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Chronic Toxicity of Ammonia to Rainbow Trout

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Abstract

The chronic effects of ammonia to rainbow trout *Salmo gairdneri* were studied in a laboratory test conducted over a 5-year period. Fish were tested at five concentrations over the range 0.01–0.07 mg/liter un-ionized ammonia; the mean pH of the test water was 7.7, and the mean temperature was 9.3 C. Parental fish were exposed for 11 months, the first filial generation (F_1) for 4 years, and the second filial generation (F_2) for 5 months. The parental fish spawned of their own volition at all ammonia concentrations tested; baskets containing crushed rock served as the spawning substrate. The F_1 fish did not spawn voluntarily at either 3 or 4 years of age, although manual spawning of 4-year-old F_1 fish produced viable eggs. There was no significant correlation between ammonia concentration and numbers of egg lots spawned, total numbers of eggs produced, numbers of viable eggs, growth of progeny, or mortality of parents or progeny in any of the generations tested. Blood ammonia concentrations were measured in F_1 fish, and proved to be positively correlated with ammonia concentrations in the test water. Histopathological lesions were common in parental and F_1 fish at un-ionized ammonia concentrations of 0.04 mg/liter and higher; in F_2 fish, which incurred a severe protozoan infection (*Costia* sp.), lesions were common at 0.02 mg/liter and higher.

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Ammonia is widely recognized as a common pollutant in aquatic environments. In aqueous solution it assumes both an ionized form and an un-ionized form; the latter is bound to at least three water molecules (Butler 1964). The equilibrium relationship is (*g*, *l*, and *aq* denoting gas, liquid, and aqueous phases)

$$NH_{3(g)} + nH_2O_{(l)} \rightleftharpoons NH_3 \cdot nH_2O_{(aq)} \rightleftharpoons NH_4^+ + OH^- + (n-1)H_2O_{(l)}.$$

In this paper the un-ionized form of ammonia is expressed as NH_3 (molecular weight 17) and the ionized form as NH_4^+ (molecular weight 18). Total ammonia refers to the sum of these ($NH_3 + NH_4^+$), but is expressed as total ammonia-nitrogen, NH_3-N (atomic weight 14), which is the element commonly measured. The ammonia equilibrium strongly depends upon pH and, to a lesser extent, upon temperature and ionic strength. As either pH or temperature increases the equilibrium shift is toward the NH_3 species. Ionic-strength increase, within the range of most freshwater systems, results in an increase in the concentration of the NH_4^+ species (Emerson et al. 1975).

Early research on the toxic effects of ammonia to fishes implicated NH_3 as the toxic form, and the toxicity of NH_3 was considered to be relatively independent of pH; $NH_4^+$was considered to be nontoxic, or appreciably less toxic than NH_3 (Chipman 1934; Wuhrmann et al. 1947; Wuhrmann and Woker 1948). More recent research has shown that NH_3 is more toxic as the hydrogen ion concentration increases, at least below pH 7.5 (Tabata 1962; Robinson-Wilson and Seim 1975; Armstrong et al. 1978; Thurston 1980; Tomasso et al. 1980; Thurston$_3$ et al. 1981). Over the pH range 7.8–9.0, the acutely toxic effects of NH_3 on rainbow trout *Salmo gairdneri* appear to be relatively constant (Thurston$_3$ et al. 1981).

At the present time, both European and United States water-quality criteria for ammo-

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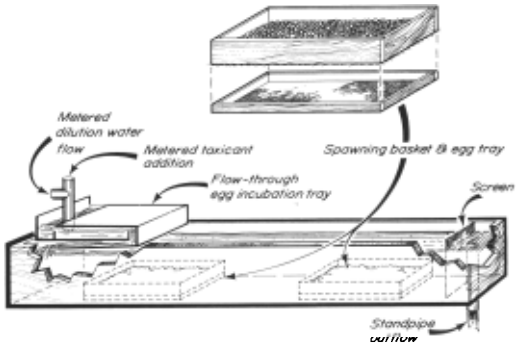

FIGURE 1.—*Linear spawning trough, egg incubation tray, and spawning baskets.*

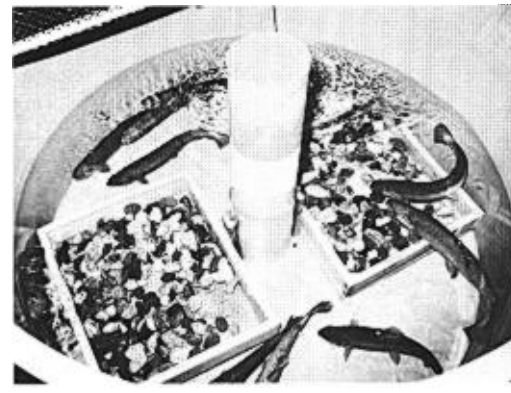

FIGURE 2.—*Circular tank with spawning baskets in place.*

nia are stated in terms of NH_3, regardless of pH. The European Inland Fisheries Advisory Commission (1970) has recommended a criterion of 0.025 mg/liter NH_3 at temperatures above 5 C and below pH 8.5, and the United States Environmental Protection Agency (US-EPA 1977) has recommended a criterion of 0.02 mg/liter NH_3. These criteria documents, and reviews by Willingham et al. (1979) and Alabaster and Lloyd (1980), summarize much of the available literature on ammonia toxicity to aquatic organisms.

Reported median lethal concentrations of NH_3 in 4-day tests range from 0.083 to 1.1 mg/liter for salmonids, and from 0.14 to 4.6 mg/liter for nonsalmonids. In 1-week to 3-month tests, fish have evidenced reduced food uptake and growth inhibition at 0.05 to 0.15 mg/liter NH_3 (Department of the Environment 1972; Robinette 1976; Schulze-Wiehenbrauck 1976; Burkhalter and Kaya 1977); swelling and diminishing of red blood cells, irreversible blood damage, inflammation and degeneration of gills and other tissues, and increased susceptibility to disease at 0.06 to 0.4 mg/liter NH_3 (Reichenbach-Klinke 1967; Flis 1968; Smart 1976); and extensive hyperplasia of the gill epithelium at 0.006 mg/liter NH_3 (Burrows 1964). In 6-month tests on rainbow trout, 0.017 mg/liter NH_3 caused both reduced growth rates and pathologic changes in gills and livers (Smith and Piper 1975).

The present study was undertaken to determine the sublethal effects of ammonia on rainbow trout throughout their life cycle. A laboratory toxicity test was started with 30-month-old fish (the parental generation) that spawned 5 to 7 months later, progeny from that spawn (the F_1 generation) were reared for more than 4 years, and their progeny (the F_2 generation) were reared for 5 months. The concentrations chosen for testing ranged from 0.01 to 0.07 mg/liter NH_3, so as to span the water-quality criteria values for NH_3 commonly adopted at the time the test was initiated. The test water was known to have a pH of approximately 7.7, and long-term variations in temperature between approximately 7.5 and 10.5 C. The entire test lasted 5 years, and data collected on the test fish included spawning success, egg viability, growth rates, and mortality. Certain blood variables also were measured, and histopathological examinations were made on selected tissues from fish of all three generations.

There are no previous reports in the literature of any full life-cycle toxicity test of ammonia to any fish species, nor are we aware of any previous report of a life-cycle toxicity test on rainbow trout for any toxicant.

Methods

Experimental Design and Test Procedure

The test was conducted between July 1974 and July 1979 at the Montana State University Fisheries Bioassay Laboratory, located at the Bozeman (Montana) Fish Cultural Development Center, United States Fish and Wildlife Service. Fish were tested in six stainless steel troughs (Fig. 1), one control trough and five test troughs each at a different concentration of ammonia. Dimensions of the troughs were 3.7 × 0.38 m and water depth was 28 cm; water inflow rate to each trough was 18 liters/minute

and water-exchange time was 22 minutes. On three occasions during the test, each lasting for about 2 months during the spawning seasons, some of the fish from each of the troughs were transferred to a companion set of six fiberglass circular tanks (Fig. 2). Concentrations of ammonia in the tanks were similar to those in the troughs. The tanks were 1.2 m in diameter and water depth was 32 cm; water-inflow rate to each tank was 18 liters/minute and the water exchange time was 20 minutes.

