Effects of prolonged exposure to ammonia on rainbow trout (Salmo gairdneri) eggs and sac fry
by Dalton Earl Burkhalter

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Zoology
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Abstract:
Laboratory experiments were conducted to determine the effects of ammonia on the eggs and sac fry of rainbow trout (Salmo gairdneri). Two experimental runs were made. Each run exposed eggs and the resulting sac fry to five concentrations of ammonia ranging up to 0.37 mg/l un-ionized ammonia as N (NH3-E). Exposure was continuous throughout the incubation period and for 42 days thereafter. Controls were maintained in essentially ammonia-free water.

A concentration of 0.1 mg/l NH3-N caused retardation of growth and development of sac fry throughout most of the test period. Evidence existed that 0.05 mg/l NH3-N caused retardation of growth and development early in the growth period.

A concentration of 0.25 mg/l NH3-N was suggested as the incipient LC50 (lethal threshold concentration) for rainbow trout sac fry. There was no differential egg mortality or effect on incubation period.

Hypertrophy of the epithelium of the secondary lamellae of gill tissue occurred at 0.19 mg/l NH3-N. Karyolysis and karyorrhexis occurred in the same tissue at 0.28 mg/l NH3-N.

Pale coloration occurred in sac fry at concentrations of 0.19 mg/l HN3-N and higher. This effect was attributed to a lack of blood coloration through possible reduction in erythrocyte numbers or reduced hemoglobin content in the erythrocytes.

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Date 31 March 1975

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ABSTRACT

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INTRODUCTION

The presence of ammonia in aquatic ecosystems has received increased attention in recent years. A growing awareness of the toxicity of ammonia to aquatic animals has fostered much of this attention.

Ammonia finds its way into the aquatic ecosystem through the municipal sewage treatment plant, agricultural and feedlot runoff, coal coking and gasification plants, and fertilizer manufacturing plants (EIFAC 1973). Organisms residing within a watercourse are a further source of ammonia but generally in such nominal quantities that natural mechanisms of degradation can maintain concentrations well below toxic levels. Introductions of ammonia by the activities of man can be so localized and concentrated that natural means of degradation are overwhelmed and ammonia concentrations can reach toxic levels.

Many studies of ammonia toxicity to warm and cold water fishes have been made. Most cold water studies have used salmonids as the test animal with the rainbow trout being used most frequently in this country. Much acute toxicity data exist but longer term toxicity data are less numerous.

This study was undertaken in an effort to obtain more toxicity data on very young cold water fish. This paper discusses a study of the toxicity of aqueous ammonia to the eggs and sac fry of rainbow trout (*Salmo gairdneri*).

CHEMISTRY OF AMMONIA IN AQUEOUS SOLUTION

The chemistry of ammonia is relatively simple in most natural waters. Ammonia gas is very soluble in water and may dissolve by simple dissolution. Ammonia may also enter water as the ammonium ion, NH_4^+, from the dissolution of such compounds as NH_4Cl, NH_4HCO_3, and $(NH_4)_2SO_4$.

Ammonia behaves in water as a Brønsted acid or base as shown in the following equations (Stumm and Morgan 1970):

$$NH_4^+ + H_2O = NH_3 + H_3O^+ \text{ (acid)}$$

$$NH_3 + H_2O = NH_4^+ + OH^- \text{ (base)}$$

The pK values at 20 C for these reactions are given below (Hodgman 1958):

$$pK_a = 9.400$$

$$pK_b = 4.767$$

In contrast to earlier concepts the NH_4OH molecule probably does not exist. Instead, the NH_3 molecule is most likely loosely bound to many water molecules through hydrogen bonding (Stumm and Morgan 1970). This ammonia hydrate ($NH_3 \cdot H_2O$) is most frequently called "un-ionized ammonia" while the NH_4^+ ion is referred to as "ionized ammonia". The sum of the two fractions is then termed "total ammonia". In this paper the terms "NH_3" and "un-ionized ammonia" will be used interchangeably as will also "NH_4^+" and "ionized ammonia".

The un-ionized fraction of ammonia has long been recognized as

the agent which is toxic to fish (Wuhrmann and Woker 1948, cited by EIFAC 1973; Downing and Merkens 1955). This fraction is not constant for a given total ammonia concentration but varies significantly with pH and temperature and slightly with ionic strength. The chemical equations above show an increase in the NH_3 fraction with increasing pH. Similarly an increase in temperature results in an increase in the NH_3 fraction (Hodgman 1958). These variations with pH and temperature are illustrated by the following representative figures (Table 1) extracted from Trussell (1972).

Table 1. Percentage of un-ionized ammonia in aqueous solution at different pH's and temperatures.

