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Reproduction of salmonid fishes in acidic water was studied using the rainbow trout (Salmo gairdneri). Adult trout were exposed to various levels of hydrogen ion (pH levels 4.5, 5.0, 5.5, and control [6.5 to 7.1]) during the final 6 weeks of reproductive maturation. Reduced survival rates of the progeny of acid-exposed females through 7 days of development, hatching, and yolksac absorption demonstrate that oogenesis is sensitive to high hydrogen levels. Similar reductions in the survival of the progeny of acid-exposed males indicate the sensitivity of spermatogenesis to low ambient pH levels. The progeny of unexposed adults had lower survival rates through 7 days of development and hatching when they were reared at pH levels 4.5, 5.0, and 5.5 than did embryos reared at the control pH level. No embryos exposed to pH 4.5 survived to the eyed stage. Plasma estradiol-17β, androgen, and

17α-hydroxy-20β-dihydroprogesterone concentrations in acid-exposed adult trout revealed no gross physiological abnormalities due to acid stress. Plasma calcium levels in adult females showed no apparent effects due to hydrogen ion exposure, but were decreased in adult males exposed to pH 4.5 for 42 days. Plasma sodium levels fell slightly in adult fish after 7 days of exposure to pH levels 4.5, 5.0, and 5.5 and continued to decline in fish exposed to pH 4.5. I conclude that salmonid gametogenesis and early ontogeny are likely to be affected by environmental acidification which results in pH changes to levels below 5.5.

INFLUENCES OF ENVIRONMENTAL ACIDIFICATION ON SALMONID REPRODUCTION

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INTRODUCTION

Surface water acidification, presumably due in part to acidic precipitation, has been documented in northern Europe (Almer et al. 1974; Leivestad et al. 1976; Wright et al. 1980) and eastern North America, including the northeastern region of the United States (Haines 1981). The occurrence of acidic precipitation has been reported in other parts of the United States, such as the Los Angeles, San Francisco, Seattle, and Denver areas (Lewis and Grant 1980; Powers and Rambo 1981), and these areas contain lakes and streams that are susceptible to acidification (Omernik and Powers 1983). Thus, current and potential impacts of acidification on aquatic biota are receiving widespread attention.

Fish populations have been eliminated as a result of acidification in some systems and are endangered in others (Jensen Snekvik 1972; Schofield 1976; Harvey 1980). Because of the commercial and recreational importance of salmonid fisheries in Europe and North America, much of the concern has focused on the impacts of acidification on salmonid populations. The literature review in this section establishes the context for a study of the reproduction of rainbow trout (Salmo gairdneri) in a low pH environment, which will be reported in the following sections.

Acidic deposition and water quality alterations

The combustion of fossil fuels results in the emission of sulfur dioxide and nitrogen oxides into the atmosphere, where they undergo a complex process of transportation and transformation into sulfuric and nitric acids (Dovland and Semb 1980). Along with other chemicals from various natural and man-made sources, these acids may reach watersheds and aquatic systems through wet precipitation, dry deposition, impaction of aerosols, and adsorption of gases (Galloway and Cowling 1978).

The alterations of surface water chemistry by acidic deposition depend upon the nature of the receiving system and the quantity and mode of acidic input. The chemical composition of precipitation is modified as it moves through the watershed and interacts with the geological substrate, soil, and vegetation (Likens et al. 1979). The acid-neutralizing capacity of the bedrock and soils, hydrologic flow characteristics, soil and mineral types, and age of the soil influence the sensitivity of particular systems to acidification (Hendrey et al. 1980).

Temporal variations in acidification processes are also important. Heavy rains and rapid snowmelt may result in sharp episodic pH depressions and metal inputs (Gjessing et al. 1976; Schofield and Trojnar 1980). Chronic low pH conditions develop in waters in which the bicarbonate buffering system is gradually depleted (Henriksen 1980).

The characteristics of watersheds that determine water quality responses to acidic inputs are sufficiently divergent to warrant caution in describing "typical" conditions in impacted systems. Gjessing et al. (1976) thoroughly reviewed this subject. They noted increased acidity, decreased alkalinity, replacement of bicarbonate by sulfate as the predominant anion, and increased concentrations of calcium, magnesium, and aluminum as common effects of acidic precipitation on the water chemistry of susceptible lakes and streams.

Fish mortalities and toxicity bioassays

Observations of fish mortalities have been correlated with sudden episodic acidification events. Jensen and Snekvik (1972) described mortalities of Atlantic salmon (Salmo salar) in southern Norway associated with snowmelt. The pH of samples taken from a river during the fishkill ranged from 3.9 to 4.2. Similarly, an acidic pulse due to snowmelt coincided with a massive kill of brown trout (Salmo trutta) in the Tovdal River of Norway (Leivestad and Muniz 1976). Schofield (1977) reported mortalities of adult, yearling, and larval brook trout (Salvelinus fontinalis) held in tanks supplied with water from Little Moose Lake, New York, during a spring snowmelt. The relatively minor change in lake water pH and significantly elevated aluminum concentrations implicated aluminum as the dominant toxic factor rather than hydrogen ion. Grahn (1980) theorized that a kill of ciscoe (Coregonus albula) in an acidified Swedish lake resulted from the exposure of fish to precipitating aluminum hydroxide.