A solution of ammonium chloride (reagent grade) was added to the dilution water by means of fixed-speed pumps (Masterflex, Barnant Corporation) as the water entered the test troughs and tanks. The rate of addition of toxicant solution was different for each of the troughs (and tanks) so as to achieve the desired range of ammonia concentrations (0.01 to 0.07 mg/liter NH_3). Dilution water was obtained from a groundwater spring located at the Fish Cultural Development Center. The chemical composition of this water (Thurston$_1$ et al. 1981) remained fairly constant, and showed only slight seasonal variations in pH, temperature, hardness, and alkalinity.

The test fish were Ennis strain rainbow trout. This strain, from the Ennis (Montana) National Fish Hatchery, had been bred over a period of years to spawn between November and January. These fish were reported to be free from any possible hybridization with cutthroat trout *Salmo clarki*. Before the test, eggs from a single pair of adults were incubated in the laboratory. The young were then reared in an outdoor raceway until adulthood; they were 30 months old at the start of the test, and had a mean weight of 604 g. Fish were fed a Fish and Wildlife Service hatchery production diet prepared either by Rangen, Incorporated (Buhl, Idaho) or Murray Elevator (Murray, Utah) before and during the test.

At the beginning of the test, approximately 10 males and 20 females, randomly selected from the outdoor raceway, were placed into each of the troughs. Four and one-half months later the fish were examined, after being anesthetized with dilute tricaine methane sulfonate solution, to determine their sex and spawning condition. Eight males and 12–14 females from each trough, all in or near spawning condition, were retained for further testing. Half of these were tested in the troughs and the other half in the corresponding tanks. Blood and tissue analyses were made on a random sample of the remaining fish from each trough prior to their being discarded. Two wooden spawning baskets and egg collection trays, modified from a design described by Benoit (1974), were placed into each of the troughs and tanks (Figs. 1 and 2). The baskets had a wire screen-cloth bottom (1.3-cm mesh) and contained a 5–6-cm-deep layer of crushed rock (2–4-cm diameter); the trays had a nylon web (0.4-cm mesh) bottom.

Periodically over the next 2 months, the fish dug redds in the rocks in the baskets and spawned, and the resultant spawn passed through the wire mesh into the egg-collection trays. Each morning any eggs produced during the previous 24 hours were transferred from the egg-collection trays to incubators. At the end of the spawning period, four to six parental fish from each trough and tank were sacrificed, and tissues from each were examined. The remaining fish in the troughs were continued under test; the fish from the tanks were pooled in a single tank and subsequently maintained for the next 4 months without toxicant addition. Before they were pooled, these fish were finclipped to designate the tank from which they had been taken. Both groups of parental fish, those that remained under test and those transferred to ammonia-free water, were sacrificed and examined 11 months into the test.

Eggs were incubated in two kinds of trays. The first several lots of eggs produced at each ammonia concentration were transferred to flow-through incubator trays (Heath Tecna) positioned at the head of the troughs in such a way that the inflowing water passed through them (Fig. 1). The trays were partitioned to accommodate six egg lots. Subsequent egg lots produced at each concentration were incubated in a wood-frame tray with a nylon-mesh bottom; the tray was partitioned to accommodate 12 egg lots. One tray was positioned, partially submerged, near the head of each trough in such a way that the flowing water passed across the bottom of the eggs. All trays were covered to protect the eggs from direct light. Egg lots produced after all available incubator compartments at any concentration were filled were counted and discarded. Fertility of egg lots was determined after 8 to 10 days by examining a

sample of eggs placed in dilute acetic acid solution; infertile egg lots were discarded. Each lot was examined for eyed eggs after an additional 18 to 21 days; live eggs were retained and dead eggs discarded at least every 2 days thereafter.

After hatching (30–34 days), the F_1 larvae were maintained in the incubator trays for an additional 20–22 days until they reached "swim-up" stage. For the next 3 to 5 months, juvenile lots in each trough were maintained separately in screen-bottomed plastic buckets (8-liter capacity) suspended within the troughs. Fish were fed beginning when they were placed in these buckets. When the F_1 fish were 6 months old, five lots in each trough were retained for further testing and all others were discarded. Lots selected for retention had all hatched during the first 2 weeks of January from eggs spawned during the previous month; this selection was made so that all fish retained would be approximately the same age. Between 6 and 14 months of age, the fish lots were maintained separately within the troughs in wooden-framed holding baskets (30 × 30 × 30 cm) with nylon-mesh sides and bottoms. At 14 months, the fish were finclipped to designate lot number, and the fish within each trough were pooled. At 27 months, 7 to 10 fish were selected at random from each lot (42 to 48 fish in each trough) and retained for further testing; the balance were discarded.

When the F_1 fish were 35 months old they were divided between troughs and tanks; two spawning baskets were placed in each trough and tank as before. Eggs produced in the spawning baskets were handled as described earlier, but only a single egg lot reached hatching stage. After 2 months the spawning baskets were removed, and the fish were combined in the troughs. When the fish were 46 months old they were examined and only those in spawning condition were retained in the troughs, to which spawning baskets were again added. The unripe fish were temporarily removed to the tanks. Some egg lots were produced, although none reached hatching stage. Because voluntary spawning by the F_1 fish was not producing the desired results, ripe females were manually stripped when they were 48 months old; milt from two or three males from the corresponding ammonia exposure concentration was used to fertilize the eggs in water from the same trough from which the fish were taken. The same procedure was repeated 1 month later. Eggs produced were incubated in flow-through trays; additional trays were added as needed.

When the F_1 fish were 52 months old they were sacrificed, and five fish of each sex from each trough were examined for tissue condition, including blood analysis. The F_2 fry were reared until they were 4 or 5 months of age and then were sacrificed. The test was concluded at that point, 5 years from the day it started.

Water Chemistry

At the start of the test, several measurements for total ammonia-nitrogen (NH_3-N) and temperature were taken simultaneously from several locations throughout each trough and tank to determine if toxicant addition and mixing were complete or if stratification might be occurring; results of all measurements in any trough or tank were the same. Throughout the test, dissolved oxygen, temperature, pH, and NH_3-N were measured in each trough and tank at least weekly for the first year and approximately every 4 days thereafter. Nitrate (NO_3-N) and nitrite (NO_2-N) were measured every 7–10 days, and hardness and alkalinity were measured in at least one trough and tank each week. Temperature was measured with a calibrated mercury thermometer, pH with a digital meter (Beckman Phasar-I), and dissolved oxygen with a meter (Yellow Springs Instrument Model 54) standardized by comparison with results of a Winkler test (APHA et al. 1971, 1976). Nitrite was determined according to the method of the USEPA (1974), and alkalinity, hardness, NO_3-N, and NH_3-N according to the methods in APHA et al. (1971, 1976); the nesslerization method was used to determine NH_3-N. Colorimetric measurements were made with a Varian-635 ultraviolet–visible spectrophotometer. Concentrations of NH_3 were calculated from NH_3-N measurements, the formulas of Emerson et al. (1975), and the table of Thurston et al. (1979).

