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The Effects of Two Acid pH Conditions on Vitellogenesis in Rainbow Trout (*Salmo gairdneri*)

Robert L. Roy

A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montreal, Quebec, Canada

July 1988

ISBN 0-315-49064-0

ABSTRACT

The Effects of Two Acid pH Conditions on Vitellogenesis in Rainbow Trout (*Salmo gairdneri*)

Robert L. Roy

The effects of sublethal acid pH exposure on vitellogenesis in rainbow trout was investigated with the indicators serum vitellogenin (Vg, measured by a homologous radioimmunoassay), total serum calcium, (SCa) and total serum phosphoprotein phosphorous, (TPP).

In August 1986, early vitellogenesis was not affected after 20 days of exposure at pH 5.5. Vg and TPP were not significantly different in control and pH 5.5 groups during the experiment, while SCa in the pH 5.5 females was significantly higher than controls on day 20.

Late vitellogenesis was not affected by sublethal acid pH exposure in two populations, differing in age and acclimation history. Vg, TPP and SCa levels were not significantly different in control, pH 5.5 and pH 4.5 groups after 20 days of exposure in September and in November - December.

In September 1986, Vg did not change over 20 days in the pH 4.5 exposed fish, but significantly increased in control fish, following the usual pattern of rapid increases in Vg during late vitellogenesis in rainbow trout. This could indicate that pH 4.5 exposure delays vitellogenesis, perhaps affecting ovulation and spawning.

SCa and TPP had low degrees of correlation with Vg in control, pH 5.5 and pH 4.5 exposed females. TPP and SCa are not precise indicators of actual Vg levels, but are useful for the differentiation of vitellogenic from non-vitellogenic trout during late vitellogenesis.

In conclusion, while sublethal pH exposure did not affect vitellogenesis in mature rainbow trout in this study, low pH may disrupt vitellogenesis during long term exposure or in soft water environments.

ACKOWLEDGEMENTS

I would like to express my deepest appreciation to my supervisor, Dr. Sylvia M. Ruby, for her guidance, support and constructive criticism during my studies. I would also like to thank the members of my commitee, Dr. Ed Maly and Dr. Gerard Leduc, for their advice.

My sincere thanks to Dr. D. Idler, Dr. Y. P. So and James Nagler of the Marine Sciences Research Laboratory, for their hospitality, and the use of the facilities, during my sojourn in St. John's.

My fellow graduate students provided moral support, especially Christine Searle, Yves Bois, Henry van Leeuwen, and Attila Szabo. I would also like to thank Dr. Arthur Hewitt and the soon to be "Dr.", Simon Pedder, for their advice and friendship.

Finally, I would like to express my indebtedness to my wife, Line McLaughlin, for her support and encouragement.

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INTRODUCTION

Acid precipitation is recognized as a major pollutant in Europe and North America. Emissions from the burning of fossil fuels combine with water vapour to form sulphuric, nitrous and nitric acids; these return to earth as acidified rain or snow and cause considerable damage to terrestial and aquatic ecosystems (Harvey et al, 1980; Haines, 1981). The disappearance of fish populations, due to acidification of their habitat, has occurred in the Northeastern United States (Schofield, 1976; Haines, 1981); in Norway (Drablos and Tollen, 1980) and in Canada (Harvey et al, 1980; Harvey, 1982;).

This threat to fish populations has lead to research on the ecological and physiological impacts of low pH stress. The habitats susceptible to acidification are soft waters, with low ion content and poor buffering capacity (Harvey, 1975; Schofield, 1976; Leivestad et al, 1976). Physiological studies have shown that resistance to low pH is increased with increased external calcium levels (Graham and Wood, 1981; Brown, 1981). The mechanism of toxicity of acid pH involves the gill, which is the major site of action of H^+ (reviewed by Wood and McDonald (1982),

and by McDonald (1983a). At very low pH, (pH < 4.0), gill tissue damage, leading to respiratory dysfunction, appears to be the major cause of death in salmonids (Fromm, 1980; McDonald, 1983a). However, at pH > 4.0, the major toxic mechanism is the disruption of ionoregulation, as plasma concentrations of Na^+ and Cl^- decrease (McDonald, 1983a; McDonald et al, 1983). External calcium levels influence this disruption, as plasma ions (chiefly Na^+ and Cl^-) are lost at a greater rate in acidified soft water (10 mg Ca^{2+}/L) than in acidified hard water (50 mg Ca^{2+}/L), even when fish have been properly acclimated to the water conditions (McDonald et al, 1980, 1983; McDonald, 1983b). In addition, external calcium has a general effect on membrane stability, possibly permitting a greater recovery of gill membrane function in acid hard water (McDonald, 1983a). Most studies suggest that adult salmonids in the laboratory can probably tolerate pH levels of 4.5 - 5.0 indefinitely (reviewed by Fromm, 1980 and by Spry et al, 1981).

