ACUTE TOXICITY OF AMMONIA TO SEVERAL DEVELOPMENTAL STAGES OF RAINBOW TROUT, *SALMO GAIRDNERI*

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ABSTRACT

Median tolerance limits derived from 24-h bioassays demonstrated that fertilized eggs and alevins of rainbow trout, *Salmo gairdneri*, were not vulnerable to 3.58 ppm un-ionized ammonia at 10°C (pH 8.3). At the end of yolk absorption, rainbow trout fry increased in susceptibility dramatically; their median tolerance limit values were about 0.072 ppm, the same as for adult trout. Fertilization of eggs was not prevented in un-ionized ammonia solutions up to 1.79 ppm, the highest exposure tested.

Much information is available on the toxicity of ammonia to juvenile and adult trout, but the paucity of information on the toxicity of ammonia to fertilized eggs and larvae of teleosts is surprising since these life stages are often assumed to be relatively sensitive. Several studies have examined ammonia toxicity to adult trout (Lloyd 1961; Ball 1967; Wilson et al. 1969), including the effects of increased ammonia toxicity to trout at lower oxygen levels (Downing and Merkens 1955) and decreased toxicity at higher carbon dioxide levels (Lloyd and Herbert 1960). Temperature, oxygen, pH, carbon dioxide, and bicarbonate alkalinity influence the toxicity of ammonia and are discussed in a report by the European Inland Fisheries Advisory Commission (1970). Exposure of juvenile or adult salmonids to ammonia has been associated with decreased growth (Brockway 1950; Burrows 1964; Larmoyeux and Piper 1973), gill damage (Burrows 1964; Reichenbach-Klinke 1967), and other sublethal physiological effects (Reichenbach-Klinke 1967; Fromm and Gillette 1968; Lloyd and Orr 1969), and similar effects may occur with salmonid eggs and alevins. Exposure to ammonia has also been associated with increased incidence of disease in juvenile and adult salmonids (Burrows 1964; Larmoyeux and Piper 1973) and in salmonid alevins (Wolf 1957).

The only study of toxicity of ammonia to eggs and larvae (Penaz 1965) involved three stages of eggs and two stages of yolk fry of *Salmo trutta*. Penaz observed an increase in sensitivity of the eggs with age to brief (120 min) exposures to ammonia at pH 8 and temperatures of 5.68° to 3.56°C. A similar pattern was observed with longer exposures (10 h) of newly hatched and 12-day-old alevins to ammonia at pH 8 and temperatures of 11° and 16.9°C. The early eggs were resistant to the highest dose he tested—50 mg/liter of un-ionized ammonia. These data suggest changes in sensitivity with development, but the changes in lengths of exposure and temperature make it difficult to compare differences between eggs and alevins.

We used a series of bioassays to determine the stage of development at which eggs and larvae of rainbow trout, *Salmo gairdneri*, were most susceptible to acute ammonia toxicity. Such information is needed to establish realistic limits for survival of eggs and larvae in both natural and hatchery environments. Knowledge of concentrations of ammonia that may limit survival is particularly important in hatchery operations where it is advantageous to maintain the greatest density of fish and eggs per unit water flow.

MATERIALS AND METHODS

Freshly fertilized rainbow trout eggs were obtained from Bowden National Fish Hatchery, W.V., (courtesy of the U.S. Bureau of Sport Fisheries and Wildlife) and transported to the laboratory within 6 h.

About 2,000 of the eggs were poured under water into 4-inch-square trays with nylon screen bottoms at about 25 to 35 eggs per tray. For incubation the trays were put into a 10°C water

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bath that recycled through both charcoal and a gravel bacterial filter at a rate of 3 gallons/min. Ammonia levels were measured periodically and never attained 0.1 ppm. All ammonia analyses were made by separating ammonia by diffusion (Conway and Cooke 1939) and followed by nesslerization of the separated ammonia. For the ammonia bioassays, the small trays were transferred directly to the experimental medium. By conducting the bioassays in the same trays in which the eggs or larvae were incubated, we did not have to pipette them to other containers—a process that might have injured them.

Two series of duplicated ammonia toxicity bioassays were conducted according to standard procedures outlined by Doudoroff et al. (1951) and results were expressed as 24-h median tolerance limits (24-h TLm)[3]. The bioassays were conducted every 4 to 7 days from fertilization to the completion of yolk sac absorption. Toxicity of ammonia to adult rainbow trout (length 7-9 inches) was also measured with static bioassays (12 fish per concentration tested, 1 fish per 10-liter aquarium) at the same water temperature and pH used with the eggs and larvae.