The 5-year mean values for the water chemistry measurements from each trough are summarized in Table 1; the 2-month mean values for measurements in the tanks during the three mid-winter spawning periods were similar. The quarterly averages for NH_3 for all six troughs

TABLE 1.—*Five-year summary of water chemistry measurements in troughs. (Mean values reported; SDs and numbers of samples are in parentheses.)*

Trough	Alkalinity as $CaCO_3$, mg/liter	Hardness as $CaCO_3$, mg/liter	NO_3-N, mg/liter	NO_2-N, mg/liter	Dissolved oxygen, mg/liter	pH[a]
1	174	197	0.17	0.00	7.5	7.72
	(5.3,153)	(7.7,155)	(0.07,200)	(0.00,204)	(0.9,440)	(−0.10,+0.13,435)
2	173	197	0.20	0.01	7.6	7.72
	(5.3,145)	(7.9,147)	(0.08,195)	(0.01,200)	(0.8,440)	(−0.10,+0.12,433)
3	173	197	0.20	0.01	7.4	7.71
	(5.3,148)	(8.0,150)	(0.09,195)	(0.02,201)	(0.8,440)	(−0.10,+0.11,434)
4	173	198	0.22	0.02	7.3	7.70
	(5.2,146)	(7.9,148)	(0.09,196)	(0.03,201)	(0.8,440)	(−0.09,+0.11,432)
5	173	198	0.21	0.02	7.4	7.69
	(5.2,147)	(7.9,149)	(0.09,195)	(0.02,200)	(0.9,440)	(−0.09,+0.12,433)
6	173	198	0.20	0.02	7.4	7.70
	(5.5,151)	(8.0,153)	(0.09,200)	(0.03,203)	(0.9,440)	(−0.09,+0.11,433)

[a] Standard deviations reported in pH units.

are plotted in Fig. 3. Throughout the 5 years of the test there were some changes upward and downward in the ammonia concentrations in all of the troughs, but most noticeably in troughs 5 and 6. These changes resulted principally from the difficulties of maintaining the toxicant metering pumps at constant flow rates, although long-term variations in water pH and temperature were also partly responsible for the fluctuations in NH_3. On three occasions during the 5 years, electrical power failures resulted in the temporary suspension of toxicant metering; the longest suspension was 14 hours. Metering pump failures resulted in suspension of toxicant addition for 1- to 3-day periods to troughs 3 and 4 when the F_1 fish were 1 year old, to trough 5 when the F_1 fish were 2 years old, and to troughs 2 and 6 when the F_1 fish were 1 and 3 years old. We believe these relatively short time periods without toxicant did not significantly affect the test results.

Hematology and Histopathology

Blood was obtained from the fish after they were immobilized by a sharp blow to the head. The caudal peduncle was severed immediately posterior to the adipose fin, and blood was collected from the caudal vessels. Blood NH_3-N was measured by the method of Seligson and Hirahara (1957), and pH was measured aerobically on an untreated sample (within 20–30 seconds after collection) with a combination electrode cooled to water temperature and standardized between pH 7 and 8. Hematocrits were measured by a microhematocrit method, and hemoglobin by the cyanamet hemoglobin method (Hycel Incorporated, Houston, Texas). Tissue samples for histopathological examinations were taken from freshly killed fish, preserved in Bouin's solution for 24 to 48 hours, and stored in 65% ethanol; paraffin sections were cut to 5-μm thickness and stained with hematoxylin and eosin.

Histopathological examinations were made on tissues of parental fish collected after 4 months of exposure to ammonia (five to nine fish/trough), after 7 months exposure (five fish/trough), and after 11 months exposure when all parental fish were sacrificed (six to eight fish/trough). In addition, the parental fish that had been moved from the tanks after the spawning season and maintained in an ammonia-free environment for the next 4 months were examined (six to eight fish from each of the prior ammonia concentrations). Tissues of F_1 fish were examined when these fish were 6 months old (50–83 fish/trough), 15 months old (25 fish/trough), and 52 months old (10 fish/trough). Tissues of the F_2 fish were examined at the conclusion of the study, when they were 4 or 5 months old (10 fish/trough).

Data Treatment

Average pH values were obtained by converting each measurement to the hydrogen-ion concentration, averaging these, then reconverting to pH units. The NH_3 values reported were computed individually from each NH_3-N measurement and the nearest (in time) measurements of pH and temperature. Consider-

TABLE 1.—*Extended.*

Temperature, C	NH_3-N, mg/liter	NH_3, mg/liter
9.3	0.11	0.001
(0.9,457)	(0.11,459)	(0.001,459)
9.3	1.16	0.013
(0.9,455)	(0.24,461)	(0.004,461)
9.3	1.95	0.022
(0.9,456)	(0.28,461)	(0.006,461)
9.3	4.01	0.044
(0.9,454)	(0.43,463)	(0.010,463)
9.3	5.76	0.063
(0.9,455)	(0.65,464)	(0.015,464)
9.3	6.71	0.074
(0.9,456)	(0.70,464)	(0.016,464)

ation was given to the length of the period (2 to 7 days) between NH_3-N measurements: for computation of average ammonia values in each trough, each measurement was weighted to cover half the time back to the next earlier measurement and ahead to the next later one. In the results section below, in which biological data are reported for different time periods during the life of the test, the NH_3-N and NH_3 values reported are the arithmetic means and standard deviations (SD) for those time periods.

Results from each portion of the study first were analyzed to determine if there were statistically significant differences among troughs; analysis-of-variance *F*-tests (Neter and Wasserman 1974), chi-squared contingency-table tests (Neter and Wasserman 1974), and Spearman rank correlation tests (Snedecor and Cochran 1980) were used. If the *P*-value obtained was less than 0.05, an analysis was conducted to determine if the differences were related to ammonia concentration. Armitage trend tests (Armitage 1971), Jonckheere trend tests (Jonckheere 1954), and linear-regression *t*-tests (Snedecor and Cochran 1980) were used for this purpose. A correlation with ammonia concentration was assumed if the *P*-value obtained was less than 0.05.

Results

Spawning and Early Development

The parental fish began spawning within 5 months from the start of the test, and within 1 week after the spawning baskets were introduced. Spawning usually took place during the night or early morning and little of this activity was observed. Some of the egg lots produced probably resulted from multiple spawnings in the same basket between collections because distinct differences in egg sizes occasionally were noticed in some of the egg lots. During the first 5 weeks of the spawning period, the combined production of eggs by the parental fish in all troughs and tanks was at the rate of one to six lots per day; during the next 2 weeks this dropped to less than one lot per day. The spawning baskets were removed after 53 days.

From 5 to 15 egg lots were produced in each trough or tank, of which 3 to 11 proved to be fertile (Table 2). There was no significant correlation ($P > 0.5$) in either troughs or tanks between ammonia concentration and number of egg lots produced, eggs produced per female, or number of fertile egg lots, nor was there a difference ($P \geq 0.5$) between troughs and tanks for any of these three variables. The overall hatching success for eggs placed in the flow-through incubators was 46% and that for eggs incubated in the wooden trays was less than 5%; inadequate water circulation through the floating trays resulted in heavy fungus accumulation and subsequent egg loss. As a result, only the progeny from eggs incubated in the flow-through incubators were carried forward in the test. There was no significant correlation between ammonia concentration and hatching success of the eggs in the flow-through incubators ($P > 0.5$).

Voluntary spawning by the F_1 fish was limited. At each ammonia concentration, 3-year-old fish produced 1 to 23 egg lots per 14 females, and 4-year-old fish produced 0 to 13 egg lots per seven females. There was no significant relationship between egg production and ammonia concentration either year, and only one lot (of about 500 eggs produced by age-3 fish) proved to be fertile; approximately half of these eggs hatched after 42 days' incubation at about 10 C, and the young were discarded after 2 months.

When the F_1 fish were manually spawned at 48 and 49 months of age, from 2 to 14 egg lots were produced at each ammonia concentration; two to seven of these lots proved to be fertile (Table 3). The hatching success of these fertile egg lots was 61% (range 29–90%). There was no significant correlation between ammonia concentration and either eggs produced per female ($P > 0.5$) or percent hatching success of those eggs ($P > 0.4$).

