THE EFFECT OF OXYGEN SUPPLEMENTATION ON THE TOXICITY OF AMMONIA (NH_3) IN RAINBOW TROUT *Oncorhynchus mykiss* (Richardson)

by

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VITA

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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

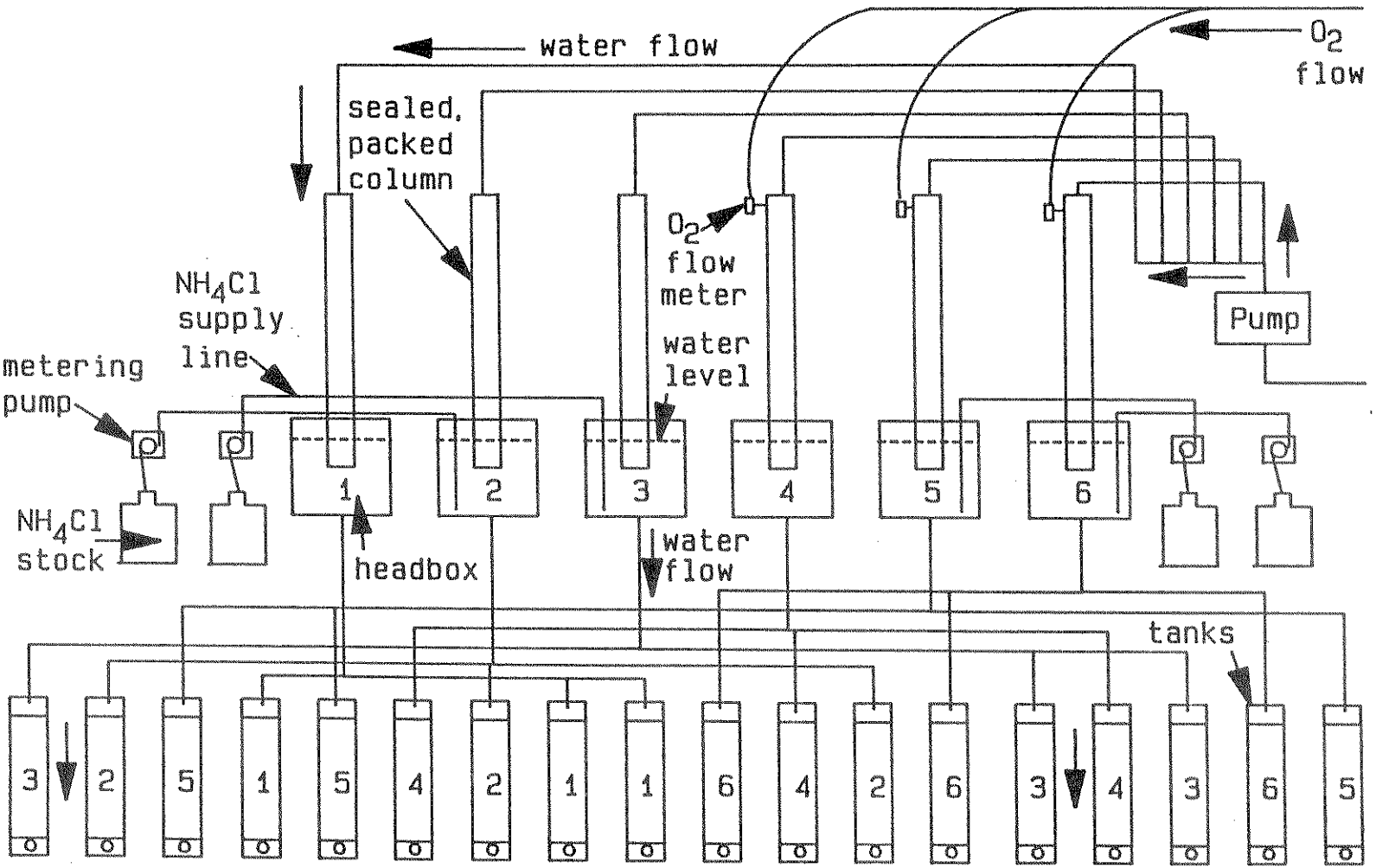

Figure 1. Schematic of experimental apparatus testing the effects of oxygen supplementation on the toxicity of un-ionized ammonia in rainbow trout. Treatment numbers are shown. Refer to Table 1 for treatment descriptions.

polyvinyl chloride (PVC) pipe and packed with 37 x 37 mm Koch rings. The columns were each 1.14 m in length. The bottom of each column was kept approximately 12.7 cm below the water surface in each headbox. Oxygen was introduced into three of the packed columns through Puritan (Model C) pressure compensated flow meters regulated to produce DO saturations of approximately 180%. Oxygen was produced by a Nitrox T/2 oxygen generator (On-Site Gas Systems, Inc., 159 John Downey Drive, New Britain, CT 06051) and was delivered through a surge tank to the flow meters at approximately 4.4 kiloPascals. To achieve 180% saturation, approximately 0.5 L/min oxygen was required. Oxygen purity was approximately 90% and was tested monthly with a Miniox I oxygen analyzer (model OXI). The remaining three packed columns received no oxygen supplementation (Figure 1).

Un-ionized ammonia concentrations (NH_3) were derived from stock ammonium chloride (NH_4Cl) solutions and metered into the appropriate headboxes using Masterflex Pump Drive metering pumps (model # 7015, Cole-Parmer Inst. Co., Chicago, IL 60648) (Figure 1). Stock NH_4Cl solutions were replenished every 3 to 4 d as needed.

A random group of three experimental tanks received water from each headbox (Figure 1). Table 1 describes the six treatments as they were tested.

Experimental fish were Arlee rainbow trout selected randomly from a production lot of fish at the Fish

Table 1. Mean (SD) water quality characteristics of each treatment during the 130 d study period. All measurements were taken at the influent of the study tanks and are weekly averages. N=19.

Treatment	NH_4^+-NH_3 (mg/L)	NH_3 (mg/L)	NH_4^+-NH_3 (mg/L)*	NH_3 (mg/L)*	pH	DO** sat. %	DO (mg/L)	N_2 sat. %	TDG*** sat. %	Water temp. °C
1	<0.01 (0.0)	<0.01 (0.0)	0.10 (0.05)	<0.01 (0.0)	7.6 (0.09)	98.10 (1.11)	9.59 (0.17)	101.90 (0.85)	100.95 (0.49)	8.4 (0.2)
2	4.50 (0.48)	0.04 (0.01)			7.6 (0.07)	96.90 (1.23)	9.48 (0.15)	101.90 (0.49)	100.75 (0.21)	8.4 (0.2)
3	6.32 (0.45)	0.06 (0.01)			7.6 (0.05)	96.90 (1.19)	9.49 (0.13)	100.90 (1.56)	100.20 (0.99)	8.4 (0.2)
4	<0.01 (0.0)	<0.01 (0.0)			7.6 (0.10)	179.10 (2.10)	17.52 (0.26)	82.35 (1.48)	102.05 (0.21)	8.4 (0.2)
5	4.05 (0.32)	0.04 (0.01)			7.6 (0.05)	185.30 (13.22)	18.15 (1.35)	81.45 (1.20)	102.30 (0.99)	8.4 (0.2)
6	6.22 (0.46)	0.06 (0.01)			7.6 (0.06)	183.80 (7.80)	18.00 (0.87)	79.95 (3.75)	101.90 (0.0)	8.4 (0.2)

* NH_4^+-NH_3 and NH_3 contributions of the control fish to the system. All measurements were taken at the effluent of the control tanks.

