Effects of depressed pH on survival, growth and reproduction of brook trout, *Salvelinus fontinalis* (Mitchill).

by

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A THESIS

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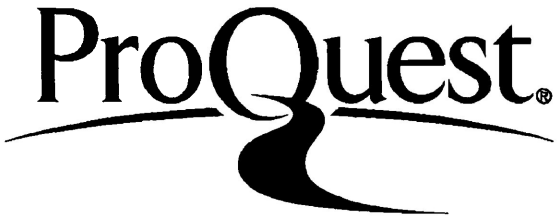

ABSTRACT

Brook trout were continuously exposed to depressed pH values from 4.0 to 6.0, at 0.5 unit intervals during a nine-month, partial-chronic bioassay. At all pH levels above 4.0, juvenile fish exhibited greater than 96 per cent survival. Although a pH of 4.0 was not acutely lethal, juvenile survival was poor. All trout exposed below pH 5.0 showed disturbances in acid-base balance and plasma electrolyte composition. Juveniles did not grow or sexually mature at pH 4.0 and exhibited slower growth at pH 4.5, compared to fish exposed at higher pH's. Inhibition of growth at pH 4.0 was attributed to starvation rather than H-ion concentration. Spawning occurred at all pH levels except 4.0. Eggs produced by females held at pH 4.5 were abnormal in shape and size, compared to those spawned by females held at or above pH 5.0. The total number of eggs produced at pH 4.5, however, did not differ significantly from the higher pH levels.

Embryos from parents exposed to pH 4.5 showed low viability and did not hatch. Viability and hatchability of eggs held in the pH range, 5.0 to 6.0, was significantly reduced ($P < .01$) when compared to eggs held at pH 7.0. Poor alevin survival occurred at all pH levels below 7.0. Data on alevin growth at low pH was highly variable and hence inconclusive.

Results of the present investigation coincide with those of other studies on fathead minnow, brook trout and flagfish, which indicate that reproductive success of these species is affected below pH 6.5.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

INTRODUCTION

Inland freshwater fisheries are threatened by industrial acidification. Declining fish populations have been directly associated with depressed water pH.

A pH of 6.0 is considered to be the lower limit for inland waters. However, higher hydrogen ion (H-ion) concentrations are frequently found in natural waters and can be related to specific chemical characteristics of the water and/or substrate.

In recent years, accelerated acidification of waterways is attributed to human influence, specifically, to industrial pollution.

Fish do exist in natural waters where the pH value is substantially less than 6.0. But many species are unable to sustain their populations when H-ion concentrations exceed this magnitude. Recruitment failure may be the result of spawning impairment. Alternatively, mass mortality of the more sensitive egg and fry stages may also cause the population declines.

To clarify the relationship between declining fish populations and waters undergoing accelerated acidification, I conducted the following partial-chronic study utilizing brook trout (*Salvelinus fontinalis*, Mitchill) continuously exposed to a constant, depressed pH regime.

LITERATURE REVIEW

The pH Limits for Freshwater Fishes.

A survey showed that the pH range of 409 rivers and lakes containing normal fish populations was 6.3 to 9.0 (Ellis, 1937). Reviewing the affects of acids and alkalies on aquatic life (Doudoroff and Katz, 1950; Vivier, 1954), the Ohio River Valley Water Sanitation Commission (ORSANCO, 1955) pointed out that although fish are found at pH values between 4.0 and 10.0, the "safe" (non-lethal) range for most fully developed freshwater fishes was 5.0 to 9.0. Further, the pH value for maximum fish productivity should lie between 6.5 and 8.5. Later studies (McKee and Wolf, 1963; Jones, 1964) support ORSANCO's findings. Hence, according to the European Inland Fisheries Advisory Commission, these criteria have become widely quoted and the "safe" range of pH 5.0 to 9.0 has been generally accepted and adopted (EIFAC, 1969).

Natural Acid Waters.

Waters having a pH below 6.1 do indeed occur in nature (Reid, 1961). Low values result from one or a combination of the following conditions: (1) poorly aerated water with an unusually high carbon dioxide content; (2) organic substrates promoting the production of humic acids; and, (3) highly mineralized groundwater sources which produce inorganic acids. The biology of such atypical freshwater systems has been extensively investigated (Jewell, 1922; Jewell and Brown, 1924 ; Pentelow, 1944; Dunson and Martin, 1973; *etc.*).

Accelerated Acidification of Natural Waters.

I am primarily concerned with accelerated acidification resulting from industrial pollution. Industrial activity depresses the pH of aquatic systems in three ways: (1) directly, by discharge of acidic wastes; and, indirectly by (2) acid mine drainage and (3) atmospheric fallout of acid.

Commercially produced mineral acids such as hydrochloric (HCl) and

sulphuric (H_2SO_4) are used in such industries as metal smelting and pulp and paper manufacturing (Doudoroff and Katz, 1950). Years ago, the effluents (liquid wastes) from such industrial operations were discharged into sewers or directly into rivers and lakes. Today, laws prohibit effluent disposal without prior treatment. In the case of acidic wastes, neutralization with an alkaline chemical such as calcium carbonate ($CaCO_3$) is mandatory. Still, industrial acids all too frequently find their way into freshwater systems.

Coal mining is another major source of industrial acid pollution. Acids are formed within the mine itself. Sulphur-rich iron pyrite (FeS_2) present in the coal-bearing rock oxidizes upon exposure to air and water to produce ferrous sulphate ($FeSO_4$) and sulphuric acid. Further oxidation produces more sulphuric acid and iron compounds (Nichols and Bulow, 1973). Heterogeneous solutions of these acid salts and additional elements leached from mine material exit the mine as acid mine water. Barnes and Romberger (1968) studied the detailed chemical reactions resulting in sulphuric acid production by acid mine drainage. The direct addition is augmented by the practice of dumping low grade coal and shale into waterways and, by washing higher grade coal prior to shipment.

The effects of acid mine drainage have been thoroughly documented in the United States. Severe destruction of aquatic biota by acid mine water are reported from West Virginia (Lackey, 1939), Kentucky (Kinney, 1964), Ohio (Steinback, 1966), and Tennessee (Carrithers and Bulow, 1973; Nichols and Bulow, 1973; Parsons, 1952). Severe habitat damage has also occurred in localized areas of Australia, England, Japan, Korea, South Africa, and the Soviet Union, and probably exists everywhere coal is mined (Porges *et al.*, 1966).

Atmospheric fallout is undoubtedly the largest and most widespread source of acidification of inland waters. Although acid mine drainage can devastate waterways, the destruction is relatively localized when compared to atmospheric fallout which has no limiting boundaries. Sulphate is a common component of the atmosphere, formed by oxidation of sulphur dioxide (SO_2). Fossil fuels used as energy sources in industrial

processes emit enormously high atmospheric concentrations of SO_2. If the air is also rich in water vapour, the oxidative conversion of SO_2 to sulphate (SO_4) is accelerated. The resulting sulphated aerosol droplets possess an extremely high acidity. Most of the acidity is attributed to sulphuric acid (Commins, 1963; Junge and Seiche, 1971) but hydrochloric acid can also be produced by atmospheric reactions (Yue *et al.*, 1976).

The exact reaction kinetics of SO_2 oxidation are poorly understood. The process does require the presence of catalysts (Junge and Ryan, 1958). In addition to minute quantities of metal ions (such as copper, manganese, or iron), trace amounts of ammonia (NH_3) enhances the conversion efficiency of SO_2 to SO_4. Scott and Hobbs (1967) worked out the mechanism of the catalytic action of NH_3 in the SO_2-NH_3-liquid H_2O system.

Sulphur dioxide can also be "dry" oxidized to sulphur trioxide (SO_3) by photochemical reactions (Gerhard and Johnstone, 1955; Cox and Penkett, 1970). In the presence of water vapour the SO_3 is subsequently converted to H_2SO_4. However, production of acid by SO_2 oxidation in solution is much greater than by the SO_2-SO_3-H_2SO_4 system.

Only recently have the effects of atmospheric fallout of acid on the chemistry and biology of freshwater been investigated. In the early 1960's, initial reports of acid "rains" in Europe were documented (Odén, 1968). Yearly acid deposition on Scandinavia alone has been calculated to be one to two grams of H_2SO_4/m^2 (Holt-Jensen, 1973).

During 1970-72, an investigation of nearly 400 lakes on the Swedish west coast revealed pH values consistently below 6.0 (Almer *et al.*, 1974). Many of these lakes temporarily drop to pH values below 5.0 at the surface during the winter and spring seasons. Such temporary pH "shocks" result when acids are released from snow and meltwater which concentrate atmospheric pollutants (Elgmork *et al.*, 1973; Hagen and Longeland, 1973).

In Canada, extensive nickel and copper smelting at Sudbury, Ontario emits more than 2.5 million tons of SO_2 yearly (Beamish and Harvey, 1972). In 1960, a survey of more than 100 lakes and ponds showed pH

values ranging from 7.6 to a low of 3.2 (Gorham and Gorden, 1960). Lakes with pH values below 6.1 were within eight km southwest of the smelter sites. More recently, pH measurements were taken from 150 lakes in the La Cloche Mountain area, located 65 km southwest of Sudbury (Beamish and Harvey, 1972). Some 33 lakes showed a pH of less than 4.5, while an additional 37 had a pH in the range, 4.5 to 5.5.

Both Scandinavian and northern Ontario lakes are typically oligotrophic. The granite lake substrates produce soft waters which are low in buffering capacity. Consequently, acid fallout dramatically affects such waters (Wright and Gjessing, 1976; Summers and Whelpdale, 1976) and their associated fisheries (Schofield, 1976).

The impact of acid rains on the biology of specific lakes and rivers has been devastating. If present rates of acidification continue, about 50 per cent of Sweden's fresh water will be in a "biologically critical" pH range of 5.5 to 5.0 in less than 50 years (Bolin *et al.*, 1971). Beamish and Harvey (1972) describe the La Cloche Mountain lakes with a pH of less than 4.5 as "critically acidic" and those in the range, 4.5 to 5.5 as "endangered lakes".

The effects of acid rains in destroying the aquatic resources of Scandinavia have been thoroughly investigated (Bolin *et al.*, 1971; Jensen and Snekvik, 1972; Holt-Jensen, 1973; Almer *et al.*, 1974; Wright *et al.*, 1976; Hendrey *et al.*, 1976; Leivestad and Muniz, 1976; Overrein, 1976). Similar investigations have been made in Canada (Beamish and Harvey, 1972; Beamish, 1974a, 1974b, 1976; Beamish *et al.*, 1975; Sprules, 1975; Lockhart and Lutz, 1977). The aquatic resources of heavily industrialized countries such as the United States, Britain, Germany, Japan, and others are undoubtedly experiencing similar destruction.

Though accelerated acidification has destroyed fisheries, the mechanisms responsible are unclear. Controlled laboratory experiments (bioassays) dealing with the toxicity of low pH to fishes may help resolve some of these problems.

The Toxicity of Low pH to Fishes.

Because of the formidable amount of bioassay literature dealing

with the acute toxicity of depressed pH on fishes, a detailed discussion of such papers is beyond the scope of this review. However, the EIFAC (1969) has summarized the relevent world literature on acute effects of extreme pH values to freshwater fishes:

pH range	Effect
3.0-3.5	Unlikely that any fish can survive more than a few hours.
3.5-4.0	A range lethal to salmonids. Roach (*Leuciscus rutilus*), tench (*Tinca tinca*), perch (*Perca fluviatilis*), and pike (*Esox lucius*) may, however, survive.
4.0-4.5	Likely to be harmful to salmonids, tench, roach, bream (*Abramis brama*), goldfish (*Carassius auratus*), and common carp (*Cyprinus carpio*), although the resistence to this range increases with size and age of the fish.
4.5-5.0	Likely to be harmful to the eggs and fry of salmonids. Can be harmful to common carp.
5.0-6.0	Unlikely to be harmful to any species unless either the concentration of free carbon dioxide (CO_2) is greater than 20 mg/1 or the water contains iron salts which are precipitated as ferric hydroxide, $Fe(OH)_2$.
6.0-6.5	Unlikely to be harmful to fish unless free CO_2 is present in excess of 100 mg/1.
6.5-9.0	Harmless to fish, although the toxicity of other poisons (particularly heavy metals) may be affected in this range.

modified from: EIFAC, 1969. Water Res. 3: p. 603.

Ellis (1937) and Westfall(1945) suggested that high concentrations

of H-ion suffocated goldfish as a result of mucus precipitation on the gill membrane. This mechanism is referred to as "coagulation film anoxia". Alternatively, anoxia may be the result of direct destruction of the gill epithelium, by precipitation of proteins within the epithelial cell (Kuhn and Koecke, 1956). However, Lloyd and Jordan (1964) found no evidence of either mechanism in similar experiments on rainbow trout (*Salmo gairdneri*). Rather, low water pH caused a reduction in blood pH, suggesting that the cause of death was acidaemia. Recent physiological investigations by Packer and Dunson (1970; 1972) with brook trout confirm that depression of blood pH does occur, but anoxia was still considered to be the ultimate cause of death. Further, these authors and others (Leivestad and Muniz, 1976) also found that fish exposed to lethal acidic pH values experience a massive efflux of sodium and chloride ions. Whether the loss of these plasma electrolytes plays a direct role in fish death at low pH is still unclear.