Bioassay studies have provided information about several factors that influence acid toxicity. Leivestad et al. (1976) pointed out that high hydrogen ion concentrations are relatively more toxic in waters with low concentrations of dissolved salts. Kwain (1975) showed a positive relationship between temperature and median lethal pH levels for rainbow trout fingerlings. Packer and Dunson (1972) and Swartz et al. (1978) determined that sulfuric acid, hydrochloric acid, and acid mine waste are not of equal toxicity.

As illustrated by the tabulation of acute and chronic pH exposure bioassays prepared by Spry et al. (1981), differences in test conditions make it difficult to generalize about the relative toxicity of acidic water to fishes. However, some important conclusions can be drawn. The effects of elevated hydrogen ion concentrations appear to be species-specific. For example, Grande et al. (1978) concluded that the relative order of tolerance to low environmental pH conditions for some salmonids is (from least tolerant to most tolerant): rainbow trout, Atlantic salmon, brown trout, and brook trout. Johansson et al. (1977) demonstrated that the early development of Atlantic salmon is more sensitive to hydrogen ion toxicity than is the early development of brown trout or brook trout. Intraspecific differences in tolerance have been demonstrated for brook trout (Robinson et al. 1976; Swartz et al. 1978) and brown trout (Edwards and Gjedrem 1979).

In addition to species specificity of tolerance to ambient acidity, the toxicity of acidic water varies among life history stages. Bioassay results (Menendez 1976; Daye and Garside 1977; Craig and Baksi 1977; Carrick 1979) reveal as a general trend that yolksac larvae are the least tolerant of acid stress followed by post-yolksac larvae, embryos, juveniles, and adults, in order of increasing tolerance.

Aluminum, leached from soils by acidic waters and carried into lakes and streams through watershed runoff, can reach high enough concentrations to be toxic to fish (Cronan and Schofield 1979). Schofield (1977) identified aluminum as the primary toxicant in snowmelt runoff into Little Moose Lake, New York, and discussed the complex interactions between aluminum and pH. Driscoll et al. (1980) determined that inorganic aluminum forms contribute most to fish toxicity, whereas organically complexed aluminum is of relatively low toxicity. Baker and Schofield (1982) further evaluated the effects of aluminum and hydrogen ions on early life history stages of white sucker (Catostomus commersoni) and brook trout. They concluded that the effects of aluminum vary for different life history stages; aluminum toxicity is dependent upon pH; and brook trout and white suckers are not equally sensitive to aluminum and hydrogen ions. Interestingly, they showed that aluminum actually enhanced survival of eggs at pH levels between 4.2 and 4.8. Damage and mucous clogging of gills due to aluminum exposure have been reported frequently (Freeman and Everhart 1971; Schofield 1977; Schofield and Trojnar 1980; Muniz and Leivestad 1980; Baker and Schofield 1982).

Physiological responses to ambient acidity

Studies of the physiological responses of fish to ambient acidity have usually involved acute exposures to very low pH. Spry et al. (1981) commented that these results may be of limited applicability in assessing chronic effects of acidification. Nevertheless, they do contribute to an understanding of some important physiological processes, especially ionoregulation and acid-base balance.

Leivestad and Muniz (1976) correlated the losses of sodium and chloride from the plasma of brown trout with acid stress in the Tovdal River of southern Norway and in tank experiments. Packer and Dunson (1970, 1972) attributed the decline in total body sodium of acid-exposed brook trout to decreased influx and increased efflux rates. Similar depletions in plasma sodium and chloride have been demonstrated with rainbow trout (McDonald and Wood 1981; Booth et al. 1982). McWilliams and Potts (1978) determined that high hydrogen ion concentrations may be associated with a shift in gill transepithelial potential from negative to positive, resulting in increased permeability of the gill epithelium to sodium. Furthermore, they reported that the presence of calcium in the water reduced the permeability of the gill epithelium to sodium. Spry et al. (1981) related this information to a field survey by Wright and Snekvik (1977) in which fish population status was positively correlated with lake calcium concentration.

McDonald et al. (1980) found that exposure of rainbow trout to high levels of hydrogen ions in soft water resulted in severe ionoregulatory dysfunction and minor acidosis. In contrast, exposure to ambient acid in hard water showed little effect on plasma ions and more serious acidosis. Lowered blood pH has been reported in several other studies of rainbow trout (Neville 1979; McDonald and Wood 1981; Booth et al. 1982), brook trout (Packer 1979), and carp (Cyprinus carpio) (Ultsch et al. 1981). Neville (1979) found increases in hemoglobin, hematocrit, and erythrocyte levels as additional effects of acid exposure on blood characteristics.