** DO = dissolved oxygen

*** TDG = total dissolved gases

Technology Center. Thirty fish, mean total length of 154.2 (+/- 4.2) mm and mean weight of 55.4 (+/- 4.0) g, were weighed, measured and placed into each of the 18 tanks. Following a 14 d acclimation period at control DO and NH_3 concentrations (approximately 97% DO and <0.01 mg/L NH_3), treatment DO and NH_3 concentrations were supplied to the appropriate tanks (Table 1). The fish were fed Zeigler Brand #38-480 Trout Grower (Zeigler Bros. Inc., P.O. Box 95, Gardners, PA. 17234-0095) in the 5/32 inch (4.0 mm) size. Feed pellet size was not changed during the study. All tanks were cleaned daily.

Water Quality

Total ammonia concentrations (NH_4^+-NH_3) and pH were monitored two to three times per week at the influent of each tank, using a Corning Model 255 ion analyzer (Corning Glass Works, Corning, New York 14831) with ammonia and pH probes. Concentrations within the calibration range were recorded. Calibration points ranged from 0.01 - 0.1 - 1.0 - 10.0 mg/L as NH_3. Samples were also collected at the effluent ends of the control tanks (treatment 1) for determination of the NH_4^+-NH_3 contribution of the fish. Total ammonia concentrations were converted to NH_3 concentrations using the tables of Thurston et al. (1977). Mean NH_4^+-NH_3 and NH_3 concentrations, pH and other water quality characteristics were calculated (Table 1).

Dissolved oxygen concentrations and water temperatures were also monitored two to three times per week using a YSI Model 50 dissolved oxygen meter and probe (YSI Co. Inc., Yellow Springs, Ohio). Dissolved oxygen concentrations were monitored at the influent and effluent of each tank to determine the DO consumed by the fish. Dissolved oxygen consumption was monitored from week 4 through week 12 of the study and was calculated using weekly tank means as follows:

$$\frac{[DO(in) - DO(out)(mg/L)] \times \text{water flow (L/min)} \times 60 \text{ min/h}}{\text{fish weight (kg)}}$$

The results were expressed as milligrams of oxygen consumed per kilogram of fish per hour (Westers 1981). Overall treatment DO consumption means were then compared for this 9 week period. The dissolved oxygen meter was calibrated periodically using the Winkler method (azide modification) for dissolved oxygen determination (APHA, 1985). Nitrogen and total dissolved gas concentrations were monitored periodically using a Weiss saturometer (Eco Enterprises, Seattle, Washington). Barometric pressure was read from a fortin type mercurial barometer. Nitrogen and total dissolved gas saturations were calculated using the formulas of Colt (1984). Water temperature was constant for each treatment throughout the study but nitrogen and total dissolved gases (TDG) varied depending upon the amount of oxygen introduced (Table 1).

Immune Response

Immune responses were determined using a microtiter agglutination technique (Anderson and Dixon, 1988). Injections and sampling techniques were similar to those used by Meade (1986). On day 38 of the study, 10 fish from each tank (30/treatment) were injected intramuscularly (IM) anterior to the dorsal fin with 0.1 mL of sterile 0.85% saline solution and served as negative controls. Another 10 fish from each tank were injected IM with 0.1 mL of a killed Aeromonas salmonicida antigen (test fish) and 10 fish from each tank received no injections. Negative controls were used to determine any natural response to the Aeromonas salmonicida antigen by the fish. All injections were made with a 1 cc tuberculin syringe and a 0.95 cm, 26 gauge needle. Fish receiving the saline injections were identified by an anal fin clip, while antigen injected fish received an adipose fin clip. Noninjected fish received no fin clip. The formalin killed Aeromonas salmonicida antigen suspension was prepared using methods described in Anderson and Dixon (1988). The injected suspension had a 26.1% transmittance as determined using a Bausch and Lomb 501 spectrophotometer.

On day 113 of the study, all antigen injected fish were sampled for immune response. The fish were carefully netted and anesthetized with Finquel brand (Argent Chemical Labs,

Inc., Redmond, Washington 98052) MS-222 (tricain methane sulfonate). Blood was collected from the caudal vein using a 3 cc tuberculin syringe and a 2.54 cm, 22 gauge needle. Blood samples were placed into individual plastic vials and refrigerated overnight to allow clotting and serum separation. On day 114, microtiter agglutinations were performed on five randomly selected serum samples from each tank. The prepared antigen used in the agglutinations had a 55% transmittance. Positive controls were performed using a lyophilized rabbit antiserum to Aeromonas salmonicida. Dilutions of 1:512 were performed on each serum sample. The plates were checked at 1, 6 and 24 h for agglutination. The results were assigned a numerical value of 1 through 8 corresponding to the highest dilution in which agglutination occurred. A numerical result of 1 would indicate agglutination occurred through a dilution of 1:4 while a result of 8 would indicate agglutination occurred through a dilution of 1:512, etc. On day 122, the saline injected fish were sampled and on day 123, agglutinations were performed in the same manner. All fish sampled for immune response (20/tank, 60/treatment) were removed from the study after sampling. Noninjected fish (10/tank, 30/treatment) were not sampled for immune response and were not removed from the study following immune response sampling.

Hematology

Hematocrit (%) and hemoglobin concentrations (g/100 cm^3) were determined for fish from each treatment on days 102 and 130 (termination) of the study. Microhematocrit (packed cell volume) levels were determined according to the methods described by Blaxhall and Daisley (1973). Hemoglobin was measured using the cyanmethemoglobin method (Blaxhall and Daisley, 1973). On day 102 of the study, five of the uninjected control fish from each tank were sampled for hematology. Fish were carefully netted and anesthetized with MS-222. Blood was collected from each fish by severing the caudal fin from the body and collecting the blood from the caudal vein. This same sampling procedure was used on day 130 of the study on the remaining five fish in each tank. Also on day 130, leucocrit (%) and plasma protein (grams/deciliter) levels were determined for each fish as part of Goede's (1989) fish health assessment.

Histology

Histopathological tissue examinations were done according to the methods described by Thurston et al. (1984). On day 130 of the study, gill, liver and kidney samples were collected from the remaining control fish in each tank (same fish sampled for hematology). Tissue samples were preserved in Bouin's solution for 24 h, then

transferred to 65% ethanol. The tissue samples were embedded in paraffin, cut to 5 micron thickness and stained with hematoxylin and eosin. These stained sections were examined microscopically. A ranking system of 0 through 4 was established to quantify pathological changes in the tissues. The ranking of pathological changes in gill tissue included the presence of increased numbers of chloride cells, as well as increased levels of edematous tissue and epithelial swelling (hypertrophy) when compared to controls. Increased presence of these parameters received a higher rank. Other changes such as aneurysms, clubbing of gill lamellae and scattered fusion of gill filaments were noted but not ranked. Liver tissue was examined for glycogen vacuolation which was ranked on the same 0 through 4 scale with increased vacuolation receiving a higher rank. Kidney tissue was examined and any pathological changes were noted but not ranked.

Growth, Feed Conversion, Condition Factors and Survival

Beginning with day 1 of the study, rainbow trout from each tank were weighed as a group every 14 d to determine growth (g/fish) and feed conversion. Feed conversion was calculated per weigh period for each tank as follows: amount of food fed (g) / change in biomass (g). These parameters were calculated using a computer program developed at the Fish Technology Center. Feeding rates for

each tank were calculated using a hatchery constant of 8.5 (Buterbaugh and Willoughby, 1967) and were adjusted every 7 d. Growth and feed conversion data were collected for 14 weeks of the study. Condition factor data were collected on day 130 as part of the fish health assessment (Goede 1989). Condition factors (K) were calculated as: weight(g) / (length (mm))^3 x 10^5 (Piper et al. 1986). Mortalities were recorded for each tank as they occurred throughout the study.