Chronic bioassays involve exposing an aquatic organism to sublethal concentrations of a toxicant for indefinite periods of time. With respect to fishes, sublethal effects include changes in behaviour (such as feeding patterns, avoidance reactions, social interaction, *etc.*) and effects on long-term survival, growth and reproduction. Effects on survival, growth and reproduction comprise the most meaningful chronic test for assessing non-acute concentrations of a specific toxicant on fishes.

Few studies on the chronic effects of low pH on fishes have been conducted. Mount (1973) investigated the chronic effects of acidic water on fathead minnow (*Pimephales promelas*). Fatheads were continuously exposed to pH levels of 4.5, 5.2, 5.9, 6.6 and 7.5 (control) during a 13-month, one generation test. Survival was not significantly affected even at pH 4.5. However, the species showed no evidence of increased resistance to pH 4.5 even after several months of exposure, refuting the previously held assumption that fish acclimate to low pH levels (EIFAC, 1969). No reliable data on growth was obtained but Mount did demonstrate a significant reduction in egg production and hatchability below pH 6.0. He concluded that H-ion concentrations need to be maintained at or above pH 6.5 because, even though sustained fish populations may exist

at lower levels, production will most likely be reduced. Mount's study supports the assumptions of the Scandinavian and Canadian field workers (Jensen and Snekvik, 1972; Beamish, 1974a), that declining fish populations are the result of lesions occurring somewhere in the reproductive cycle.

Menendez (1976) studied the chronic effects of low pH on brook trout. All developmental stages of the fish were continuously exposed over an 11-month period to pH levels of 4.5, 5.0, 5.5, 6.0, 6.5 and control (pH 7.1). The number of viable eggs spawned were significantly reduced at pH 4.5 and 5.0 and, to a lesser extent, at all higher levels except control. Hatchability was also reduced at all levels below pH 6.5. No trout survived at pH 4.5, and growth of both juvenile-adults and fry-alevins was reduced in the pH range, 5.0 to 6.0. Menendez concluded that brook trout, like fatheads, require a pH at or above 6.5 for successful maintainance of a population.

Craig and Baksi (1977) studied the effects of depressed pH on the life cycle of American flagfish (*Jordanella floridae*). Breeding communities of flagfish were subjected to pH levels of 4.5, 5.0, 5.5, 6.0 and 6.8 (control). Egg production, egg viability and fry growth were impaired at all pH levels below control. Fry survival was reduced at pH 5.5 and 5.0, and no fry survived at pH 4.5. The results of this investigation support earlier findings that successful reproduction of freshwater fishes requires a pH above 6.4.

Although adult spawning impairment has been suggested as the point of lesion in the reproductive cycle (Beamish *et al.*, 1975), other field workers (Jensen and Snekvik, 1972) suggest that the lesion occurs at the more sensitive egg and fry stages. The Scandinavian theory finds support in laboratory studies of low pH effects on the embryos and fry of zebra fish, *Brachydanio rerio* (Johansson *et al.*, 1973), pike (Johansson and Kihlstrom, 1975), rainbow trout (Kwain, 1975), brook trout and white sucker, *Catostomus commersoni* (Trojnar, 1977a, 1977b) and Atlantic salmon, *Salmo salar* (Daye and Garside, 1977).

MATERIALS AND METHODS

I employed the general bioassay procedure for brook trout outlined by the United States Environmental Protection Agency (1971).

The Physical System.

I used untempered municipal water, routed through a 4000-liter charcoal and sand dechlorinator. Wide spectrum fluorescent tubes, controlled by clock timer, simulated sunlight conditions. The dawn/dusk phenomena of the natural environment was simulated by incandescent, low-intensity lighting, also controlled by clock timer. Dawn to dusk intervals reflected local latitude position. Length of photoperiod was adjusted twice a month.

The flow-through system incorporated three major components: (1) header tank; (2) dosing apparatus; and, (3) test chambers.

The 380-liter polypropylene header tank performed three functions: (1) fed water to the dosing apparatus; (2) refilled by drawing water from the dechlorinator; and, (3) increased the dissolved oxygen (DO) content of the water. A pump and solenoid valve connected to a dual-phase timer controlled the water delivery and refill operations. A mechanical-electrical counter (also controlled by the timer) registered the cycle number. Cycle time was 15 minutes. The DO content of the header tank water was elevated by continuous aeration.

The dosing apparatus consisted of two integrated subsystems: (1) a 6-cell, equal-volume diluter (DeFoe, 1975); and, (2) a multichannel pneumatic injector (Smith *et al.*, 1977). Each cell delivered 20 *l* of water per cycle. Sulphuric acid (1N) was added by the injector. All five channels of the injector drew from a common 60-liter polypropylene toxicant reservoir. Injected acid volumes were calibrated to establish a pH gradient of 4.0, 4.5, 5.0, 5.5, and 6.0. The control (pH 7.0) received no acid.

The dosing apparatus fed 24 glass test chambers, consisting of duplicate spawning and progeny tanks. Positional effects were minimized by random assignment of the duplicates on either side of the diluter system.

This arrangement resulted in the designation, "Right" and "Left" bank tanks. All test chambers were enclosed with screened covers. Adequate DO levels were maintained by constant aeration. The spawning tanks (91 cm x 30 cm x 45 cm high) held 80 l. Progeny tanks measured 37 cm x 25 cm x 10 cm high and held 15 l. Ninety per cent of the tank volume was replaced at 5.5-hour intervals in the spawning chambers and at 4.5-hour intervals in the progeny chambers (Sprague, 1969). Debris and algae were removed from the test tanks three times a week. During reproduction, the spawning tanks were shielded by curtains to prevent disturbances from outside activities.

Eggs were deposited on artificial spawning substrates (Benoit, 1974) placed in stainless steel boxes (30 cm x 25 cm x 15 cm high). Egg cups were used to incubate the embryos in the progeny tanks. Cups were constructed from 120 cc glass jars (5 cm OD) with the bottoms removed and replaced with #40 mesh stainless steel screen. A rocker arm apparatus (Mount, 1968) oscillated the cups in the progeny tank waters, simulating stream conditions. Embryos and fry were protected from direct light by a combination of plywood and black plastic covers.

An alarm system connected to the university's security office monitored electrical power and pH. The alarm enabled 24-hour surveillance for power failure and/or lethal pH (< 3.8) throughout the bioassay.

The Chemical System.

Water quality was monitored by taking monthly samples of the dechlorinated water from September, 1975 to March, 1976. Seventeen parameters were measured: conductivity, turbidity, hardness, alkalinity, total phosphorus, sulphate, chloride, ammonia nitrogen, Kjeldahl nitrogen, nitrites, nitrates, copper, zinc, iron, cadmium, lead, and nickel. Tests for free chlorine and chloramines were also conducted on three occasions during the bioassay. In addition, monthly water samples from each of the six pH levels were collected from alternate duplicate tanks and analysed for: alkalinity, hardness, calcium, magnesium, sodium, potassium, chloride, and sulphate. These samples were taken from the spawning tanks from September to December, 1975, and from the progeny tanks during the per-

iod, January to March, 1976. All water chemistry measurements were made in accordance with Standard Methods (American Public Health Association *et al.*, 1971).

Temperature, DO and pH were recorded daily for each pH level from alternate, duplicate test chambers. Temperature readings were taken with a standard mercury thermometer. DO measurements were made with an IBC dissolved oxygen analyzer. A pH-monitoring system (Radiometer) consisting of one PHM 63 pH meter, one SAS-1 six-channel switch, and six combination electrodes continuously measured pH. The system also included the sensing components of the emergency alarm. All three parameters were monitored in the spawning tanks until December 31, 1975 and in the progeny tanks, from January 1 to April 30, 1976.

The Biological System. A. Juvenile-Adult Exposure.

On June 12, 1975, yearling brook trout were obtained from the Dorion Fish Hatchery, Ontario Ministry of Natural Resources. Fifteen randomly selected trout were distributed in each of the 12 spawning tanks. The following day, weights and standard lengths were determined on all test fish. Prior to measurement, individuals were anesthetized with MS-222 (100 mg/l).

Fish were fed a diet of raw diced beef liver daily, throughout the prespawning period. All tanks received the same ration at each feeding.

Test trout were exposed and acclimated for one week to an initial pH of 7.0 (control water value). Depression of pH began on June 19. All test tanks (except control duplicates) were taken down to pH 6.0. After stabilization for 24 hours, all tanks (except 7.0 and 6.0 pH duplicates) were lowered to pH 5.5. This procedure was again repeated, depressing the last three levels to pH 5.0. After 48 hours, depression to pH 4.5 and 4.0 followed. Thus, all test tanks were stabilized at their nominal pH values by June 30, 1975. Chronic exposure therefore began on July 1, 1975.

From July 4 to October 3, I measured weight and standard length on weekly random samples of five fish per pH level. The samples were alternated, from the Right and Left bank duplicates. All measured fish were

anesthetized with MS-222. On October 9, all surviving trout were sexed and measured for weight and standard length, following anesthetization.

Surviving fish were culled for reproduction on October 16. Except for the pH 4.0 level, six trout per duplicate (four females and two males) were randomly selected for spawning. In the case of pH 4.0, only one pair per duplicate was saved. Discarded trout which most nearly approximated the October 9th mean weight (±10 g) for each test level served as a photographic record. Selected fish were photographed alongside a control specimen for comparison.

Blood samples were collected on seven discarded fish from all duplicate spawning tanks. Each fish was anesthetized (MS-222) and approximately two cc of blood was withdrawn by caudal puncture. I employed a three cc syringe (22G needle), prerinsed with aqueous sodium heparin (10%). A 255 µl capillary tube was filled with blood from the syringe. Blood was transferred to the capillary tube by using a short length of PVC tubing (2 mm ID). All capillary samples were iced immediately, while the remaining portion of each blood sample was centrifuged for five minutes at approximately 2000 rpm. Resulting plasma layers were then transferred into separate five cc Vacutainer tubes and stored at 8 ^{0}C.

Within one-half hour after collection the whole blood capillary samples were measured for pH and carbon dioxide tension (pCO_2). Measurements were made at 37 ^{0}C with a Corning 165 Blood pH/gas Analyzer. Utilizing the Henderson-Hasselbach equation (Siggaard-Andersen, 1963) and the hemoglobin value (measured with a Coulter F Hemoglobinometer), the instrument automatically calculated values for total carbon dioxide, bicarbonate and base excess. All values were corrected for the prevailing water temperature (10 ^{0}C) by calculation (Severinghaus, 1966). Pooled plasma samples from each pH duplicate were analysed for major electrolytes (sodium, potassium, calcium, magnesium and chloride) within 24 hours after collection. One sample per pH duplicate was obtained by pooling equal volumes of plasma from equal numbers (seven) of fish. An IL 143 Flame Photometer measured both sodium and potassium ions. Calcium and magnesium were measured by atomic absorption, utilizing a Pye-Unicam SP-90A Spectrophotometer. Chloride was determined on a Corning 920

Chlorimeter. Assayed serum controls (Hyland I and II), as well as an unassayed human sample, were run with all electrolyte determinations.

On October 23 the selected, sexually mature females and males were separated and placed in duplicate spawning tanks at their respective pH levels. One week later the sexes were reunited and one spawning box was added to each tank. Spawning commenced within 48 hours, on November 2, 1975.

All spawning boxes were checked daily and deposited eggs removed and counted. To prevent excessive exposure to direct light the fluorescent lights were turned off during egg removal and counting. The incandescent lights provided sufficient illumination. Each spawning box was removed and placed into a shallow stainless steel tray (68 cm x 48 cm x 3 cm high) filled with test water (2 cm deep) from the same spawning tank. After removal of the spawning substrate, eggs were gently flushed into the tray with test water. Eggs were separated into two groups: (1) "potentially" viable (translucent) eggs; and, (2) dead (white) eggs. All translucent eggs were pipetted into incubation cups in their corresponding progeny tanks. Each cup was numbered and sequentially loaded to a maximum of 250 potentially viable eggs. If more were spawned in one day, the overflow was placed into subsequent cups. Potentially viable eggs obtained from a recognized spawning were incubated for 12 days to determine "actual" viability. Viable eggs developed a neural keel after 12 days incubation at 8 to 9 ^{0}C. A "spawn" was defined as a 24-hour period during which 50 or more eggs were deposited. When less than 50 eggs were present, they were simply counted and discarded.

Spawning continued in each tank until a three week period passed during which no potentially viable eggs were deposited. Using these criteria, spawning ended by January 26, 1976, and all spawning boxes were then removed. One week later, all surviving adults were terminated (overdosed with MS-222). Their gonads were inspected for maturity and reproductive condition.

All mortalities were recorded throughout the juvenile-adult exposure period. Dead fish were autopsied for gonadal development and gross

abnormality of internal organs.

The Biological System. B. Embryo-Alevin Exposure.

After determining viability at each pH level, eggs from the first five cups in both duplicates were retained to determine hatchability (60 days incubation at 8 to 9 ^{0}C). The rest were discarded. Only incandescent lighting was used during daily observations on development and removal of dead embryos from the cups. Additional incubation data were obtained by transferring two groups of 100 eggs (12 days old) to different pH levels. One group of pH 7.0 eggs was transferred into pH 4.5; one group of pH 5.0 eggs was transferred into pH 7.0.

Upon hatching, I determined growth and survival of fry exposed for 90 days to the prescribed pH regime. Because low numbers of hatched fry were obtained in the five test levels, these individuals were saved. In control, 65 fry were retained for growth and survival. I saved another 100 fry from pH 7.0 for later transfer experiments. After swim-up (21 days posthatch) the alevins were released from the cups. They were fed brine shrimp (*Artemius*) nauplii two to three times daily. Alevin lengths were determined by photography (Martin, 1967) at 21, 30, 60 and 90 days after hatching. The alevins were terminated (overdosed with MS-222) at 90 days posthatch and final weights determined. I recorded all alevin mortality during exposure.