Daye and Garside (1976) studied the histopathology of brook trout surficial tissues in low pH waters. Their findings included hypertrophy of mucous cells in the gills, nares, and integument; and epithelial damage in the gills, corneae, integument, and esophagus. Fritz (1980) speculated that such injuries to corneal and olfactory tissues could affect salmonid imprinting, homing, and spawning. Integumental damage in prehatching embryos and gill epithelial alterations in alevins contributed to acid-induced mortalities of young Atlantic salmon (Daye and Garside 1980).

Falk and Dunson (1977) observed behavioral indications of stress in brook trout at pH 5.0 and 5.8. Johnson and Webster (1977) determined that spawning female brook trout avoided upwellings of pH 4.0 and 4.5. They suggested that this behavior could aid in increasing egg and larval survival in systems where such an avoidance response is possible.

Population extinction and reproductive failure

Occasional fish kills provide dramatic evidence of detrimental effects of acidification, and physiological studies of acute acid and metal toxicity add to our understanding of some relevant processes. However, the persistence of resources in impacted systems is of greater concern from a fisheries perspective.

In a survey of southern Norway, for example, the percentage of lakes devoid of trout populations increased gradually from 3.8% to 60.0% as lake pH decreased from 5.5 or more to 4.0 (Jensen and Snekvik 1972). A later study of 700 lakes in southern Norway (Wright and Snekvik 1977) revealed that about 40% of the lakes were devoid of fish and another 40% had sparse populations. Brown trout is the principal species of the region.

Fish population surveys of 50 Swedish lakes that ranged in pH from 4.40 to 7.45 were reported by Almer et al. (1974). The roach (Leuciscus rutilus) was missing from ten of the low pH lakes and reproductive failure was apparent below pH 5.5. Populations of perch (Perca fluviatilis), pike (Esox lucius), minnow (Phoxinus phoxinus), arctic char (Salvelinus alpinus), brown trout, ciscoe, and eel (Anguilla vulgaris) were also sparse or nonexistent.

Concern for possible acidification and metal deposition in the La Cloche Mountain Lakes of Ontario caused by a smelter prompted several studies of fish population status. Beamish and Harvey (1972) chronicled the gradual reductions and losses of populations of seven

species as the pH of Lumsden Lake dropped from 6.8 in September 1961 to 4.4 in August 1971. Beamish (1974) described the decline of populations in Ontario Society of Artists (O.S.A.) and Muriel Lakes. The average pH of O.S.A. Lake during the study was 4.5 and the average pH of Muriel Lake was 4.7. Beamish et al. (1975) studied the fish populations of George Lake, where the pH ranged from 4.8 to 5.3 between February 1972 and August 1973. They reported changes in the average size of age classes, reduction of population sizes, and disappearance of some species.

A survey of 217 high elevation lakes in the Adirondack Mountains of New York (Schofield 1976) showed that 51% of the lakes had pH values below 5.0. No fish were present in 90% of the low pH lakes. Schofield and Trojnar (1980) determined that in 53 acidified lakes of the Adirondacks, mean aluminum concentrations were inversely correlated with presence of stocked brook trout. The mean concentrations were 0.29 mg Al/liter for lakes in which trout were absent and 0.11 mg Al/liter for lakes in which trout were present.

Reproductive failure has been cited as a major contributor to the gradual extinction of fish populations in acidic waters (Jensen and Snekvik 1972; Beamish et al. 1975; Beamish 1976; Ryan and Harvey 1977, 1980). Of the many factors that determine reproductive success, investigators have specified mortality of early life history stages (Jensen and Snekvik 1972: Leivestad et al. 1976) and physiological disruptions during gametogenesis (Beamish et al. 1975; Lockhart and Lutz 1977) as the causes of reproductive failure due to acidic conditions.

As noted earlier, bioassays have established that early life history stages are relatively more sensitive to hydrogen ion than are juveniles and adult fish. Other studies have demonstrated impairment of adult reproductive physiology due to acid stress. Craig and Baksi (1977) showed reduced egg production and egg fertility in flagfish (Jordanella floridae) at low pH. Similarly, Lee and Gerking (1980) exposed adult desert pupfish (Cyprinodon nevadensis nevadensis) to acidic conditions and observed decreased egg production, egg laying, and egg viability. Ruby et al. (1977) determined that exposure to high levels of hydrogen ion of adult female flagfish inhibited the deposition of secondary yolk in the cytoplasm of oocytes. Beamish et al. (1975) and Lockhart and Lutz (1977) concluded that acidic water may impair plasma calcium regulation associated with vitellogenesis in reproductively maturing female fishes.

The goal of the research described here was to increase understanding of current and potential consequences of low environmental pH for the reproduction of salmonid fishes. This goal was addressed by studying final reproductive maturation and progeny survival of rainbow trout, a relatively acid-sensitive species among the salmonids (Grande et al. 1978), in a low pH environment. Plasma ion and sex hormone levels were evaluated as physiological indicators of reproductive stress in acid-exposed adult trout. Estradiol-17β is involved in vitellogenesis and calcium regulation in maturing female salmonids (Whitehead et al. 1978) and may serve as an indicator of reproductive stress early in the reproductive cycle. Similarly, the

androgens are the primary gametogenic hormones in male salmonids and they co-vary with estradiol-17β in females (Sower and Schreck 1982).

