Zar 1974). The quadratic polynomial model that describes the relationship among the variables was:

$$\hat{Y} = 261.2\ X_1 - 115.3\ X_2 - 18.1\ X_1^2 + 14.5\ X_2^2 - 22.3\ X_1X_2$$

where $\hat{Y}$ is the predicted mortality (%), and X1 and X2 are the independent variables: nitrite (mg $N\text{-}NO_2^-$/L) and chloride (mg Cl^-/L) concentrations, respectively. The estimators of the model were: $R^2 = 0.96$, $F = 17.9$ and $P = 0.008$. Results can be visualized (Fig. 1) through the response surface (Montgomery and Peck 1982). Although chloride was added as NaCl we have taken into account that cations have very little effect on nitrite toxicity (Lewis and Morris 1986); thus, any changes observed on this can be attributed to chloride. Our results show that the fish mortality decreased as the chloride concentration in the media increased, which supports the hypothesis of nitrite uptake by the competence of monovalent anions through the sites of ionic uptake on the gill surface (Williams and Eddy 1986). Considering this latter, we can say that the decrease in fish mortality can be attributed to low uptake of nitrite due to high concentrations of chloride in the media.

Table 2. Mortality percentage of grass carp exposed to nitrite-chloride concentration for 96 hr. M′ are the codified values of mortality.

Nitrite (mg/L)	Chloride (mg/L)	Mortality (%)	M′
1.7	5.0	0	0.00
1.7	6.0	10	18.44
1.7	6.5	20	26.56
2.7	5.0	80	63.44
2.7	6.0	70	56.79
2.7	6.5	20	26.56
4.5	5.0	100	90.00
4.5	6.0	40	39.23
4.5	6.5	10	18.44

Moreover, our results show that the chloride protection to the herbivorous carp exposed to a low nitrite concentration was less than that observed in fish exposed to high concentration of the contaminant. Thus, when fish were exposed to 4.5 mg $N\text{-}NO_2^-$/L, 1.5 mg of chloride decreased the mortality in 90 % which indicates that there is an inverse relationship between the nitrite toxicity and the chloride concentration in the environment; however, this relation was not linear as it was reported by some authors (Lewis and Morris 1986). Likewise these results support the hypothesis that suggests the nitrite could be a competitive

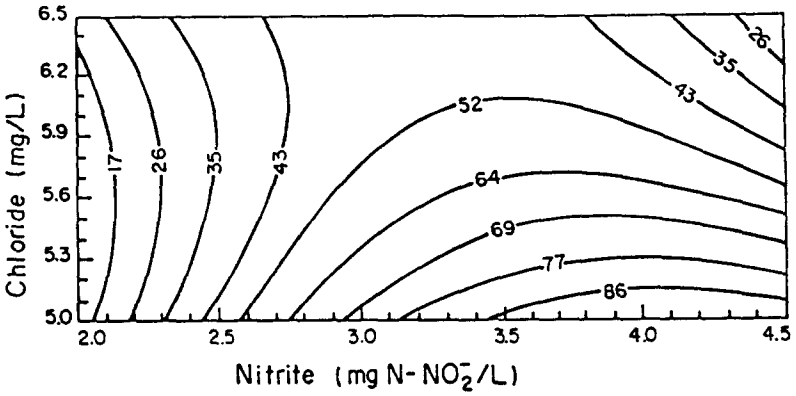

Figure 1. Response surface that describes the relationship between mortality of grass carp and the nitrite (mg N-NO_2^-/L) and chloride (mg Cl^-/L) environmental concentration.

inhibitor of active chloride uptake through the gills (Williams and Eddy 1986). Thus, when the environmental chloride concentration is high, the presence of nitrite does not have a significant influence on the chloride uptake which in our results is revealed by a low mortality of organisms; moreover, when the chloride concentration is low, its uptake is significantly inhibited by nitrite; consequently, the fish mortality increases. Furthermore, it is important to consider that the tolerance of nitrite-exposed fish is highly dependent on the environmental chloride concentration. The low value of LC 50 for the pollutant, obtained in this work, for the cyprinid C. idella proves this statement.

Acknowledgments. We are gratefull to the Centro Piscícola de Tezontepec, personnel for supplying the fish. The translation to English by María Antonieta Arizmendi is greatly appreciated.

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Received June 30, 1992; accepted June 29, 1993.