	Temperature, C				
pH	8	10	12	14	16
7.0	0.16	0.19	0.21	0.25	0.29
7.5	0.50	0.59	0.68	0.80	0.92
8.0	1.58	1.83	2.12	2.48	2.86
8.5	4.82	5.55	6.40	7.45	8.52

VARIABLES AFFECTING TOXICITY

The toxicity of ammonia to fishes depends upon several factors including dissolved oxygen, free carbon dioxide, pH, temperature, prior exposure to ammonia, physical stress, general physiological status, and

the presence of other toxic substances or mitigating parameters (Willingham 1973). In this study these factors were either eliminated or closely controlled. The more salient of these factors will be discussed in the following paragraphs.

pH and Temperature

An increase in pH and/or temperature increases the toxicity of a given total ammonia concentration to fish due to the previously discussed pH and temperature dependence of the NH_3 fraction. This correlation with temperature may be offset, however, by a greater susceptibility of fish to ammonia poisoning at low temperatures. Burrows (1964) found reduced recovery in chinook salmon fingerlings exposed to ammonia at about 6 C compared with those exposed at about 14 C. Brown (1968) indicated a linear relationship showing a decreasing 48-hr LC50 for NH_3 with decreasing temperature. For rainbow trout the 48-hr LC50 at 3 C was less than one-third the value at 20 C.

Free Carbon Dioxide

Lloyd and Herbert (1960) found that ammonia toxicity decreased with decreasing carbon dioxide concentration. They reasoned that the respiratory water increased in carbon dioxide content and hence decreased in pH as it passed over the gill surfaces. This caused a lowering of the NH_3 concentration at the gill surfaces. When initial carbon dioxide levels were high, the lowering of pH would be less than when initial carbon dioxide levels were low (when added carbon dioxide

would be relatively greater).

Dissolved Oxygen

Downing and Merkens (1955) found that survival periods of rainbow trout exposed to ammonia increased with increasing dissolved oxygen levels ranging from 1.5 to 8.5 mg/l. The increase in survival time as a function of dissolved oxygen was greatest at the lower concentrations of ammonia. Oxygen effects were observed throughout the range but were most pronounced in the lower extremes.

Lloyd (1961a, 1961b) also found an increased toxicity of ammonia at low dissolved oxygen levels. For example he found that the threshold LC 50 at 40% dissolved oxygen saturation was half that at 100% saturation (Lloyd 1961b). He reasoned that lower oxygen levels resulted in lower output of carbon dioxide at the gill surface which lessened the downward change in pH.

Alkalinity and Hardness

No direct influence on the toxicity of ammonia to fish has been observed for alkalinity and its effect is limited to the extent that it helps determine pH (EIFAC 1973).

Herbert (1962) found no effects on ammonia toxicity to rainbow trout from variations in calcium water hardness.

AMMONIA TOXICITY TO FISHES

Acute Toxicity Studies

Lloyd and Herbert (1960) showed that the 500-minute LC 50 for

rainbow trout was approximately 0.4 mg/l un-ionized ammonia as N (NH_3-N) at the gill surface. Merkens and Downing (1957) found a threshold LC50 of approximately 1.7 mg/l NH_3-N in the presence of very low carbon dioxide (less than 4 mg/l).

Lloyd (1961b) graphically relates the threshold LC50 to pH, alkalinity, dissolved oxygen, temperature, and carbon dioxide. When applied to a typical water of pH 7.6, alkalinity 200 mg/l as $CaCO_3$, temperature 18 C, dissolved oxygen 60% of air saturation, and carbon dioxide 11 mg/l, the threshold LC50 for rainbow trout is 0.4 mg/l NH_3-N.

Ball (1967) found the threshold LC50 for rainbow trout to be 0.41 mg/l NH_3-N. Lloyd and Orr (1969) found a similar threshold LC50 of 0.39 mg/l NH_3-N for rainbow trout.

Rice (1971) found rainbow trout eggs and early sac fry unaffected by concentrations of 3.0 mg/l NH_3-N. During absorption of the yolk sac the resistance to NH_3 decreased and near completion of absorption the 24-hr lethal concentration (LC50?) had dropped to approximately 0.08 mg/l NH_3-N.

Herbert and Shurben (1963) found a 24-hr LC50 of 0.3-0.4 mg/l NH_3-N for rainbow trout. A later study (Herbert and Shurben 1965, cited by EIFAC 1973) showed 24-hr LC50 values of 0.40-0.58 mg/l NH_3-N.

Penaz (1965) showed that brown trout eggs were resistant to two-hour exposures to concentrations of 41 mg/l NH_3-N, however, some

evidence existed that hatching success was reduced if exposure occurred during later stages of development. Other work by Penaz (1965) suggested that the threshold LC50 for brown trout fry was 0.33 mg/l NH_3-N.

Wuhrmann and Woker (1948, cited by EIFAC 1973) found a threshold for trout spawn (fry?) of 0.25-0.33 mg/l NH_3-N. Liebmann (1960, cited by EIFAC 1973) found a threshold value of 0.2 mg/l NH_3-N for rainbow trout fry.

