Bull. Environ. Contam. Toxicol. (1985) 34:369–376

Bulletin of Environmental Contamination and Toxicology

Nitrite Toxicity to the Crayfish *Procambarus clarkii*

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The toxicity of nitrite to freshwater fishes has been studied intensively in recent years (see review by Russo and Thurston 1977, Tomasso et al. 1981). Reported median lethal concentrations (LC-50) of nitrite to various species of fishes range from 0.36 mg/L for rainbow trout, Salmo gairdneri, (Russo et al. 1981) to 460.40 mg/L for largemouth bass, Micropterus salmoides (Palachek 1984). Toxicity of nitrite to fishes has been shown to be dependent on water quality conditions such as calcium concentrations (Wedemeyer & Yasutake 1978), pH (Russo et al. 1981) and chloride concentrations (Crawford and Allen 1977; Perrone and Meade 1977).

While much information is available concerning the toxicity of nitrite to freshwater fishes, the effects of environmental nitrite on freshwater invertebrates have been largely ignored. The purpose of this study was to determine the effects of acute nitrite exposure to the crayfish Procambarus clarkii (Decapoda). Specific objectives of this study included (1) determining the 24-, 48-, 72- and 96-h LC-50's of nitrite to crayfish of different weights and genders in freshwater, (2) determining the LC-50's of nitrite to crayfish in water with elevated chloride concentrations, and (3), in order to gain insight into the mechanisms of nitrite toxicity in crayfish, determining hemolymph nitrite concentrations in crayfish exposed to nitrite in freshwater and water with elevated chloride concentrations.

MATERIALS AND METHODS

Crayfish were seined from the culture ponds at Southwest Texas State University, San Marcos, Texas. Animals were held at least 48-h prior to testing in 252-L fiberglass tanks supplied with well water at the San Marcos National Fish Hatchery and Technology

Center (temperature = 23° C; pH = 7.2; dissolved oxygen > 6.0 mg/L; hardness = 310 mg/L as calcium carbonate (magnesium = 18 mg/L); chloride = 22 mg/L) at a flow rate of 6-7 turnovers per hour. Animals were fed a formulated fish food (40% protein) when first placed in the 252-L tanks. Feeding was suspended 24-h before crayfish were placed in the experimental aquaria for testing.

All tests were conducted in 15-L glass aquaria placed in a 23 C water bath to maintain constant temperature. Crayfish were allowed to acclimatize to the aquaria for 24-h prior to nitrite exposure. Test water was from the same source as holding water and was aerated constantly to maintain dissolved oxygen levels near saturation. Reagent grade sodium nitrite was used to obtain desired nitrite concentrations. Sodium chloride was used to increase environmental chloride concentrations in the chloride tests. Water quality characteristics were monitored every 24-h in selected tanks during all tests (Table 1).

Five crayfish per aquarium were used to determine LC-50's according to the method of Thompson (1947). Concentrations of nitrite in experimental aquaria were increased from aquarium to aquarium by a geometric progression factor of 1.7 (from 10 to 83.5 mg/L for gender and weight tests and from 29 to 242 mg/L for chloride inhibition tests). Every 24-h dead crayfish were removed from aquaria and weighed to the nearest 0.1g. No movement by their appendages and no response to prodding by a glass rod was considered an indication of death. Twenty six trials were conducted. Nineteen trials tested effect of crayfish weight on tolerance to nitrite; 14 trials tested effect of gender on tolerance to nitrite (conducted simultaneously with size trials); and 7 trials tested the effect of the addition of 100 mg/L environmental chloride on tolerance to nitrite toxicity. Each trial consisted of one control aquarium and five experimental aquaria. Mean crayfish weights per trial in each of the gender and size trials ranged from 0.5 $\pm$ 0.03 to 12.4 $\pm$ 1.76 g, and from 1.57 $\pm$ 0.22 to 17 $\pm$ 1.65 g (mean $\pm$ S.E.) in the chloride inhibition tests.

The relationship of environmental nitrite to hemolymph nitrite concentrations was investigated by exposing crayfish to nitrite concentrations ranging from 10-160 mg/L (geometric progression factor of 2, 10 crayfish/concentration) for 24-h in freshwater and in water containing 100 mg/L chloride. After 24-h, crayfish were removed from aquaria, weighed to the nearest 0.1 g., and a 20 µL hemolymph sample obtained

Table 1. Water quality characteristics during exposure of Procambarus clarkii to nitrite. Values represent ranges, means or mean ± S.E. Numbers of observations are given in parentheses.