Prior to development of the spawning-basket

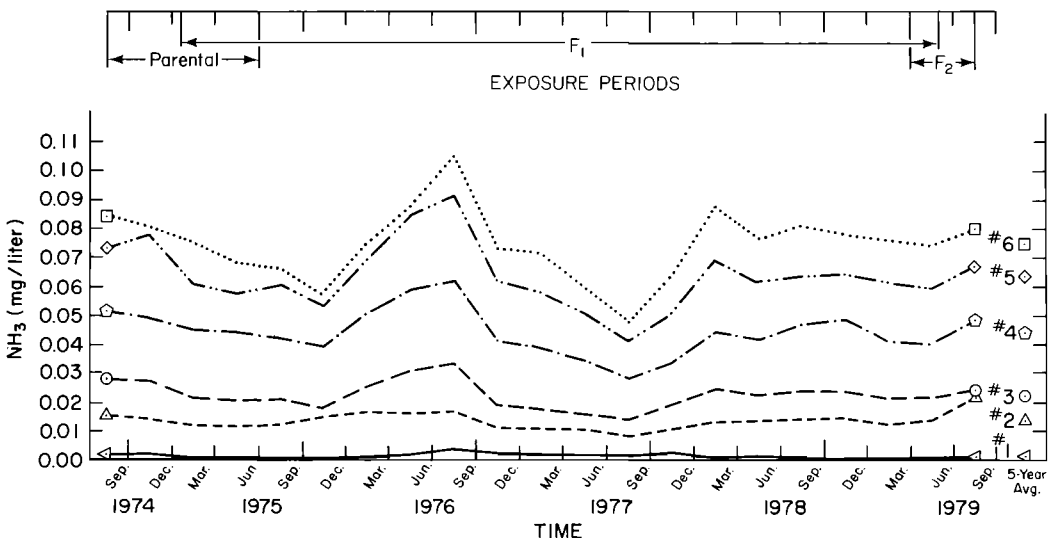

FIGURE 3.—*Quarterly averages of un-ionized ammonia in linear troughs, Jul 1974–Jul 1979.*

design used in this experiment, several other methods were tried to induce spawning of wild rainbow trout collected by electrofishing in local streams during early spring and of hatchery (Ennis strain) fish. One of the successful methods involved simulation of natural stream-bottom conditions. A 6–8-cm-thick substrate of rock, sand, and gravel was placed directly on the bottom of a trough. Water was introduced from perforated pipes that ran the length of the trough under the spawning substrate. Three pairs of wild fish, collected just 4 hours earlier, were each placed in separate sections of the trough. One pair spawned within 24 hours; the eggs were left in the gravel to incubate and the gravel was removed when larvae were observed. Over 2,000 larvae were counted, but of this number approximately 75% died within 24 hours, apparently injured during the process of removing the gravel and rocks. The substrate was not disturbed in the other two sections, and from one of these, over 1,500 "swim-up" young eventually were collected; in the other, the fish did not spawn. A fourth pair of wild fish were placed in a trough with only rocks as the substrate. This pair also spawned within 24 hours after collection, resulting eventually in over 1,000 swim-up young.

An important observation is that collection by electrofishing did not impair the ability of the fish to spawn. A second observation is that a substrate of rocks alone proved adequate to stimulate the spawning process.

Mortality and Growth

There was no significant relationship between ammonia concentration and mortality for any of the three generations tested (Table 4). Mortality rates for parental fish were determined for three separate time periods, and for F_1 fish for seven separate time periods; in none of these was there a significant difference among troughs ($P = 0.14$ to 0.95). During one of these periods, when the F_1 fish were between 15 and 19 months old, the mean NH_3 concentration in trough 6 was 0.11 mg/liter for 98 days; this included a peak of 0.15 mg/liter NH_3, which we estimate was sustained for 2 days, when the fish were 18 months old. Statistically significant differences in mortality among troughs were detected for the F_2 progeny, although these were not related to ammonia concentration ($P > 0.6$). P-values for the data in Table 4 are >0.5 for both the parental and F_1 generations, and >0.2 for the F_2 generation.

There was no statistically significant relationship between ammonia concentration and growth for either the F_1 or F_2 fish (Table 5); determination of such a relationship for the parental fish was not possible because these fish had not been weighed either individually or collectively by trough at the start of the test. Al-

TABLE 2.—*Production and hatching success of eggs from parental rainbow trout.*

Trough or tank	Spawning: Exposure concentration,[b] mg NH_3/liter, mean (SD,*N*)	Spawning: Number of females	Spawning: Egg lots produced	Spawning: Eggs produced per female	Spawning: Fertile egg lots produced	Egg incubation[a]: Exposure concentration,[c] mg NH_3/liter, mean (SD,*N*)	Egg incubation[a]: Fertile egg lots incubated	Egg incubation[a]: Total eggs incubated	Egg incubation[a]: Percent hatching success
				Troughs					
1	0.000 (0.001,9)	7	11	1,077	10	0.000 (0.000,12)	5	4,382	10
2	0.012 (0.002,9)	6	5	744	5	0.012 (0.001,12)	2	1,379	13
3	0.022 (0.003,9)	6	8	893	7	0.022 (0.003,12)	4	2,581	86
4	0.044 (0.009,9)	7	11	955	9	0.044 (0.008,12)	5	3,200	97
5	0.059 (0.008,9)	7	15	1,233	11	0.060 (0.008,12)	5	2,407	29
6	0.072 (0.011,9)	7	6	493	3	0.073 (0.009,12)	0[d]		
				Tanks					
1	0.001 (0.000,9)	7	7	827	7	0.000 (0.000,12)	1	635	96
2	0.011 (0.002,8)	6	7	870	7	0.012 (0.001,12)	4	4,299	22
3	0.019 (0.003,9)	6	9	1,056	4	0.022 (0.003,12)	2	1,781	82
4	0.034 (0.005,9)	7	12	1,177	10	0.044 (0.008,12)	1	1,149	22
5	0.070 (0.011,9)	7	5	629	5	0.060 (0.008,12)	1	387	76
6	0.080 (0.012,9)	6	11	1,228	9	0.073 (0.009,12)	6	4,151	66

[a] Only egg lots incubated in flow-through trays are reported.
[b] Exposure concentrations are those for the 7-week spawning period.
[c] Exposure concentrations are those for the 9-week period during which these egg lots were incubated.
[d] None of the fertile egg lots spawned in trough 6 were incubated in flow-through tray.

though there were significant differences in weight and length among troughs at both 15 and 21 months, these were not related to ammonia concentration ($P \geq 0.18$); there were no significant differences among troughs at either 10 or 52 months ($P > 0.09$). Measurements of the F_2 fish at the conclusion of the test, when the lots of fish were either 4 or 5 months old, also showed no significant correlation between ammonia concentration and either weight or length ($P \geq 0.07$).

Hematology

Hematocrit and hemoglobin measurements on blood of parental fish after 4 months' exposure showed no significant differences among troughs ($P \geq 0.12$). The mean hematocrit and hemoglobin values were 36.2% (SD = 8.2; N = 42) and 7.9 g/100 ml (SD = 2.2; N = 42), respectively. Some values obtained were below those expected for healthy fish, and it was evident these fish were anemic. Examination of blood smears and of liver and kidney tissues showed that the fish from all troughs suffered from microcytic anemia and ceroidosis. Such conditions are frequently caused by rancid feed (Smith 1979), so the suspect lot of feed was discarded. Hematocrit and hemoglobin measurements of parental fish taken after the spawning season (7 months' total exposure) also

TABLE 3.—*Hatching success of eggs from F_1 rainbow trout.*

Trough	Exposure concentration,[a] mg NH_3/liter, mean (SD,*N*)	Females spawned and egg lots produced	Eggs produced per female	Fertile egg lots produced and incubated	Total eggs incubated	Percent hatching success
1	0.000 (0.000,24)	9	939	6	5,557	72
2	0.012 (0.002,24)	14	878	7	6,535	90
3	0.020 (0.003,24)	4	1,592	3	4,946	39
4	0.040 (0.006,24)	2	1,316	2	2,632	29
5	0.060 (0.008,24)	8	899	4	3,514	64
6	0.077 (0.010,24)	7	977	7	6,837	50

[a] Exposure concentrations are those for the 12-week period during which all egg lots were incubated.