Alevin data were supplemented by randomly dividing 100 control alevins (50 days old) into five groups of 20 individuals, and transferring them into the five test levels. Daily mortality of the transfers was recorded. All surviving transfers were terminated in the usual way and final weights determined, after 120 days posthatch.

Statistics.

Growth curves of the juvenile-adult trout during prespawning exposure were constructed using linear regression analysis. All other biological data were subjected to independent one-way analysis of variance (ANOVA) with an F-test at significance levels of .05 and .01. If a significant F-ratio resulted the ANOVA was followed by a multiple range test

(Duncan, 1955) to determine which pH levels yielded significantly different results. When applicable, independent t-tests were employed to test for significant differences ($P < .05$ or $.01$) between duplicate tanks at the same pH level.

RESULTS

The Physical/Chemical System.

The natural, seasonal water temperatures were similar to EPA's recommended thermal regime. Mean monthly temperatures varied little between pH levels (Table 1). Mean DO content was consistently high at all pH levels (Table 2). After culling adult fish, DO's increased about two mg/1 during November, 1975. The oxygen demand of the fry was less than that of the adult fish and resulted in higher DO readings in the progeny tanks after January 1, 1976.

The dosing apparatus maintained good pH control (Table 3). Deviations from the nominal values rarely exceeded ±0.15 pH unit. No major system malfunctions occurred throughout the 11-month bioassay.

The Biological System. A. Juvenile-Adult Exposure.

Prior to pH depression, no significant differences were found between pH levels for trout mean weight or length (Table 4). Nor were significant differences revealed between duplicates at pH levels of 5.5, 5.0, 4.5, and 4.0. Differences between duplicates were found, however, in mean weight and length at pH 7.0 ($P < .05$) and in mean length at pH 6.0 ($P < .01$).

Growth during juvenile-adult exposure (from July to October, 1975) was not affected at or above pH 5.0 (Figures 1 to 3). Rate of growth was somewhat reduced by chronic exposure to pH 4.5 (Figure 4). At pH 4.0, the trout exhibited complete inhibition of growth during the entire bioassay (Figure 5).

After four months exposure, differences were found in trout weight and length at some pH levels (Table 5). Both mean weight and length of the trout held in pH 4.0 and 4.5 water were significantly different from each other and from the fish in the higher pH levels ($P < .01$). Except for the pH 4.5 trout, there were no differences in mean weight and length between duplicates. In the case of the pH 4.5 trout, these differences were significant ($P < .01$).

Sex determination of the pH 4.0 trout was difficult because the

secondary sexual characteristics were poorly developed in all individuals (Table 5). Most of these fish (73.3 per cent) were classified as immature, as well as one fish in pH 4.5. All trout matured at the higher pH levels. Hence, although poor growth occurred at both pH 4.5 and 4.0, secondary sexual development was inhibited only at pH 4.0 (Figures 6 and 7).

Survival was not substantially affected above pH 4.0 (Table 5). Two fish died in pH 5.5. One was accidentally killed by an overdose of anesthetic during a weight determination. The other pH 5.5 death resulted from unknown causes. One death occurred in pH 4.5. Again, cause of death was unknown. At pH 4.0, better than seventy-three per cent of the fish survived four months of prespawning exposure. None of the trout that died at pH 4.0 during this period showed any gross abnormalities of the internal organs. However, all possessed immature gonads. Mortality was reasonably equal between duplicates at pH 4.0. None of the fish selected for spawning at this pH level survived the spawning period (November, 1975 to February, 1976). No selected fish died at any of the higher pH levels during spawning.

The whole blood analyses are presented in Table 6. Significant differences in mean blood pH was found only between the pH 4.5 trout and those in pH 5.5 and 6.0 ($P < .01$). Mean pCO_2 of the pH 4.5 fish was significantly different from the pH 5.0, 6.0 and 7.0 trout ($P < .01$). Total carbon dioxide (CO_2), bicarbonate (HCO_2^-) and base excess (blood alkalinity) for both pH 4.0 and 4.5 fish had significantly different means from the trout in pH 5.5, 6.0 and 7.0 ($P < .01$). With respect to these latter three parameters, the pH 4.0 and 4.5 fish were not significantly different from each other or from the trout in pH 5.0.

Duplicates differed in mean blood pH at pH 6.0 ($P < .01$) and in mean pCO_2 at pH 5.5, 6.0 and 7.0 ($P < .05$). Control duplicates differed in blood alkalinity ($P < .05$) and in total CO_2 and HCO_2^- ($P < .01$). Duplicates at pH 4.0 also differed significantly in total CO_2, HCO_2^- and blood alkalinity ($P < .05$).

No significant differences were found between pH levels for potassium, calcium and magnesium (Table 7). However, both sodium and

chloride means in the pH 4.0 trout differed significantly from the fish in the higher pH levels ($P < .01$). Mean plasma sodium of the trout in pH 4.5 only differed significantly from the pH 5.5, 6.0 and 7.0 groups ($P < .01$). Mean plasma chloride in the pH 4.5 trout was significantly lower than all other groups.

The pH 4.0 trout did not spawn and all four selected fish in this group died during the spawning period. Subsequent autopsies performed on these fish revealed an error in sex determination. Three males and one female had been chosen instead of what was thought to be one pair per duplicate tank. The single female and all three males were sexually immature. Selected males in the higher pH levels all possessed mature testes when autopsied after the spawning period. Immature ovaries were found in one pH 4.5 female. All other females in pH 4.5 and the higher levels were found to possess either mature or spent ovaries upon termination of spawning.

Spawning did occur at and above pH 4.5 (Table 8). Significant differences were found in the mean number of viable eggs per spawning between pH 4.5 trout and those in pH 6.0 and 7.0 ($P < .01$). No differences were found in the number of viable eggs per spawning between pH 6.0, 5.5 and 5.0, nor between pH 5.0 and 4.5. The number of viable eggs per spawning was significantly different between duplicates at pH 7.0 and 5.5 ($P < .05$) and at pH 4.5 ($P < .01$).

The Biological System. B. Embryo-Alevin Exposure.

Eggs failed to hatch at pH 4.5 (Table 9). Mean hatchability per spawn was significantly different between pH 7.0 and pH 6.0, 5.5 and 5.0 ($P < .01$). Mean incubation time at pH 5.0 was significantly shorter than at pH 7.0 and 6.0 ($P < .01$). Incubation time for the pH 5.5 group, however, was not different from either pH 5.0 or pH 7.0 and 6.0.

Eggs transferred into different pH levels did not hatch. The 50 per cent median survival time (MST) of the pH 7.0 embryos transferred to pH 4.5 was 25.5 days. The 50 per cent MST of the pH 5.0 embryos transferred to pH 7.0 was only 11 days.

Figure 8 illustrates growth (length) of the alevins during 90 days

of posthatch exposure. Lengths of the pH 7.0 alevins were significantly greater than the alevins in pH 6.0, 5.5, and 5.0 after 21 days of exposure ($P < .01$). No differences in alevin lengths between pH levels were found after 30 and 60 days. At termination of exposure (90 days), mean length of the pH 5.5 alevins differed significantly from all other groups ($P < .01$).

Ninety-eight per cent of the alevins survived 90 days at pH 7.0 and better than 70 per cent survived 90 days at pH 6.0 (Table 10). Survival at pH 5.5 was slightly better (60 per cent) than at pH 5.0 (56 per cent). Only the mean final weight of the pH 5.5 alevins differed significantly from the other groups ($P < .01$).

The final weights and per cent survival of the control alevins transferred at 50 days posthatch into the five test levels are given in Table 11. Survival at pH 6.0 and 5.5 was better than 80 per cent. A substantially lower survival (55 per cent) occurred at pH 5.0. No transferred alevins survived the 70-day exposure period at pH 4.5 and 4.0. The final mean weight of the pH 5.0 transfers differed significantly from that of the pH 6.0 transfers ($P < .01$). Final mean weight of the alevins transferred to pH 5.5 did not differ from either pH 5.0 or pH 6.0.

Table 1. Monthly water temperatures (^{0}C). Data presented as $\bar{X}$ ± S.D., July 1, 1975 to April 30, 1976.

Month	pH 7.0	pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
July	13.5±0.87	13.3±0.86	13.4±0.93	13.3±0.89	13.1±0.94	13.3±0.94
August	14.4±0.86	14.2±0.86	14.3±0.87	14.2±0.88	14.0±0.89	14.2±0.94
September	14.4±0.92	14.3±0.83	14.3±0.85	14.2±0.85	14.2±0.83	14.4±0.85
October	11.6±0.98	11.6±0.98	11.7±0.98	11.7±0.99	11.6±0.90	11.7±0.99
November	9.7±0.87	9.7±0.83	9.7±0.82	9.6±0.83	9.6±0.84	9.6±0.85
December	7.8±0/43	7.8±0.45	7.8±0.44	7.8±0.45	7.8±0.43	7.8±0.47
January	7.8±0.25	7.9±0.20	7.8±0.25	7.8±0.25	7.7±0.27	7.8±0.32
February	8.0±0.16	7.9±0.18	7.9±0.18	8.0±0.16	8.0±0.16	8.0±0.19
March	7.5±0.32	7.6±0.26	7.6±0.26	7.7±0.27	7.5±0.32	7.5±0.34
April	7.2±0.28	7.7±0.33	7.8±0.30	7.7±0.41	7.1±0.23	7.7±0.53

Table 2. Monthly dissolved oxygen (mg/l). Data presented as $\bar{X}$ ± S.D., July 1, 1975 to April 30, 1976.

Month	pH 7.0	pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
July	7.3±0.18	7.4±0.20	7.3±0.18	7.3±0.21	7.4±0.23	7.5±0.15
August	7.2±0.81	7.2±0.79	7.3±0.23	7.3±0.25	7.5±0.19	7.6±0.25
September	7.4±0.28	7.4±0.25	7.3±0.34	7.3±0.26	7.6±0.27	7.7±0.25
October	7.6±0.27	7.6±0.25	7.7±0.23	7.7±0.18	7.9±0.19	7.8±0.21
November	10.2±0.53	10.1±0.52	10.1±0.42	10.2±0.42	10.1±0.39	9.9±0.54
December	9.5±0.81	9.5±0.92	9.4±0.80	9.7±0.82	9.3±1.06	9.5±0.96
January	9.9±0.39	10.2±0.42	9.6±0.54	9.6±0.63	9.8±0.62	10.0±0.45
February	10.4±0.35	10.2±0.07	10.5±0.14	10.5±0.14	10.7±0.14	10.7±0.14
March	9.1±1.27	8.6±1.41	8.7±1.14	8.8±1.34	8.4±1.13	8.3±0.85
April	9.8±0.82	9.9±0.93	9.6±0.81	9.6±0.83	9.7±1.05	9.8±0.97

Table 3. Monthly pH values (pH units). Data presented as $\bar{X}$ ± S.D., July 1, 1975 to April 30, 1976.

Month	pH 7.0	pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
July	7.58±0.066	6.06±0.068	5.59±0.107	5.07±0.136	4.54±0.112	4.03±0.070
August	7.28±0.095	5.94±0.174	5.28±0.250	4.84±0.105	4.35±0.139	4.00±0.063
September	7.18±0.096	6.04±0.162	5.46±0.371	4.89±0.356	4.45±0.134	3.99±0.054
October	7.19±0.076	5.99±0.135	5.47±0.127	4.91±0.129	4.40±0.010	3.95±0.033
November	7.04±0.049	5.95±0.116	5.46±0.089	4.95±0.115	4.44±0.072	4.00±0.036
December	7.06±0.035	5.85±0.049	5.58±0.052	5.08±0.060	4.40±0.084	4.02±0.100
January	6.88±0.031	5.86±0.038	5.51±0.078	5.05±0.185	4.44±0.106	3.96±0.022
February	6.87±0.036	5.90±0.048	5.54±0.065	5.08±0.046	4.55±0.082	4.02±0.050
March	6.90±0.025	5.92±0.041	5.47±0.056	5.04±0.107	4.54±0.068	4.02±0.003
April	6.95±0.021	5.99±0.048	5.52±0.065	4.98±0.114	4.55±0.062	4.08±0.054

Table 4. Initial weights (g) and lengths (cm) of trout on June 13, 1975. Data presented as $\bar{X}$ ± S.D. (N=30). Numbers in parentheses indicate mean value per duplicate test chamber.

pH level	Weight	Length
7.0	44.4 ± 8.65 (47.1/41.7)[b]	14.5 ± 0.92 (14.9/14.2)[b]
6.0	43.9 ± 7.80 (44.4/43.5)	14.4 ± 0.94 (14.5/13.4)[a]
5.5	40.7 ± 10.53 (41.5/39.8)	14.2 ± 1.17 (14.3/14.0)
5.0	42.8 ± 8.36 (42.1/43.5)	14.5 ± 1.13 (14.5/14.5)
4.5	45.4 ± 8.98 (46.5/44.2)	14.5 ± 0.93 (14.6/14.5)
4.0	42.4 ± 7.52 (41.9/42.8)	14.3 ± 0.84 (14.3/14.2)

a ($P < .01$)

b ($P < .05$)

