

Effects of Prolonged Exposure to Ammonia on Fertilized Eggs and Sac Fry of Rainbow Trout (*Salmo gairdneri*)

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ABSTRACT

Effects of ammonia on fertilized eggs and resulting sac fry of rainbow trout (*Salmo gairdneri*) were tested at concentrations of un-ionized ammonia ranging from 0.05 to 0.37 mg/liter (as $\text{NH}_3 - \text{N}$). Exposure was continuous throughout the incubation period and for 42 days thereafter. There was no differential egg mortality or effect on incubation period. The lowest concentration of 0.05 mg/liter $\text{NH}_3 - \text{N}$ caused some retardation of early growth and development and 0.1 mg/liter caused similar but more severe effects throughout most of the test period. Hypertrophy of secondary gill lamellae epithelium occurred at 0.19 mg/liter. Karyolysis and karyorrhexis occurred in the same tissue at 0.28 mg/liter. Pale coloration and blue-sac disease occurred in sac fry at concentrations of 0.19 mg/liter and higher. The estimated incipient LC50 (lethal threshold concentration) for rainbow trout sac fry was 0.25 mg/liter $\text{NH}_3 - \text{N}$.

The occurrence of ammonia in natural waters and its effects on fishes have been extensively documented. Ammonia is a naturally occurring product of biological metabolism, but high concentrations are often associated with human sources such as sewage treatment plants, agricultural and feedlot runoff, coal coking and gasification plants, and fertilizer manufacturing plants (EIFAC 1973). Ammonia behaves in water as a Brønsted acid or base existing in the ionized (NH_4^+) and un-ionized (NH_3) forms, with the NH_3 fraction probably occurring as an ammonia hydrate (Stumm and Morgan 1970). The un-ionized fraction has long been recognized as the agent toxic to fishes (Wuhrmann and Woker 1948, cited by EIFAC 1973; Downing and Merkens 1955).

The toxicity of a given amount of total ammonia to fishes depends on several factors. The fraction of un-ionized ammonia increases essentially by a factor of ten for each unit increase in pH and to a lesser extent with temperature (Trussell 1972; Emerson et al. 1975). Other factors which increase toxicity of NH_3 to fishes include depressed levels of dissolved oxygen, elevated carbon dioxide, and physical stress (EIFAC 1973).

Much information exists on acute toxicity to fishes, but information on long-term effects is less common. Most lethal threshold concentrations reported have been in the

range 0.2–0.4 mg/liter $\text{NH}_3 - \text{N}$ (Loyd 1961; Ball 1967; Lloyd and Orr 1969; Penaz 1965; Wuhrmann and Woker 1948, cited by EIFAC 1973; Liebmann 1960, cited by EIFAC 1973). Studies of long term toxicity have reported tissue damage to gills, skin, and internal organs (Burrows 1964; Flis 1968; Larmoyeux and Piper 1973; Smart 1976), reduction in numbers of red blood cells (Reichenbach-Klinke 1967), and inhibition of growth (Robinette 1976). Brockway (1950) correlated decreased oxygen carrying capacity of fish blood with the presence of ammonia in the surrounding water. Embryonic and early larval stages of fishes are often considered to be especially sensitive to toxicants, but there have been no studies of continuous exposure of these stages of salmonids to ammonia. Some data are presently available from short-term exposure to ammonia of eggs and fry of rainbow trout (Rice and Stokes 1975) and of eggs of brown trout (Penaz 1965). The present paper documents effects of continuous exposure to ammonia on the fertilized eggs and resulting sac fry of rainbow trout (*Salmo gairdneri*).

METHODS

Fertilized rainbow trout eggs and sac fry were exposed to controlled concentrations of reagent grade NH_4Cl . Fertilized eggs were obtained from the Ennis National Fish Hatchery (U.S. Fish and Wildlife Service), Ennis, Montana. Exposure to ammonia

TABLE 1.—Chemical and physical properties of dilution water.

Alkalinity, mg/liter as CaCO ₃	100-110
Hardness, mg/liter as CaCO ₃	106-123
Ca ⁺⁺ , mg/liter as CaCO ₃	74-82
Mg ⁺⁺ , mg/liter as CaCO ₃	32-41
Dissolved oxygen, mg/liter	>8
pH	7.4-7.6
Controlled temperature, C	10-12
Conductivity, micromhos/cm	202-262
NO ₃ ⁻ , mg/liter as N	<1
NO ₂ ⁻ , mg/liter as N	0.07 max.
NH ₃ - N, mg/liter	0.001 max.
Residual chlorine	0.0

commenced within 24 hours after fertilization.