Fish Health Assessment

On day 130 (termination) of the study, Goede's (1989) fish health assessment (Table 2) was performed on the remaining five fish in each tank (15/treatment) prior to sampling for hematology and histopathology. As the fish health assessment was being performed, data were also collected for quantifying the degree of fin erosion present in fish from each treatment. Caudal and dorsal fin erosion were quantified using a method described by Kindschi (1987). Caudal fin length (mm) was determined by subtracting the standard length of the fish (mm) from the total length (mm). The results of the fish health assessment were analyzed using the accompanying Lotus 123 spreadsheet template (Goede 1989).

Table 2. Description of fish health assessment performed on fish from each treatment on day 130 of the study. Taken from Goede (1989).

AUTOPSY CLASSIFICATION	
Length:	Total length in millimeters
Weight:	Weight in grams
Ktl:	$= W \times 10^5 / L^3$
Eyes:	Normal (N); Exopthalmia (E1, E2); Hemorrhagic (H1, H2); Blind (B1, B2); Missing (M1, M2); Other (OT)
Gills:	Normal (N); Frayed (F); Clubbed (C); Marginate (M); Pale (P); Other (OT)
Pseudobranchs:	Normal (N); Swollen (S); Lithic (L); Swollen and Lithic (S&L); Inflamed (I); Other (OT)
Thymus:	No Hemorrhage (0); Mild Hemorrhage (1); Severe Hemorrhage (2)
Mesentery Fat:	0 - None 1 - Little, where less than 50% of each cecum us covered 2 - 50% of each cecum is covered 3 - More than 50% of each cecum is covered 4 - Ceca are completely covered by fat
Spleen:	Black (B); Red (R); Granular (G); Nodular (NO); Enlarged (E); Other (OT)
Hind Gut:	No inflammation (0); Mild inflammation (1); Severe inflammation (2)
Kidney:	Normal (N); Swollen (S); Mottled (M); Granular (G); Urolithiasis (U); Other (OT)
Liver:	A - Red B - Light red C - "Fatty" Liver; "coffee with cream" color; "greasy" to feel D - Nodules in liver E - Focal discoloration F - General discoloration OT - Other

Table 2. Continued.

Bile:	0 - Yellow or straw color; bladder empty or partially full 1 - Yellow or straw color; bladder full, distended 2 - Light green to "grass" green 3 - Dark green to dark blue-green
Blood:	Hematocrit - Volume of red blood cells (erythrocytes) expressed as percent of total blood volume. Centrifuged 5 min.
	Leucocrit - Volume of white blood cells (leucocytes) expressed as percent of total blood volume.
	Plasma Protein - Amount of protein in plasma, expressed as gram percent (grams per 100 mL). Determined with hand-held protometer.

Statistical Analysis

Data collected were analyzed as a 2 x 3 factorial treatment design using the General Linear Models Procedure of the Statistical Analysis System (SAS, 1982). The effect of DO (% saturation), NH_3 concentration and the interaction of the two effects was determined. Differences were considered significant when $P<0.05$. Orthogonal comparisons were used to detect a difference between treatment means.

RESULTS

Water Quality

Weekly mean NH_4^+-NH_3 contributions from the control fish (treatment 1) ranged from 0.04 mg/L to 0.19 mg/L (mean = 0.10 mg/L). When these values were converted to NH_3, the NH_3 contributions by rainbow trout to the system in the control tanks were found to be <0.01 mg/L (Table 1).

Dissolved oxygen concentrations and NH_3 concentrations affected overall DO consumption by rainbow trout over the 9 week study period. Fish exposed to high DO (treatments 4, 179.2%; 5, 185.3%; and 6, 183.8%) consumed significantly more (about 15%) oxygen than those exposed to control levels (treatments 1, 98.1%; 2, 96.9%; and 3, 96.9%; Table 3).

Dissolved oxygen consumption of rainbow trout also increased at higher NH_3 concentrations (Table 3). Fish exposed to 0.04 mg/L and 0.06 mg/L consumed significantly more DO than controls (<0.01 mg/L NH_3). However, DO consumption did not increase progressively with NH_3 concentration. Orthogonal comparisons showed that rainbow trout exposed to 0.04 mg/L NH_3 (treatments 2 and 5) consumed more DO than rainbow trout exposed to less NH_3 (<0.01 mg/L; treatments 1 and 4), and to more NH_3 (0.06 mg/L; treatments

Table 3. Mean (SD) DO consumption (mg O_2/kg fish/h) of rainbow trout treatments exposed to control and high DO levels, and <0.01, 0.04 and 0.06 mg/L NH_3, for weeks 4 through 12 of the study. Values without a superscript in common are significantly different ($P<0.05$). Refer to Table 1 for treatment descriptions.

Treatment	DO Consumption
1, 2 and 3 (97.3% Sat.)	146.0[a] (24.2)
4, 5 and 6 (182.7% Sat.)	168.1[b] (16.7)
Treatment	**DO Consumption**
1 and 4 (<0.01 mg/L NH_3)	140.3[1] (20.2)
2 and 5 (0.04 mg/L NH_3)	175.6[2] (14.9)
3 and 6 (0.06 mg/L NH_3)	155.2[3] (20.8)

3 and 6) over the 9 week period (Table 3). No effect of the interaction between DO and NH_3 concentration on DO consumption was found.

Immune Response

High NH_3 levels or DO concentrations did not influence the immune response of rainbow trout to the Aeromonas salmonicida antigen and no significant differences in immune response were found between treatments for either the antigen-injected fish or saline-injected fish (negative

controls). A strong agglutination response (Figure 2) was found in all antigen-injected fish sampled from each treatment (Table 4). Also, a strong agglutination response (through dilutions of 1:64) to the Aeromonas salmonicida antigen was found in all saline injected fish (negative controls) sampled from each treatment. This response was not affected by exposure to high NH_3 or DO levels.

Figure 2. Positive response to Aeromonas salmonicida antigen (agglutination) found in antigen and saline-injected fish from each treatment. In a negative response none of the dark, clumped areas would be evident.

Table 4. Comparison of mean (SD) microtiter agglutination values from antigen and saline injected rainbow trout. Refer to Table 1 for treatment descriptions.

Treatment	Antigen Injected	Saline Injected
1	7.7 (0.2)	4.5 (0.5)
2	7.5 (0.4)	4.5 (0.8)
3	7.3 (0.6)	5.1 (0.5)
4	7.8 (0.2)	4.8 (0.6)
5	7.6 (0.4)	4.6 (0.7)
6	6.9 (0.5)	5.7 (1.0)

Hematology

Elevated NH_3 levels and DO concentrations had no effect on hematocrit and leucocrit percent or hemoglobin and plasma protein concentrations in rainbow trout tested. No significant differences were found between treatments for any of these parameters on either days 102 or 130 (Table 5). Blood chemistry characteristics of fish from all treatments were within the normal ranges reported for rainbow trout (McCarthy et al. 1973).

Table 5. Mean (SD) blood chemistry characteristics of rainbow trout sampled from the six treatments on days 102 and 130 of the study. Refer to Table 1 for treatment descriptions.