The performances of the gametes from the acid-exposed fish were compared to those from unexposed trout to demonstrate effects of high levels of hydrogen ion on oogenesis and spermatogenesis. Specifically, I was interested in the ontogenetic effects of low environmental pH on reproductive success and in evaluating the potential for maternal and paternal effects.

METHODS

Adult rainbow trout (age 2^{+}), averaging 57.4 ± 3.2 cm in length and 2.2 ± 0.5 kg in weight, were obtained in June 1982 from the Roaring River Hatchery (Oregon Department of Fish and Wildlife, Scio, Oregon) and transported to the Western Fish Toxicology Laboratory of the United States Environmental Protection Agency, Corvallis, Oregon. Blood samples were obtained (prior to October 5, 1982) by caudal puncture with a needle and ammonium-heparinized syringe, and gender of the fish was determined by the Ouchterlony Immunodiffusion Assay Technique through which vitellogenin is specifically detected in the plasma of female fish (Terry Owen, Helix Biotech, Ltd., Richmond, British Columbia, Canada). The fish were distributed on October 27, 1982 into eight 757-liter circular fiberglass tanks, with three females and four males in each tank. The tanks were set up outdoors to provide a natural photoperiod.

The inflowing well water was gradually softened by reverse osmosis (Purification Techniques, Inc., Brielle, New Jersey) from 23 mg/liter as $CaCO_3$ to 10 mg/liter over a period of five days. After an additional 3 days of acclimation, reagent grade sulfuric acid was introduced into the system over 5 days until pH levels of 4.5, 5.0, and 5.5 were reached in duplicate treatment tanks, while two control tanks (pH range 6.5 to 7.1) remained on softened water.

Total water hardness, determined at least twice daily by the EDTA titrimetric method (American Public Health Association et al. 1980), was 9.2 ± 1.2 mg/l as $CaCO_3$ during the experiment. A stock solution of

softened, vigorously aerated water was acidified with sulfuric acid and held at pH 4.0 ± 0.1 with a Beckman Model 942 pH Monitor (Beckman Instruments, Inc., Fullerton, California). The acidified stock solution was vigorously aerated to remove carbon dioxide that may have been generated by the addition of acid, and pumped to the head tank of a gravity-flow diluter. The acidic stock solution was diluted with softened well water to attain appropriate pH levels and delivered at a rate of 4.5 liters/minute to the tanks in the flow-through system. Treatment pH levels were automatically monitored and recorded every 3 hours and the diluter was manually adjusted as necessary in order to maintain exposure levels within ± 0.1 pH units of 4.5, 5.0, and 5.5. Water temperatures during the experiment ranged from 10.9 to 14.0 C and alkalinity, determined by Gran titration as described by Stumm and Morgan (1981), was 252 ± 11 µeq/l prior to addition of acid. Metal levels were below limits of detection by atomic absorption flame spectrophotometry.

Blood samples from each fish were drawn from the caudal artery with a needle and ammonium-heaparinized syringe under anesthesia (MS222, 50 mg/liter) and the plasma was stored at -20 C. Anesthetic solutions were titrated to treatment pH levels with 0.1 M NaOH to minimize stress. The first blood sample was taken following tank acclimation, just prior to the addition of acid to the system. The second sample was taken 7 days after treatment pH levels were reached, and the final sample was drawn at the time of spawning.

Fish were examined for reproductive maturity weekly, beginning with the fourth week of exposure. After 42 days of exposure (December 21, 1982), the ripe fish were anesthetized for blood sampling, killed with a blow to the head, and manually spawned. The gametes were collected separately from each fish and matings were conducted according to the following scheme, employing dry fertilization and 1-hour water hardening:

A) Unexposed females X unexposed males

Eggs from unexposed females were fertilized with sperm from unexposed males. Water hardening and rearing took place at each of the pH levels used in the prespawning exposure in order to assess the performance at low pH of the progeny of unexposed adults.

B) Acid-exposed females X unexposed males

Eggs from females exposed to each pH level were fertilized with sperm from unexposed males. Water hardening and rearing took place at the control pH level to determine the effects of acidic water on oogenesis.

C) Acid-exposed males X unexposed females

Eggs from unexposed females were fertilized with sperm from males from each pH exposure group. Water hardening and rearing took place at the control pH level to determine the effects of acidic water on spermatogenesis.

D) Acid-exposed females X acid-exposed males

Eggs from females exposed to each pH level were fertilized with sperm from males exposed to the same pH level. Water hardening and rearing took place at the respective parental exposure pH levels to assess reproductive performances when both parents and the progeny were exposed to low pH.