Fish populations in acid environments tend to decrease in numbers and eventually disappear at pH levels that are not found to be toxic in laboratory studies (Haines, 1981). Surviving acid stressed fish

populations show signs of recruitment failure, with altered age class structures (an abscence of young individuals and few older fish). These have been documented for white sucker, Castostomus commersoni, (Beamish et al, 1975; Harvey, 1982), rock bass, Ambloplites rupestris, and yellow perch, Perca flavescens, (Ryan and Harvey, 1977, 1980), in several acid lakes in Ontario; and for European perch, Perca fluviatilis, in an acid lake in Norway (Rosseland et al, 1980).

This recruitment failure may be due to spawning failures, which have been reported for populations of white sucker, brown bullhead (Ictalurus nebulosus), rock bass, pumpkinseed sunfish (Lepomis gibbosus) and northern pike (Esox lucius), in acid Ontario lakes (Beamish, 1974; Beamish et al, 1975). These fish had not spawned when examined after their normal spawning period. Exposure to acid conditions, especially in oligotrophic or soft water environments, has also been associated with impairments in calcium regulation (Beamish et al, 1975; Lockhart and Lutz, 1977). Female white suckers in acid lakes (pH range 4.8 - 6.0) had lower serum calcium (SCa) levels, compared to fish in neutral lakes, when sampled just before their

spawning period (Beamish et al,1975; Weiner et al, 1985). SCa levels are usually high prior to spawning, when females are producing eggs. However, Weiner et al (1985) suggest that the lower SCa in their female white suckers may be due to low pH, low waterborne calcium, or both of these factors, since their acid lakes also had lower calcium levels than the neutral lakes used for comparison.

From laboratory studies, it is clear that low pH can affect spawning and egg production in some species. Fathead minnows (Pimephales promelas) exposed for one generation, did not spawn at pH 5.2 and egg production was significantly reduced at pH 5.9 (Mount, 1973). Over a 20 day exposure period, flagfish (Jordanella floridae) egg production was significantly decreased at pH 6.0 and below, and spawning completely failed at pH 4.5 (Craig and Baski, 1977). Lee and Gerking (1980) exposed desert pupfish (Cyprinodon nevadensis), a species found in alkaline waters, to several acid pH levels for 20 days. Egg production was significantly reduced at all pHs below the control (8.3), and egg laying ceased at pH 5.0 (Lee and Gerking, 1980).

In salmonids, low pH has not been found to disrupt egg production, but egg quality may be affected. In chronic (6 - 9 month) studies with brook trout (Salvelinus fontinalis), egg viability was decreased at pH 5.0 (Menendez, 1976); ovulation was delayed at pH 4.5 and pH 5.0 (Tam and Payson, 1986). Weiner et al (1986) exposed mature rainbow trout (Salmo gairdneri) females for 42 days to acid pH. At pH 4.5 and 5.5, eggs had reduced viability, and the report concluded that oogenesis was affected.

Low pH does affect the formation of mature oocytes in flagfish and desert pupfish. Ruby et al (1977), exposed flagfish to several acid pHs and found that the ability to form mature oocytes was reduced at all pHs below the control. At pH 4.5, yolk deposition was severely affected. Lee and Gerking (1980) found fewer mature oocytes in ovaries of desert pupfish exposed to acid pH. Since the formation of yolk involves the yolk precursor vitellogenin, the production of this protein may be affected by low pH, leading to reduced yolk deposition and an inability to form mature oocytes.