TABLE 4.—*Mortality rates of parental, F_1, and F_2 rainbow trout.*

Trough	Parental fish: 11 months (Jul 1974–Jun 1975)		F_1 fish: 52 months (Jan 1975–May 1979)		F_2 fish: 5 months (Mar 1979–Jul 1979)	
	Exposure concentration, mg NH_3/liter, mean (SD,*N*)	Percent mortality per month	Exposure concentration, mg NH_3/liter, mean (SD,*N*)	Percent mortality per month	Exposure concentration, mg NH_3/liter, mean (SD,*N*)	Percent mortality per month
1	0.001 (0.001,47)	3.4	0.001 (0.001,433)	2.8	0.000 (0.000,42)	24.0
2	0.013 (0.003,49)	0.8	0.013 (0.004,434)	2.2	0.016 (0.005,42)	12.3
3	0.024 (0.005,49)	0.9	0.022 (0.006,434)	2.4	0.021 (0.003,42)	24.6
4	0.047 (0.007,51)	2.9	0.044 (0.010,434)	2.2	0.042 (0.007,42)	18.1
5	0.067 (0.014,51)	2.3	0.062 (0.014,435)	2.3	0.061 (0.008,42)	14.6
6	0.076 (0.013,51)	2.3	0.073 (0.016,435)	2.9	0.076 (0.009,42)	17.3

showed no significant differences among troughs ($P \geq 0.33$), nor had the fish recovered from their anemic condition. The mean hematocrit and hemoglobin values were 31.0% (SD = 8.3; N = 30) and 7.2 g/100 ml (SD = 2.2; N = 29).

Measurements after 11 months of exposure showed that the parental fish had made significant recovery from their anemic condition; mean hematocrit and hemoglobin values for the fish from troughs 1 through 4 were at or near normal (Larsen and Snieszko 1961; Snieszko 1961). These were 44.5% (SD = 6.3; N = 28) and 10.1 g/100 ml (SD = 1.6; N = 29). There was no correlation between these values and ammonia concentration ($P \geq 0.6$). The fish from troughs 5 and 6, however, did have significantly lower mean hematocrit ($P < 0.03$) and hemoglobin ($P = 0.05$) values than those in troughs 1 through 4. The hematocrit values for the fish from troughs 5 and 6 were 36.1% (SD = 4.6; N = 7) and 36.2% (SD = 6.4; N = 6); the hemoglobin values were 8.3 g/100 ml (SD = 1.2; N = 7) and 8.3 g/100 ml (SD = 1.4; N = 6). Measurements on the blood of parental fish exposed to ammonia for 7 months and then placed in a recovery environment for 4 months did not reveal any correlation with the ammonia concentration to which they had been exposed for the 7-month period ($P \geq 0.6$). Mean hematocrit value for these fish was 46.6% (SD = 6.2, N = 28), and mean hemoglobin value was 10.3 g/100 ml (SD = 1.7; N = 38).

Hematocrit measurements of F_1 fish after 52 months' exposure to ammonia showed no significant differences among troughs ($P = 0.49$); the mean value was 50.8% (SD = 8.9; N = 38).

TABLE 5.—*Weights and lengths of F_1 rainbow trout at 10, 15, 21, and 52 months. (Mean values reported; SDs and numbers of samples are in parentheses.)*

Trough	Exposure concentration, mg NH_3/liter	Age 10 months[a] Weight, g	Age 15 months[a] Weight, g	Age 21 months[b] Weight, g	Age 21 months[b] Length, cm	Age 52 months[b] Weight, g	Age 52 months[b] Length, cm
1	0.001	23.9	52.5	251	25.7	1,343	47.1
	(0.001,433)	(2.6,150)	(3.7,147)	(70,44)	(2.5,44)	(328,10)	(4.0,10)
2	0.013	24.1	47.3	218	24.2	1,093	44.3
	(0.004,434)	(2.2,147)	(3.9,146)	(46,45)	(2.1,45)	(320,10)	(4.8,10)
3	0.022	23.4	47.0	207	23.9	1,413	45.3
	(0.006,434)	(0.8,150)	(1.2,150)	(51,48)	(2.2,48)	(370,10)	(3.7,10)
4	0.044	23.1	50.7	209	24.5	1,240	44.9
	(0.010,434)	(0.8,150)	(2.3,149)	(55,50)	(2.2,50)	(287,10)	(2.5,10)
5	0.062	23.2	47.9	207	24.7	1,367	47.7
	(0.014,435)	(0.9,150)	(2.0,149)	(48,47)	(2.3,47)	(294,10)	(3.1,10)
6	0.073	23.7	52.1	232	25.0	1,114	44.3
	(0.016,435)	(1.5,150)	(2.5,149)	(55,48)	(2.3,48)	(158,10)	(2.0,10)
1–6		23.6	49.6	220	24.7	1,262	45.6
		(1.7,897)	(3.8,890)	(56,282)	(2.3,282)	(313,60)	(3.6,60)

[a] Fish in each trough were weighed in five lots of 26–30 fish/lot. Mean and SD values reported are those of lots, weighted to consider number of fish in each lot.

[b] Fish were weighed and measured individually.

TABLE 6.—*Blood ammonia, pH, and hematocrit measurements for F_1 rainbow trout at 52 months.*

	Water measurements[a]				Blood measurements			
Trough	Temperature, C	pH	NH_3-N, mg/liter	NH_3, mg/liter	pH mean (SD,*N*)	NH_3-N, mg/liter, mean (SD,*N*)	NH_3,[b] mg/liter, mean (SD,*N*)	Hematocrit, % whole blood, mean (SD,*N*)
1	7.5	7.71	0.02	0.000	7.44 (−0.17,+0.27,10)	7.19 (0.81,9)	0.032 (0.012,9)	50.8 (9.6,10)
2	7.5	7.73	1.31	0.013	7.50 (−0.13,+0.17,9)	8.47 (1.69,8)	0.038 (0.010,7)	54.8 (12.2,10)
3	7.5	7.73	2.21	0.022	7.56 (−0.09,+0.12,10)	8.24 (2.33,9)	0.047 (0.017,9)	50.7 (5.7,10)
4	7.5	7.74	3.81	0.040	7.49 (−0.10,+0.13,10)	10.9 (3.86,9)	0.051 (0.019,9)	48.9 (7.2,10)
5	7.5	7.73	5.59	0.057	7.50 (−0.07,+0.08,10)	14.5 (3.89,10)	0.070 (0.020,10)	52.2 (6.9,10)
6	7.4	7.75	7.12	0.076	7.55 (−0.15,+0.24,9)	13.8 (4.79,10)	0.072 (0.034,9)	47.1 (10.2,10)

[a] The water measurements reported are those taken during a 3-day period when fish were sacrificed for blood measurements. Water temperature range for all troughs was 7.3–7.8 C. Other reported water values were the same each time measured.

[b] NH_3 was calculated from total NH_3-N by formulas of Emerson et al. (1975) corrected for ionic strength of trout plasma (about 0.152 mM).

Hemoglobin was not measured in the blood of F_1 fish.

Ammonia concentrations, both total and unionized, in the blood of F_1 fish (Table 6) were significantly different among troughs ($P \leq 0.0006$). The Jonkheere trend test showed that these differences were correlated with NH_3 concentrations in the test water ($P \leq 0.0001$).

Histopathology

Examination of tissues from parental fish after 4, 7, and 11 months' exposure to ammonia revealed mildly to moderately scattered fusion of gill lamellae, separation of epithelia from underlying basement membrane, and telangiectasia (aneurysms). Large accumulations of melanin were dispersed throughout hematopoietic tissue of kidneys of most fish; some fish had glomerulosclerosis. Amorphous eosinophilic material was present around renal tubules in some instances. Various amounts of ceroid pigment were present in livers and hematopoietic tissues of some fish from all troughs. Hearts and spleens of all fish were normal. There appeared to be no correlation between the above changes and ammonia concentration.