Table 5. Weights (g) and lengths (cm), sex, and per cent survival of trout on October 9, 1975. Weight and length data presented as $\bar{X}$ ± S.D. Numbers in parentheses indicate mean value per duplicate tank.

pH level	Weight[a]	Length[a]	Males	Females	Immatures	Per cent survival
7.0	233.3 ± 58.81	22.9 ± 1.64	21	9	0	100.0
	(244.5/222.0)	(23.4/22.5)	(11/10)	(4/5)	(0/0)	(100.0/100.0)
6.0	245.0 ± 55.47	23.3 ± 1.90	19	11	0	100.0
	(258.1/231.9)	(23.4/23.1)	(8/11)	(7/4)	(0/0)	(100.0/100.0)
5.5	254.4 ± 70.90	23.5 ± 2.31	19	9	0	93.3
	(259.0/249.7)	(23.9/23.1)	(10/9)	(4/5)	(0/0)	(93.3/93.3)
5.0	230.1 ± 59.98	22.9 ± 1.91	19	11	0	100.0
	(248.9/211.2)	(23.1/22.5)	(9/10)	(6/5)	(0/0)	(100.0/100.0)
4.5	130.4 ± 54.64	19.7 ± 2.01	18	10	1	96.7
	(164.7/94.1)[a]	(20.8/18.5)[a]	(9/9)	(6/4)	(0/1)	(100.0/93.3)
4.0	38.9 ± 13.7	15.1 ± 1.32	3?	2?	17	73.3
	(39.3/38.4)	(15.1/15.0)	(3?/0)	(0/2?)	(9/8)	(80.0/66.7)

a ($P < .01$)

Table 6. Blood pH and gases of brook trout following three months exposure to an acidic pH gradient. Data presented as $\bar{X} \pm$ S.D. (N=14). Numbers in parentheses indicate mean value per duplicate test tank.

pH level	pH[a] (pH units)	pCO_2[a] (mm Hg)	Total CO_2[a] (mMol/1)	Bicarbonate[a] (mEq/1)	Base excess[a] (mEq/1)	Hemoglobin (g/100 ml)
7.0	7.46 ± 0.071 (7.47/7.44)	7.6 ± 2.04 (8.6/6.5)[b]	9.0 ± 1.92 (10.2/7.7)[a]	8.4 ± 1.94 (9.7/7.2)[a]	-14.0 ± 2.69 (-12.4/-15.6)[b]	9.5 ± 1.02 (9.1/9.8)
6.0	7.47 ± 0.090 (7.52/7.41)[a]	7.1 ± 1.82 (6.2/8.1)[b]	8.7 ± 2.53 (9.0/8.4)	8.3 ± 2.32 (8.6/8.0)	-14.2 ± 3.82 (-12.8/-15.5)	9.2 ± 0.96 (9.4/9.1)
5.5	7.47 ± 0.070 (7.48/7.45)	6.8 ± 1.26 (5.9/7.7)[a]	8.5 ± 1.47 (7.9/9.1)	8.0 ± 1.48 (7.4/8.5)	-14.4 ± 2.59 (-14.7/-14.4)	9.0 ± 0.88 (8.9/9.1)
5.0	7.40 ± 0.075 (7.36/7.43)	7.2 ± 2.18 (7.9/6.5)	7.3 ± 1.68 (7.2/7.4)	6.7 ± 1.66 (6.6/6.8)	-17.0 ± 2.54 (-17.7/-16.4)	8.9 ± 1.18 (9.1/8.7)
4.5	7.38 ± 0.061 (7.37/7.39)	6.7 ± 0.85 (6.8/6.7)	6.4 ± 1.45 (6.2/6.7)	5.9 ± 1.51 (5.7/6.1)	-18.0 ± 2.68 (-18.4/-17.6)	10.4 ± 1.30 (10.5/10.3)
4.0	7.41 ± 0.080 (7.40/7.43)	5.2 ± 1.58 (4.5/6.0)	5.8 ± 1.99 (4.6/7.0)[b]	5.2 ± 1.90 (4.1/6.4)[b]	-18.6 ± 3.27 (-20.3/-16.9)[b]	10.0 ± 1.46 (10.0/9.9)

a ($P < .01$) b ($P < .05$)

Table 7. Plasma electrolytes (mEq/l) of brook trout following three months exposure to an acidic pH gradient. Data presented as $\bar{X} \pm$ S.D. (N=2). Numbers in parentheses denote pooled electrolyte value per duplicate tank. Insufficient plasma sample for calcium and magnesium determinations at pH 4.0.

pH level	Sodium[a]	Potassium	Calcium	Magnesium	Chloride[a]
7.0	165.5 ± 6.36	2.7 ± 0.28	5.7 ± 0.07	1.6 ± 0.07	124.0 ± 2.83
	(170/161)	(2.5/2.9)	(5.6/5.7)	(1.6/1.5)	(122/126)
6.0	157.0 ± 7.07	3.0 ± 0.71	5.1 ± 0.07	1.7 ± 0.42	124.0 ± 0
	(152/162)	(2.5/3.5)	(5.1/5.0)	(2.0/1.4)	(124/124)
5.5	159.5 ± 2.12	2.1 ± 0.71	6.5 ± 1.06	1.5 ± 0.14	126.5 ± 2.12
	(161/158)	(2.0/2.2)	(5.7/7.2)	(1.4/1.6)	(128/125)
5.0	151.5 ± 0.71	2.3 ± 0.28	6.8 ± 0.78	1.7 ± 0.64	125.0 ± 0
	(152/151)	(2.1/2.5)	(6.2/7.3)	(1.4/1.3)	(125/125)
4.5	136.0 ± 1.41	2.2 ± 0.28	6.3 ± 1.41	1.4 ± 0.07	98.0 ± 2.83
	(137/135)	(2.4/2.0)	(7.3/5.3)	(1.4/1.3)	(100/96)
4.0	110.0 ± 8.49	2.5 ± 0			59.5 ± 2.12
	(104/116)	(2.5/2.5)			(58/61)

a ($P < .01$)

Table 8. Spawning of brook trout following four months exposure to an acidic pH gradient. Total and viable eggs per spawning presented as $\bar{X} \pm$ S.D. Numbers in parentheses denote values per duplicate tank.

Parameter	pH 7.0	pH 6.0	pH 5.5	pH 5.0	pH 4.5
Immature females	0	0	0	0	1(0/1)
Mature females	4(2/2)	5(2/3)	3(1/2)	3(2/1)	1(1/0)
Spent females*[1]	4(2/2)	3(2/1)	5(3/2)	5(2/3)	6(3/3)
Spawnings*[2]	33(12/21)	19(11/8)	31(16/15)	26(17/9)	23(11/12)
Total eggs*[3]	6127(2470/3657)	3754(2190/1564)	6434(3005/3429)	5475(3791/1684)	4707(2216/2491)
Viable eggs*[4]	5188(2015/3173)	3095(1668/1427)	3205(1072/2133)	2538(1839/699)	1536(267/1269)
Per cent viable	85(81.6/86.7)	83(72.2/91.2)	50(35.7/62.2)	46(48.5/41.5)	33(12.1/50.9)
Spawnings/female	8(6.0/10.5)	6(5.5/8.0)	6(5.3/7.5)	5(8.5/3.0)	4(3.7/4.0)
Total eggs/female	1532(1235/1829)	1251(1095/1564)	1287(1002/1715)	1095(1896/561)	785(739/830)
Viable eggs/female	1297(1008/1587)	1032(834/1427)	641(357/1067)	508(920/233)	256(89/423)
Total eggs/spawning	182±137(193/179)	171±174(170/169)	193±123(173/214)	204±178(223/169)	195±145(191/199)
Viable eggs/spawning[a]	200±44(183/211)	155±69(152/159)	134±71(107/152)	115±61(123/100)	96±68(53/115)

*[1] Females retaining <100 large, loose eggs at termination.

*[2] Twenty-four hour egg depositions, yielding >49 eggs.

[3] All eggs deposited, from spawnings[2].

*[4] Viable eggs after 12 days incubation at 8 to 9 ^{0}C.

a ($P < .01$)

Table 9. Hatchability of brook trout eggs spawned in and exposed to an acidic pH gradient. Hatchability per spawn and incubation time presented as $\bar{X} \pm$ S.D. Numbers in parentheses indicate the number of spawnings in which hatching occurred.

pH level	Total eggs set to hatch	Per cent reaching eyed-stage	Per cent hatched	Hatchability per spawn[a]	Incubation time in days[a]
7.0	1846	58.8	35.9	66.2 ± 81.62	60 ± 1.8(6)
6.0	1775	23.4	1.0	1.8 ± 2.94	61 ± 1.7(3)
5.5	1310	10.5	1.2	1.5 ± 3.17	59 ± 0(2)
5.0	1305	15.3	1.9	2.5 ± 3.78	57 ± 2.2(5)
4.5	830	2.7	0		

a ($P < .01$)

Table 10. Per cent survival and ninety-day final weights (g) of brook trout alevins spawned in various acid pH's. Weight data presented as $\bar{X}$ ± S.D.

pH level	Number of exposed alevins	Per cent survival	Final weight[a]
7.0	65	98.5	0.179 ± 0.0737(N=64)
6.0	18	72.2	0.192 ± 0.0754(N=13)
5.5	15	60.0	0.310 ± 0.0879(N=9)
5.0	25	56.0	0.166 ± 0.0698(N=14)

a ($P < .01$)

Table 11. Per cent survival and one hundred and twenty-day final weights (g) of control alevins (fifty days old) transferred into various acid pH's. Weight data presented as $\bar{X}$ ± S.D.

pH level	Per cent survival	Final weight[a]
6.0	80.0	0.203 ± 0.0774(N=16)
5.5	85.0	0.246 ± 0.0965(N=17)
5.0	55.0	0.328 ± 0.0924(N=11)
4.5	0(after 48 days)	
4.0	0(after 12 days)	

a ($P < .01$)

Figure 1. Growth of juvenile-adult brook trout during four months exposure to an acidic pH gradient: pH 6.0 versus control (pH 7.0) regression line.

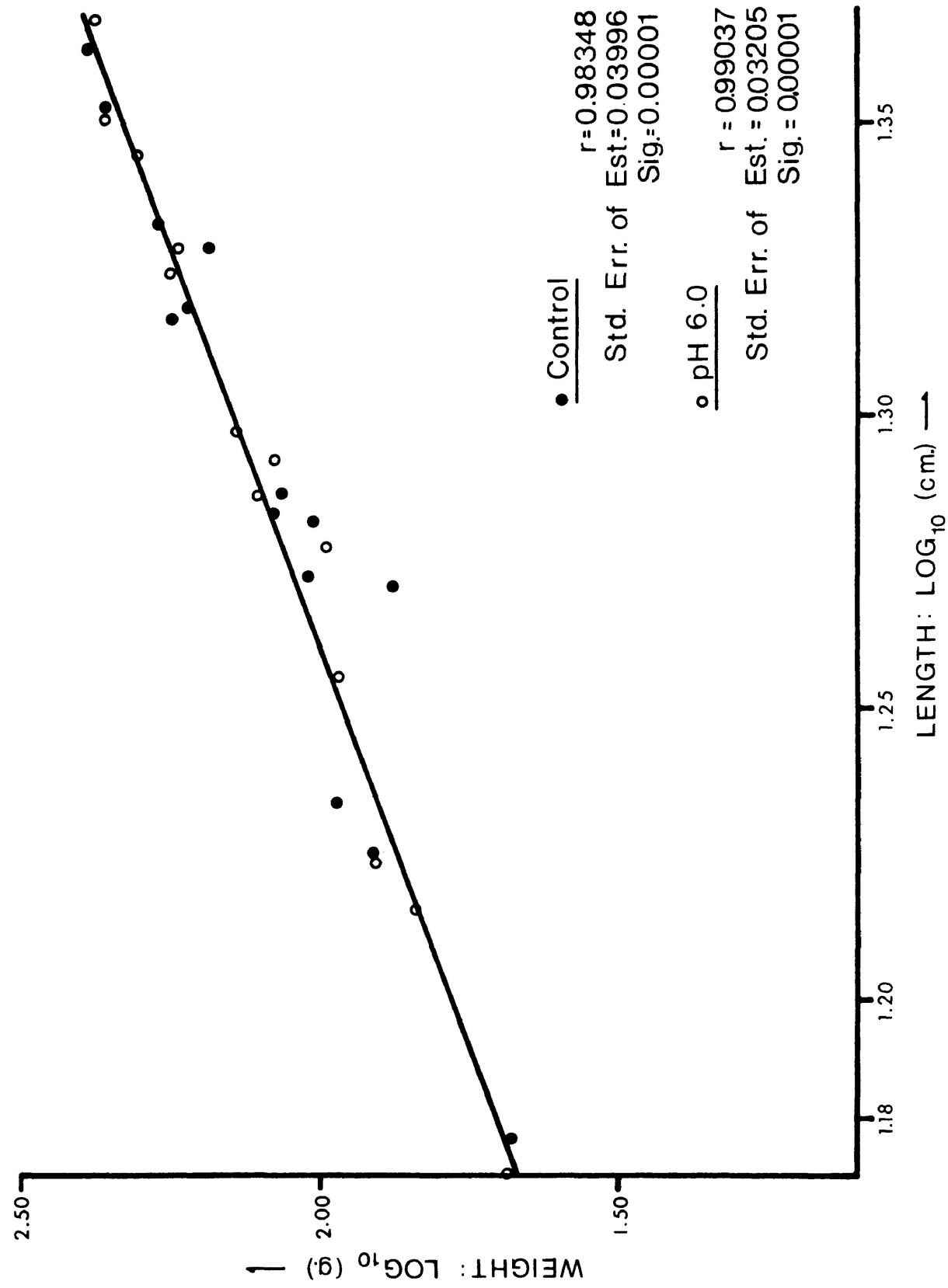

WEIGHT: LOG10 (g) ⟶
2.50
2.00
1.50
1.18
1.20
1.25
1.30
1.35
LENGTH: LOG10 (cm.) ⟶
• Control
r=0.98348
Std. Err. of Est.=0.03996
Sig.=0.00001
o pH 6.0
r = 0.99037
Std. Err. of Est. = 0.03205
Sig. = 0.00001

Figure 2. Growth of juvenile-adult brook trout during four months exposure to an acidic pH gradient: pH 5.5 versus control (pH 7.0) regression line.

