CALCIUM ANTAGONISM TOWARD NITRITE TOXICITY IN CHINOOK SALMON

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Abstract. Percent of methemoglobin (MHb) in the blood of fingerling chinook salmon (Oncorhynchus tshawytscha) acclimated to fresh water and sea water was observed after 48 hours of exposure to nitrite in a standing bioassay. Percent mortality was also recorded. MHb in fresh water (32 mg/l Ca^{++}) with 30 mg/l nitrite increased from 16.2% to 44.6% with a corresponding mortality of 80%. MHb in sea water (400 mg/l Ca^{++}) remained near 19% with 100 mg/l nitrite and no mortalities were observed.

Increasing the calcium concentration from 32 to 200 mg/l in fresh water with 30 mg/l nitrite decreased the mortality from 80% to 0% while the MHb level remained near 40%. Decreasing the calcium concentration from 400 to 0 mg/l in artificial sea water with 100 mg/l nitrite increased mortalities from 0% to 90% while the level of MHb remained at 20%.

The data presented here suggest that calcium acts antagonistically toward the toxicity of nitrite in chinook salmon fingerlings.

INTRODUCTION

The generally deleterious effects of ammonia on salmonids have been well documented (Brockway 1950, Burrows 1964, Reichenbach-Klinke 1967) but little has been done on the effects of nitrite, the oxidative product of ammonia in aerobic bacterial nitrification.

While nitrite has been described by Klingler (1957) as a slow-acting fish poison, much species variation in the symptoms and susceptibility to nitrite has been observed (Jaffe 1964, van Duijn 1973). The recognized acceptable maximum nitrite concentration in fish management lies in the range of 1-20 mg/l as NO_2^- (Reichenbach-Klinke 1973, van Duijn 1973).

Smith and Williams (1974) observed an increase in the percentage of methemoglobin (oxidized hemoglobin) in the blood of chinook salmon (Oncorhynchus tshawytscha) and rainbow trout (Salmo gairdneri) exposed to nitrite for 24 hours. Weber (no date) reported that increasing water hardness

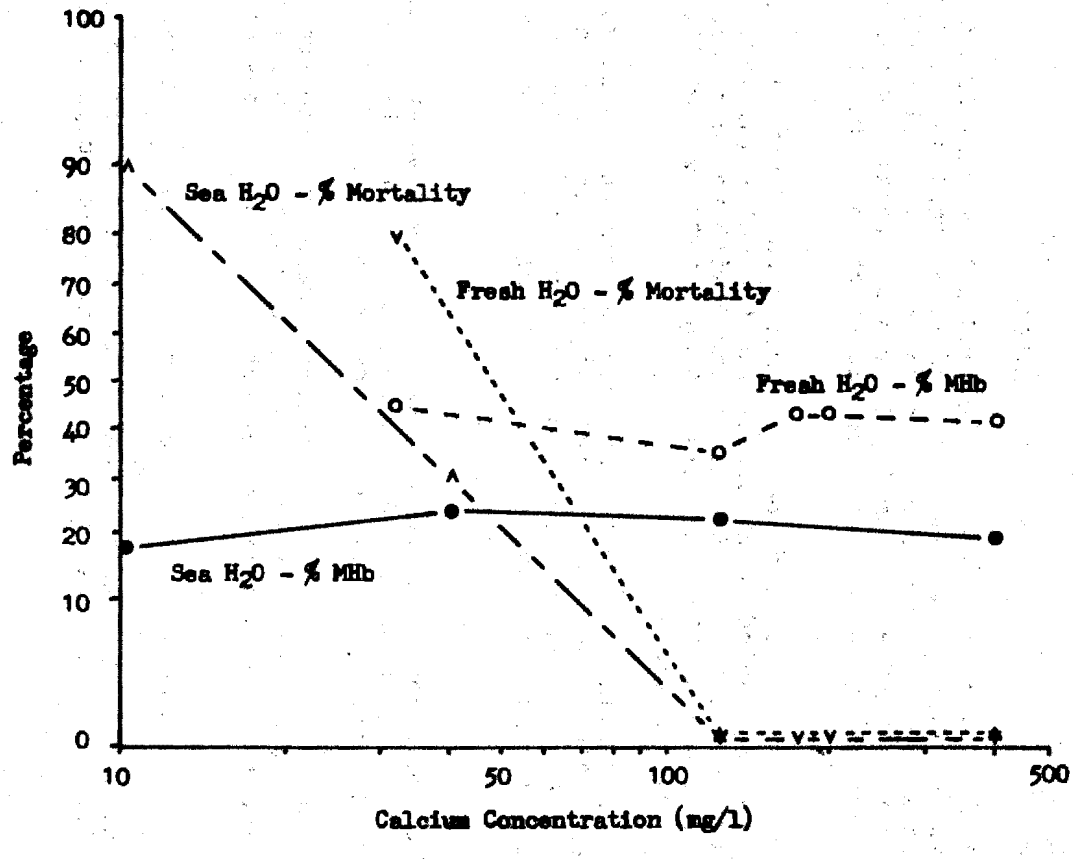

Figure 1. Effect of calcium on the percentage of methemoglobin (MHb) and the percentage of mortality in freshwater-acclimated chinook salmon exposed to 30 mg/l NO_2^- for 48 hours and seawater-acclimated (32 °/oo) chinook salmon exposed to 100 mg/l NO_2^- for 48 hours.

increased survival time for the guppy (Lebistes reticulatus) when exposed to sodium nitrite.