Toxicant was introduced to dilution water at a 1:100 ratio through a constant flow dilution apparatus patterned after those described by Brungs and Mount (1967), Grenier (1960), and McAllister et al. (1972). The dilution water was dechlorinated tap water. Water temperature was controlled within ± 0.5 C of the 10 and 12 C test temperatures. Chemical and physical characteristics of the dilution water are presented in Table 1. The ions of calcium and magnesium accounted for approximately 85% of the conductivity. The chemical and physical properties were determined by standard laboratory procedures (APHA et al. 1971). Ammonia nitrogen was measured by direct Nesslerization (APHA et al. 1971) and the fraction of un-ionized ammonia was calculated from Trussell (1972).

Five test concentrations (0.05, 0.10, 0.19, 0.28, 0.37 mg/liter NH₃ - N with a 95% confidence interval of $\pm 10\%$) and a control were maintained during each of two runs. Each run began with 310 fertilized eggs at each concentration. A flow rate of 400 ml/min exceeded the minimum rates recommended by APHA et al. (1971) and Sprague (1969) in order to minimize any decline in dissolved oxygen or contribution of ammonia from the specimens themselves.

Fertilized eggs were incubated in glass tubes patterned after those described by Hurley (1972). Eggs were undisturbed during incubation and were transferred to polyethylene rearing pans at the time of hatching. Dead eggs were removed and recorded at this time. Sac fry were inspected regularly and mortalities recorded. Al-

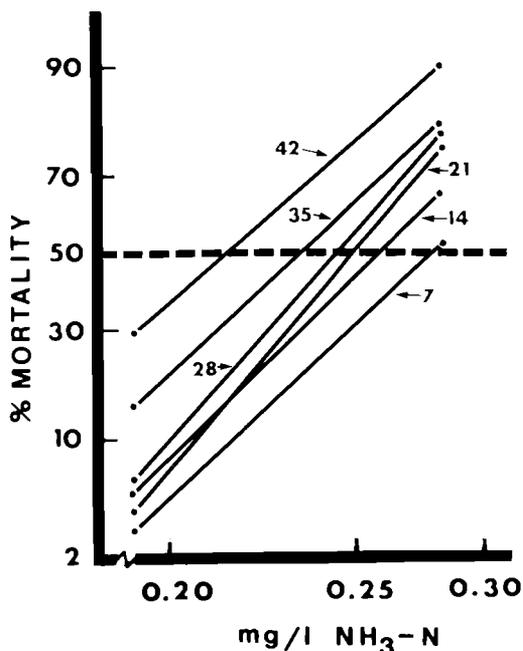


FIGURE 1.—Toxicity curves for rainbow trout sac fry at given exposure times in days based upon concentrations of 0.19 and 0.28 mg/liter NH₃ - N (log-probability).

though some sac fry developed to the point where feeding normally commences, no feeding was done.

Total lengths of fry were determined from specimens fixed in aqueous Bouin's solution and preserved in 70% ethanol. Specimens for histological examination were processed according to procedures of Luna (1968). Staining was done with hematoxylin and eosin.

RESULTS

Egg mortality was not affected in either run by the ammonia concentrations used in this study. Mortalities at each concentration ranged from 2.3 to 6.8% during run 1 and from 28.4 to 35.8% during run 2. There was no correlation with concentration (chi-square test). The higher mortalities among eggs during run 2 may be attributable to reduced viability of these eggs obtained at the end of the spawning period (Davis 1953). Incubation periods of 25 days at 12 C and 33 days at 10 C for runs 1 and 2, respectively, corresponded to expected values (Embry

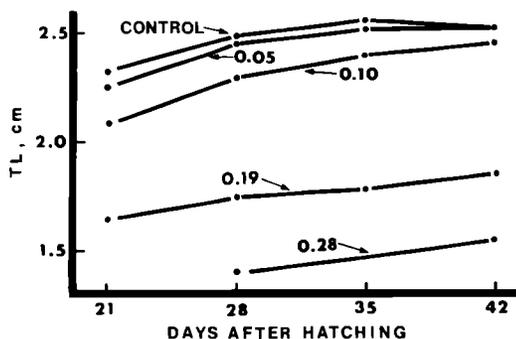


FIGURE 2.—Growth of rainbow trout sac fry at given concentrations of $\text{NH}_3 - \text{N}$ (mg/liter). TL is total length. Standard error = 0.02 cm; $N = 29 - 40$.