Vitellogenin (Vg), is the yolk precursor produced by oviparous vertebrates including birds, amphibians

and fish (Follet and Redshaw, 1974; Wallace, 1978; Ng and Idler, 1983). It is a lipophosphoprotein, and when in the serum is bound to calcium (Follet and Redshaw, 1974; Hori et al, 1979). For these reasons, serum total calcium (SCa) and serum total phosphoprotein phosphorous (TPP), have been used as indirect indicators of Vg (Ng and Idler, 1983). A direct method of measuring Vg in teleosts has been developed using a radioimmunoassay, which is more sensitive and precise than previous methods (Ng and Idler, 1983; So et al,1985). The production of vitellogenin can also be induced in immature females and in males by the administration of estrogen, such as estradiol (Emmersen and Petersen, 1976; Nath and Sudarajaj, 1981; Campbell and Idler, 1980).

Vitellogenin appears in the serum after pituitary gonadotropins stimulate the secretion of estrogen by the ovary. This in turn induces the production of vitellogenin by the liver. Vg is then transported in the blood, where it may reach levels of tens of mg/ml during late vitellogenesis. It is sequestered by the oocytes and split into lipovitellin and phosvitin. Further gonadotropin stimulation leads to oocyte

maturation, and the cycle ends with spawning (Scott and Sumpter, 1983; van Bohemen and Lambert, 1981).

Vitellogenesis in fish is influenced by environmental factors, such as temperature and photoperiod (reviewed by De Valming, 1972). Acid conditions in the wild have caused recruitment failure of fish populations, possibly due to a failure to spawn. It is possible that low pH exposure interferes with vitellogenesis, leading to delayed maturation or spawning failure. Thus, this study was designed to ask the following questions:

* Is vitellogenesis in rainbow trout affected by exposure to sublethal acid conditions, using serum Vg as a parameter ?

* Are early and late stages of vitellogenesis affected by sublethal pH exposure ?

* Is there a correlation between serum Vg, as measured by RIA, and the indirect indicators serum phosphoprotein phosphorous and serum calcium ? Is this correlation affected by exposure to acid conditions ?

MATERIALS AND METHODS

1) MATERIALS

A) Test organisms

One group of immature (17 - 27 g), and two groups of mature rainbow trout (200 - 450 g) were obtained from La Pisciculture Mont Sutton (R.R.# 1, Sutton, Quebec) in April 1986, September 1985, and October 1986. Upon arrival, they were transferred to 250 L (immature fish), or 620 L (mature fish), holding tanks and kept at 12 +/- 1 °C. Montreal city water, dechlorinated by filtration through activated charcoal, was fed continuously into the holding tanks at a rate providing > 90 % renewal every 4 hours. A 12 hour light : 12 hour dark photoperiod was maintained using an automatic timer. Fish were fed ad libidum with the appropriate grade Trout Chow (Martin Feed Mills) every afternoon. The composition of this feed is shown in Table 1.

Table 1: Composition of trout feed grower pellets. All values are minima, except for * (maximum).

Crude protein	:	40.0 %
Crude fat	:	8.0 %
Crude fibre	:	3.0 % *
Vitamin A	:	5,000 i.u./kg
Vitamin D	:	3,000 i.u./kg
Vitamin E	:	100 i.u./kg
Ascorbic acid	:	800 mg/kg

B) Experimental Apparatus

The acidification apparatus consisted of a headbox, two 1000 L reservoirs, two Teflon solenoid valves, a pH controller, two pH meters with appropriate probes and three test basins.

Incoming water entered the bottom of each reservoir at a rate of 4.2 - 4.6 L/ min., permitting a 90 % replacement time of > 9 hours. Flowrates were controlled by PVC spigot valves. The stock acid solution (1.4 N H_2SO_4,made up from reagent grade concentrated acid), was stored in a 60 L covered polyethylene headbox and delivered to the solenoid valves by Tygon tubing. The pH controller (Concordia Technical Center) had settings for two pH conditions and was connected to the pH meters (Concordia Technical Center) and to the solenoid valves (Galtec,model 203-1214). Each pH meter was equipped with a reference electrode (Fisher, calomel sleeve junction, #13-639-62) and a glass electrode (Fisher, universal, #13-639-3 or Corning, #476024).

Acidification was accomplished by adjusting the set points of the pH controller. When engaged, this would open the solenoid valves to allow the acid stock

solution to enter the bottom of the reservoir, until the desired pH was reached. Vigorous aeration mixed and decarbonated the acidified water, which exited by standpipe and was directed to the test basin.