Treatment	Day 102 Hematocrit %	Day 102 Hemoglobin (g/100cm^3)	Day 130 Hematocrit %	Day 130 Hemoglobin (g/100cm^3)	Day 130 Leucocrit %	Day 130 Plasma protein (g/dL)
1	42.00 (4.90)	10.43 (0.97)	43.87 (2.77)	10.63 (1.10)	1.53 (0.46)	6.73 (0.15)
2	41.73 (3.27)	9.33 (0.55)	40.77 (1.75)	9.03 (0.67)	1.80 (0.46)	6.57 (0.57)
3	40.47 (2.00)	10.10 (0.53)	39.00 (2.61)	9.23 (0.55)	1.70 (0.50)	6.37 (0.60)
4	36.20 (3.63)	9.40 (0.26)	40.60 (1.18)	9.63 (0.51)	1.77 (0.40)	6.30 (0.44)
5	39.10 (0.78)	9.40 (0.70)	40.50 (2.10)	9.50 (0.10)	1.80 (0.20)	6.67 (0.40)
6	36.53 (1.38)	9.33 (0.45)	30.00 (1.49)	9.00 (0.52)	1.33 (0.12)	6.27 (0.35)

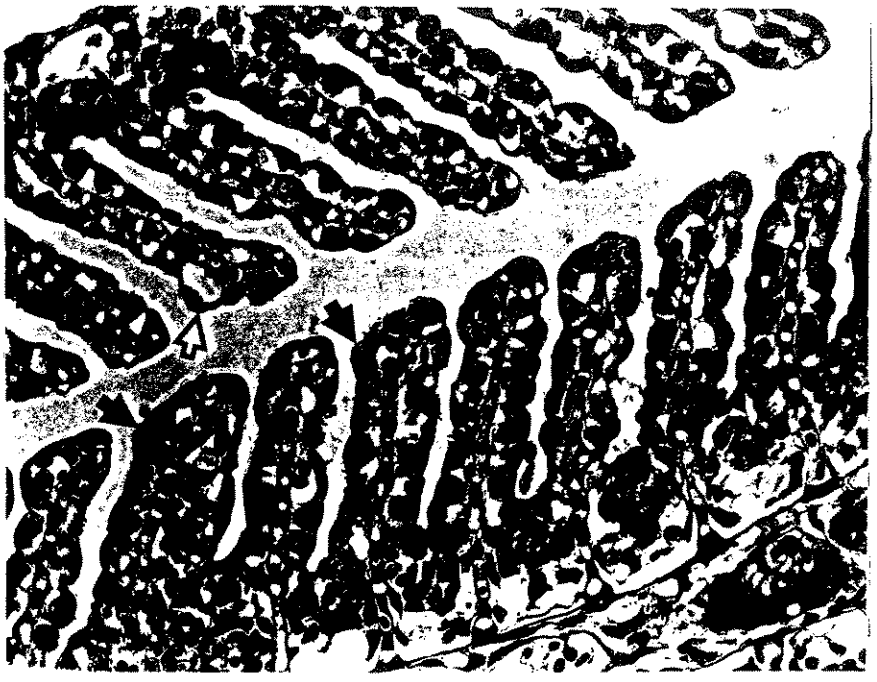

Figure 3. Histological section of gill tissue showing increased numbers of chloride cells (arrows) and epithelial swelling (open arrow) representative of fish from treatments 2, 3, 5 and 6 (x400).

Histology

Rainbow trout gill tissues from fish exposed to elevated NH_3 concentrations (0.04 - 0.06 mg/L) had increased numbers of chloride cells (Figure 3), as well as increased incidence and severity of epithelial swelling (Figure 3) and edematous tissue (Figure 4) compared to controls (<0.01 mg/L NH_3; Figure 5). Significant differences due to NH_3 concentration were found in the rankings of number of chloride cells and in the degree and severity of edematous tissue and epithelial swelling

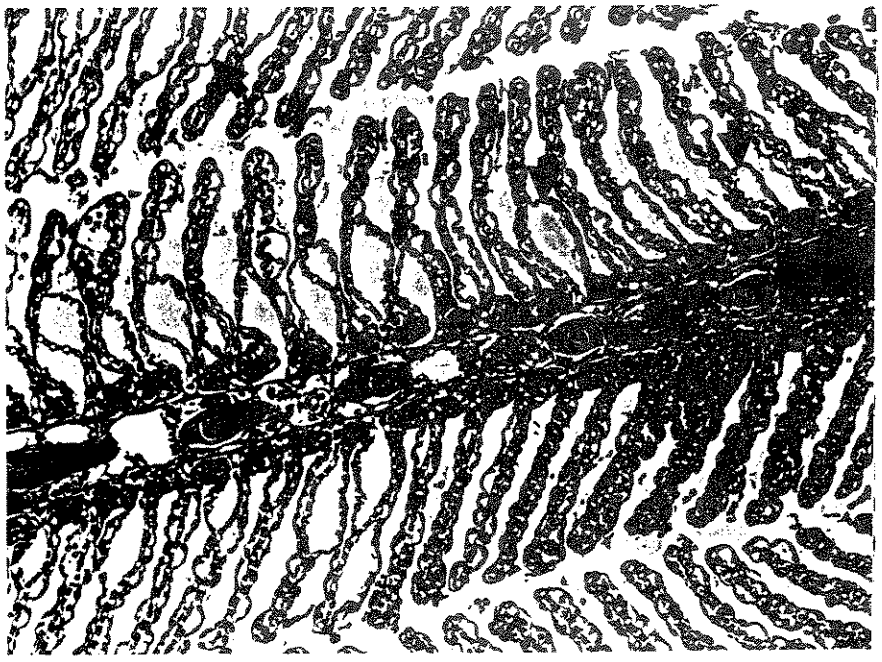

Figure 4. Histological section of gill tissue showing edematous tissue (arrows). This was found in all fish from treatments 2, 3, 5 and 6 (x160).

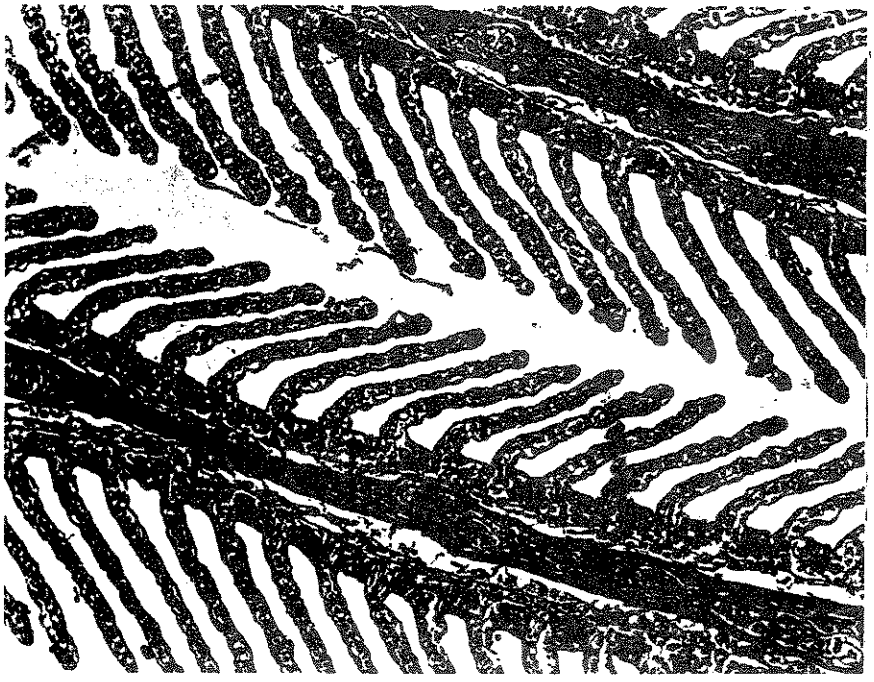

Figure 5. Histological section of normal gill tissue taken from treatment 1 (control) fish (x160).

Table 6. Comparison of mean (SD) differences in assigned rankings of gill histological parameters and glycogen vacuolation between control (treatments 1 and 4) and test (treatments 2, 3, 5, and 6) rainbow trout sampled on day 130 of the study. Values without a letter in common are significantly different ($P<0.05$). Refer to Table 1 for treatment descriptions.