Long-term Toxicity Studies

Burrows (1964) found no mortality among chinook salmon fingerlings after 6-week exposures to ammonia concentrations of 0.005-0.007 mg/l NH_3-N. Pathological symptoms were, however, observed. Severe hyperplasia of the secondary lamellae resulted at the lower concentration while hyperplasia and fusion of the secondary lamellae occurred at the higher concentration. Flis (1968) found tissue damage in gills, skin, intestine, liver, and kidneys of carp resulting from exposure to sublethal levels of ammonia.

Larmoyeux and Piper (1973) found that an 8-month exposure of rainbow trout to 0.01 mg/l NH_3-N caused hyperplasia and fusion of the secondary lamellae of the gills. Evidence of tissue damage was also noted in the liver, spleen, and thyroid. These results were obtained at dissolved oxygen levels ranging down to 3.3 mg/l.

Reichenbach-Klinke (1967) found that concentrations up to

0.4 mg/l NH_3-N caused hyperplasia, swelling, and inflammation of the gills in rainbow trout. In addition, he found that rainbow trout suffered a distinct and irreversible decrease in the number of erythrocytes in the blood.

MECHANISM OF TOXICITY

The exact mechanism of toxicity of NH_3 to fishes is not known at this time. Several studies have been done in an attempt to identify this mechanism.

Brockway (1950) correlated ammonia in the surrounding water with reduced oxygen carrying capacity of trout blood. He found that ammonia concentrations of 1 mg/l (assumed as total ammonia) reduced the oxygen content of the blood to about one-seventh the normal value and increased the carbon dioxide content of the blood by 15%. He concluded that ammonia reduced the ability of fish hemoglobin to combine with oxygen or to liberate carbon dioxide. Suffocation occurred as a result.

Fromm and Gillette (1968) correlated blood ammonia and nitrogen excretion with ambient ammonia. They found that blood ammonia increased with increasing ambient ammonia while at the same time total nitrogen excretion decreased. Ammonia excretion decreased by a greater percentage than did total nitrogen excretion. They also found that the ability of hemoglobin to combine with oxygen in vitro was not altered by ammonia concentrations up to 0.05 mg/l NH_3-N.

Lloyd and Orr (1969) found that urine excretion in rainbow trout increased with increasing ambient ammonia levels. They suggest that this diuresis results from an increase in permeability of the fish to water in the presence of ammonia. They suggest that ammonia concentrations may increase until urine excretion reaches a plateau at which time permeability exceeds excretion and death results.

Olson and Fromm (1971) correlated a decrease in total nitrogen excretion by rainbow trout with an increase in ambient ammonia. They suggested that the decrease in ammonia excretion was due to a decrease in gradient between blood and water. No compensatory excretion of urea or protein nitrogen was noted to offset the increased blood ammonia.

Reichenbach-Klinke (1967) maintains that ammonia acts on the respiratory organs and the blood of the fish. His findings that rainbow trout suffered an irreversible decline in erythrocyte numbers led him to hypothesize that erythrocyte numbers could become so low that the fish could no longer maintain life.

Some workers currently feel that ambient ammonia produces toxicity in fishes due to the increase in blood ammonia which results. Since ammonia is toxic the fish must either detoxify or excrete it. Forster and Goldstein (1969) state that teleost fishes lack two enzymes which mammals utilize in the ornithine urea cycle for urea synthesis. They believe these fishes produce urea in small quantities from purines

and amino acids. Urea synthesis in the teleost cannot keep pace with rising blood ammonia and the fish becomes poisoned. Fromm and Gillette (1968) believe increased levels of blood ammonia stimulate glycolysis in neural tissue. Their work did not show that ammonia inhibited exchange of respiratory gases or decreased the affinity of hemoglobin for oxygen.

MATERIALS AND METHODS

Two test runs were performed during the time period December, 1973, through March, 1974. Testing was performed in a laboratory on the campus of Montana State University, Bozeman, Montana. Rainbow trout eggs and sac fry were exposed to controlled concentrations of reagent grade NH_4Cl.

DILUTION WATER

The test water was from the domestic supply to the city of Bozeman, Montana. Treatment of this water before distribution is limited to settling and chlorination. The water was dechlorinated by filtering through activated charcoal. Test water was heated and maintained at constant temperature by an electric flow-through heater controlled by a voltage regulator.

The chemical and physical characteristics of the test water remained quite constant throughout the test periods. The values for the characteristics monitored are presented in Table 2. The ions of calcium, magnesium, and bicarbonate accounted for approximately 85% of the conductivity measured. Chemical measurements are described later in this section.