The test basin were 500 L rectangular polyethylene tanks with covers; half of each tank was draped with dark plastic sheeting to provide shading within the tank. Water entered each basin through PVC piping or Tygon tubing, at one end, and exited through a standpipe,at the other.

The experimental area was lit by overhead fluorescent tubes (40 watt) set on a 12 hour light :12 hour dark photoperiod and was also separated from the rest of the laboratory by dark plastic curtains.

II) METHODS

A) Experimental protocols

i) Acclimation and feeding

Before each experiment, individual fish were anaesthetized and transported to the experimental weighing room. Each fish was then blotted dry, weighed, and labelled with a plastic numbered tag threaded with a nylon cord (Floy Tag and Mfg.Inc.,

Seattle). A needle was sterilized in 90 % ethanol, passed through the dorsal musculature posterior to the dorsal fin, and discarded; the thread was tied and trimmed. Fish were randomly assigned to a test basin for at least two weeks of acclimation. Feeding was continued at 1.5 % wet body weight for each tank during this period.

ii) Estradiol treated fish

A preliminary experiment was performed from June 18 - July 1, 1986, to investigate the effects on vitellogenesis of a short term exposure (10 days) to 2 sublethal acid pH conditions (pH 4.9 and pH 5.4; Table 4). The production of vitellogenin in immature rainbow trout was induced by the injection of estradiol (E-2), using the model system described by Cyr (1984). This model has been used previously in toxicological studies (Cyr, 1984; Nagler, 1985). The aim of this experiment was to determine the pH conditions and exposure periods for later experiments with mature fish.

The immature fish in this experiment were obtained in April, 1986 and weighed 30.1 +/- 1.0 g. After acclimation, the labelled fish were given 2 injections

of estradiol; the first on June 18 and the second 3 days later, on day 0 of the experiment. The following protocol was used : a fish was transported to the weigh room, where it was anaesthetized, blotted dry, weighed and had the tag number recorded. The injection was performed and the fish was allowed to recover before being returned to the test tank. After all fish had received the second injection on day 0, the pH exposure was begun. Blood was sampled on the same day for SCa and TPP levels in non induced fish, using a group of fish from the holding population. The estradiol treated fish were sampled after 10 days of acid exposure.

iii) Experiments with mature fish

The aim of the experiments with mature fish was to determine the effects of sublethal acid conditions on serum Vg, SCa and TPP levels in female rainbow trout during early and late vitellogenesis. These experiments involved exposure to two acid pH conditions (nominally pH 4.5 and pH 5.5; Table 3), and an exposure period of 20 days. Four experiments were performed: in August (August 9 - August 29, 1986); in September (September 23 - October 13, 1986); in

November (October 29 - November 18, 1986);and in December (December 3 - December 23, 1986).

The first experiment,in August 1986, was performed during early vitellogenesis in rainbow trout, with fish weighing 580.1 +/- 23.3 g. The second experiment,in September, took place during late vitellogenesis; when fish weighed 725.1 +/- 29.5 g. Both of these experiments involved fish which had been maintained in the laboratory since September 1985. Some fish had been identified as mature females, based on their Vg levels.

In September, 1986 most of this population died, due to low oxygen levels and a rise in pH in the water supplying the holding tanks. For the third and fourth experiments, a younger population was obtained (in October, 1986) and had less time to acclimate to laboratory conditions than the previous population. These fish weighed 342.8 +/- 14.5 g in November and 396.1 +/- 15.4 g in December.

Following acclimation, the same protocol was followed for all experiments. On day 0, the fish was transported to the weigh room, weighed and had the tag number recorded. Blood was withdrawn from the dorsal blood vessels and the animal was placed in a recovery

tank with flowing laboratory water. Once all fish in a treatment group had recovered, they were returned to the proper test basin and the pH exposure was begun. A similar sampling procedure was followed on day 10. On day 20, following blood sampling, the fish were killed by a blow to the head and the ovary and liver were weighed and preserved.