All bioassays were conducted in aged tap water (total hardness 5.94 ppm as calcium carbonate at pH 7.8) adjusted to pH 8.3 with tris buffer (final concentration 0.05 M). Ammonia, in the form of ammonia sulfate, was added to arrive at the various test concentrations. The resulting conditions made the ammonia toxicity assays more severe than would normally be encountered because the toxicity of ammonia increases as pH increases due to the conversion of ionized NH_4^+ into the un-ionized NH_3 form. At 10°C and pH 8.3, 3.58% of the ammonia in water is un-ionized, considerably more than the 0.19% un-ionized ammonia at pH 7 (Trussell 1972).

Since the un-ionized form of ammonia has been identified as the toxic form, we report our results in units of un-ionized ammonia rather than total ammonia.

Several water quality parameters were measured at the beginning and end of the bioassays, since changes could affect the results. Ammonia levels never dropped below 93% of the initial bioassay concentrations during the course of the 24-h experiments. Very low levels of un-ionized ammonia (0.011 ppm) were detected in the control exposures after 24 h. The tris buffer prevented any changes in pH from occurring during the 24-h tests. Dissolved oxygen remained above 91% saturation in the shallow egg-alevein bioassay containers and above 88% saturation in the adult bioassays (measured with YSI oxygen probe).[4] Carbon dioxide was not measured in any of the bioassays.

We tested the influence of ammonia on egg fertilization and viability during the water-hardening stage by exposing some eggs to ammonia at Bowden Hatchery on the day our experimental eggs were collected. Approximately 200 to 300 eggs from one female were stripped into each of several pans containing tris buffered water (pH 8.3, temperature 8°-10°C), some with added ammonia at concentrations up to 1.79 mg/liter of un-ionized ammonia. Milt from at least two young males was stripped into each pan of water and eggs 15 to 30 s later. Buss and Corl (1966) determined that fertilization must be completed within the first 1 or 2 min because the sperm are viable in water for only a few seconds. By replacing the ammonia solutions with fresh water in one-half of the pans after 2 or 3 min of ammonia exposure and in the remaining pans after 1 h, we hoped to separate the effects of ammonia on fertilization per se from the effects on the viability of the fertilized eggs during the water-hardening stage of the first hour. The effects were measured by determining the percentage of eggs that hatched.

RESULTS AND DISCUSSION

Neither fertilized eggs, embryos, nor alevins (embryo after hatching) were susceptible to a 24-h exposure of un-ionized ammonia (3.58 mg/liter) until about the 50th day of development (Figure 1). At that time, susceptibility increased dramatically and continued to increase until most of the yolk was absorbed (when alevins became fry). The median tolerance limits (24-h TLm) for 85-day-old fry were 0.068 mg/liter, slightly less than the 0.097 mg/liter value we observed for adult trout; in the bioassays for both the fry and the adults, temperature was 10°C and pH was 8.3.

Buss and Corl (1966) found that the viability of eggs of brook trout, *Salvelinus fontinalis*, and

[3]24-h TLm = the concentration resulting in 50% survival after 24-h exposures.

[4]YSI = Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio. Reference to trade name does not imply endorsement by the National Marine Fisheries Service, NOAA.

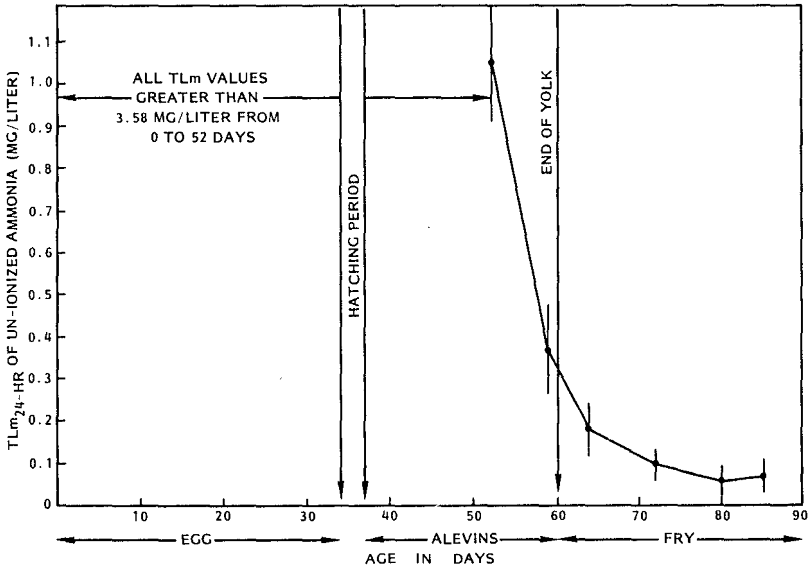

FIGURE 1.—Twenty-four-hour median tolerance limits (TLm) of un-ionized ammonia to eggs and alevins of rainbow trout (10°C, pH 8.3). Points indicate mean of two bioassays; bars indicate the range. Adult trout 24-h TLm was 0.097 mg/liter (10°C, pH 8.3).