Character	24h	48h	72h	96h
pH[a]	7.9 - 8.3 (180)	7.9 - 8.3 (161)	7.9 - 8.1 (137)	7.9 - 8.3 (128)
D.O. (mg/L)[b]	6.9 ± 0.1 (36)	7.5 ± 0.1 (54)	7.1 ± 0.1 (21)	7.2 ± 0.1 (34)
Temperature ^{o}C [b]	23 (40)	23 (40)	23 (20)	23 (21)
Alkalinity (mg/L, as $CaCO_3$)[c]	196 ± 3 (46)	182 ± 3 (82)	165 ± 4 (47)	153 ± 4 (61)
Hardness (mg/L, as $CaCO_3$)[c]	232 ± 3 (69)	220 ± 3 (75)	198 ± 4 (43)	198 ± 6 (26)
Nitrite (% Nominal)[d]	95 ± 1 (144)	94 ± 1 (126)	95 ± 1 (116)	94 ± 1 (116)

a. pH meter
b. dissolved oxygen/temperature meter
c. Hach Chemical Company (1973)
d. Azo-dye method (USEPA 1974)

in a disposable pipet after breaking off a periopod or cheliped. Mortality at each concentration ranged from 0 to 30 percent. Those crayfish exposed to nitrite and chloride simultaneously weighed 7.1 ± 0.5 g and those exposed to nitrite only weighed 8.4 ± 0.9 g. Hemolymph nitrite concentrations were determined by a modification of the USEPA (1974) method for water nitrite analysis (Palachek 1984).

A two-tailed t-test, one way analysis of variance (ANOVA), and Student-Newman-Keuls multiple range test (SNK) were used where appropriate. A probability level of $\leq .05$ was considered significant.

RESULTS AND DISCUSSION

Neither gender (two-tailed t-test) nor weight (ANOVA) significantly affected LC-50 of nitrite to crayfish size. Combined gender and size data resulted in 24-, 48-, 72- and 96-h LC-50's of 46 ± 6.0, 37 ± 1.0, 32 ± 1.5 and 28 ± 1.5 mg/L nitrite, respectively (Fig. 1). Our LC-50 values are intermediate between a 96-h LC-50 of 6.1 mg/L reported for *Procambarus simulans* (Beitinger and Huey 1981) and a 48-h LC-50 of approximately 600 mg/L reported for *Procambarus acutus* (Johnson 1983). No reports of gender affecting tolerance to nitrite were found in the literature; however smaller fathead minnows, *Pimephales promelas*, ($\leq$ 0.8 g) have been shown to be more tolerant of nitrite than larger ones ($\geq$ 0.9 g) (Palachek and Tomasso 1984).

Environmental chloride significantly increased the LC-50 values at all time periods tested (ANOVA)(Fig. 1). These results indicate that chloride inhibits nitrite toxicity in crayfish as it does in freshwater fishes, probably by competitively excluding nitrite from active transport sites on gill cells that normally transport chloride into the fish (Perrone and Meade 1977, Tomasso et al. 1979). Increasing environmental chloride concentrations have been shown to increase resistance times to nitrite in *Procambarus simulans* (Beitinger and Huey 1981).

Hemolymph nitrite concentrations in crayfish exposed to 10-80 mg/L nitrite without chloride for 24-h were significantly higher than controls (unexposed crayfish) (ANOVA and SNK). At all concentrations, except 10 mg/L nitrite hemolymph nitrite concentrations in water with no additional chloride were significantly higher (ANOVA) than those animals simultaneously exposed to similar nitrite concentrations and 100 mg/L chloride (Fig. 2). This

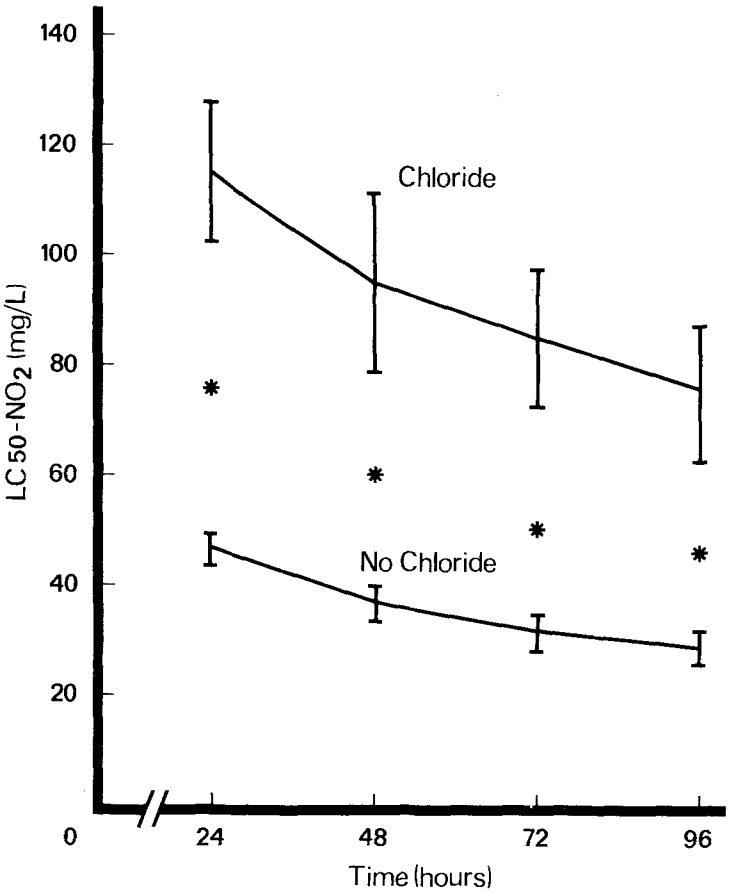

Figure 1. Median lethal concentrations (mean ± S.E.) in crayfish Procambarus clarkii exposed to environmental nitrite in freshwater and in freshwater plus 100 mg/L chloride ion. Asterisks represent significant differences between LC-50 values at same time periods.