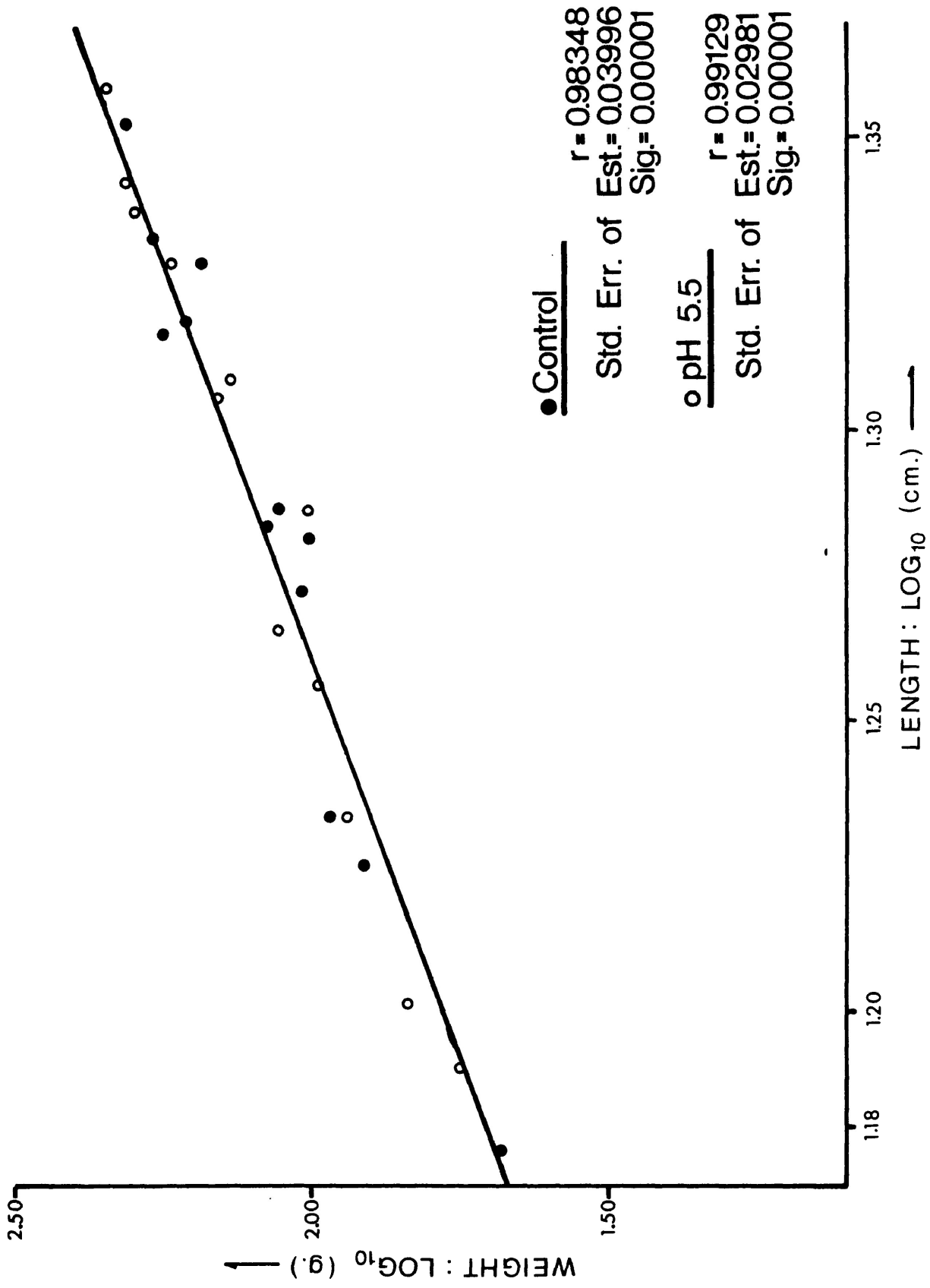

●Control
r = 0.98348
Std. Err. of Est.= 0.03996
Sig.= 0.00001
o pH 5.5
r = 0.99129
Std. Err. of Est.= 0.02981
Sig.= 0.00001
WEIGHT : LOG10 (g.)
LENGTH : LOG10 (cm.)
2.50
2.00
1.50
1.18
1.20
1.25
1.30
1.35

Figure 3. Growth of juvenile-adult brook trout during four months exposure to an acidic pH gradient: pH 5.0 versus control (pH 7.0) regression line.

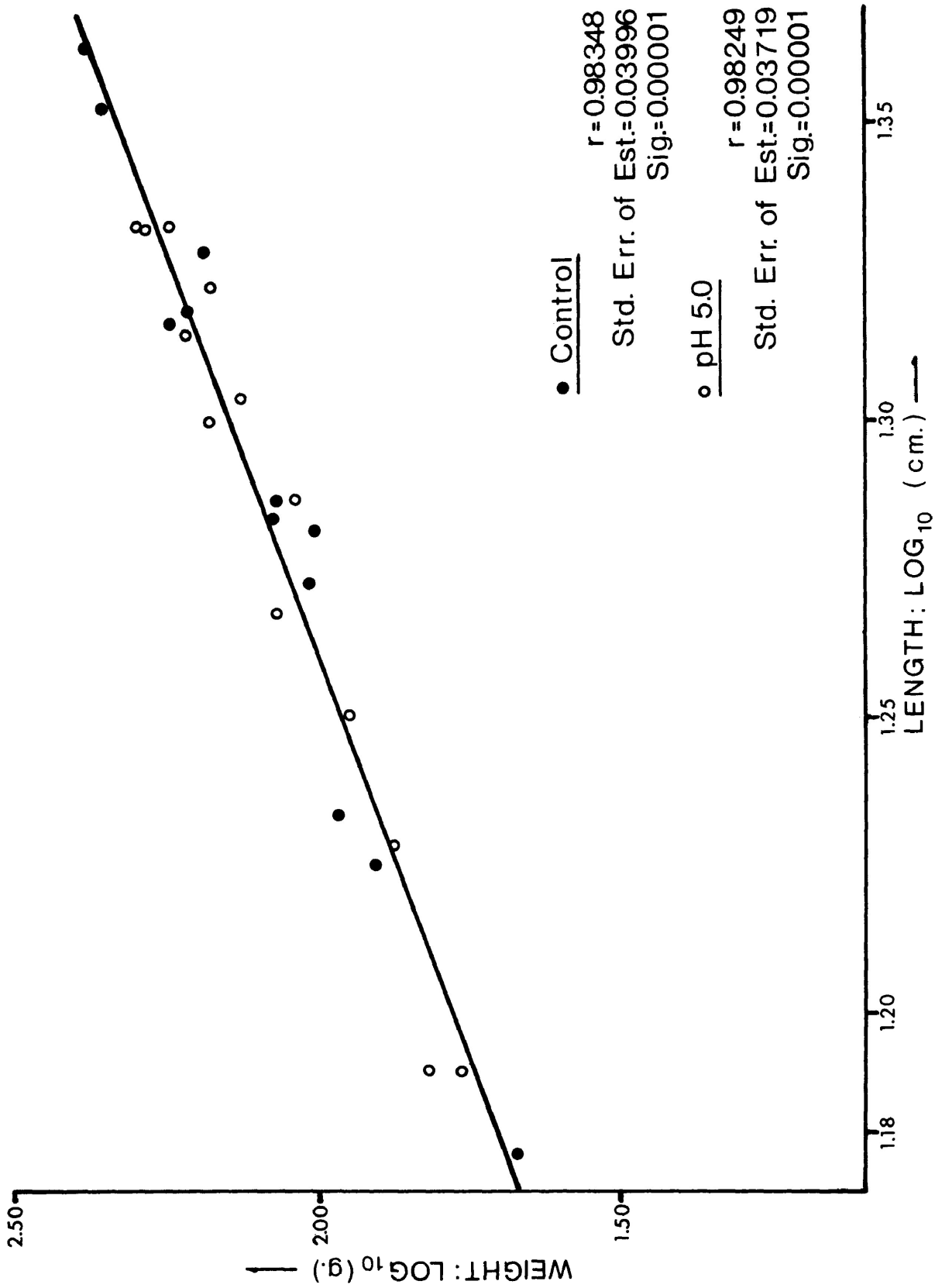

• Control
r=0.98348
Std. Err. of Est.=0.03996
Sig.=0.00001
° pH 5.0
r=0.98249
Std. Err. of Est.=0.03719
Sig.=0.00001
WEIGHT: LOG10 (g.)
LENGTH: LOG10 (cm.)
2.50
2.00
1.50
1.18
1.20
1.25
1.30
1.35

Figure 4. Growth of juvenile-adult brook trout during four months exposure to an acidic pH gradient: pH 4.5 versus control (pH 7.0) regression line.

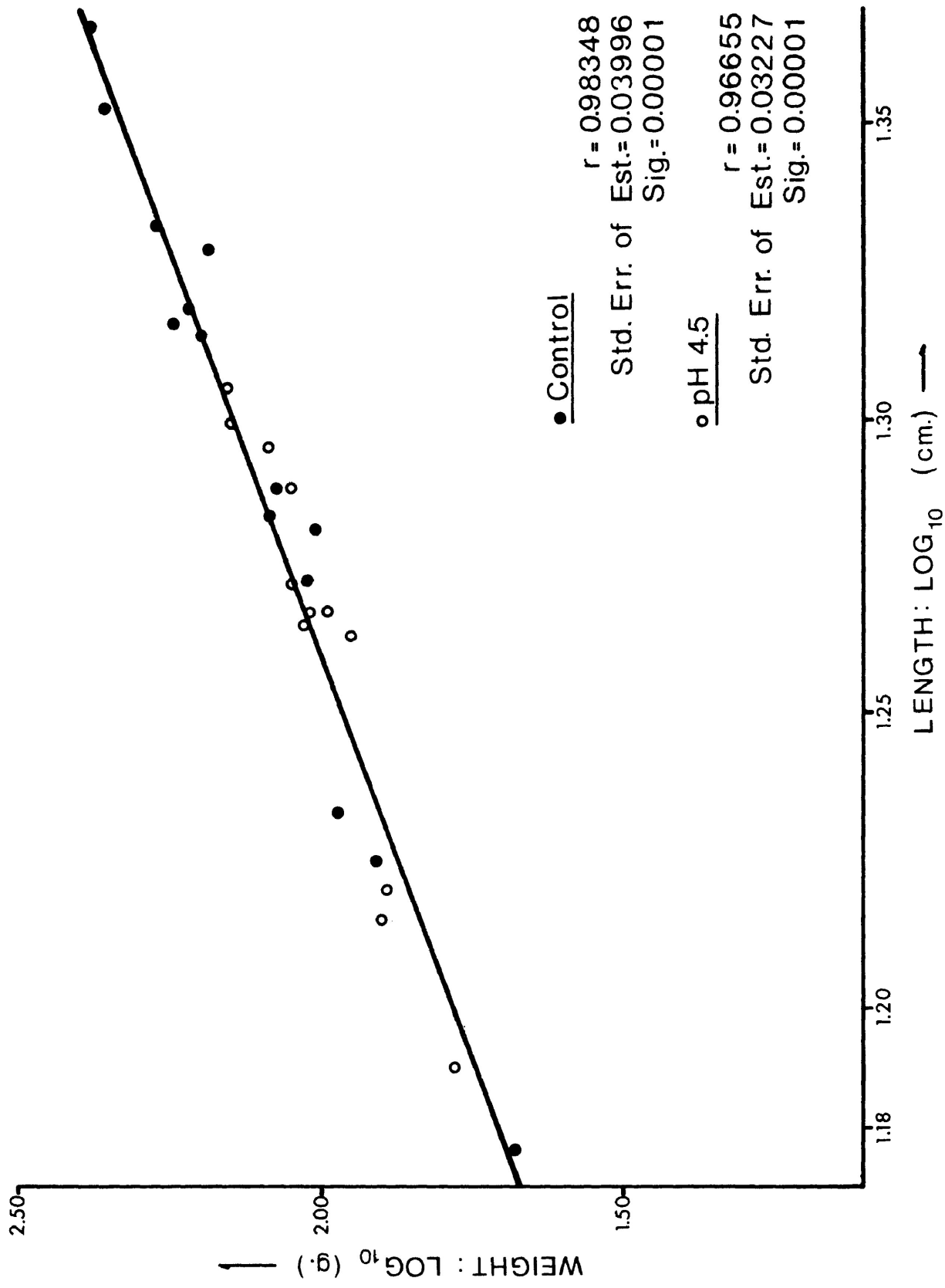

● Control
r = 0.98348
Std. Err. of Est.= 0.03996
Sig.= 0.00001
○ pH 4.5
r = 0.96655
Std. Err. of Est.= 0.03227
Sig.= 0.00001
WEIGHT : LOG10 (g.)
LENGTH : LOG10 (cm.)
2.50
2.00
1.50
1.18
1.20
1.25
1.30
1.35

Figure 5. Growth of juvenile-adult brook trout during four months exposure to an acidic pH gradient: pH 4.0 versus control (pH 7.0) regression line.