This paper describes an experiment which assessed the effects of different calcium concentrations on the percentage of methemoglobin (MHb) and the percentage of mortality due to nitrite poisoning of chinook salmon fingerlings. Both fresh water and sea water acclimated salmon were utilized in this work.

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MATERIALS AND METHODS

In 48-hour standing bioassays, fresh water and salt water (32 o/oo) acclimated chinook salmon fingerlings were exposed to 30 mg/l and 100 mg/l nitrite, respectively. The tests were conducted in 10 gallon all-glass aquaria which were loaded at 3.3 gm/l (10 fish per tank). Water temperature was 10° C and the fish were not fed during the experiment. Water was changed daily.

Calcium content in the fresh and sea water media was controlled as follows. Calcium, as $CaSO_4$, was added to dechlorinated tap water (32 mg/l Ca^{++}); artificial sea water (Harvey 1955) was prepared with various calcium concentrations. Single distilled tap water (0 mg/l Ca^{++}) was utilized in the artificial sea water mixture.

After 48 hours, surviving fish were examined for percentage of MHb according to Dubowski (1960). Due to the small volume of blood available, 1200 ul cuvettes in a Beckman DU Spectrophotometer were used. Appropriate adjustments to the method were made to allow for the small sample size.

RESULTS AND DISCUSSION

Nitrite was much more toxic in fresh water than in sea water. Experimental values for the percentage of MHb and the respective percentages of mortality at the tested nitrite concentrations are presented in Table 1.

In both fresh water and salt water, increasing calcium concentrations decreased the toxicity of the nitrite solution to chinook salmon (Figure 1). However, the amount of calcium had no effect on the percentage of methemoglobin.

Modern aquacultural management practices recognize the many benefits resulting from the addition of calcium to waters utilized for fish production. Reduction of the toxicity of nitrite can be added to the list of improvements derived from the practice of supplementing water supplies with calcium.

Table 1. Percentage of methemoglobin (MHb) and percentage of mortality in chinook salmon fingerlings exposed to 0, 30, and 100 mg/l nitrite (NO_2^-) in fresh water and salt water for 48 hours. Values are mean ± 2 S.D. Figures in parentheses are sample size.

	Fresh water acclimated fish (32 mg/l Ca^{++})		Sea water acclimated fish (400 mg/l Ca^{++})	
(NO_2^-)	% MHb	% mortality	% MHb	% mortality
Controls	16.2 ± 0.21 (5)	0	18.3 ± 0.82 (21)	0
30 mg/l	44.6 ± 0.15 (4)	80	no data	no data
100 mg/l	no data	no data	19.1 ± 0.76 (5)	0

LITERATURE CITED

Brockway, D. R. 1950. Metabolic products and their effects. Prog. Fish-Cult. 12(3):127-129.

Burrows, R. E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. U.S. Fish and Wild. Serv., Res. Rep. #66. 12 pp.

Dubowski, K. M. 1960. Measurements of hemoglobin derivatives. Pages 49-60 in F. W. Sunderman and F. W. Sunderman Jr., eds. Hemoglobin, its precursors and metabolites. Lippincott Co., Phil.

Harvey, H. W. 1955. The chemistry and fertility of sea waters. Cambridge Univ. Press. 224 pp.

Jaffe, E. R. 1964. Metabolic processes involved in the formation and reduction of methemoglobin in human erythrocytes. Pages 397-422 in C. Bishop and D. Surgenor, eds. The red blood cell. Academic Press, N.Y.

Klingler, K. 1957. Sodium nitrite, a slowly acting fish poison. Water Pollution Abs. 32(3):142.

Reichenbach-Klinke, H. H. 1967. Studies regarding the effect of ammonia on fish. Arch. Fischereiwiss 17:122-131.

Reichenbach-Klinke, H. H. 1973. Fish pathology. T. F. H. Publications, Inc., Ltd. 512 pp.

Smith, C. E. and W. G. Williams. 1974. Experimental nitrite toxicity in rainbow trout and chinook salmon. Trans. Amer. Fish. Soc. 103(3):389-390.

van Duijn, C. Jr. 1973. Diseases of fishes. Iliffe Books, London. 372 pp.