1934) and were thus also not affected by the experimental ammonia concentrations.

Differences in fry mortalities among concentrations became evident at or after hatching. Many mortalities occurred when fry were unable to complete the hatching process and died with only their heads protruding from the ruptured eggs. Cumulative mortalities at concentrations of 0.28 mg/liter $\text{NH}_3 - \text{N}$ and 0.37 mg/liter $\text{NH}_3 - \text{N}$ were significantly different (chi-square test) from the control at all ages ($P < 0.05$). Fry mortalities at a concentration of 0.19 mg/liter $\text{NH}_3 - \text{N}$ were significantly different ($P < 0.05$) from the control fry mortalities during the latter parts of the runs. Data from both runs were combined and the runs considered as replications. Toxicity curves (Fig. 1) based upon mortalities at concentrations of 0.19 and 0.28 mg/liter $\text{NH}_3 - \text{N}$, show a 21-day LC50 (lethal threshold concentration, Sprague 1969) of 0.25 ± 0.044 mg/liter $\text{NH}_3 - \text{N}$ (95% confidence interval using the t -distribution). This value was used as the estimate of the incipient LC50 (Sprague 1969).

Evaluation of growth was based on total lengths attained by the sac fry. The mean total lengths (TL) are plotted in Fig. 2. Due to high, early mortality, data from a concentration of 0.37 mg/liter were not available. Mean lengths of fry from all test concentrations were significantly different from those of the controls ($P < 0.05$, least squares analysis of variance and Newman-Keuls method of differences) at 21 days after hatching, and remained significantly differ-

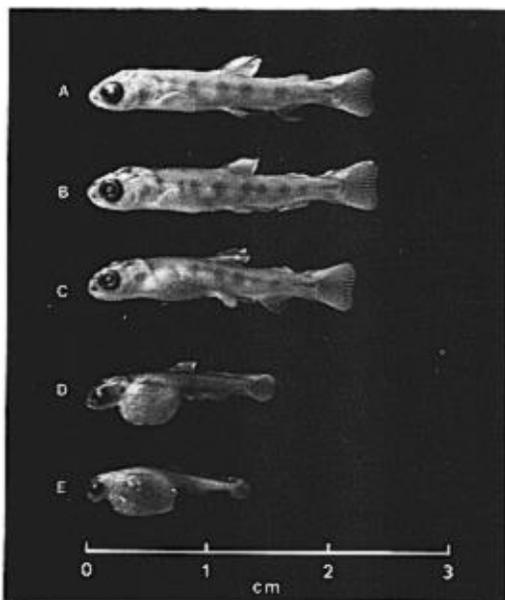


FIGURE 3.—Rainbow trout sac fry development at 28 days after hatching. Exposure concentrations (mg/liter $\text{NH}_3 - \text{N}$): A, control; B, 0.05; C, 0.10; D, 0.19; E, 0.37.

ent to the end of the test period at 42 days after hatching, with the exception of those from 0.05 mg/liter $\text{NH}_3 - \text{N}$ at 35 and 42 days, and 0.10 mg/liter at 42 days.

The physical appearance of the fish at 28 days age is illustrated by Fig. 3. The subjects represent the mean length from each sample. The photograph is representative of fish from both runs and the subject from a concentration of 0.37 mg/liter $\text{NH}_3 - \text{N}$ is representative of specimens from a concentration of 0.28 mg/liter $\text{NH}_3 - \text{N}$ also. The photograph illustrates that retardation of growth at the higher ammonia concentrations is accompanied by a general inhibition of development and failure to absorb the yolk sac. Blue-sac disease (confirmed by Charlie E. Smith, Fish Cultural Development Center, Bozeman, Montana) was common in fry from concentrations of 0.19 mg/liter $\text{NH}_3 - \text{N}$ and higher (Fig. 3, D and E).

Inhibition of development was also evident in the length of time required to reach a free-swimming state, with a delay of 1 week at a concentration of 0.10 mg/liter $\text{NH}_3 - \text{N}$ and complete failure to attain this state at

concentrations of 0.28 and 0.37 mg/liter $\text{NH}_3 - \text{N}$. Obvious differences in coloration were also observed, with fry from a concentration of 0.19 mg/liter $\text{NH}_3 - \text{N}$ and higher noticeably pale in comparison to the control fish.