DILUTION APPARATUS

The dilution apparatus shown in Figures 1 and 2 was used to introduce the test water containing toxicant to the trout eggs and/or sac fry. This apparatus combined some of the concepts of Brungs and Mount (1967), Grenier (1960), and McAllister et al. (1972). I added

Table 2. Chemical and physical properties of dilution water.

Alkalinity, mg/l as $CaCO_3$	100-110
Hardness, mg/l as $CaCO_3$	106-123
Ca^{++}, mg/l as $CaCO_3$	74-82
Mg^{++}, mg/l as $CaCO_3$	32-41
Dissolved oxygen, mg/l	> 8
pH	7.4-7.6
Controlled temperature, C	10-12
Conductivity, micromhos/cm	202-262
NO_3^-, mg/l	< 1
NO_2^-, mg/l	0.07 max.
Total ammonia, NH_4^+-N	0.15 max.
Residual chlorine	0.0

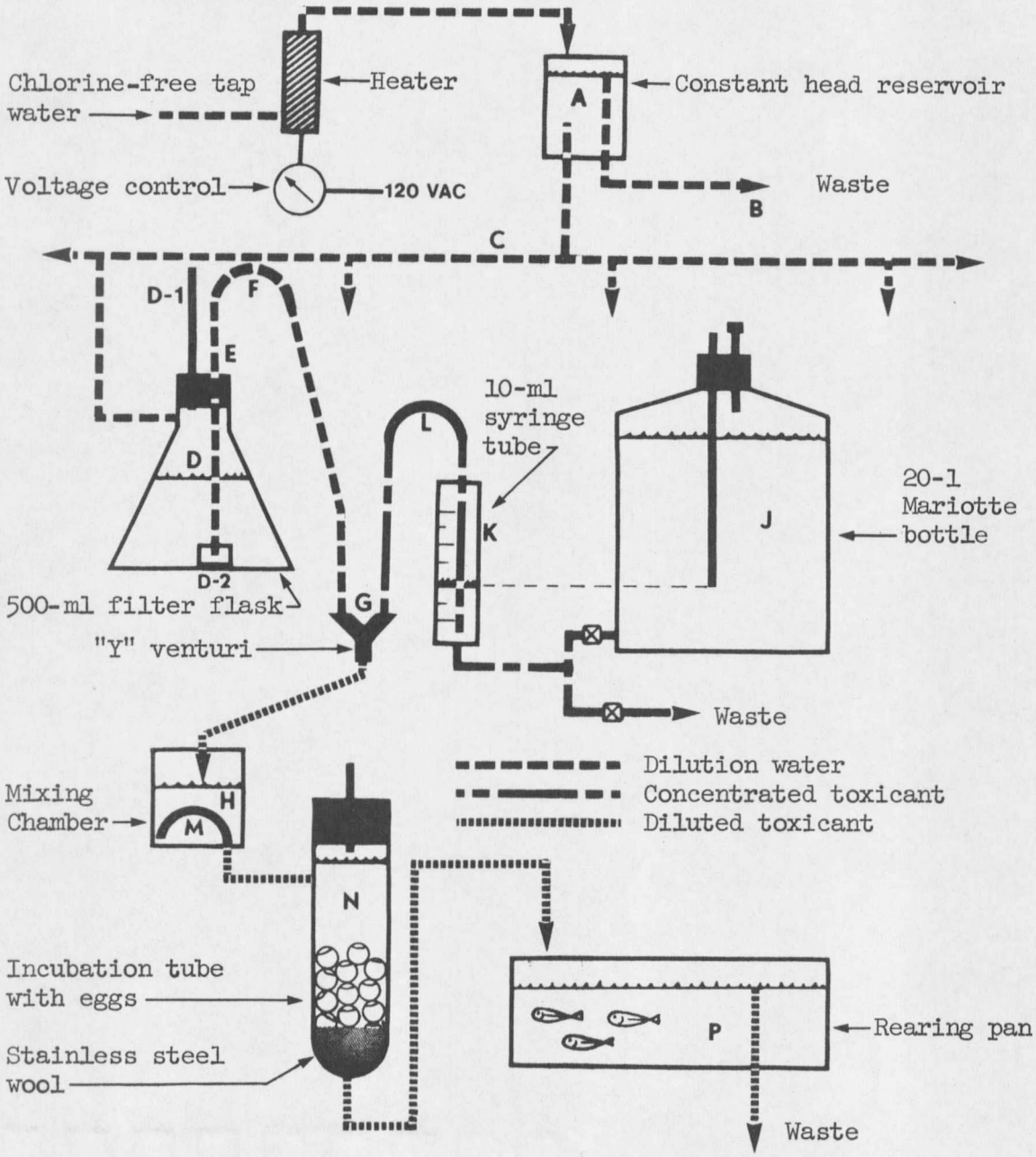

Figure 1. Schematic diagram of constant flow toxicant dilution apparatus.