B) Experimental techniques

i) Water chemistry

Physico-chemical parameters of each test condition were measured every morning of the experimental period and are presented in Table 2. Water temperature was read with a graduated mercury thermometer (Canlab, 110 C max.); dissolved oxygen was measured with a D.O. probe and meter (Y.S.I.Co., Yellow Springs) which was calibrated before use. Alkalinity was measured every 3 days in control tanks. The pH measurements (Table 3) were made 4 - 5 times during a 10 hour period, using a digital pH meter (Concordia Technical Center) equipped with both reference (Fisher) and glass (Fisher or Corning) electrodes. Dissolved CO_2 was measured daily, and water samples from each test tank were taken every 3 days for measurements of hardness

and sulfate concentrations. Water samples were collected in acid washed and dH_2O rinsed polyethylene bottles, and were stored at 4°C until the sample was analyzed (weekly for sulfates and at the end of each experiment for calcium and magnesium ions for hardness). Alkalinity, dissolved CO_2, hardness and sulfates were analyzed according to Standard Methods (1977).

Table 2: Dissolved oxygen and temperature in control and acid exposure tanks, and alkalinity in control tanks during experiments in June, August, September, November and December 1986. (mean +/- S.D.).

Dissolved Oxygen (mg/L)	: 9.2 +/- 0.6, n = 285
Temperature (°C)	: 12.3 +/- 1.1, n = 285
Alkalinity (mg/L $CaCO_3$)	: 86.5 +/- 3.6, n = 37

Table 3 : Mean pH levels, +/- (S.D.), in control and acid exposure tanks during experiments in 1986. (n = number of measurements).

Experiment	Control	pH 5.4	pH 4.9
June	7.65 (0.06) (n = 31)	5.37 (0.53) (n = 31)	4.90 (0.36) (n = 31)
	Control	**pH 5.5**	**pH 4.5**
August	7.65 (0.09) (n = 92)	5.58 (0.21) (n = 91)	4.49 (0.33) (n = 89)
September	7.53 (0.08) (n = 92)	5.59 (0.29) (n = 91)	4.61 (0.45) (n = 91)
November	7.81 (0.11) (n = 89)	5.53 (0.24) (n = 89)	4.56 (0.24) (n = 89)
December	7.68 (0.08) (n = 92)	5.48 (0.32) (n = 91)	4.54 (0.38) (n = 91)

ii) Estradiol induction of vitellogenin in immature fish

The experiment involving estradiol treatment of immature fish used crystalline beta-estradiol-3-benzoate (Sigma, E2), dissolved in peanut oil (Sigma) and stirred for 24 hours prior to use. A dosage of 5 ug/kg was chosen, based on previous studies (Nagler, 1986)

Before being injected, the fish were individually anaesthetized in 3-amininobenzoic acid ethyl ester (0.1 g/L, Sigma) and weighed as described. The injection was made into the ventral midline posterior to the pectoral fins, using a 50 ul Hamilton syringue and a disposable 26G 13 mm Yale needle. The solution was slowly introduced into the organism, then the fish was placed in a 200 L polyethylene recovery tank for 1 hour before being returned to the test basin.

iii) serum and tissue sampling

On the sampling days of each experiment, individual fish were anaesthetized in 3-aminobenzoic acid ethyl ester (0.1 g/L for immature fish, 0.16 - 0.24 g/L for mature fish) and weighed as described above. The blood from immature fish was sampled by

caudal severance; blood from mature fish was drawn from the dorsal blood vessels into 1 ml tuberculin syringues fitted with a 26G Yale needle, which had been previously rinsed in 25 % sodium citrate to prevent coagulation. In both cases, blood was transferred to 1.5 ml centrifuge tubes and placed on ice. On day 20, mature fish were sampled for hematocrit by caudal severance; liver and ovary samples were frozen in liquid nitrogen. An ovarian midsection was preserved in Bouin's Fixative for histological analysis.

iv) Serum preparation.