Histopathological changes interpreted to be caused by ammonia were apparent in gills of parental fish from troughs 3–6 after 4 months' exposure, and from troughs 5 and 6 after 7 and 11 months' exposure. These alterations included extensive hypertrophy of lamellar epithelium and mild diffuse necrosis of individual epithelial cells (Fig. 4). Nephrosis was apparent in parental fish from all troughs at each examination; severity of nephrosis was directly correlated with ammonia concentration. Extensive hyaline droplet degeneration was apparent in kidney tubules of fish from the two highest ammonia concentrations (Fig. 5). After 4, 7, and 11 months, various amounts of ceroid pigment were present in livers and hematopoietic tissues of some parental fish from each group, including controls. The pigment was more abundant in kidneys of fish from troughs 4 and 5 and tanks 4–6 at 4 months, and troughs 5 and 6 after 7 and 11 months.

Examination of tissues from parental fish exposed to ammonia for 7 months but not for the next 4 months revealed some degree of the nonspecific tissue conditions present in parental fish that had remained under test for 11 months. However, the degenerative changes seen in the gill epithelium and kidney tubules of the fish under test for the longer period were not apparent in the fish from the recovery environment.

Gill tissues of F_1 fish at 6 months demonstrated a progressive increase (troughs 1–6) in scattered fusion of gill epithelium, hyperplasia of gill epithelium at bases of lamellae, hypertrophy of epithelial cells, and telangiectasia (Figs. 6 and

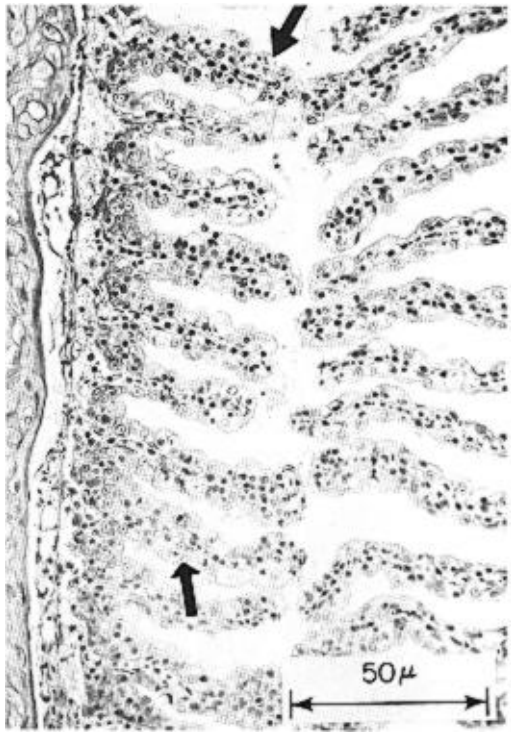

FIGURE 4.—*Extensive hypertrophy of epithelium covering gill lamellae of parental fish from trough 6. Note also mild scattered necrosis (arrows).*

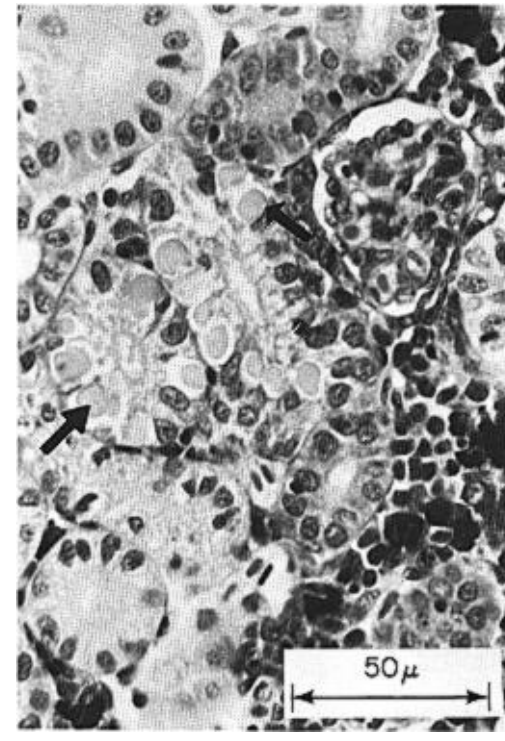

FIGURE 5.—*Hyaline droplet degeneration of epithelium of renal tubules of the first proximal segment (arrows) of parental fish from trough 5.*

7). Hyaline droplet degeneration was apparent in the first proximal segment of kidney tubules of fish from each trough; it was minor in the fish from trough 1 and extensive in tubules of fish from troughs 4–6. In addition, mild nephrosis was apparent in kidneys of fish from troughs 4–6. Livers of fish from all troughs were normal.

Gill tissues of F_1 fish after 15 months' exposure showed scattered fusion of lamellae, the severity of which increased progessively in troughs 1–6; occasionally fusion of several lamellae on a filament was evident. Hypertrophy of gill epithelium was apparent in fish from troughs 3–6, increasing in severity as the ammonia concentrations increased. Mild nephrosis was apparent in kidneys of fish from troughs 4–6; the incidence was directly correlated with ammonia concentration and was characterized by swollen epithelia with finely granular cytoplasm. Occasionally, lumina of such tubules were almost completely occluded (Fig. 8). There was no marked difference in livers of test fish when compared with controls.

The most consistent pathologic change in F_1 fish after 52 months' exposure to ammonia was telangiectasia in gills of fish from troughs 3, 4, and 6, but not from trough 5. Proliferated epithelium often surrounded the lesion. There was little difference in kidneys of controls and test fish. Kidneys of females from all troughs showed moderate to severe glomerulosclerosis; many of these kidneys had eosinophilic debris that was being phagocytized by macrophages scattered in hematopoietic tissue surrounding renal tubules. Occasionally, the material was present in tubule lumena.

Prior to examination at 4 and 5 months, the F_2 fish incurred a severe infestation of a protozoan parasite, *Costia* sp.; histological examination of gill tissues confirmed this diagnosis. Because of the pathologic changes resulting from this infestation, it was not possible for us to discern any effects in gills caused by elevated

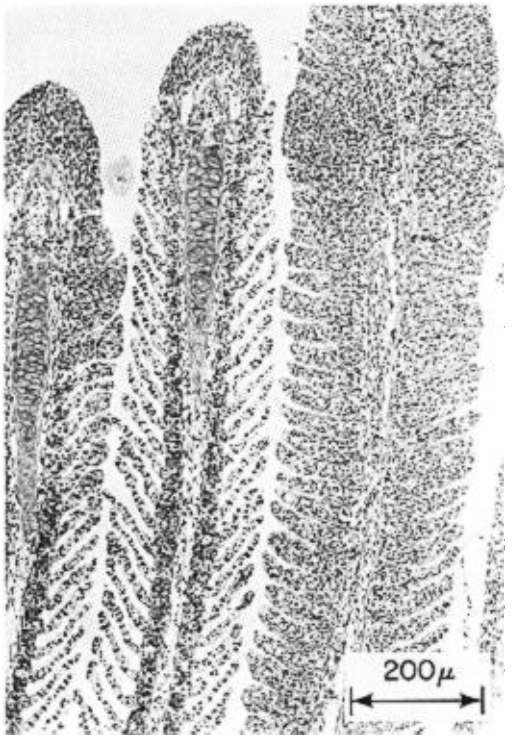

FIGURE 6.—*Hypertrophy and hyperplasia of lamellar epithelium resulting in fusion of several lamellae of F_1 fish from trough 5.*