Aliquots of embryos from each fertilization group were placed in incubation cells within drawers of Heath incubators for rearing. The cells consisted of 4.6 cm sections of 6.4 cm diameter PVC pipe with plastic mesh screen attached across one end, forming a cup. Similar cups, constructed of 1.3 cm sections of 7.6 cm diameter PVC pipe, were inverted over each cell to confine the fish after hatching. The flow rate through the incubators was 2 liters/minute. The incubators were covered with black plastic sheets to exclude light. Dead eggs and larvae were counted and removed daily except as noted subsequently. A different incubator was used for each test pH level (4.5, 5.0, 5.5, and ambient). Within a treatment, cells of eggs were randomly distributed throughout the incubator.

Plasma estradiol-17β, androgen (testosterone and 11-ketotestosterone) and 17α-hydroxy-20β-dihydroprogesterone concentrations were determined by radioimmunoassay, following the general method of Korenman et al. (1974). Details of the procedure are described by Sower and Schreck (1982). Estradiol-17β was determined in 10 µl of plasma for males and 25 µl of plasma for females. Androgens were determined in 10 µl of plasma and

17α-hydroxy-20β-dihydroprogesterone was determined in 25 µl of plasma. Samples were extracted twice with diethyl ether and extraction efficiency was about 90% for all assays. Antiestradiol-17β and antitestosterone were provided by Dr. G. Niswender (Colorado State University, Fort Collins, Colorado) and anti-17α-hydroxy-20β-dihydroprogesterone was provided by Dr. A. P. Scott (Ministry of Agriculture and Fisheries, Lowestoft, Suffolk, United Kingdom). Antibody characteristics are given by Sower and Schreck (1982) and Scott et al. (1982). Plasma calcium concentrations were determined by atomic absorption flame emission spectrophotometry, and sodium concentrations were measured by flame photometry.

Progeny survival rates were statistically analyzed with contingency tables (Cochran and Cox 1957). Plasma calcium and sodium concentrations were evaluated by analysis of variance and Duncan's new multiple-range test for comparisons among means when appropriate (Steel and Torrie 1980).

RESULTS

Survival rates of the progeny of acid-exposed and unexposed trout appear in Table 1. On the eighth day of development, some eggs became infected with Saprolegnia fungi. Thus, mortalities through the last day before the occurrence of infection (7 days of development) were tallied, the infected eggs were removed, and the remaining eyed eggs were monitored for survival through hatching. Survival through yolksac absorption was then determined among the hatched fish.

The progeny of unexposed adults that were reared at pH 5.5 and 5.0 (groups B and C) had lower survival rates through 7 days and slightly lower hatching success than those reared in unacidified water (group A). No embryos reared at pH 4.5 (groups D and M) survived through 7 days.

Influences of acid-exposure on oogenesis are indicated by the reduced hatching percentages among the progeny of females that had been exposed to pH levels 5.5 and 4.5 (groups E and G). The progeny of females that had been exposed to pH 5.5 (group E) had lower survival from hatching through yolksac absorption than did controls (group A).

Impaired spermatogenesis at low pH is evident in the performances of the progeny of acid-exposed males (groups H, I, and J). The progeny of males exposed to pH 4.5 prior to spawning (group J) had lower survival through 7 days of development and through hatching than did the progeny of unexposed males (group A). The progeny of males exposed to pH levels 5.5 and 5.0 (groups H and I) had lower survival rates through hatching and yolksac absorption than did controls (group A).

Table 1. Survival rates of the progeny of acid-exposed and unexposed adult rainbow trout. Total numbers of eggs or larvae in each case are in parentheses.

	Parental prespawning exposure pH level		Progeny rearing pH level	Percent survival through 7 days	Percent of eyed eggs successfully hatched	Percent of larvae surviving to yolksac absorption	[d] Cumulative percent survival
	Female	Male					
A	control	control	control	91.3(689)	93.8(465)	86.2(436)	73.8
B	control	control	5.5	[a]64.2(749)	[a]89.2(381)	92.4(340)	52.9
C	control	control	5.0	[a]54.7(766)	[a]80.1(294)	81.5(238)	35.7
D	control	control	4.5	[a] 0 (761)	--	--	0
E	5.5	control	control	91.9(211)	[a]57.8(154)	[a]70.8(89)	37.6
F	5.0	control	control	[a]99.1(439)	91.1(426)	88.9(388)	80.3
G	4.5	control	control	93.1(189)	[a]26.4(148)	89.7(39)	22.0
H	control	5.5	control	93.8(689)	[a]80.8(386)	[a]79.5(312)	60.2
I	control	5.0	control	93.2(883)	[a]75.0(641)	[a]82.7(481)	57.8
J	control	4.5	control	[a]87.4(933)	[a]58.6(534)	86.6(313)	44.4
K	5.5	5.5	5.5	60.3(136)	[b]57.8(71)	[b]82.9(41)	28.9
L	5.0	5.0	5.0	[c]76.8(555)	84.0(374)	77.7(314)	50.1
M	4.5	4.5	4.5	0 (244)	--	--	0

[a] Different from group A at the corresponding stage of development, $P < 0.05$.

[b] Different from group B at the corresponding stage of development, $P < 0.05$.

[c] Different from group C at the corresponding stage of development, $P < 0.05$.

[d] Calculated as the product of the survival rates in the three preceding columns.