Histological analysis of gill tissue revealed some occurrences of hypertrophy of the gill secondary lamellae epithelium in fish from concentrations of 0.19 mg/liter $\text{NH}_3 - \text{N}$ and higher after 28 days exposure. Karyolysis and karyorrhexis were sometimes noted in the same tissue in fish from concentrations of 0.28 and 0.37 mg/liter $\text{NH}_3 - \text{N}$ after 28 days exposure.

DISCUSSION

The ammonia concentrations used in this study produced no significant differences in mortalities of fertilized eggs or of incubation times to hatching of rainbow trout. Similar results were reported by Rice and Stokes (1975) and Penaz (1965).

The 21-day LC50 was selected to represent the incipient LC50 since this time period reflected extended exposure but preceded indications that feeding should commence. The value of 0.25 mg/liter $\text{NH}_3 - \text{N}$ is lower than the threshold values of 0.3–0.4 mg/liter $\text{NH}_3 - \text{N}$ generally found for larger rainbows (Lloyd 1961; Ball 1967; Lloyd and Orr 1969; Penaz 1965) and intermediate compared with values of 0.2–0.33 mg/liter $\text{NH}_3 - \text{N}$ found for rainbow and brown trout fry (Wuhrmann and Woker 1948, cited by EIFAC 1973; Liebmann 1960, cited by EIFAC 1973).

This study demonstrated that growth and development of rainbow trout sac fry are inhibited by long term exposures to concentrations of ammonia as low as 0.05 mg/liter $\text{NH}_3 - \text{N}$. The decreasing slope of the control growth curve after 21 days (Fig. 2) is likely due to depletion of yolk and the absence of supplementary feeding. Inhibition of physical activity corresponded well with retarded growth and development. The presence of blue-sac disease at the higher ammonia concentrations confirmed the results obtained by Wolf (1957) at similar unionized ammonia levels.

The pale coloration noted in fish from a concentration of 0.19 mg/liter $\text{NH}_3 - \text{N}$ and

higher may be attributable to a reduction in red blood cell numbers as observed by Reichenbach-Klinke (1967) or depressed oxygen levels in the blood (Brockway 1950). These possibilities and the histological changes noted in the gills could have lowered the rate of oxygen uptake and transport by the blood to a point where growth and development were inhibited. This speculation is supported by experimental evidence that low levels of dissolved oxygen cause similar retardation of growth in young salmonids (Warren 1971) and young northern pike (Siefert et al. 1973). The condition of sac fry dying as they were attempting to emerge from the egg was also noted by Siefert et al. (1973) in northern pike maintained at low dissolved oxygen levels.

Some implications of retardation of growth and development and inhibition of physical activity deserve attention. Such effects would prolong the period when sac fry are most vulnerable to predators such as other salmonids, squaw-fish, sculpins (Ricker 1941) and stonefly nymphs (Claire and Phillips 1968). Herting and Witt (1967) observed that bowfin showed preferential predation toward centrarchids with impaired physical condition.

The lowest ammonia concentration in this study (0.05 mg/liter $\text{NH}_3 - \text{N}$) produced sublethal effects of retardation in growth and development. Various sublethal effects on rainbow trout have been observed by other investigators at ammonia concentrations of 0.01 mg/liter $\text{NH}_3 - \text{N}$ (Larmoyeux and Piper 1973) and 0.038 mg/liter $\text{NH}_3 - \text{N}$ (Lloyd and Orr 1969); and on other species at concentrations ranging from 0.006 to 0.212 mg/liter $\text{NH}_3 - \text{N}$ (Burrows 1964; Robinette 1976; Flis 1968).

Recommended allowable levels of ammonia in a fishery are 0.02 mg/liter $\text{NH}_3 - \text{N}$ (EIFAC 1973; Willingham 1973) and 0.017 mg/liter $\text{NH}_3 - \text{N}$ (National Academy of Sciences and National Academy of Engineering 1972). These recommended maxima are less than the lowest level shown by this study to have sublethal effects. Based upon this study and previous work, ammonia concentrations in natural waters exceeding the maximum recommended values may, depending on condi-

tions, inhibit growth and effect mortality in rainbow trout sac fry.

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