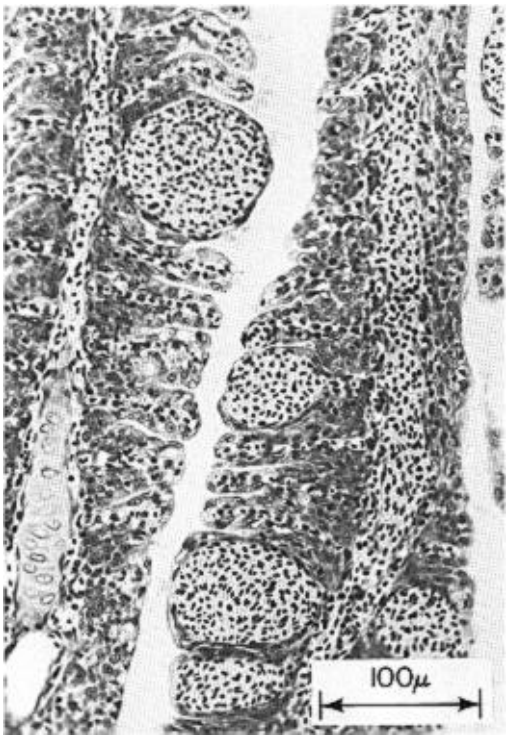

FIGURE 7.—*Telangiectasia of gill lamellae of F_1 fish from trough 6.*

ammonia concentrations. The most significant histopathologic change in F_2 fish was that found in the epidermis, primarily on the abdomens of fish exposed to ammonia and consisted of excessive vacuolation of epidermal cells. Severity of this condition increased with increasing ammonia concentrations. It was absent in fish examined from troughs 1 and 2 (Fig. 9), and was present in only 3 of the 10 fish examined from trough 3. It was, however, apparent in all 10 fish examined from each of troughs 4–6 (Fig. 10). This vacuolation was also present in the epidermis covering the lower jaws of the fish from trough 6, and occasionally in the oral cavity as well. Staining for mucopolysaccharides by the periodic-acid–Schiff method revealed that there was not a substantial increase in goblet cells and, therefore, vacuolation was probably hydropic. The only other histological change noted in F_2 fish was the occurrence of mild to moderate hyaline droplet degeneration in renal-tubule epithelia of fish from troughs 3–6.

Discussion

Spawning

The technique we used in our test for voluntary spawning by rainbow trout proved to be satisfactory for the parental fish, although not for the F_1 fish at either 3 or 4 years of age; we have no satisfactory explanation for this. One difference in their prespawning history is that the F_1 fish had been reared entirely in the laboratory, whereas the parental fish had been reared in an outdoor raceway, under more natural climatic and seasonal light conditions, from the time they were early juveniles until 4 months prior to spawning.

Although the "artificial-stream" methods we tried in our preliminary experiments successfully induced voluntary spawning, it was not possible to determine larva:egg ratios because eggs could not be counted without injury to them. It is reasonable to assume, however, that the numbers of larvae hatched per spawn in these preliminary experiments were higher than the numbers produced in the experimental de-

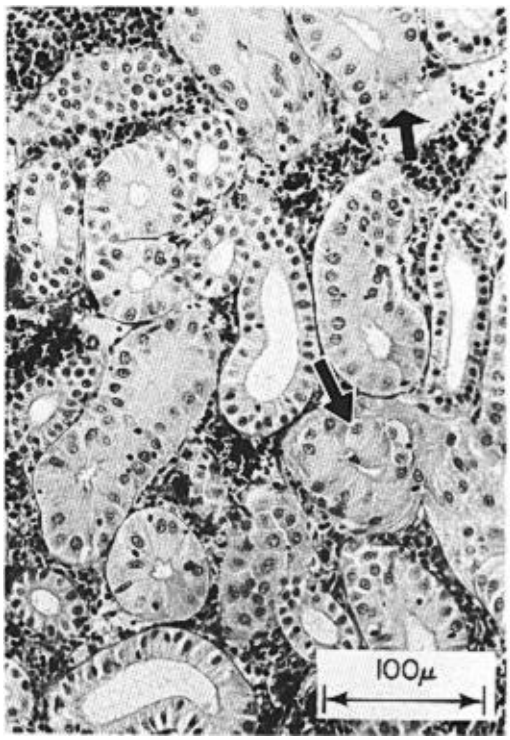

FIGURE 8.—*Swelling and cytoplasmic degeneration of renal tubule epithelium of the second proximal segment (arrows) of F_1 fish from trough 6.*

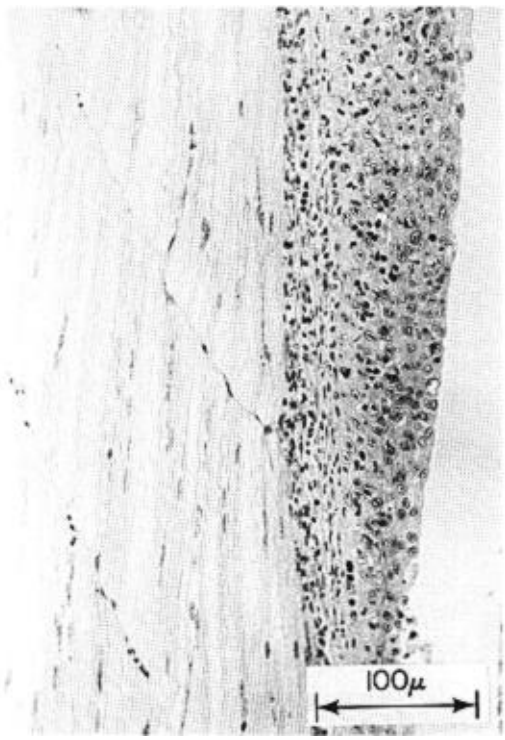

FIGURE 9.—*Normal epidermis on abdomen of F_2 control fish from trough 1.*

sign we eventually chose for our study. An artificial-stream design, similar to that described, might prove useful for both spawning and incubation during toxicity tests if the objectives do not require quantitative data prior to the swim-up stage.

Mortality and Growth

The high percentage mortality in certain lots of swim-up progeny during the time they were confined to the buckets and baskets corresponded to those lots containing the greatest numbers of young. We believe much of this mortality was caused by overcrowding and resultant disease conditions, although this was not always confirmed. Especially heavy mortality occurred among the F_2 juveniles during the final 2 months of the test, the major cause of which we attribute to the protozoan *Costia* sp. which was identified as being present in considerable degree on both live and dead fish. Of special note was the finding that the F_1 fish, between 15 and 19 months of age, were able to withstand a mean concentration of NH_3 as high as 0.11 mg/liter for a 3-month period, and a peak of 0.15 mg/liter NH_3 for approximately a 2-day period, without suffering significant mortality.

Growth differences related to ammonia concentration were not demonstrated in our test. Burkhalter and Kaya (1977), however, have reported retardation in growth of rainbow trout larvae during a test lasting 42 days at concentrations of un-ionized ammonia-nitrogen as low as 0.05 mg/liter (0.06 mg/liter NH_3), which was the lowest concentration they tested. Our highest concentration tested averaged 0.073 mg/liter NH_3 during the same stage of development, and 0.067 mg/liter NH_3 over the 10-month period from hatching to when the lengths and weights of the F_1 fish were first measured. We conclude that any early growth retardation that may have occurred as a result of ammonia exposure was compensated for during the next several months.

Hematology

A correlation between ammonia concentration in the blood of the F_1 test fish and concentration of ammonia in the water has been demonstrated. The concentrations of total ammonia we measured in the blood of the F_1 control fish are comparable to those reported for rainbow trout by Hillaby and Randall (1979), although the un-ionized ammonia concentrations we calculated from these are lower than those reported by them. The blood pH measurements we report are also lower than those generally accepted as normal for rainbow trout, and possibly reflect a sudden drop in pH as a result of stress placed on the fish during the half-minute between their removal from the troughs and their deaths for sampling purposes. If we assume our fish had a blood pH of 7.8 before being disturbed, rather than the mean value of 7.5 we measured after the fish were handled and sacrificed, this difference of 0.3 pH units would account for the lower un-ionized ammonia values we calculated. If the resting pH was 7.8, the corrected value for the amount of un-ionized ammonia in the blood of our control fish would be 0.064 mg/liter; this value is more in line with accepted values. By the same reasoning, fish in the water with the highest ammonia concentration would have a mean blood ammonia concentration of 0.14 mg/liter NH_3, markedly higher than that calculated for the control fish.

Regardless of the blood pH assumed, it is clear that both the total and un-ionized ammonia in the blood of the test fish was higher at the higher ammonia concentrations tested. This would indicate a physiological stress was being placed on the fish, although the consequences of this stress were not evident from this study.