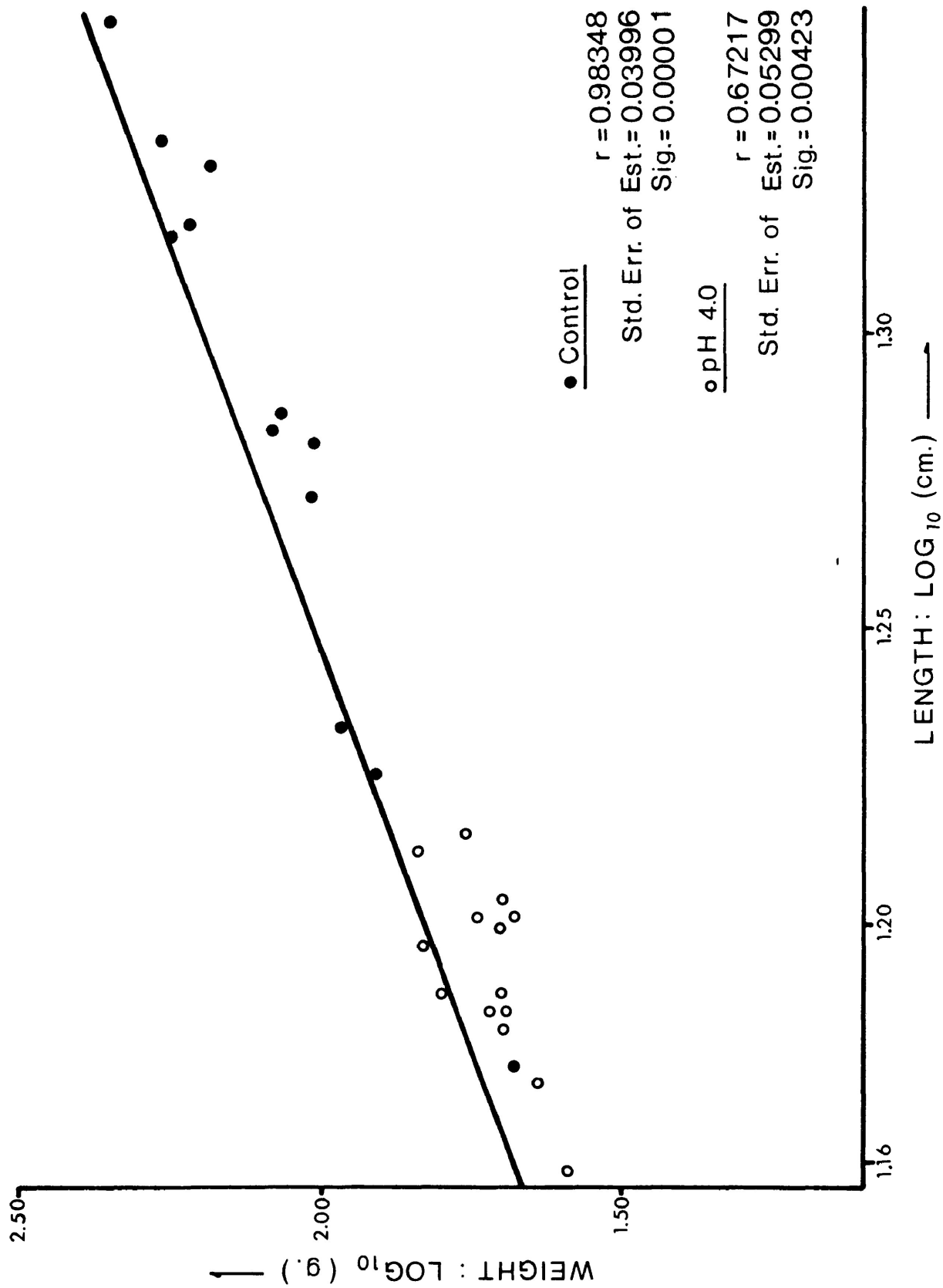

● Control
r = 0.98348
Std. Err. of Est. = 0.03996
Sig. = 0.00001
○ pH 4.0
r = 0.67217
Std. Err. of Est. = 0.05299
Sig. = 0.00423
WEIGHT : LOG10 (g.) ——
LENGTH : LOG10 (cm.) ——
2.50
2.00
1.50
1.16
1.20
1.25
1.30

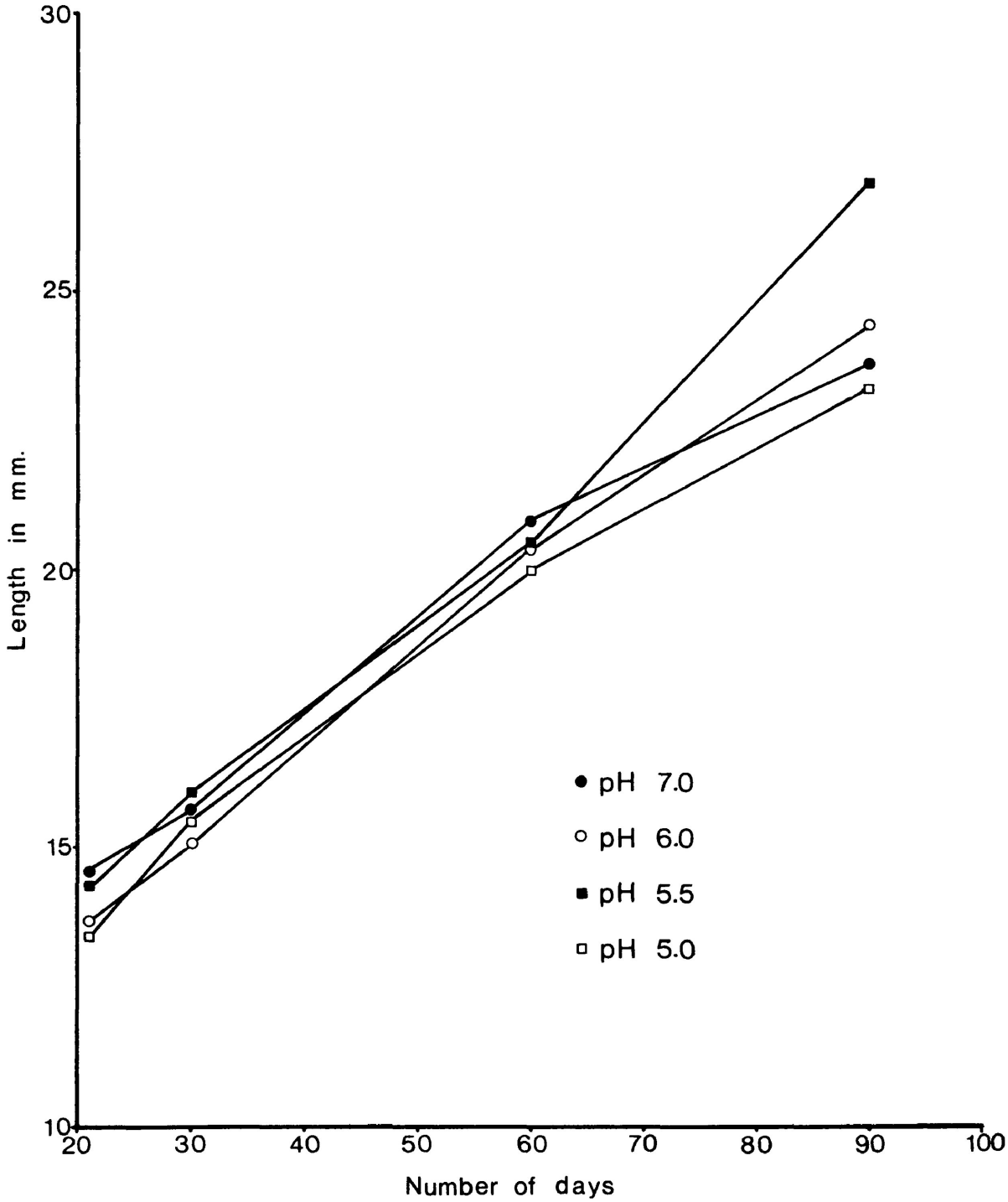
Figure 6. Comparison of growth in adult brook trout following four months exposure to an acidic pH gradient: pH 4.5 and control.


pH 7.0
CONTROL
232.9m.
pH 4.5
130.9m

Figure 7. Comparison of growth and secondary sexual development in brook trout following four months exposure to an acidic pH gradient: pH 4.0 and control.


pH 7.0
CONTROL
232 gm.
pH 4.0
47 gm.

Figure 8. Ninety day growth expressed as length (mm) of brook trout alevins spawned in and exposed to an acidic pH gradient.


30
25
20
15
10
Length in mm.
20
30
40
50
60
70
80
90
100
Number of days
pH 7.0
pH 6.0
pH 5.5
pH 5.0

DISCUSSION

A. Juvenile-Adult Exposure. 1. Survival.

Juvenile and adult brook trout did not survive chronic exposure to pH 4.0. This particular H-ion concentration, however, is not acutely lethal. Under similar water conditions, Daye and Garside (1975) estimated the lethal pH for yearling brook trout to be 3.5 in approximately four days of exposure. In the present study mortality at pH 4.0 first occurred at seven weeks and one fish survived seven months.

Lloyd and Jordan (1964) attribute death at low environmental pH to acidaemia. A pH of 3.15 was shown to be lethal to rainbow trout yearlings in two to four hours. These fish exhibited lowered blood pH prior to death. When exposed for acute periods to pH values below 3.6, brook trout suffered a decrease in oxygen (O_2) consumption concurrent with massive plasma sodium loss and acidaemia (Packer and Dunson, 1970; 1972). Depression of blood pH reduced O_2 uptake at the gills and death was ultimately due to anoxia. Loss of body sodium was considered to be of secondary importance at this pH.

The blood chemistry analyses were performed to ascertain whether death at pH 4.0 was a direct or indirect effect of H-ion concentration. If water of pH 4.0 caused death the brook trout should show signs of acidaemia. However, acidaemia was not evident at any of the environmental pH levels tested. Even though statistically significant differences were found in mean blood pH between the trout held at pH 6.0 and those held at pH's of 4.5 and 5.5, and also between duplicate tanks at pH 6.0, these differences are probably not relevant. Control blood pH was not significantly different from that of the pH 4.0 trout. The blood pH range of "normal" (control) brook trout has been reported to be 7.31 to 7.47 at 30 ^{0}C (Packer and Dunson, 1970). According to the calculations of Rosenthal (1948) this range is actually pH 7.54 to 7.70, when corrected for the temperature at which the fish were held (14 to 15 ^{0}C). My controls had a somewhat lower range (pH 7.39 to 7.53) at 10 ^{0}C. However, because of differences in environmental temperature (and in blood sampling techniques) the two studies are not directly comparable. Howell *et al.*

(1970) clearly demonstrated the importance of water temperature on real blood pH values in poikilothermic vertebrates. At a common water temperature of 10 ^{0}C, a range of pH 7.47 to 7.84 has been reported for rainbow trout blood, utilizing similar sampling methods (Wedemeyer and Nelson, 1975). These values for salmonid blood agree well with the overall range determined for brook trout (pH 7.35 to 7.51) in the present study. Hence, the blood pH values of my brook trout are most likely normal.

The pCO_2 values are difficult to assess. Statistically significant differences generated by the pCO_2 data are probably not relevant. No differences were found between the trout exposed to pH 4.0 and those exposed to pH 5.5, nor between the trout in pH 4.5 and those in the higher pH levels. Wedemeyer and Chatterton (1971) determined the pCO_2 range for coho salmon (*Oncorhynchus kisutch*) to be 2.6 to 6.1 mm Hg at 10 ^{0}C. Although my range for brook trout is wider (4.96 to 8.57 mm Hg at 10 ^{0}C) there is still good agreement between the two ranges. Hence, the pCO_2 values are also probably normal.

Freshwater fishes usually do not alter pCO_2 levels to regulate acid-base balance; rather, they depend primarily upon adjustments in plasma bicarbonate (Randall and Cameron, 1973). My brook trout bicarbonate values, as well as total CO_2 and blood alkalinity parameters are significantly lower for both the pH 4.0 and 4.5 groups, compared to those held in pH's above 5.0. Yet blood pH values for all groups were normal. Diminished levels for these latter three acid-base measurements indicate compensated acidosis as environmental pH decreases. Below a water pH of 5.0 this metabolic adjustment occurs in order to maintain normal blood pH.

Significantly lower plasma sodium and chloride values in the trout exposed below pH 5.0 indicates severe ionic imbalance. In an effort to maintain normal blood pH, brook trout alter their plasma sodium chloride level, in addition to alterations in their acid-base balance. Freshwater fishes exchange sodium for hydrogen ions and chloride for bicarbonate through the gill epithelium (Evans, 1975). Packer and Dunson (1970; 1972) showed that low environmental pH impairs active sodium uptake. Low plasma chloride also occurs when fishes are exposed to water of low pH (Leiv-

estad and Muniz, 1976).