Treatment	Chloride cells	Edematous tissue	Epithelial swelling	Glycogen vacuolation
1	0.8^a (0.5)	0.8^a (0.6)	0.7^a (0.6)	2.4^a (0.6)
2	2.3^b (0.4)	2.2^b (0.2)	2.5^b (0.2)	2.0^a (0.0)
3	3.1^b (0.6)	2.9^b (0.8)	2.8^b (0.7)	2.2^a (0.5)
4	0.4^a (0.2)	0.6^a (0.4)	0.8^a (0.4)	2.4^a (0.6)
5	3.0^b (0.9)	2.9^b (0.2)	2.7^b (0.1)	2.4^a (0.6)
6	2.5^b (0.5)	2.5^b (0.5)	2.5^b (0.5)	2.4^a (0.6)

present in fish between treatments (Table 6). Orthogonal comparisons showed a significant difference between control treatments 1 and 4 (<0.01 mg/L NH_3) and treatments 2 and 5 (0.04 mg/L NH_3) for the number of chloride cells present. No differences were found between treatments 2 and 5 and treatments 3 and 6 (0.06 mg/L NH_3). Similar significant differences were found in degree and severity of edematous

tissue and epithelial swelling (Table 6). Other abnormalities noted but not ranked included aneurysms, clubbing, and scattered fusion of lamellae. These were found in gill tissue from fish in all treatments but no consistent differences were found between control and test fish.

High NH_3 levels and DO concentrations had no effect on glycogen vacuolation in livers of rainbow trout tested and no differences were found in rankings between treatments (Table 6). Similar degrees of moderate glycogen vacuolation were found in livers from all fish sampled (Figure 6).

High NH_3 levels and DO concentrations did not affect kidney tissues. Some hyaline droplet degeneration and swollen kidney tubules were found in fish from each treatment. However, all kidney tissues were considered normal (Figure 7).

Growth, Feed Conversion, Condition Factors and Survival

Rainbow trout reared in elevated NH_3 levels gained significantly more weight than the control fish over the 14 week period (Table 7). No significant difference was found in weight gain between fish in treatments 2 and 5 (0.04 mg/L NH_3) and fish in treatments 3 and 6 (0.06 mg/L NH_3). Based on covariate analysis, initial weight of the fish in each treatment did not influence results.

Figure 6. Histological section of liver tissue representative of fish from all treatments showing moderate glycogen vacuolation (arrow) (x400).

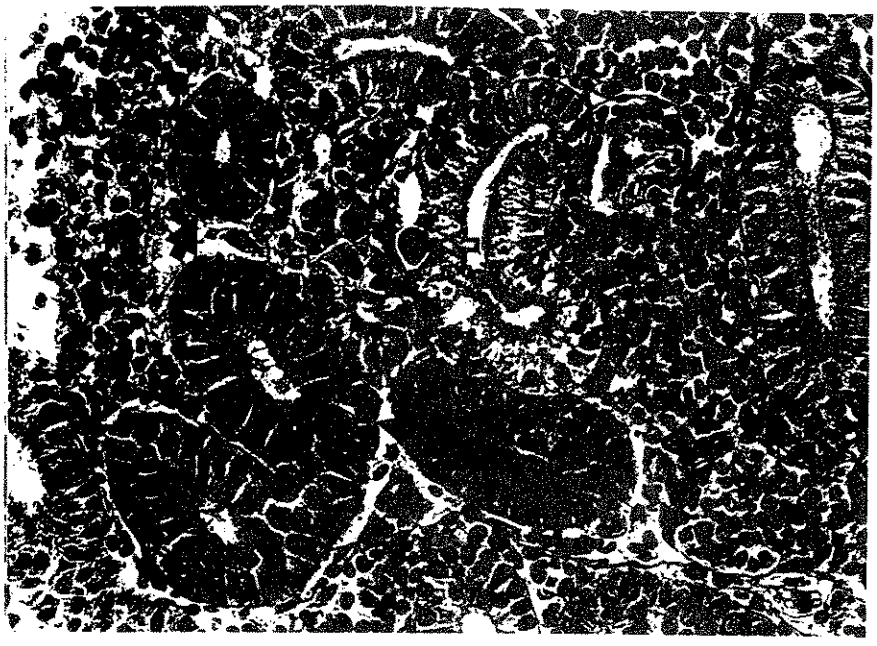

Figure 7. Histological section of kidney tissue from one fish in treatment 3 showing hyaline droplet degeneration (open arrow). The closed arrows show normal kidney tubules found in fish from all treatments (x400).

Table 7. Comparison of mean (SD) rainbow trout weight gain, feed conversion and condition factors (K) between treatments at the end of 14 weeks (day 98). Weight gain, feed conversions and percent survival are for the 14 week period. Condition factors are from fish sampled on day 130. Values without a letter in common are significantly different ($P<0.05$). Refer to Table 1 for treatment descriptions.

Treatment	Weight gain (g/fish)	Feed conversion	Condition factor ($K \times 10^5$)*	Survival (%)
1	41.34[a] (2.74)	2.03 (0.14)	1.12 (0.07)	98.90 (0.00)
2	51.96[b] (4.67)	1.86 (0.34)	1.13 (0.02)	96.70 (0.00)
3	53.56[b] (5.38)	1.70 (0.14)	1.17 (0.09)	100.00 (0.00)
4	43.37[a] (5.52)	1.99 (0.13)	1.03 (0.02)	100.00 (0.00)
5	54.14[b] (2.39)	1.66 (0.02)	1.08 (0.08)	100.00 (0.00)
6	48.18[b] (6.17)	1.80 (0.05)	1.13 (0.06)	100.00 (0.00)

*Condition Factor = weight(g) / (length(mm))^3 x 10^5

High NH_3 levels and DO concentrations had no significant effect on total feed conversions in rainbow trout tested and no differences were found in total feed conversions between treatments for the overall 14 week period (Table 7). Feed conversions were very high during the first 2 weeks, for control treatments 1 and 4. However,

there were no significant differences in feed conversions between treatments for the remaining six 2-week periods (Figure 8).

Condition factors (K) of rainbow trout were not affected by exposure to high NH_3 levels or high DO concentrations (Table 7). Rainbow trout from all six treatments maintained good condition throughout the study and no significant differences were found in condition factors between treatments on day 130.

Only four fish died during the study. One of these was from treatment 1 (control) and three were from treatment 2 (0.04 mg/L NH_3). These mortalities were believed to be unrelated to the test conditions. There were no significant differences in percent survival between treatments (Table 7).

Fish Health Assessment

High NH_3 levels and DO concentrations had no significant effect on thymus index, fin condition, opercle condition, or hind gut inflammation in rainbow trout tested (Table 8). However, fish in the high NH_3 treatment (0.06 mg/L) had a significantly lower mesenteric fat index (a measure of the percentage of the cecum covered with fat). Orthogonal comparisons showed no difference in mesenteric fat index in fish from control treatments 1 and 4 (<0.01 mg/L NH_3) and fish from treatments 2 and 5 (0.04 mg/L NH_3).