There is less evidence that hematocrit and hemoglobin values in the fish we tested were affected by ammonia. Although there were significant differences for both these measurements between parental fish exposed to 0.067 mg/liter NH_3 and higher and those exposed to 0.047 mg/liter NH_3 and lower, these differences were not confirmed by measurements taken on the blood of F_1 fish. Hematocrit readings for the F_1 fish at all test concentrations (up to 0.076 mg/liter NH_3) showed no significant relationship to test concentration, and hemoglobin in the blood of F_1 fish was not measured.

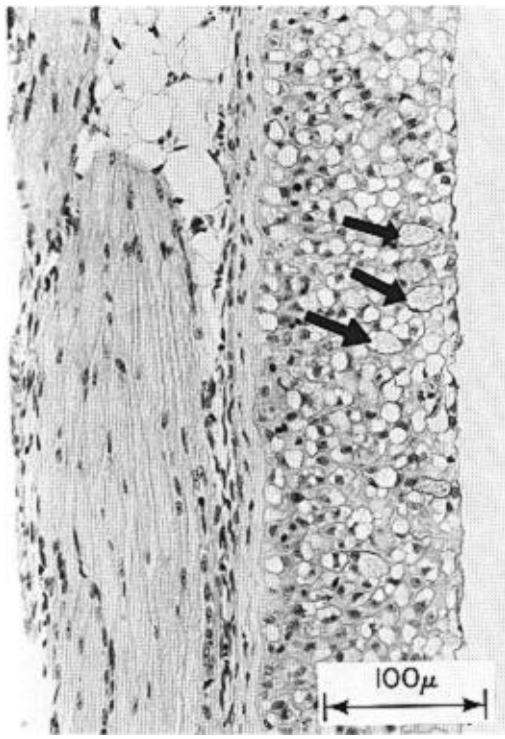

FIGURE 10.—*Thickening and excessive vacuolation of epidermis of F_2 fish from trough 6. Note some goblet cells scattered throughout (arrows).*

Histopathology

The gill lesions we noted are typical of those found by other workers studying the effects of ammonia on fishes (Burrows 1964; Flis 1968; Smith and Piper 1975; Smart 1976). Resulting gill lesions may cause reduced oxygen diffusion across membranes and predispose fishes to bacterial infections. Fishes exposed to increased metabolic ammonia in hatcheries are known to be more susceptible to bacterial gill disease (Burrows 1964; Bullock 1972; Larmoyeux and Piper 1973; Snieszko 1974; Smith and Piper 1975). Smart (1976) found an increase in bacteria-associated fin and tail rot in rainbow trout exposed to ammonia. Nephrosis and accumulation of melanin in hematopoietic tissue of fish, including controls, are probably related to age and spawning condition. Severity, however, appears to be exacerbated by increased ammonia concentrations. Flagg and Hinck (1979) found that channel catfish *Ictalurus punctatus* exposed

to 0.02–0.04 mg/liter NH_3 were more susceptible to invasion by the bacterium *Aeromonas hydrophila*. They also found that exposure time at these concentrations was correlated with lowered host resistance.

In our study, hyperplasia of hematopoietic tissue was apparent in fish from all troughs, and was probably a response to the severe anemia that occurred near the beginning of the test. The accumulation of ceroid in the liver and hematopoietic tissues, first noticed when the parental fish were examined 4 months into the test, was probably a consequence of their consuming rancid feed (Smith 1979). Although blood hemoglobin and hematocrit values in control fish and fish at the lower ammonia concentrations returned to normal within 6 months after the change to new feed, ceroid in livers and kidneys of fish at all concentrations persisted and appeared to be metabolized quite slowly. This was especially true in fish from troughs 5 and 6. Only trace amounts of ceroid pigment were apparent in livers of fish that had been removed from the highest ammonia concentration and transferred to ammonia-free water, compared to increased amounts in livers of fish that had been retained under test at that concentration.

The cause of glomerulosclerosis and eosinophilic debris around renal tubule epithelium of some female, but not male, F_1 adult fish is apparently sex-related and probably age-related. Negative results were obtained when the debris was stained for acid-fast material, fat, amyloid, and hemoglobin. It is possible that the material was a result of an autoimmune response and a buildup of antigen–antibody complexes. This kidney condition had not been observed in any of the younger F_1 fish but had been observed during all three examinations of parental fish. There was essentially no difference between livers of test fish and those of controls.

The increased vacuolation (presumably hydropic) in the epidermis of F_2 fish exposed to ammonia may have resulted from increased tissue permeability. A comparison cannot be made between the F_2 fish and either the parental or F_1 fish because epidermal tissues of the latter were not examined. However, similar histological changes have been reported in the epidermis of rudd *Scardinius erythrophthalmus* exposed to 0.08 and 0.16 mg/liter un-ionized ammonia-nitrogen (Department of the Environment 1972).

The degenerative changes we observed occurred in parental and F_1 fish at concentrations of 0.04 mg/liter NH_3 and greater, and in F_2 fish at 0.02 mg/liter NH_3 and greater. Reduced growth, and lesions in gill and liver tissues, of rainbow trout exposed to concentrations of 0.017 mg/liter NH_3 for periods of 9 to 12 months have been reported by Smith and Piper (1975), although the dissolved oxygen concentration in their test water averaged 5.8 mg/liter. In the present study, the dissolved oxygen concentrations averaged approximately 7.5 mg/liter, and seldom dipped below 6.5. It is known from a previous study on rainbow trout by Thurston$_2$ et al. (1981) that any significant reduction in dissolved oxygen below saturation can increase the acutely toxic effects of ammonia. It logically follows that any tissue impairment that reduces the rate of intake of oxygen to rainbow trout might also increase the toxic effects of ammonia.

Summary and Implications

Conventional end-point indices for aquatic-toxicity tests include reduced growth and survival, impaired reproduction, and gross anatomical deformities. These abnormalities are clinical expressions of altered structure and function that originate at the cellular level. By using these conventional indices, we saw no significant effects of ammonia exposure to the test fish in this study. However, histological examination of the test fish showed that adverse sublethal effects were abundant.

Alterations of gill and kidney tissues were obvious, and their occurrence and severity were directly related to ammonia concentration. Pathologic conditions were most apparent in test fish exposed to concentrations of 0.04 mg/liter NH_3 and greater. The gill tissues of parental and F_1 fish evidenced hypertrophy of the gill lamellae with accompanying basal hyperplasia, separation of epithelia from the underlying basement membranes, necrosis, aneurysms, and mild to moderate fusion of gill lamellae. These alterations no doubt impair the performance of fish under conditions of reduced ambient dissolved oxygen, as demonstrated by Smith and Piper (1975) and Thurston$_2$ et al. (1981). Alterations observed in the kidney

tissues of parental and F_1 fish exposed to 0.04 mg/liter NH_3 and greater included generalized nephrosis, degeneration of renal tubule epithelia, hyaline droplet degeneration, and, in some instances, partially occluded tubule lumens. These histopathological alterations invariably result in impaired glomerular blood flow and filtrations, and eventually may induce renal failure.

It is not evident to what extent the type of tissue damage and blood-ammonia increase observed in this laboratory study may lead to organ dysfunction in, or impaired functional behavior of, fish in a natural environment and, in turn, to their early death or greater susceptibility to predation. It can be conjectured, however, that the dysfunctions would be similar. Further, it is reasonable to assume that diseased fish are more susceptible than are healthy fish to chronic effects of ammonia exposure inasmuch as the protozoan-infected F_2 fish evidenced pathologic conditions at 0.02 mg/liter NH_3, this concentration being lower than those in which these same conditions were observed in parental and F_1 fish.

Given the strong positive correlations observed between ambient ammonia concentrations and tissue damage, we believe that agencies responsible for water-quality management should carefully weigh the potential that histopathology offers in development of water-quality criteria.

Acknowledgments

This paper is dedicated to the late C. J. D. Brown, Professor of Fisheries at Montana State University until his retirement in 1972. Dr. Brown helped plan this study before he retired, and after retirement assisted in the research; he lived to see the study completed. Throughout his life he directed many graduate research projects with the selfless policy of never adding his name to the resultant publications. We have taken the liberty of including his name as coauthor of this publication in recognition of his contributions. This, then, is his final manuscript.

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