When both parents and the progeny were exposed to pH 5.5 (group K), hatching success and survival through yolksac absorption were reduced in comparison with the performances at pH 5.5 of the progeny of unexposed adults (group B). Females exposed to pH 5.0 produced gametes of high quality as reflected in the survival of their progeny in unacidified water and at pH 5.0 (groups F and L) through seven days of development in comparison with the performances of the progeny of unexposed females (groups A and C).

Calcium concentrations in the plasma of the adult trout are presented in Table 2. No clear indications of acid stress are apparent in the calcium levels of the females. Plasma calcium decreased in males that were exposed to pH 4.5 for 42 days ($P < 0.05$). Plasma sodium levels in the adults tended to decrease after 7 days of exposure to each treatment pH level (Table 2). Fish exposed to pH 4.5 for 42 days had sharply reduced sodium concentrations in comparison with unexposed fish ($P < 0.05$). Estradiol-17β, androgen, and 17α-hydroxy-20β-dihydroprogesterone levels in adult trout revealed no differences between treatments (Table 3). There was no apparent effect of the acid exposure on rate of sexual development since the fish all ovulated at the same time. When pressure was applied to the abdomen, eggs flowed easily from the ovipore. No eggs appeared to be atretic. In two of the females held at pH 4.5, approximately 10% of the eggs in the ovaries had not ovulated and were held tightly in the skein, although the remainder of the eggs were fully mature and ovulated. All males were ripe, having copious quantities of sperm at the time of spawning.

Hatching occurred synchronously among embryos reared in unacidified water and at pH levels 5.5 and 5.0. Three adult mortalities occurred at pH 4.5. One adult mortality occurred in unacidified water.

Table 2. Calcium and sodium concentrations (mean ± standard error) in plasma of acid-exposed adult rainbow trout.

Sex/Exposure pH	Prior to acidification (57 days prespawning)	7 days of acid exposure (35 days prespawning)	42 days of acid exposure (spawning)
		Calcium (milliequivalents/liter)	
Females			
4.5	7.6 ± 0.7	6.2 ± 0.5	5.5 ± 0.8
5.0	6.4 ± 0.5	7.0 ± 0.4	7.5 ± 1.5
5.5	5.8 ± 0.6	6.3 ± 0.6	5.7 ± 0.8
control	8.2 ± 0.5	8.7 ± 0.7	7.3 ± 1.6
Males			
4.5	5.5 ± 0.2	5.5 ± 0.3	[a]4.8 ± 0.1
5.0	5.4 ± 0.1	5.5 ± 0.2	5.3 ± 0.1
5.5	5.2 ± 0.1	5.2 ± 0.2	5.8 ± 0.3
control	5.3 ± 0.2	5.8 ± 0.2	5.2 ± 0.1
		Sodium (milliequivalents/liter)	
Male and Female			
4.5	153 ± 2	145 ± 4	[a]129 ± 4
5.0	153 ± 1	146 ± 3	147 ± 4
5.5	152 ± 3	143 ± 4	143 ± 6
control	153 ± 1	152 ± 1	154 ± 2

[a]Different from mean value in same exposure group prior to acidification and mean value in control group at spawning, $P < 0.05$.

Table 3. Estradiol-17β, androgen, and 17α-hydroxy-20β-dihydroprogesterone concentrations in plasma of acid-exposed adult rainbow trout.

Sex/Exposure pH	Prior to acidification (57 days prespawning)		7 days of acid exposure (35 days prespawning)		42 days of acid exposure (spawning)	
	mean	range	mean	range	mean	range
			Estradiol - 17 β (ng/ml)			
Females						
4.5	2.9	1.1-4.1	3.3	1.1-4-8	0.7	<0.1-1.5
5.0	3.0	1.0-5.5	4.0	1.7-5.4	0.7	0.3-0.8
5.5	3.4	1.4-7.6	3.7	2.4-5.5	0.3	0.1-0.5
control	2.9	1.2-5.6	3.4	1.3-5.8	0.6	<0.3-1.3
Males						
4.5	0.1	<0.1-0.2	0.1	<0.1-0.2	0.1	<0.1-0.1
5.0	0.1	<0.1-0.3	0.1	<0.1-0.3	0.1	<0.1-0.1
5.5	0.1	<0.1-0.2	<0.1	<0.1-0.1	<0.1	<0.1-0.1
control	0.1	<0.1-0.2	<0.1	<0.1-0.1	<0.1	<0.1-0.1
			Androgens (ng/ml)			
Females						
4.5	21	16-26	27	20-33	14	13-15
5.0	19	13-23	26	10-67	18	11-29
5.5	18	14-24	25	15-42	18	13-23
control	23	15-31	26	13-48	16	13-18
Males						
4.5	26	18-37	31	19-64	33	14-61
5.0	33	16-59	19	17-25	21	10-34
5.5	24	21-27	31	17-67	27	15-35
control	29	17-48	22	13-35	21	13-46
			17α-hydroxy-20β-dihydroprogesterone (ng/ml)			
Females						
4.5	<0.1	all <0.1	1.2	0.4-5.8	37	36.2-39.5
5.0	<0.1	<0.1-0.3	0.6	<0.1-1.0	26.7	6.8-50.9
5.5	0.1	<0.1-0.7	0.7	<0.1-2.4	32.1	21.1-43.1
control	0.2	<0.1-0.7	0.6	<0.1-2.9	24.6	15.3-33.9