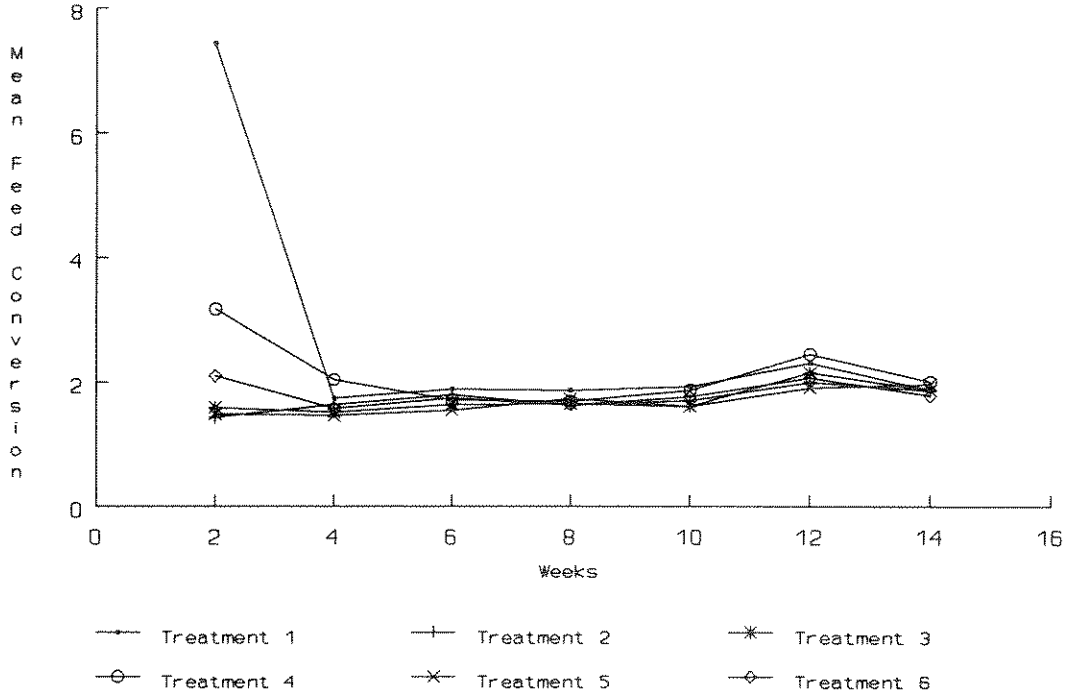

Figure 8. Mean rainbow trout feed conversions by 2 week periods for each of the six treatments. Refer to Table 1 for treatment descriptions.

There were however differences between fish from treatments 2 and 5 and fish from treatments 3 and 6 (0.06 mg/L NH_3).

Both NH_3 level and DO concentration had an effect on the bile index (bile color) in rainbow trout (Figure 9). Rainbow trout in treatments 4 (179.1% O_2), 5 (185.3% O_2) and 6 (183.8% O_2) had higher bile index values (more green in color) than the fish in treatments 1 (98.1% O_2), 2 (96.9% O_2) and 3 (96.9% O_2) (Table 9). Fish in treatments 2 and 5 (0.04 mg/L NH_3) and 3 and 6 (0.06 mg/L NH_3) had lower bile index values than the fish in treatments 1 and 4 (<0.01 mg/L NH_3) indicating the bile color was more normal (Table 9).

Table 8. Comparison of mean (SD) rainbow trout thymus index, fin condition, opercle condition, mesenteric fat index and hind gut inflammation between treatments for fish sampled on day 130 of the study as part of the fish health assessment. Values without a letter in common are significantly different ($P<0.05$). Refer to Table 1 for treatment descriptions.

Treatment	Thymus index	Fin condition	Opercle condition	Mesenteric fat index	Hind gut inflammation
1	0.00 (0.00)	0.63 (0.06)	0.00 (0.00)	3.23[a] (0.06)	0.00 (0.00)
2	0.00 (0.00)	0.87 (0.23)	0.00 (0.00)	2.83[a] (0.47)	0.00 (0.00)
3	0.13 (0.23)	0.57 (0.40)	0.00 (0.00)	2.43[b] (0.32)	0.00 (0.00)
4	0.33 (0.23)	0.87 (0.23)	0.13 (0.23)	3.13[a] (0.23)	0.00 (0.00)
5	0.33 (0.42)	0.53 (0.12)	0.00 (0.00)	3.07[a] (0.12)	0.00 (0.00)
6	0.27 (0.31)	1.00 (0.00)	0.00 (0.00)	2.60[b] (0.20)	0.00 (0.00)

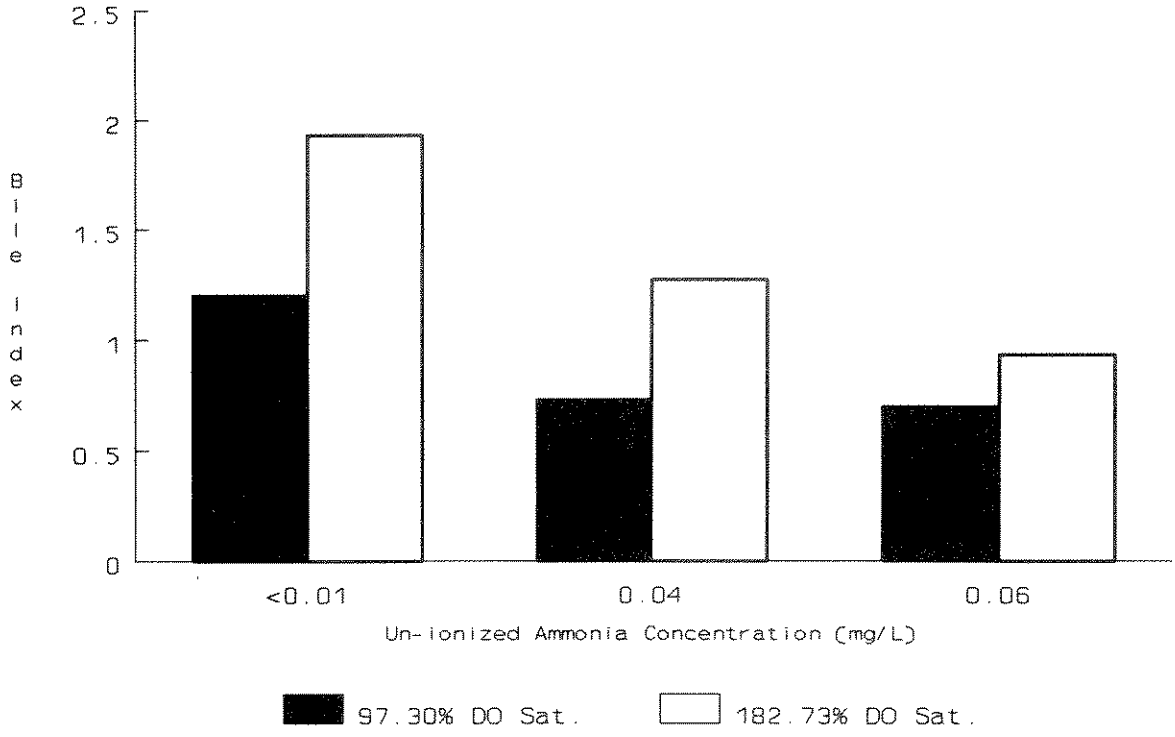

Figure 9. Mean bile index values of test rainbow trout at the three NH_3 and two DO levels tested.

No differences, however, were found due to the interaction between DO concentration and NH_3 concentration. Orthogonal comparisons for the differences due to NH_3 concentration showed a significant difference in bile index in fish from treatments 1 and 4 (<0.01 mg/L NH_3) and fish from treatments 2 and 5 (0.04 mg/L NH_3). No differences were found in the bile index of fish from treatments 2 and 5 and treatments 3 and 6 (0.06 mg/L NH_3). High NH_3 and DO concentrations had no effect on the "% normal" parameters and no differences were found in rainbow trout between treatments (Table 10). Treatments also had no effect on either the caudal or dorsal fin factor (Table 11). The caudal and dorsal fins of

rainbow trout from all treatments were in good condition and no severe erosion was noted.

Table 9. Mean (SD) bile index values of rainbow trout sampled on day 130 of the study as part of the fish health assessment. Values without a superscript in common are significantly different ($P<0.05$). Refer to Table 1 for treatment descriptions.