brown trout, *Salmo trutta*, drops significantly after 15 s in water—in our experiments this was about the minimum time lapse between stripping eggs into the water and introduction of sperm. Because we did not control the time lapse before sperm introduction precisely enough, we cannot evaluate any subtle effects of ammonia on prevention of fertilization. It was obvious, however, that high ammonia concentrations did not cause complete loss of eggs or sperm (Table 1) because more than half of the eggs were fertilized at all ammonia exposures. No obvious differences in the percentages of eggs that hatched were noticed between ammonia exposures of 2 or 3 min and 1 h, even at the highest concentrations of un-ionized ammonia (1.79 mg/liter) we tested. The fertilization and water-hardening stages are similar to later stages (before 50 days of development) in their relative insensitivity to ammonia when compared with older fry with absorbed yolks (after about 60 days of development).

Our observations of great resistance of eggs and alevins of rainbow trout to ammonia toxicity are consistent with results of ammonia toxicity studies of Penaz (1965) and with other studies of other toxicants. Trout eggs and sac fry were only slightly susceptible to endrin at concentrations that seriously affected adults (Wenger 1973). Burdick et al. (1964) observed that a high proportion of lake trout, *Salvelinus namaycush*, fry from normal appearing eggs containing 2.95 ppm DDT or more died. The sensitive fry died at the completion of yolk absorption when feeding would normally begin. Eggs of "common trout" were less susceptible to anionic detergent toxicity (sodium alkylsulphate) than alevins, whose sensitivity continued to increase for 6 wk (Wurtz-Arlet 1959). Eggs of two salmonids were about one-tenth as sensitive to a commercial formulation of rotenone and derivatives as fry at the same temperature (Garrison 1968). A study of zinc toxicity by Skidmore (1965) showed that eggs of zebrafish, *Brachydanio rerio*, were relatively less susceptible than newly hatched fish.

It appears then that eggs and developing embryos are resistant to several toxicants, including ammonia. One obvious explanation for the resis-

TABLE 1. — Effect of ammonia on fertilization.

Concentration of un-ionized ammonia	Percentage[1] hatch at exposure of 2-3 min	1 h
0 mg/liter	66.8	68.4
0.0358 mg/liter	74.3	70.1
1.79 mg/liter	58.2	68.8

[1]Percentage hatch of each group of 250 eggs.

tance may be the protection afforded the embryo by the surrounding egg membranes which separate the internal from the external environment. However, in a second study of zinc toxicity to zebrafish embryos, Skidmore (1966) found no evidence of protection of the embryo by the egg membranes. He found that embryos with ruptured outer membranes actually survived longer in a zinc sulphate solution than embryos of the same age with an intact membrane. If the outer egg membrane impermeability were a major factor in preventing ammonia toxicity, all alevins would be instantly vulnerable at hatching. No sudden susceptibility to toxicants in newly hatched fish was observed in this study or in several others.

We can see no satisfactory explanation for the observed high resistance to ammonia and other toxicants during early developmental stages of teleosts. The higher resistance of sac fry than eggs to toxicants indicates that the egg membranes are not always protective barriers and that the explanation is more complex.

In our study, the susceptibility to ammonia developed during the transition from alevin to fry, toward the end of yolk absorption. This transition, although gradual, is probably more of a physiological change than the changes that occur at hatching. The newly hatched alevins are more "embryo" than "juvenile." They normally reside in the incubation gravels, have few voluntary responses to changes in their environment, and continue to develop by catabolizing their yolk. As the alevin develops and becomes prepared for emergence, susceptibility to some toxicants increases. The alevins are now more juvenile than embryo, even to the point of preemergent feeding as concluded by Dill (1967) for sockeye salmon alevins.

Our results indicate that rainbow trout embryos and alevins are safer from ammonia toxicity than are older salmonids (Burrows 1964; Larmoyeux and Piper 1973). A dramatic increase in the excretion of ammonia (Rice and Stokes in press) and sensitivity to ammonia appears to begin about the time the fry complete absorption of their yolk. Chronic exposure to ammonia would probably exert its greatest effects beginning at this stage also.

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