DISCUSSION

Reproductive success of salmonids in acidic water is influenced by gamete quality and the developmental environment of the early life history stages. Exposure of adult rainbow trout to pH levels 5.5, 5.0, and 4.5 during final maturation affected gamete quality as demonstrated by the reduced survival in non-acidic water of the progeny of acid-exposed fish. Both oogenesis and spermatogenesis were impaired. Eggs from females that had been exposed to pH levels 5.5 and 4.5 prior to spawning had lower survival rates than did eggs from unexposed females, indicating detrimental effects of ambient acid during oogenesis. In addition, two of the five females in the pH 4.5 exposure group that survived through final maturation were incompletely ovulated and it is possible that the eggs that were ovulated at this level were not fully developed. In addition, two of the five females in the pH 4.5 exposure group that survived through final maturation were incompletely ovulated; in salmonid fishes, all eggs ovulate synchronously, suggesting an aberrant condition in the females with two distinct egg stages.

The high survival rates of the progeny of females exposed to pH 5.0 prior to spawning suggest that some physiological aspects of final reproductive maturation may actually be enhanced at this pH level. Physiological processes are sensitive to alterations in acid-base balance due to the pH-dependence of protein function (Spry et al. 1981). Thus, it is possible that oxygen exchange at the ovary or enzymatic reactions during ovulation, for example, are

facilitated at some pH levels, but the actual effects of acid observed at this level remain enigmatic. The response of eggs from females exposed to pH 5.0 prior to spawning appears not to be an experimental artifact. The proportions of survival of eggs from individual females within and among replicate pre-spawning exposure groups were compared and found to be similar in each case before they were pooled for comparisons between treatments. Thus, the performance of eggs from the females exposed to pH 5.0 cannot be explained by individual variability in adult sensitivity to acid-exposure during gametogenesis. This performance also cannot be accounted for by error in the acid delivery system, since there were no unusual responses by the males that were exposed to pH 5.0 prior to spawning, and plasma sodium levels in adults of both genders declined at this pH level. The enhancement of oogenesis at pH 5.0 should not be interpreted as indicating that reproductive success of salmonids will be increased by acid exposure at this pH level. Impaired spermatogenesis is apparent in the decreased survival rates of the progeny of male trout held at pH 5.5 and lower before spawning. Effects of acid exposure on gametogenesis have previously been demonstrated histologically and through determinations of egg production and fertility in flagfish at pH 6.0 and lower (Craig and Baksi 1977, 1978). Similarly, Lee and Gerking (1980) showed reductions in egg quality and quantity in desert pupfish below pH 7.0. The present study provides evidence that salmonid reproductive physiology is sensitive to environmental acidity as well. Examination of the cumulative percent survival of the offspring of acid-exposed fish suggests that oogenesis is affected more seriously than is

spermatogenesis at pH levels 5.5 and 4.5 in rainbow trout, while the apparent enhancement of oogenesis at pH 5.0 remains unexplained.

Direct effects of environmental acidity on early life history stages are shown by the increased mortality at pH 5.5, 5.0, and 4.5 of the progeny of unexposed trout. No embryos that were exposed to pH 4.5 survived to the eyed stage. Embryos that were exposed to pH levels 5.5 and 5.0 were most sensitive to the acid during the first 7 days of development and during hatching. These sensitive periods have been identified for toxicants in general (Rosenthal and Alderdice 1976).

Plasma calcium levels are known to increase in conjunction with vitellogenesis in many teleosts, including salmonids (Bromage et al. 1982). Vitellogenesis is generally completed prior to the final 6 weeks of reproductive maturation and calcium levels decline gradually during this period in trout (Whitehead et al. 1978). Plasma calcium levels in the acid-exposed female trout tended to decrease as expected, but variability among individuals precluded any clear indication of reproductive stress based on calcium regulatory failure. Observations of low female to male plasma calcium ratios among mature fish in acid-stressed populations were ascribed to disruption of calcium regulation associated with vitellogenesis (Beamish et al. 1975; Lockhart and Lutz 1977). However, these observations occurred after vitellogenesis was probably completed. Perhaps episodic exposure to low pH earlier in the reproductive cycle or chronic exposure would result in discernible calcium regulatory dysfunction. Indeed, plasma calcium levels in male trout tend to be relatively invariant

(Whitehead et al. 1978), but they were reduced after exposure to pH 4.5 for 42 days.