Treatment	Bile Index
1, 2 and 3 (97.3% Sat.)	0.9^{a} (0.4)
4, 5 and 6 (182.7% Sat.)	1.4^{b} (0.5)

Treatment	Bile Index
1 and 4 (<0.01 mg/L NH_3)	1.6^{1} (0.6)
2 and 5 (0.04 mg/L NH_3)	1.0^{2} (0.4)
3 and 6 (0.06 mg/L NH_3)	0.8^{2} (0.2)

Table 10. Comparison of mean (SD) rainbow trout percent normal eyes, gills, pseudobranchs, thymus, spleen, hind gut, kidney and liver in fish sampled on day 130 of the study as determined from the fish health assessment. Refer to Table 1 for treatment descriptions.

Treatment	Percent normal eyes	Percent normal gills	Percent normal pseudobranchs	Percent normal thymus	Percent normal spleen	Percent normal hindgut	Percent normal kidney	Percent normal liver
1	93.0 (12.0)	100.0 (0.0)	80.0 (20.0)	100.0 (0.0)	87.0 (23.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
2	100.0 (0.0)	100.0 (0.0)	64.0 (34.0)	100.0 (0.0)	87.0 (23.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
3	92.0 (14.0)	86.0 (23.0)	72.0 (30.0)	87.0 (23.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
4	100.0 (0.0)	93.0 (12.0)	67.0 (12.0)	67.0 (23.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
5	87.0 (12.0)	100.0 (0.0)	93.0 (12.0)	67.0 (42.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)
6	100.0 (0.0)	100.0 (0.0)	60.0 (20.0)	80.0 (20.0)	93.0 (12.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)

Table 11. Comparison of mean (SD) rainbow trout dorsal and caudal fin factors (%) between the six treatments on day 130 of the study. Refer to Table 1 for treatment descriptions.

Treatment	Caudal fin factor (%)*	Dorsal fin factor (%)**
1	11.57 (0.21)	9.80 (0.52)
2	12.00 (1.21)	9.90 (0.26)
3	11.50 (2.04)	10.50 (0.20)
4	12.50 (1.30)	9.83 (0.21)
5	11.17 (1.88)	10.13 (0.45)
6	12.17 (0.74)	10.00 (0.61)

*Caudal fin factor = (caudal fin length (mm) x 100)/ total length (mm)

**Dorsal fin factor = (dorsal fin length (mm) x 100)/ total length (mm)

DISCUSSION

Water Quality

The observed increase in oxygen consumption by rainbow trout exposed to DO above saturation has not previously been reported. Dissolved oxygen was not limiting in any of the treatments and effluent DO remained well above the recommended 5-6 mg/L (Westers and Pratt 1977) in all treatments throughout the study. The high DO exposed fish did not "perform" any better than the fish exposed to control DO even though their DO consumption was greater. Apparently there was no physiological advantage to exposing rainbow trout to supersaturated DO. It is unclear as to why the fish exposed to supersaturated DO would consume more DO than controls when DO was not limiting in the control treatments. It is possible that some of the DO thought to be consumed by the high DO exposed fish may have diffused out of the water as it travelled from the influent to the effluent of the tanks. This was not measured but could explain what appeared to be an increase in DO consumption in the high DO exposed fish.

The observed 25% increase in DO consumption related to NH_3 exposure supports results reported by Smart (1978). He

found that rainbow trout exposed to acutely lethal NH_3 concentrations (0.73 mg/L) had a three-fold increase in DO consumption compared to controls. Unionized ammonia concentrations were much lower in my study, resulting in the smaller increase in DO consumption of test fish. Changes in histopathology of gill tissue in rainbow trout reared in elevated NH_3 levels probably did not increase DO consumption. Smart (1976) reported that histopathological changes in gill tissues related to acutely lethal NH_3 concentrations did not affect oxygen uptake in rainbow trout. Smart (1978) also suggested that reduced gas exchange due to gill damage is not a major mode of NH_3 toxicity.

A possible explanation for the increased DO consumption in fish exposed to elevated NH_3 concentrations was provided by Lloyd and Orr (1969). They suggested that increased DO consumption may be due to increased energy expenditure related to correcting an imbalance in osmoregulation caused by the increased permeability of the fish to water.

The quadratic effect of NH_3 concentration on DO consumption in this study is puzzling. It is not clear why trout exposed to 0.06 mg/L NH_3 had lower DO consumption than fish exposed to 0.04 mg/L NH_3. Since both groups of fish "performed" equally well over the study period with respect to weight gain/fish, feed conversions, etc., it appears that differences in oxygen consumption were unimportant.

dilutions could only be carried out to 1:512 (8 dilutions) on serum from the antigen-injected fish. Because of this, no cut-off could be determined for agglutination to this antigen. Meade (1986) carried out dilutions to 1:8192 (12 dilutions) and found a reduced immune response in lake trout. If similar dilutions had been used in my study, a reduced immune response may have occurred in fish exposed to elevated NH_3 levels. Although results were inconclusive, I believe immune response could be used as a tool for evaluating NH_3 related stress in rainbow trout. This type of information could be important in serial reuse culture operations where exposure to elevated NH_3 concentrations may result in a reduced immune response in fish being reared in reused water. This could result in lower survival in these fish following stocking into the wild.

Hematology

Hematological data obtained from rainbow trout from each of the six treatments were normal based on standards reported by McCarthy et al. (1973). Neither elevated NH_3 or DO concentrations negatively affected or changed the hematological parameters examined. If DO and NH_3 affect hematological characteristics, DO saturations of greater than 180% and NH_3 levels of greater than 0.06 mg/L are required for a significant effect in rainbow trout. Thurston et al. (1984) found no differences in either

hematocrits or hemoglobin concentrations between controls and rainbow trout exposed to 0.08 mg/L NH_3. Smart (1978) reported similar results for rainbow trout exposed to 0.73 mg/L NH_3. Thurston et al. (1981) suggested that rainbow trout may "acclimate" to sublethal concentrations of NH_3. Thus it is possible that my experimental fish had acclimated to the test conditions prior to the time of the first sampling (day 102). In future studies, hematological samples should be collected soon after exposure to elevated NH_3 and DO levels to determine if any immediate changes in blood chemistry occur. This, along with subsequent sampling, would demonstrate any "acclimation" of fish to test conditions.

Histology

The increased presence of chloride cells in the gills of rainbow trout exposed to elevated NH_3 concentrations (both 0.04 and 0.06 mg/L NH_3) may have been a response to increased presence of chloride ions (Cl^-) in test water. Ammonium chloride (NH_4Cl) was used to achieve the test NH_3 levels. Excess Cl^- ions absorbed or ingested by fish are excreted through the gills via chloride cells (Bond, 1979). Since chloride levels were not measured, it is not known what concentrations of Cl^- may have produced this effect.

Smith and Piper (1975) reported an increase in epithelial swelling and edematous tissue in rainbow trout

exposed to 0.007 - 0.017 mg/L NH_3 for up to 12 months. Morrison and Piper (1983) reported similar results with steelhead trout exposed to 0.001 - 0.013 mg/L NH_3 for 225 days. The observed increase in degree and severity of epithelial swelling and increase in edematous tissue in the gills of rainbow trout exposed to 0.04 and 0.06 mg/L NH_3 in my study supports these findings. Some changes in gill tissue occurred in control fish but not nearly as much as in test fish. Therefore, NH_3 levels between <0.01 mg/L and 0.04 mg/L can be expected to cause these histopathological changes and DO saturations as high as 185.3% will have no alleviating effects. It is possible that the changes noted in the control fish resulted from the day to day stress (daily tank cleaning, etc.) and periodic handling (biweekly weighing, etc.) during the study.

Glycogen vacuolation in rainbow trout was not affected by either NH_3 exposure or high DO saturations. This is contrary to the reported reduction in glycogen vacuolation in rainbow trout exposed to sublethal (0.007 - 0.017 mg/L and 0.008 - 0.049 mg/L) NH_3 levels by Smith and Piper (1975) and by Soderberg (1985), respectively. The lack of reduction in glycogen vacuolation in my study may have been related to diet. An effect of diet on liver glycogen vacuolation has been shown and discussed by Edsall and Barrows (1989).