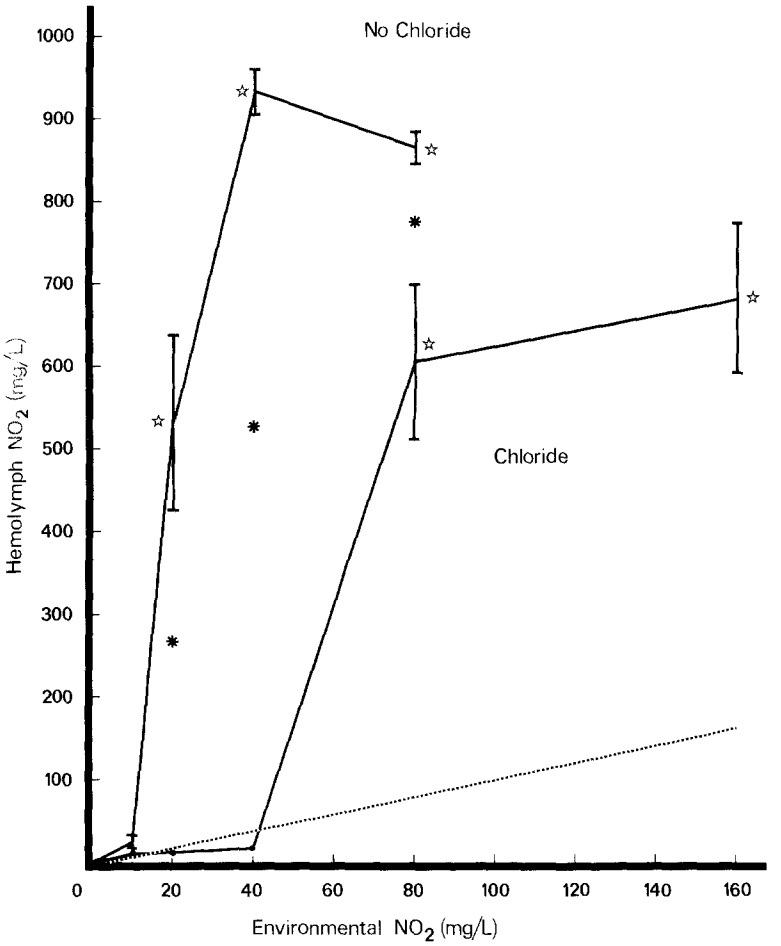

Figure 2. Hemolymph nitrite concentrations (mean ± S.E.) of crayfish Procambarus clarkii exposed to environmental nitrite alone and in combination with 100 mg/L Chloride ion for 24-h. Stars indicate significant differences from unexposed animals and asterisks indicate significant differences between animals exposed to nitrite alone and those exposed to nitrite and chloride. The dashed line at the bottom represents an equivalent environmental and hemolymph nitrite concentrations.

demonstrates that chloride does partially inhibit nitrite from entering the circulatory system. Fish have been shown to actively concentrate nitrite in their blood (Bath and Eddy 1980; Meade and Perrone 1980) with rainbow trout concentrating it as much as 60 times the environmental concentrations (Margiocco et al. 1983). Our results show that without chloride, nitrite was concentrated in hemolymph as much as 23 times in crayfish exposed to 40 mg/L environmental nitrite.

Results of this study indicate that nitrite is toxic to crayfish at concentrations similar to those which are toxic to many freshwater fishes. The primary mechanism of nitrite toxicity to crayfish, however, has not yet been identified. In vertebrates, nitrite causes the oxidation of hemoglobin to methemoglobin, a form not capable of binding and transporting oxygen. The animal then suffers from a functional hypoxia. Crayfish contain the respiratory pigment hemocyanin rather than hemoglobin. Hemocyanin regularly goes through oxidation-reduction reactions as it binds and releases oxygen (Connant et al. 1933; Needham 1961). The reversibility of hemocyanin oxidation-reduction coupled with the observation of extremely high levels of nitrite in the hemolymph (up to 929 mg/L) may indicate that nitrite toxicity in crayfish is not related to oxidation of the respiratory pigment as it is in vertebrates.

Acknowledgments. Wet lab facilities were provided by the San Marcos (Texas) National Fish Hatchery and Technology Center. A Southwest Texas State University Faculty Research Grant to the second author provided financial support.

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Received April 17, 1984; accepted May 4, 1984