The low plasma sodium levels in adult trout exposed to pH 4.5 for 6 weeks are consistent with current theory on the effects of acidic conditions on ionoregulation. Packer and Dunson (1970) showed that low pH conditions decrease sodium influx rates and increase efflux rates. Ambient acidity is believed to increase the permeability of the gill epithelium to sodium, accounting for the increased efflux rates (McWilliams and Potts 1978). McDonald et al. (1980) determined that ionoregulation and acid-base balance in acid-exposed rainbow trout are influenced by water hardness. Acute acid exposure in soft water produces severe ionoregulatory dysfunction, but only minor acidosis. However, in hard water, ionoregulation is slightly affected and profound acidosis may occur. Sublethal effects on sodium regulation of adult trout at pH 4.5 may have little meaning in the reproductive process, since no embryos survived to the eyed stage at this pH level.

Sex hormone concentrations did not reveal any gross physiological abnormalities due to acid exposure. Hormone profiles in the acid-exposed fish followed patterns typical for this species (Scott and Sumpter 1983). Estradiol-17β decreased just prior to spawning in the females and remained low throughout final maturation in the males. Androgen levels gradually declined in both males and females. 17α-hydroxy-20β-dihydroprogesterone concentrations were low in the females until ovulation, when they rose dramatically. Because these hormone levels are dynamically regulated and highly variable between

individuals (Schreck et al. 1972; Hille 1982), they are unlikely to provide useful warnings of reproductive impairment at sublethal pH levels.

Hatching was synchronous among the embryos reared at pH levels 5.5 and 5.0 and in unacidified water. Delayed hatching has been noted in Atlantic salmon and rainbow trout embryos that were exposed to low pH after they had reached the eyed stage (Peterson et al. 1980; Nelson 1982). Atlantic salmon embryos that were continuously exposed to low pH from fertilization through hatching, however, were not delayed (Peterson et al. 1980). Delays that have occurred in some studies may be due to inhibition of the hatching enzyme, chorionase, or to reduced activity of embryos just before hatching (Peterson et al. 1980; Peterson and Martin-Robichaud 1983).

Lethargic behavior was characteristic of rainbow trout yolksac larvae at pH levels 5.5 and 5.0. Rombough (1982) observed similar behavior in buttoned-up pink and chum salmon at sublethal pH levels. In an unprotected environment, such behavior might have important implications for the survival of young fish. For instance, salmonid larvae that remain inactive in the gravel may be vulnerable to predation by benthic invertebrates or other fish species, like some sculpins which are known to prey upon salmonid eggs and fry (Scott and Crossman 1973). This vulnerability to predation would depend, of course, on the tolerance of the potential predators for acidic conditions as well.

Salmonid reproductive success in acidic surface waters is determined by an array of water quality factors and biological responses. The experiment reported here demonstrates that salmonid reproduction is endangered at pH levels 5.5 and lower through effects on gamete quality and direct exposure of developing embryos and fry to acidic conditions. However, several other considerations are necessary for approaching a broad understanding of impacts of low environmental pH on salmonid reproduction.

One such consideration is the possible influence on reproductive success of acute or chronic exposure of adult fish to acidic water earlier in the reproductive cycle. In this study, continuous exposure to low environmental pH for 6 weeks prior to spawning affected gamete quality. Yet, ovarian maturation generally commences as early as 6 months before spawning in rainbow trout (Whitehead et al. 1978). Acidic conditions throughout this period of maturation or acute episodic exposures could exert additional influences on gamete quality. For example, disruptions of calcium balance in maturing female fishes as described by Beamish et al. (1975) may become important if acid-exposure occurs at the onset or peak of vitellogenetic activity, but were not observed as a consequence of acid-exposure during final maturation when vitellogenesis had probably been completed.

Studies of salmonid reproduction at low pH under controlled conditions may show physiological effects that are reflected in gamete quality, but they do not address potential effects on spawning behavior. Female brook trout avoided areas at pH 4.0 and 4.5 in

selecting sites for redd construction (Johnson and Webster 1977). Regardless of gamete quality, if female rainbow trout are unable to locate suitable spawning habitat, reproductive failure may occur. As yet, pH levels at which spawning behavior would be inhibited have not been investigated for rainbow trout.

This study partitions out the direct effects of acidic water on gamete quality and progeny survival in salmonids. As such, these data are useful for assessing impacts on salmonid reproduction in systems in which a high concentration of hydrogen ions is the dominant toxic factor. However, another factor that may be important in some acidic systems is metal toxicity. Aluminum, leached from soils by acidic water and transported into surface waters, may reach concentrations that are toxic to fish (Cronan and Schofield 1979). It cannot be assumed that aluminum toxicity and hydrogen-ion toxicity will simply be additive. Baker and Schofield (1982) found that the presence of aluminum was antagonistic to hydrogen-ion toxicity between pH 4.2 and 4.8. Interactions between acids and metals could conceibably alter gamete quality as well as survival of early life history stages.

Acidic waters of pH 5.5 and lower pose a threat to salmonid reproduction and, therefore, valuable fishery resources may be endangered. Prudent evaluations of current impacts or predictions of future impacts of low environmental pH on fish populations must recognize that many physiological and behavioral aspects of fish reproduction may be affected by low pH conditions and high metal concentrations. Caution should be taken to avoid overly simplistic models and techniques for assessment purposes.

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