

Acute Toxicity of Ammonia, Nitrite, and Nitrate to the Guadalupe Bass, *Micropterus treculi*

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The Guadalupe bass, *Micropterus treculi*, is native to streams and rivers of central Texas (Lee et al. 1980). In recent years the range and populations of Guadalupe bass have been decreasing due to impoundment of habitat, poor water quality (Edwards 1979) and hybridization with smallmouth bass, *Micropterus dolomieu* (Whitmore 1983). As a result, the Guadalupe bass has been listed as a species of special concern by Deacon et al. (1979) and placed on the Watchlist of Endangered, Threatened and Peripheral Vertebrates of Texas (TOES 1984).

The U.S. Fish and Wildlife Service is presently developing techniques for the intensive culture of Guadalupe bass (Carmichael and Williamson in press) in order to be prepared to augment natural populations with hatchery fish, should it become necessary. As a part of this development project, this study was conducted to determine the acute toxicity of the common nitrogenous wastes found in aquatic environments (ammonia, nitrite, and nitrate) to the Guadalupe bass. This information will be used to (1) set hatchery water quality criteria for this species, and (2) help determine the suitability of potential stocking waters which may contain high levels of these metabolites.

MATERIALS AND METHODS

Experimental animals were taken from a group of fish produced in two 0.04 hectare earthen ponds each stocked with 20 broodfish. The broodfish were produced in a similar manner the previous year except that only five brood fish in one pond were used. Three of the original broodfish were captured by electrofishing in Lake Buchanan, Texas, and two of the brood fish were captured by angling in the Llano River, Texas. After hatching and

rearing in ponds to approximately 0.1 g, the experimental fish were placed in raceways receiving a constant supply of well water (temperature 23°C; pH 7.2, hardness 310 mg/L as CaCO₃; alkalinity 316 mg/L as CaCO₃; dissolved oxygen > 6.0 mg/L; chloride 22 mg/L) and trained to accept pelleted food (Biodiet¹). Fish were then placed in indoor holding tanks receiving a constant supply of well water and maintained on pelleted food until needed for the study.

Acute toxicity was estimated by determining the 96-h median lethal concentrations (LC50) of Guadalupe bass for the individual nitrogenous metabolites. All tests were conducted in glass aquaria containing 15 L of aerated well water (5 fish/aquaria). When needed for testing, fish were transferred directly from the holding tank to the experimental tanks.

Eight trials were conducted to determine ammonia toxicity, and four trials each were conducted to determine the toxicity of nitrite and nitrate. Each trial consisted of a control aquarium and five experimental aquaria. Concentrations of the metabolites in the experimental aquaria were increased from aquarium to aquarium by a geometric progression factor of 2.0 within each trial. Desired ammonia, nitrite and nitrate concentrations in experimental aquaria were developed by the addition of ammonium chloride, sodium nitrite and sodium nitrate, respectively. Dead fish were counted, removed, and weighed every 24-h. Median lethal concentrations were estimated according to Thompson (1947). The mean and standard error (S.E.) of the LC50's presented in the results were determined by taking the mean and S.E. of the replicate trials for each metabolite. Fish weighed 6.5 ± 0.3 g.

Ammonia and nitrite concentrations in experimental tanks averaged $132 \pm 6\%$ (mean \pm S.E.) (Nesslerization, APHA 1971) and $97 \pm 3\%$ (diazotation of sulfanilamide by nitrite, USEPA 1974) of nominal, respectively, after 96-h. Nitrate concentrations were not monitored; however, no precipitate was observed in the bottoms of the tanks indicating that the sodium nitrate was in solution. In all tests the temperature was 22°C. The pH ranged from 8.0-8.3 at the beginning of the test to 7.9-8.4 after 96-h.

¹ Mention of commercial products or firms does not imply endorsement by the United States Fish and Wildlife Service.

Alkalinity decreased from 183 ± 6 mg/L (as calcium carbonate) at the beginning of the tests to 163 ± 8 mg/L after 96-h. Total hardness also decreased from an initial 222 ± 6 mg/L (as calcium carbonate) to 203 ± 10 mg/L by the end of the test.

RESULTS AND DISCUSSION

The 96-h LC50 for ammonia nitrogen to Guadalupe bass fingerlings was 12.7 ± 0.9 mg/L of total ammonia nitrogen. This estimate was based on the calculated concentrations in the test aquaria and is probably low due to the gradual buildup of ammonia during the course of the study. The toxicity of ammonia to fishes increases with increasing environmental pH and temperature due to the increasing percentage of the total ammonia in the un-ionized form (Emmerson et al. 1975). At 22°C and a pH of 8.0, the 96-h LC50 for Guadalupe bass was 0.56 mg/L un-ionized ammonia. This is slightly lower than values reported for other warmwater fishes. Examples of the toxicity of un-ionized ammonia to warmwater fishes include 0.67 mg/L (72-h LC50) for largemouth bass, Micropterus salmoides (Tomasso and Carmichael, unpublished data), 3.80 mg/L (96-h LC50) for channel catfish, Ictalurus punctatus (Colt and Tchobanoglous 1976), and 2.80 mg/L (96-h LC50) for red shiners, Notropis lutrensis (Hazel et al. 1979).

The 96-h LC50 for nitrite-nitrogen to Guadalupe bass was 187.6 ± 12.1 mg/L. This concentration is similar to other centrarchids such as largemouth bass (Palachek and Tomasso 1984) and green sunfish, Lepomis cyanellus, (Tomasso, unpublished data) under similar conditions. The value is also much higher than other warmwater noncentrarchid fishes such as channel catfish (23.3 ± 6.5 mg/L) and fathead minnows, Pimephales promelas (147.4 ± 4.0 mg/L) (Palachek and Tomasso 1984). Nitrite toxicity is also affected by environmental characteristics such as pH, temperature and especially chloride concentrations (Tomasso et al. 1979). However, Tomasso (unpublished data) has shown that high resistance to nitrite is a genetically based characteristic of all centrarchids tested thus far and not a water quality based characteristic.

The 96-h LC50 of nitrate nitrogen to Guadalupe bass was $1,261 \pm 142.1$ mg/L. This observation is consistent with other reported values to different

species (Westin 1974, Colt and Tchobanoglous 1976) indicating that nitrate is by far the least noxious of the three nitrogen containing substances tested.

In summary, (1) the Guadalupe bass appears to be slightly less resistant to ammonia than most warmwater species tested, (2) it is more resistant to nitrite than all noncentrarchids tested and as resistant as other centrarchids, and (3) nitrate appears to have no toxic effect at reasonable concentrations. Criteria developed by fish hatcheries and environmental protection agencies for protecting other warmwater fishes from ammonia, nitrite and nitrate should be adequate to protect the Guadalupe bass.

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