The effect of oxygen supplementation on the toxicity of ammonia (NH□3□) in rainbow trout Oncorhynchus mykiss (Richardson)

by Todd David Hanna

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Fish and Wildlife Management
Montana State University

Abstract:
Oxygen supplementation has been shown to have useful applications in aquaculture. Production capacities can be increased and nitrogen gas supersaturation problems can be alleviated by using oxygen. This study was conducted to determine effects of supersaturated dissolved oxygen concentrations (DO) on un-ionized ammonia (NH3) toxicity. Arlee rainbow trout (Oncorhynchus mykiss) were tested at three NH3 levels (<0.01, 0.04, and 0.06 mg/L) and two DO levels (97.0 and 180.0% saturation). Controls were exposed to <0.01 mg/L NH3 and 97.0% DO. Trout exposed to high DO and elevated NH3 consumed more oxygen than controls during a 9 week period. Immune response and hematology were not affected by either NH3 or DO concentration. Gills from trout exposed to 0.04 and 0.06 mg/L NH3, at both DO concentrations, had an increased number of chloride cells and an increased incidence and severity of epithelial swelling and edematous tissue. Liver and kidney tissues were not affected by either NH3 or DO concentration. Trout exposed to 0.04 and 0.06 mg/L NH3 gained more weight/fish than controls during the 14 week study period. Feed conversion, condition factors and survival were not affected by either NH3 or DO concentration. Trout exposed to 0.06 mg/L NH3 had significantly less mesenteric fat than controls or fish at 0.04 mg/L NH3. Bile color of trout exposed to high DO indicated that these fish were not feeding as well as controls. Bile color of trout exposed to elevated NH3 indicated that these fish were feeding more often than controls. High NH3 or DO levels had no effect on either dorsal or caudal fin condition in rainbow trout. Rainbow trout in this study were not severely stressed by elevated NH3 concentrations. The negative effects of elevated NH3 exposure were not alleviated by increased DO. Further research on DO consumption, with respect to supersaturated DO concentrations, and immune response with respect to NH3 exposure is needed.

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A thesis submitted in partial fulfillment
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of

Master of Science

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Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 1992

APPROVAL

of a thesis submitted by

Todd David Hanna

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

22 April 1992
Date

Robert D. White
Chairperson, Graduate Committee

Approved for the Major Department

22 April 1992
Date

Robert E. Moore
Head, Major Department

Approved for the College of Graduate Studies

April 24, 1992
Date

Henry L. Parsons
Graduate Dean

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Date April 18, 1992

ACKNOWLEDGEMENTS

I would very much like to acknowledge and thank the following people for their assistance throughout this study. I sincerely thank Mr. Charlie E. Smith, Mr. William P. Dwyer, Dr. Frederick T. Barrows, Mr. Greg Kindschi, Mr. Robert Koby, Mr. Donald Edsall, Ms. Elizabeth MacConnell, Dr. David Erdahl and the rest of the staff of the U.S. Fish and Wildlife Service Fish Technology Center, Bozeman, Montana for all their help, guidance, advice and the use of their facilities and materials throughout this study. Without their help, this study would not have been possible. Dr. Robert G. White critically reviewed the manuscript and provided guidance and assistance throughout the study. Drs. Calvin Kaya and Lynn Irby and Mr. William P. Dwyer also reviewed the manuscript. I would like to thank the personnel at Zeigler Bros., Inc., Gardners, PA for funding this study.

I thank the faculty and staff of the Biology Department and the Montana Cooperative Fishery Research Unit at Montana State University for their help and assistance. I would especially like to thank my wife Deb for all her patience, understanding and support throughout this study and for all her help in preparing this manuscript.

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ABSTRACT

Oxygen supplementation has been shown to have useful applications in aquaculture. Production capacities can be increased and nitrogen gas supersaturation problems can be alleviated by using oxygen. This study was conducted to determine effects of supersaturated dissolved oxygen concentrations (DO) on un-ionized ammonia (NH_3) toxicity. Arlee rainbow trout (Oncorhynchus mykiss) were tested at three NH_3 levels (<0.01, 0.04, and 0.06 mg/L) and two DO levels (97.0 and 180.0% saturation). Controls were exposed to <0.01 mg/L NH_3 and 97.0% DO. Trout exposed to high DO and elevated NH_3 consumed more oxygen than controls during a 9 week period. Immune response and hematology were not affected by either NH_3 or DO concentration. Gills from trout exposed to 0.04 and 0.06 mg/L NH_3, at both DO concentrations, had an increased number of chloride cells and an increased incidence and severity of epithelial swelling and edematous tissue. Liver and kidney tissues were not affected by either NH_3 or DO concentration. Trout exposed to 0.04 and 0.06 mg/L NH_3 gained more weight/fish than controls during the 14 week study period. Feed conversion, condition factors and survival were not affected by either NH_3 or DO concentration. Trout exposed to 0.06 mg/L NH_3 had significantly less mesenteric fat than controls or fish at 0.04 mg/L NH_3. Bile color of trout exposed to high DO indicated that these fish were not feeding as well as controls. Bile color of trout exposed to elevated NH_3 indicated that these fish were feeding more often than controls. High NH_3 or DO levels had no effect on either dorsal or caudal fin condition in rainbow trout. Rainbow trout in this study were not severely stressed by elevated NH_3 concentrations. The negative effects of elevated NH_3 exposure were not alleviated by increased DO. Further research on DO consumption, with respect to supersaturated DO concentrations, and immune response with respect to NH_3 exposure is needed.