In rainbow trout exposed to 0.044 - 0.074 mg/L NH_3,

hyaline droplet degeneration and nephrosis in kidney tissue were reported (Thurston et al. 1984). Larmoyeaux and Piper (1973) reported similar results in rainbow trout exposed to 0.001 -0.009 mg/L NH_3. In my study, hyaline droplet degeneration and swollen kidney tubules were noted in some fish, but no consistent differences were found between treatments, and the kidney tissues from all fish sampled were considered normal for hatchery reared fish. It is possible that the day to day stress and periodic handling of the fish resulted in the few pathological changes noted in the kidney tissue. Soderberg (1985) also found no histological changes in kidney tissue due to NH_3 exposure.

Growth, Feed Conversion, Condition Factors and Survival

Control rainbow trout gained less weight than fish exposed to elevated NH_3 concentrations. This is contrary to the results of other studies. No studies were found in which NH_3 exposure was associated with an increased weight gain.

Morrison and Piper (1983) reared rainbow trout in water reused seven times. Growth was reduced in the fish reared in the sixth and seventh reuses, where NH_3 concentrations reached 0.013 mg/L compared to 0.001 mg/L in the first use. Thus it would appear that NH_3 concentration had an effect on growth in their study. However, DO's decreased from 7.2 mg/L in the first use to 3.3 and 3.1 mg/L in the sixth and

seventh reuses, respectively. Westers and Pratt (1977) recommend 5-6 mg/L DO as a minimum effluent for salmonids. Low DO's may have had as great or greater an effect on growth than did NH_3 concentration. The NH_3 concentrations tested in my study were much higher; however, DO was not limiting in any treatment and an increased weight gain resulted in the NH_3 exposed fish.

Lang et al. (1987) found an initial reduction in growth rate in fish exposed to 0.25 mg/L NH_3 for 4 weeks. However, the fish adapted to the NH_3 exposure after the first week and only small differences were noted in growth after that point between NH_3 exposed fish and controls.

Thurston et al. (1984) found no differences in growth between two generations of rainbow trout exposed to 0.01 to 0.07 mg/L NH_3 for up to 52 months. Dissolved oxygen concentrations were not discussed in their study.

Based on results of other studies, the cause of increased weight gain in the NH_3 exposed fish in my study is unclear. Several researchers have reported that elevated NH_3 concentrations increased feed conversions in salmonids. A high feed conversion (>1.0) is the result of fish being overfed or not converting the feed they are being fed efficiently to body weight. This indicates that food is being wasted. In rainbow trout exposed to 0.007 - 0.017 mg/L NH_3 (Smith and Piper 1975) and 0.001 - 0.013 mg/L NH_3 (Morrison and Piper 1983) feed conversions increased. Lang

et al. (1987) found that rainbow trout exposed to 0.25 mg/L NH_3 initially had reduced food intake (high feed conversion) when compared to controls. After what they termed an "adaptation" of the exposed fish to the NH_3, food intake returned to that of the unexposed fish. My findings were contrary to this. Initially, control fish had a reduced food intake and very high feed conversions compared to test fish. After 4 weeks, fish from all NH_3 treatments appeared to have "adapted" to test conditions and feed conversions were not different from controls during the remaining 10 weeks of the test.

Condition factors for rainbow trout from all treatments were good as defined by Piper et al. (1986). Neither NH_3 or DO concentration improved or reduced the overall "condition" of rainbow trout in my study.

Fish Health Assessment

The fish health assessment provided little clarification of the observed relation between NH_3, DO and rainbow trout growth. Control rainbow trout and those exposed to 0.04 mg/L NH_3 had more mesenteric fat than rainbow trout exposed to 0.06 mg/L NH_3. In contrast, control fish gained less weight/fish than either NH_3 group, even though feed conversions were not different between treatments. Less mesenteric fat among trout in the 0.06 mg/L NH_3 group could be indicative of stress. If this was

the case, however, fish exposed to 0.04 mg/L NH_3 should have shown some intermediate response, yet mesenteric fat content was similar to controls. Based on other parameters examined, elevated NH_3 concentrations tested seem to have caused little stress in rainbow trout.

Bile color in rainbow trout was affected by both NH_3 and DO concentration. A possible explanation for the darker green bile in fish exposed to elevated levels of DO is that these fish were not eating as often as controls (Goede and Barton 1990). It is unclear why DO of approximately 180% saturation would have this effect. Food amounts and availability were the same for all treatments. Total gas pressures, associated with DO saturations of approximately 180%, did not exceed 102.3% and should not have negatively affected the high DO exposed fish. Also, high DO exposure was beneficial with respect to nitrogen gas saturations. Nitrogen saturation was reduced to 82.4% in the high DO treatments compared to approximately 102% in the control treatments.

A more likely explanation for the greener colored bile in the high DO exposed fish was reported by Goede and Barton (1990). The green color of bile is the result of an oxidation of bilirubin to biliverdin. It is possible that this oxidation was occurring in the high DO exposed fish as a result of their being reared in an oxygen rich environment rather than being due to reduced feeding.

Unionized ammonia exposure resulted in test rainbow trout having bile which was more normal (less green) in color than in controls. This indicates that control fish were not eating as often as the NH_3 exposed fish and helps explain their reduced weight gain. This indication of reduced feeding did not, however, result in a difference in feed conversion when compared to rainbow trout exposed to elevated NH_3 concentrations.

In summary, it appears that rearing rainbow trout in DO saturations of as high as 180% does not alleviate the effects of elevated (sublethal) NH_3 exposure. Neither the DO or NH_3 concentrations tested had any effect on hematological parameters, feed conversions, condition factors, survival or most of the parameters tested in the fish health assessment. Dissolved oxygen consumption was affected by both DO and NH_3 concentrations. Further study is needed with respect to DO consumption and rearing fish in waters with supersaturated DO concentrations.

Exposure to elevated NH_3 and DO concentrations had no effect on the immune response of rainbow trout to the Aeromonas salmonicida antigen. Since immune response has been reported to be a sensitive test for evaluating stress, further study is needed with respect to NH_3 exposure. In future studies, dilutions should be performed beyond 1:512.

Gill tissues were affected by NH_3 exposure, with the pathological changes being similar to those reported in

previous studies. Exposure to DO saturations of 180% had no alleviating effects on these changes.

Weight gain was positively affected by NH_3 exposure in this study, with fish exposed to elevated NH_3 concentrations gaining more weight than controls. Weight gain was not affected by DO concentration.

Within the fish health assessment, mesenteric fat content was affected by exposure to elevated NH_3 concentrations but not DO. The bile index (color) was affected by both NH_3 and DO concentration.

Rainbow trout in this study were relatively unaffected by the elevated NH_3 concentrations tested, even though concentrations were well above recommended maximums for NH_3 exposure in salmonids. It is possible that the negative effects attributed to sublethal NH_3 exposure in previous studies, may have been due to other parameters.

The "strain" of rainbow trout used in this study may help explain the few effects of elevated NH_3 exposure reported. Arlee rainbow trout are a "domesticated" strain of rainbow trout and are used extensively in fish cultural operations in Montana. It is possible that over generations of artificial propagation, these fish have developed a tolerance to elevated NH_3 concentrations. If a "wilder" strain of rainbow trout had been used in this study, results could have been different.

The effects of elevated NH_3 exposure found in this

study (increased DO consumption and pathological changes in gill tissue) were not alleviated by DO saturations of as high as 180%. If NH_3 becomes a limiting factor (where fish performance, health etc. are negatively affected) in fish culture operations where supplemental oxygen is being used to increase production, exposure of the fish to supersaturated DO concentrations will probably not alleviate the effects of NH_3 exposure.