The range for plasma sodium levels in the control (pH 7.0) trout approximated 159 to 172 mEq/1. These values are higher than the "normal" range previously reported for chars: 137 to 143 mEq/1 for arctic char, *Salvelinus alpinus* (Natochin and Lavrova, 1974); 122 to 128 mEq/1 for brook trout (Housten *et al.*, 1971). Use of the sodium salt of heparin in the blood-collecting equipment gave elevated sodium values. Plasma chloride values (121 to 127 mEq/1) in my controls agrees with earlier reported normal values for salmonids: 84 to 132 mEq/1 for rainbow trout (Wedemeyer and Chatterton, 1971); 118 to 120 mEq/1 for brook trout (Housten *et al.*, 1971).

Even though the plasma electrolyte composition and acid-base balance of the trout in pH 4.5 was indicative of metabolic disruption, survival was good (97 per cent). Indeed, brook trout do occur in natural waters where the pH is as low as 4.5 (Creaser, 1930; Dunson and Martin, 1973). Curiously, Menendez (1976) found that brook trout do not survive chronic exposure to pH 4.5. Free CO_2 concentrations above 20 mg/1 are known to increase the toxicity of acid waters to trout (Lloyd and Jordan, 1964). However, the acidity values of the test waters reported by Menendez (1976) indicate that free CO_2 was well below that shown to increase H-ion toxicity. Lloyd and Jordan (1964) have also demonstrated that the resistance of rainbow trout to low pH increases with water hardness. But the hardness of water employed by Menendez (70 mg/1 as $CaCO_3$) was twice as hard as mine (25 mg/1 as $CaCO_3$). Fathead minnows chronically exposed to pH 4.5 exhibited relatively good survival (80 per cent) in water of 200 mg/1 hardness (Mount, 1973). At the same pH value, flagfish showed 64 per cent survival in water of 28 mg/1 hardness (Craig and Baksi, 1977). However, water hardness may only play a secondary role in determining fish survival at low pH. As shown in the present study, water which is relatively low in total salt content (Appendix 1) severely affects normal sodium chloride levels in brook trout when environmental pH drops below 5.0. Trojnar (1977b) has further demonstrated that the lethal pH level for eggs and fry of the white sucker can be effectively lowered by increasing the salt concentration of the water. He suggests that the

presence of calcium salts may facilitate the uptake of sodium and chloride ions. Therefore, the availability and uptake efficiency of sodium chloride (not water hardness) may explain the pH 4.5 survival differences between the present study and that of Menendez (1976). Alternatively, this survival difference may be related to differences in pH tolerance among strains of brook trout. Recent work by Robinson *et al.* (1976) provides strong evidence that acid tolerance in brook trout is hereditary.

Even at pH 4.0, the biochemical data clearly indicates that brook trout are indeed able to maintain normal blood pH for prolonged periods. The poor survival for this pH level is not directly the result of H-ion concentration. Rather, poor survival results from some other form of stress. Based on casual observations, the chronic physiological stress of low water pH produces profound behavioral changes in brook trout, resulting in diminished feeding intensity and/or food consumption. The acid-stressed trout in pH 4.0 simply starved to death. The growth data supports this hypothesis.

A. Juvenile-Adult Exposure. 2. Growth.

Juvenile brook trout grow poorly in extreme acid water conditions. Growth is diminished at pH 4.5 and below. A pH of 4.0 has a dramatic effect on trout growth. As expected, the growth rate of fishes in acid waters is usually less than that under alkaline conditions (EIFAC, 1969). However, evidence is less clear as to whether or not reduced growth rates are the result of a direct effect of low pH. Campbell (1961), Pentelow (1944) and others attribute poor growth of fishes in acid waters to a reduced food supply. EIFAC (1969) suggested that the reduced growth results from unavailability of food, particularly reduced primary productivity. But Menendez (1976) observed slow trout growth at low pH even though they were adequately fed. His conclusion that reduced growth is not entirely due to a sparse food supply is supported by my study. The decreased feeding intensity of the trout at pH 4.5 and 4.0 was observed throughout the juvenile-adult exposure period. Indeed, the fish at pH 4.0 ceased feeding entirely after two to three weeks exposure, even

though they received the same daily ration as the trout in the higher pH levels. Hence, a physiologically-induced behavioural change in feeding intensity was a direct effect of H-ion concentration at pH 4.5 and 4.0. Reduced growth, therefore, was directly related to food utilization and only indirectly related to pH. White sucker also show a decrease in feeding intensity at low pH (Beamish, 1972).

The trout in pH 4.0 actually exhibited a decrease in body weight during the four month, prespawning exposure period. Loss of body weight, lack of food utilization and eventual cessation of activity confirmed that these fish literally starved to death despite an adequate food supply.

A. Juvenile-Adult Exposure. 3. Reproduction.

Secondary sexual development is severely inhibited in juvenile brook trout chronically exposed to a pH of 4.0. These sexually immature fish cannot reproduce. Their arrested sexual development directly results from nutritional deficiency, caused by diminished feeding intensity at very low pH.

Even though brook trout grow slower at pH 4.5, sexual development is not significantly affected; nor does pH 4.5 inhibit spawning. A single female in Menendez's study (1976) survived into the spawning period at pH 4.5, and was found to possess mature ovaries. Whether or not this female would have spawned successfully in the presence of a male was not determined. Fathead minnows (Mount, 1973) failed to spawn at pH 4.5 and flagfish (Craig and Baksi, 1977) showed reduced egg production at the same pH. Perhaps brook trout are less sensitive to pH 4.5 than either fatheads or flagfish. Indeed, total egg production in my study was not affected in the pH range, 7.0 to 4.5. However, casual observations during daily clearing of the spawning trays revealed that eggs produced at pH 4.5 were inferior in quality, compared to those spawned at higher pH levels. Large numbers of dead eggs were removed from the pH 4.5 trays, and more egg "shells" were found at this pH. Potentially viable eggs at pH 4.5 were more adhesive, oblong in shape, and less turgid than those produced at higher pH levels. Similar abnormalities in fathead minnow eggs spawned

at pH 5.9 have also been observed (Mount, 1973). The flaccid, jelly-like consistency of the pH 4.5 eggs appeared similar to salmonid ova prior to water-hardening. An inhibition or, at least retardation of, the water-hardening process may possibly explain the apparent poor quality of these eggs. Alternatively, their inferior quality may also be related to disruptions in ovarian maturation. Beamish *et al.* (1975) found abnormally low serum calcium concentrations in female white suckers taken from an acidified lake (George Lake, pH 4.7) near Sudbury, Ontario, Canada. During secondary sexual development (ovarian maturation), serum calcium levels in female fish are normally twice that of males (Fleming *et al.*, 1964; Oguri and Takada, 1967). George Lake female suckers exhibited serum calcium concentrations similar to that of males. The sucker population at that time was not reproducing and the authors speculated that reproductive impairment was the result of the female's inability to produce ova, due to inadequate levels of serum calcium. Even though the pH 4.5 females spawned in the present study, insufficient incorporation of calcium may have contributed to the poor quality of the eggs. Unfortunately the plasma calcium levels determined in my experiments were conducted on pooled samples, comprised mainly from male plasma. I did not find any significant differences in blood calcium between the pH 4.5 trout and those in the higher pH levels.

The spawning data demonstrates the reduced viability of brook trout eggs spawned at low pH. Viability falls to less than 50 per cent at and below pH 5.0. The viability data at pH 5.0 was similar to that of Menendez (1976).

The largest variability in the spawning data between Right and Left bank duplicates occurred at pH 4.5. A pH of 4.5 is probably critical with respect to brook trout spawning.

B. Embryo-Alevin Exposure. 1. Survival.

At low environmental pH, brook trout embryos and alevins show variable survival. Less than three per cent of the viable eggs produced by the pH 4.5 spawners reached the eyed-stage of development, and none hatched. Further, eggs transferred from pH 7.0 to 4.5 did not hatch. Clearly, a pH

of 4.5 arrests development and hatching of brook trout embryos. The data coincides with similar depressed pH studies on fish embryos. For example, rainbow trout eggs would not hatch at and below pH 4.5 (Kwain, 1975) and also, brook trout and white sucker embryos do not survive below pH 4.5 (Trojnar, 1977a, 1977b). Acid tolerance, however, is also species specific. Considerably lower lethal limits of acidic pH have been reported for embryos of zebra fish (Johansson *et al.*, 1973) and Atlantic salmon (Daye and Garside, 1977).

Poor hatchability occurred at all pH levels above 4.5, suggesting less than ideal incubation conditions during embryonic development. The rocker arm apparatus may have caused excessive physical abrasion of the eggs, which partly contributed to the poor hatching data. However, hatchability in the pH range, 5.0 to 6.0 was significantly reduced when compared to pH 7.0. Reduced egg hatchability for fathead minnow (Mount, 1973), brook trout (Menendez, 1976; Trojnar, 1977a), white sucker (Trojnar, 1977b), and American flagfish (Craig and Baksi, 1977) has also been reported.

The embryo transfer experiments suggest that high H-ion concentrations are immediately toxic to newly-spawned brook trout eggs. Eggs spawned and held at pH 5.0 for 12 days, then transferred to pH 7.0 survived only half as long as eggs spawned in neutral water and later transferred to pH 4.5. The hatching results for zebra fish (Johansson *et al.*, 1973) and for Atlantic salmon (Daye and Garside, 1977) suggests that younger embryos may indeed be more sensitive than older ones. But none of my transferred brook trout eggs survived to the eyed-stage of development. Most likely, the chorion provides little protection of the embryo at any stage of development against high H-ion concentrations.

Alevins spawned and hatched in acid waters demonstrate poor survival during the first 90 days of life. Menendez (1976) also found brook trout alevin survival below pH 6.1 reduced by 50 per cent. Both brook trout studies support the generalized statement that alevins are more sensitive than embryos. Similar findings have been found for larval stages of white sucker (Trojnar, 1977b), Atlantic salmon (Daye and Garside, 1977), flagfish (Craig and Baksi, 1977) and other species.

Slightly better survival occurred for alevins spawned and held in neutral water for seven weeks after hatching and then transferred to low pH water. Seven-week old alevins can tolerate a minimum pH of about 5.0. Below this level, death is relatively rapid. Thus, my survival data at low pH for brook trout alevins from unexposed parents confirms Menendez's results.

B. Embryo-Alevin Exposure. 2. Growth.

My incubation data indicates that a pH of 5.0 enhances embryonic development. But Menendez (1976) found no differences in incubation times in his brook trout study and extended hatching periods have been reported for fathead minnows at pH 5.2 (Mount, 1973) and white suckers below pH 6.0 (Trojnar, 1977b). Most likely, my incubation results are erroneous, due to the small sample sizes.

Large variability in the alevin growth data prevents definitive conclusions. Although pH 5.5 alevins were substantially larger in both final (90-day) weight and length than the pH 6.0 and 7.0 alevins, these differences are not likely to be pH-related. Fewer alevins in the same progeny tank space and more food per fish would account for the obtained results. This explanation would also account for the better growth (final weight) achieved by the transfers at pH 5.0, compared to those at pH 5.5 and 6.0. However, Menendez (1976) found slower growth at all pH levels below control (pH 7.1). His results coincide with larval growth studies of flagfish at low pH (Craig and Baksi, 1977).

CONCLUSIONS

A. Juvenile-Adult Exposure.

Yearling brook trout maintain normal blood pH when chronically exposed to depressed environmental pH's as low as 4.0. However, in the pH range, 4.0 to 5.0, significant alterations occur in the overall acid-base chemistry of the blood. These changes are an adaptive response to physiological stress and can be described as compensated acidosis.

The abnormal acid-base balance is complemented by drastic reductions in plasma sodium chloride. Other important electrolytes (potassium, calcium and magnesium) are unaffected.

When the water is low in total salt content, as was the case in this study, survival at and above pH 4.5 is unaffected. Survival is limited, however, at pH 4.0. Limited survival at pH 4.0 is primarily the result of starvation and only indirectly related to H-ion concentration.

Total lack of food utilization (i.e. starvation) of trout exposed to pH 4.0 inevitably results in complete inhibition of growth. At pH 4.5, reduced feeding activity is reflected by slow growth. Trout exposed at or above pH 5.0 grow at a normal rate.

Brook trout will not mature sexually at pH 4.0 and, of course, will not spawn. Even though trout held at pH 4.5 mature and will spawn with a normal degree of fecundity, the ova produced by the females are of poor quality. Normal maturation, spawning and sex products occur at and above pH 5.0.

B. Embryo-Alevin Exposure.

At pH 4.5, fertilization and embryonic development (viability) is poor and hatching is unsuccessful. Compared to pH 7.0, within the pH range of 5.0 to 6.0, hatchability is substantially reduced. Very young embryos may be more susceptable to low pH than older ones.

Alevins may even be more sensitive to low pH than embryos, and younger alevins more sensitive than older ones. However, my alevin growth data is inconclusive.

Whether declines in brook trout populations at low pH are the exclusive result of spawning impairment or, alternatively, of high mortality of the embryo-alevin stages, was not determined. Collectively, the data indicates that recruitment failure may in fact be the result of a combination of both mechanisms.

The present study coincides with other investigations on fathead minnow, brook trout and flagfish, indicating that reproductive success of these species is affected below pH 6.5.

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APPENDIX

Appendix 1. Water quality data from September, 1975 to March, 1976.

