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Acute Toxicity of Nitrite in Juvenile Grass Carp Modified by Weight and Temperature

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Nitrite accumulation is a serious problem in intensive fish culture. Nitrite is an intermediate product in the biological oxidation of ammonia to nitrate. An increase in nitrite concentration can be attributed to nitrogen excretion by organisms, food excess in ponds or disturbances in the nitrifying bacteria population. When disturbances in the nitrification process do not surpass the maximum capacities of the system, low nitrite levels are attain in short-term periods (Diab and Shilo 1988).

The toxicity of nitrite is partly due to its ability to oxidize hemoglobin to methemoglobin; however, this is not the primary cause of fish death (Almendras 1987). Nitrite toxicity is modified both by abiotic and biotic factors. Temperature has multiple effects in both water characteristics and fish physiology; high temperatures increase the diffusion rate of molecules, the gill permeability and the metabolic rates of fish. Huey et al. (1984) suggest that high temperatures increase the nitrite uptake in <u>Ictalurus punctatus</u> and thus the nitrite toxicity.

The relationship between size and nitrite toxicity remains a controversial point. Some authors report that smaller fish are more tolerant to nitrite than bigger ones (e.g. Almendras 1987). In contrast, Wedemeyer and Yasutake (1977) and Hilmy et al. (1987) suggest that the large-size fish are less sensitive to the pollutant.

The capacity to predict the impact of pollutants in aquaculture systems is very important to avoid unfavorable effects in yield. Chemical toxicity is modified both by abiotic and biotic factors, thus in this work we studied the effect of acclimation temperature and fish weight on the nitrite median lethal concentration of juvenile <u>Ctenopharyngodon idella</u>.

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MATERIALS AND METHODS

Juveniles of grass carps (*C. idella*) (0.02 to 7.6 g wet weight) were obtained from Centro de Produccion Piscicola de Tezontepec de Aldama, Hidalgo, Mexico. The carps were held in glass aquaria with tap water (alkalinity of 120 mg $CaCO_3$/L, pH of 7.0-7.4 and chloride of 80 mg/L) at a similar temperature as that registered in the culture tanks (24 C). Small fishes (0.02 g) were fed with egg yolk and the bigger ones with balanced food pellets and lettuce; photoperiod was established at 12 hr light and 12 hr dark. Daily, one third of aquaria water was changed and the non-ingested food was withdrawn. Fish were maintained under these conditions for 15 d. After this period, water temperature of two containers was increased at a rate of 1 C/d until 29 and 32 C were reached, a third one was maintained at 24 C. Carps were acclimated at these temperatures for 2 wk.

At the end of the acclimation period, fish were transferred to six aquaria with synthetic fresh water (SFW; U.S. EPA 1989; alkalinity of 60-70 mg $CaCO_3$/L, pH of 7.4-7.8 and chloride of 5 mg/L), where they were maintained for 24 hr before nitrite was added. Density was 0.5 g wet weight per liter. Fish were fasted for 24 hr before the experimental phase.

Nitrite was used as $NaNO_2$ (Merck Mexico, 99 %) in concentrations that produced mortality between 0 and 100 % in 96 hr, established in previous essays (Alcaraz 1993). The toxicity test was conducted as a static bioassay (Buikema et al. 1982). Ten fish were exposed to each of six nitrite concentrations. The mortality percentage was registered each 24 hr. All fish were weighed when they died or at the end of the experimental period; smaller fish were weighed in an analytical balance (Sauter + 0.5 mg) and the bigger ones in a plate balance (OHAUS + 0.01 g). The exposure period lasted 96 hr. One group of 10 organisms were kept in SFW without nitrite as control group. One replicate was used for each experimental and control group. Water temperature, pH and nitrite were measured every 24 hr. Nitrite concentration was determined by azo-dye technique (APHA 1985). Similar experiments were performed with juvenile carps weighing 0.02, 0.45 and 7.60 g w.w., acclimated to 24, 29 and 32 + 1 C.

The values of 96-hr LC50 of nitrite were obtained by means of a computer program (Ramirez 1989).

RESULTS AND DISCUSSION

Mortality was not observed during the maintenance or the acclimation periods. Neither the physico-chemical characteristics of water nor the nitrite concentration were modified during the experimental period.

In all the experimental conditions, the median lethal concentration of nitrite (96-hr LC50) was estimated using the Probit model. This model was selected according to the determination coefficient (R2), proportionated by the DORES computer program (Ramirez 1989). All the determination coefficients were greater than 0.90. Standard errors among the nine experimental groups (n=20) were found in an interval from 0.51 to 1.50.

Non mortality was observed in control group. Nitrite lethal median concentration was influenced by weight and temperature ($P < 0.05$). In the groups acclimated to 29 C smaller fish were more sensitive than the largest, with 96-hr LC50 values 3.4 times higher in the latter group. The same trend was observed in fish acclimated at 32 C but differences were not significant ($P > 0.05$). Thus, bigger fish acclimated to 29 C were more tolerant to nitrite than smaller ones (Fig. 1).