INTRODUCTION

Oxygen supplementation has been shown to be of significant value for fish production. It can increase production capacity and reduce nitrogen gas supersaturation, a cause of gas bubble disease (Westers et al. 1987). Oxygen supplementation may allow hatcheries to meet or exceed production goals which were previously unattainable. This will become more important as the demand for hatchery fish increases.

While some effects of oxygen supplementation have been documented (Westers et al. 1987), the effects of supersaturated dissolved oxygen concentrations (DO) on un-ionized ammonia (NH_3) toxicity have not been reported. In flowing-water systems where reaeration is possible (Soderberg et al. 1983), the accumulation of NH_3 usually limits fish production (Westers and Pratt 1977). Accumulations of NH_3 would be expected in waters with high fish loadings where supplemental oxygen is used to maintain adequate DO.

Thurston et al. (1981) showed that NH_3 toxicity to rainbow trout (Oncorhynchus mykiss) increases as DO decreases below saturation. Westers and Pratt (1977) stated

that the significance of this relationship to fish culture was unknown and that knowledge of it should be valuable in the design and operation of hatcheries. It is not known however, if DO above saturation will reduce the effects of harmful levels of NH_3. More recently, Colt et al. (1988) again pointed out the need for this type of information along with the "reassessment of un-ionized ammonia criteria for DO's in the range of 10-30 mg/L."

According to Meade (1985), a clearly safe, maximum allowable concentration of un-ionized, or total ammonia (NH_4^+-NH_3) for fish culture is not known. Many parameters affect NH_3 toxicity in fish. It is extremely variable and depends on factors other than the mean or maximum concentration of NH_3. Dissolved oxygen concentration, pH, water temperature and free carbon dioxide affect NH_3 toxicity. Many studies have shown that exposure to elevated NH_3 levels can cause problems such as: reduced growth rates (Morrison and Piper 1988), poor feed conversions (Smith and Piper 1975), increased incidence of gill histopathology (Larmoyeux and Piper 1973), increased ventilation frequencies (Lang et al. 1987), increased susceptibility to pathogens (Burrows 1964), and death in high concentrations (Thurston et al. 1978). These effects have been shown for many salmonid species including rainbow trout. Additionally, weakened immune responses have been reported for lake trout (Salvelinus namaycush) exposed to high NH_3

concentrations for 85 days (Meade 1986). Rearing fish under these conditions may lead to reduced survival after stocking.

This study was designed to determine if rainbow trout reared under hyperoxic conditions are better able to withstand the toxic effects of elevated concentrations of NH_3. Specific objectives were to:

1. Determine if supersaturated DO concentrations will reduce the effects of elevated (sublethal) concentrations of NH_3 on rainbow trout.
2. Determine if the immune response in rainbow trout is adversely affected by elevated (sublethal) concentrations of NH_3 and if supersaturated DO will help reduce these effects.

METHODS

This study was conducted at the U.S. Fish and Wildlife Service Fish Technology Center, Bozeman, Montana from August 28, 1989 to January 5, 1990. The study was designed as a 2 x 3 factorial, testing three levels of NH_3 (<0.01, 0.04 and 0.06 mg/L) and two levels of DO saturation (97.0% and 180.0%). Control fish were exposed to <0.01 mg/L NH_3 and approximately 97.0% DO saturation. Test NH_3 levels were greater than the recommended upper limit of 0.0125 mg/L (Piper et al. 1986) but less than lethal concentrations (0.3 - 0.8 mg/L; Thurston et al. 1978). Each set of test conditions was tested in triplicate. Eighteen experimental tanks were used, each having a volume of 83 L, a flow rate of 3.8 L/min and a water exchange rate of 2.7 times per hour. Water from a cold spring (mean water temperature of 8.4°C and pH of 7.6) was introduced to the system through a centrifugal pump. Chemical characteristics of the water supply were described by Thurston et al. (1978 and 1986).

Water was pumped to each of six headboxes in which a constant hydraulic head was maintained (Figure 1). Before entering the headboxes, water passed through sealed, packed columns similar to those described by Boerson (1985) and Dwyer et al. (in press). Columns were made of 10.2 cm