Parameter*[1]	$\bar{X}$ ± S.D.	
conductivity (μmhos/cm)	57.571 ± 1.9024	
tubidity (turbidity units)	0.375 ± 0.9024	(6)*[2]
hardness as $CaCO_3$	24.857 ± 1.9518	
total alkalinity as $CaCO_3$	19.714 ± 1.2536	
total phosphorus	0.005 ± 0.0035	(5)
sulphate	7.143 ± 1.2536	
chloride	2.429 ± 0.5345	
ammonia nitrogen	0.026 ± 0.0127	
Kjeldahl nitrogen	0.218 ± 0.0264	(6)
nitrites	0.002 ± 0.0009	
nitrates	0.030 ± 0.0208	
copper	0.012 ± 0.0154	
zinc	0.008 ± 0.0028	
iron	0.058 ± 0.0376	
cadmium	0.003 ± 0.0012	
lead	0.009 ± 0.0019	
nickel	0.005 ± 0.0019	
free chlorine	0	(3)
combined chlorine	0	(3)

*[1]All analyses reported in mg/l unless otherwise indicated.

*[2]Numbers in parentheses indicate number of samples, if <7.

Appendix 2. Photoperiod employed for brook trout study, 1975 - 1976. Time expressed in hours and minutes.

Date	Dawn to dusk time	Day-length
January 1	9:00 - 5:30	8:30
15	9:00 - 5:45	8:45
February 1	9:00 - 6:30	9:30
15	9:00 - 7:15	10:15
March 1	9:00 - 8:00	11:00
15	9:00 - 8:45	11:45
April 1	9:00 - 9:45	12:45
15	9:00 - 10:45	13:45
May 1	9:00 - 11:30	14:30
15	9:00 - 12:15	15:15
June 1	9:00 - 12:45	15:45
15	9:00 - 1:00	16:00
July 1	9:00 - 1:00	16:00
15	9:00 - 12:45	15:45
August 1	9:00 - 12:00	15:00
15	9:00 - 11:15	14:15
September 1	9:00 - 10:30	13:30
15	9:00 - 9:30	12:30
October 1	9:00 - 8:45	11:45
15	9:00 - 7:45	10:45
November 1	9:00 - 7:00	10:00
15	9:00 - 6:15	9:15
December 1	9:00 - 5:45	8:45
15	9:00 - 5:15	8:15

Appendix 3. Chemical characteristics of the test tank waters. Data presented as $\bar{X}$ ±S.D. for the period, September, 1975 to March, 1976.

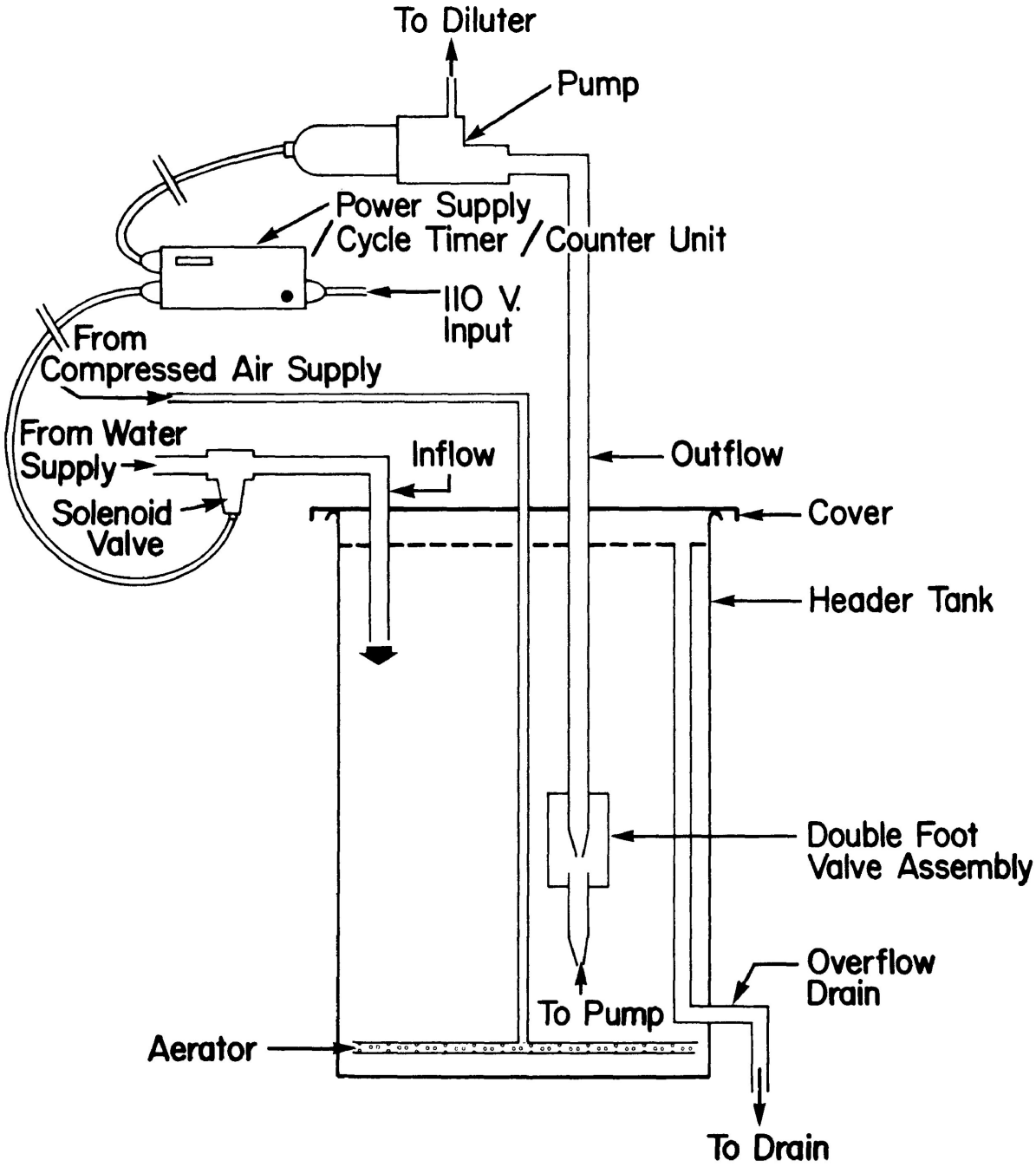
Parameter*[1]	pH 7.0	pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
hardness*[2]	25.29±1.704	26.14±5.786	25.00±1.291	26.71±5.851	26.14±3.761	25.29±2.360
alkalinity*[3]	19.14±1.865	7.29±3.592	5.86±2.734	4.00±3.055	2.57±2.992	1.67±2.338
calcium	6.29±0.488	6.29±0.488	6.29±0.488	6.57±0.976	6.14±0.378	6.14±0.378
magnesium	2.29±0.488	2.29±0.756	2.29±0.488	3.00±1.155	2.57± 1.272	2.57±0.787
sodium	1.40±0.383	1.81±1.022	1.20±0.306	1.35±1.139	1.09±0.180	1.15±0.441
potassium	0.90±0.228	1.16±0.815	0.78±0.253	0.86±0.621	0.66±0.146	0.86±0.356
chloride	2.86±0.900	2.57±0.535	2.57±0.535	2.57±0.535	2.57±0.535	2.43±0.535
sulphate	6.21±1.286	17.29±1.976	18.29±1.890	19.00±2.582	20.71±2.215	23.83±2.927

*[1] All analyses reported in mg/l.

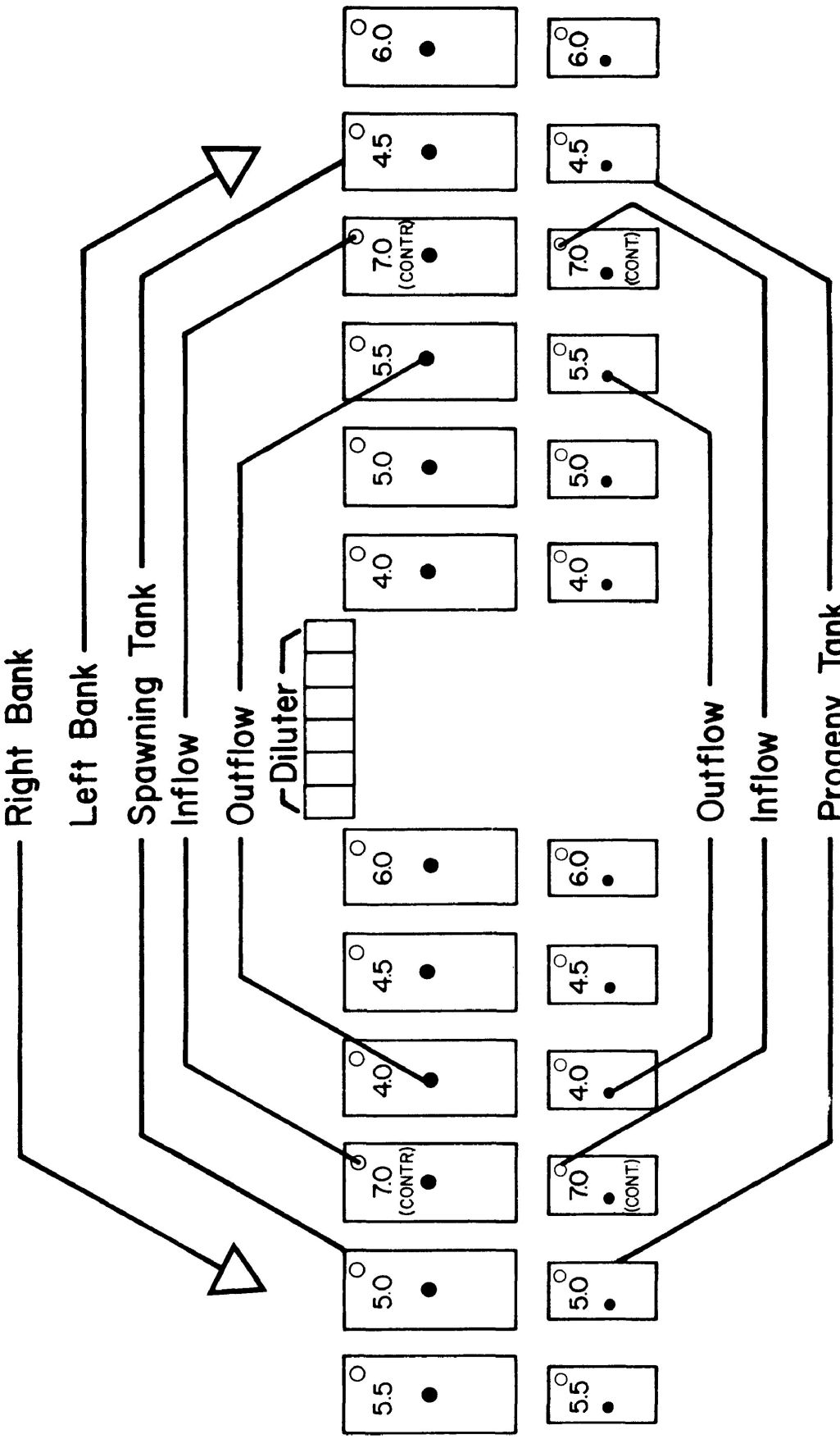
*[2] Hardness as $CaCO_3$.

*[3] Total alkalinity as $CaCO_3$.

Appendix 4. Schematic of the header tank system.


To Diluter
Pump
Power Supply
/Cycle Timer /Counter Unit
110 V.
Input
From
Compressed Air Supply
From Water
Supply
Solenoid
Valve
Inflow
Outflow
Cover
Header Tank
Double Foot
Valve Assembly
Overflow
Drain
To Pump
Aerator
To Drain

Appendix 5. Schematic illustrating the random assignment of duplicate test tanks on either side of the diluter (dosing apparatus).


Right Bank
Left Bank
Spawning Tank
Inflow
Outflow
Diluter
Outflow
Inflow
Progeny Tank
6.0
4.5
7.0 (CONTR)
5.5
5.0
4.0
6.0
4.5
4.0
7.0 (CONTR)
5.0
5.5
6.0
4.5
4.0
7.0 (CONT)
5.0
5.5
6.0
4.5
4.0
7.0 (CONT)
5.0
5.5

Appendix 6. Summary of Duncan multiple range tests performed on data yielding a significant F-ratio ($P < .01$). Underlining denotes non-significance at the .01 level.

Data from	Parameter	Duncan test
Table 5	weight	4.0 4.5 <u>5.0 7.0 6.0 5.5</u>
	length	4.0 4.5 <u>5.0 7.0 6.0 5.5</u>
Table 6	pH	<u>4.5 5.0 4.0</u> <u>7.0 6.0 5.5</u>
	pCO_2	<u>4.0 4.5 5.5</u> <u>6.0 5.0 7.0</u>
	total CO_2, HCO_3^-, base excess	<u>4.0 4.5 5.0</u> <u>5.5 6.0 7.0</u>
Table 7	sodium	4.0 <u>4.5 5.0</u> <u>6.0 5.5 7.0</u>
	chloride	4.0 4.5 <u>7.0 6.0 5.0 5.5</u>
Table 8	viable eggs per spawning	<u>4.5 5.0 5.5</u> <u>6.0 7.0</u>
Table 9	hatchability per spawn	<u>5.5 6.0 5.0</u> 7.0
	mean incubation	<u>5.0 5.5</u> <u>7.0 6.0</u>
Table 10	weight	<u>5.0 7.0 6.0</u> 5.5
Table 11	weight	6.0 <u>5.5 5.0</u>
Figure 8	21-day length	<u>5.0 6.0 5.5</u> <u>7.0</u>
	90-day length	<u>5.0 7.0 6.0</u> 5.5