Cairns et al. (1975) reported that the water temperature is an important factor that determines the chemical toxicity; however, the temperature-toxicity interaction is complex being difficult to predict it effect. Elevated temperature can increase nitrite toxicity directly since it increases the lamellar water flux and the ionic exchange through the gill; in consequence, nitrite accumulation in fish blood increases (Huey et al. 1984). Thus, in the present work the higher nitrite toxicity caused by elevated temperatures could be attributed to higher nitrite uptake by smaller fish.

Temperature of 29 C had a protective effect against nitrite toxicity in the largest organisms. In this sense it is important to consider that nitrite toxicity does not depend only on the nitrite uptake, but also depends on the internal detoxification process of the animals, mainly through the methemoglobin reductase system. It is know that the biochemical and the enzymatic processes are sensitive to temperature, being more efficient in the thermal interval corresponding to the preferred temperature of the species (Hochachka and Somero, 1973). Particularly, have been reported than juvenile grass carp acclimated to 29 ^{o}C acumulated less nitrite in relation to 24 and 32 C (Alcaraz 1993).

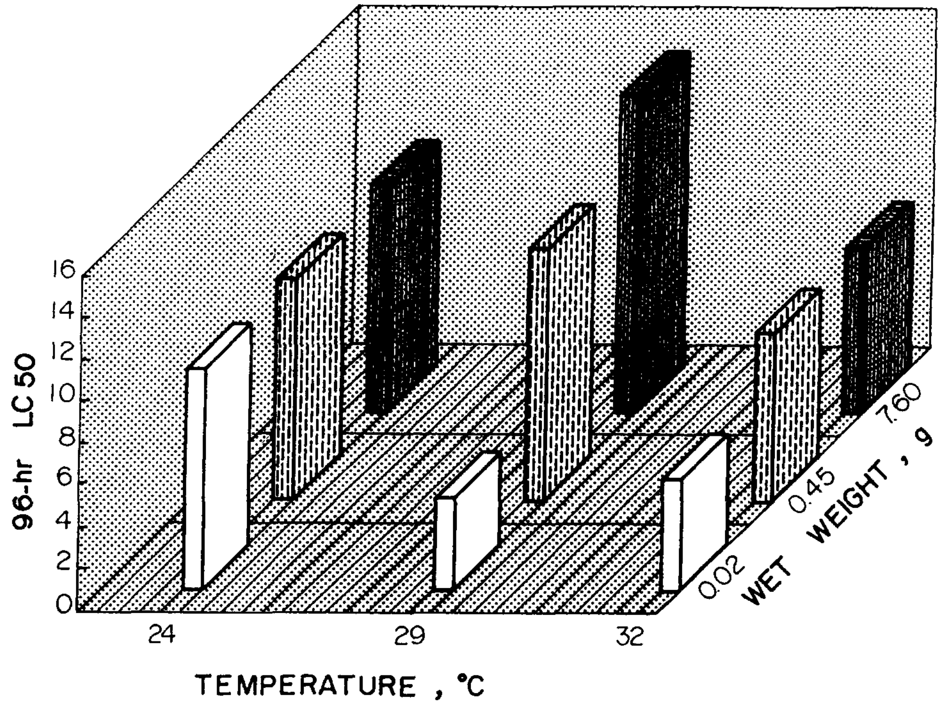

Figure 1. Nitrite lethal media concentration of C. idella acclimated to 3 temperatures.

On the other hand, it is probable that in small fish the methemoglobin reductase system was little efficient in relation to the bigger ones, maybe because the smaller were so young. Probably in the fish of the former group, nitrite toxicity could only depend on the increase of nitrite uptake which increases with water temperature, as mentioned above; while in the latter group, although the elevated temperatures trend to increase the nitrite uptake, the methemoglobin reductase system could reduce the blood methemoglobin and so the toxicity of nitrite to fish.

Nitrite toxicity depends on many factors, including fish weight. A great number of papers report that smaller fish are more tolerant to nitrite than bigger ones (e.g. Almendras 1987). However, it is important to consider that chemical toxicity is influenced by the interaction of many factors, and does not depend on only one. In this sense we atributted the minor damage caused by nitrite to the protective effect of the temperature, in the assumption that there is a thermal interval for optimum biochemical and physiological function (Hochachka and Somero 1973). However, although 29 C is the optimum temperature of grass carp of 1.03 to 3.20 g

(Alcaraz et al. 1993), the response to temperature changes in individuals of different weight. In this sense, it is possible that smaller fish have a different interval of preferred temperature, in which they display their function more efficiently, or also maybe because the acclimation mechanisms in smaller fish had not been completely developed, like has been suggested by McCormick et al. (1972) for small-size Salvelinus fontinalis (0.04-0.05 g).

One important aim in the study of chemical toxicity in aquaculture is to predict the effect of some important toxicants to prevent deleterious effects to the organisms. The results obtained in this work emphatically suggest the importance of considering both extrinsic and intrinsic factors to establish the safe concentration of pollutant in culture ponds.

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