Blood collected was centrifuged at 6000 g using a benchtop microcentrifuge (Eppendorf); the serum was siphoned off and split into centrifuge tubes for serum Ca^{2+}, serum phosphoprotein P and, in the case of mature fish, vitellogenin assays. The blood samples were then frozen in liquid nitrogen and stored at - 80 C until assayed.

v) Serum calcium analysis

Serum Ca^{2+} was determined by flame atomic absorption, according to Bhattacharya (1977). The

sample was thawed at room temperature, then 100 or 50 ul were diluted in a 5 ml volumetric flask with 2000 mg/L KCl solution. Standards (0.5 - 4 mg/L) were prepared in the same salt solution. The analysis was carried out on a Perkin - Elmer spectrophotometer (model 503) using a nitrous oxide flame. The sample concentrations were read from the standard curve and then corrected for dilution.

vi) Serum phosphoprotein phosphorous analysis

Serum phosphoprotein P was determined using volumes of 100 or 50 ul of thawed serum. The proteins were precipatated with 5 ml of cold 10 % trichloroacetic acid. After centrifugation, the pellet was successively washed with solvents (hot alcohol, chloroform:petroleum ether:alcohol in a 1:2:2 ratio, acetone and finally ether) to remove lipids (Wallace and Jared, 1968). The dried pellet was assayed for total phosphoprotein P according to the Boehringer - Mannheim Test Handbook (Boehringer - Mannheim Corp., 1970). Standards were prepared from reagent grade KH_2PO_4 and covered the range 10 - 400 mg/L. The absorbance at 390 nm was read on a Bausch and Lomb Spectronic 70 spectrophotometer. Sample

concentrations were calculated from the linear regression equation of the standard curve.

vii) Vitellogenin radioimmunoassay.

Serum vitellogenin in 10 ul serum samples was measured using a homologous radioimmunoassay for rainbow trout, identical to one developed for salmon.(So et al, 1985). This assay uses I^{131} labelled rainbow trout vitellogenin as the tracer, unlabelled vitellogenin as the standard, and rabbit antibody to rainbow trout vitellogenin as the ligand.

Serial dilutions of the standard and samples were made in barbitol buffer, to which had been added 0.5 % bovine serum albumin and 0.01 % thimersal. The standard curve covered the range 0.5 ng/ml to 1042.6 ng/ml and was reliable for the range 1 - 261 ng. Sample dilutions ranged from 1:100 to 1:1,000,000. Standards and samples were incubated with 200 ul of antibody and 200 ul of radiolabelled vitellogenin at 4 C for 3 days. Normal rabbit serum and goat anti - rabbit antibody were added 24 hours before counting.

Data reduction was done using a log - logit transformation which averaged replicate counts. The log of each standard concentration was plotted with

the logit of the per cent binding at that concentration to obtain a dose - response curve. The regression lines of these curves had r^2 values ranging from 0.96 - 0.99, intercepts of 1.11, 0.94, and 1.14, slopes of -1.94, -1.87 and -1.99 and B/B_0 (50 %) of 3.83, 3.77 and 2.84 ng. Sample concentrations were obtained from averages of dilutions which fell in the range of greatest sensitivity of the standard curve.

viii) Histological analysis

The mature females chosen for comparison in this study had large oocytes (350 um in diameter), which coalesced upon preservation and could not be sectioned. To overcome this difficulty, measurements of preserved oocytes from each ovary were made in order to obtain a crude estimate of mean oocyte diameter for each treatment group. The method used was to obtain 50 oocytes from each mature female and measure them on a millimeter scale ruler.

ix) Growth rates

Day 0 and day 20 wet weights of both male and female fish were used to calculate growth rates.

Growth rates were calculated according to Ricker (1979), using the following formula:

$$G = \frac{\ln w\ (2) - \ln w\ (1)}{t_2 - t_1}$$

where: G = instantaneous growth rate, w (1) = fish weight on day 0, w (2) = fish weight on day 20, t_1 = day 0 and t_2 = day 20.

x) Statistical analysis

The day 20 and day 0 serum values were subtracted and the effect of pH was tested using a single factor ANOVA on the differences, with one exception noted below. Serum data were log transformed (X = log(X+10)) before statistical comparisons, to allow equality of variances. For experiments with mature fish, trends within each group were examined by comparing day 0 and day 20 values using a correlated t-test. All means and standard errors were calculated without transforming the data.

In the August experiment, an equipment failure in the pH 4.5 acidification reservoir led to an extreme drop in pH (to pH 3.3), killing most of the fish on day 9 and leaving only 2 mature females. A statistical comparison (t test) was possible only

between the control and pH 5.5 groups. One fish in the August pH 5.5 group had a GSI of 20.2 and was judged to be in late vitellogenesis; this individual was excluded from the analysis.

The third and fourth experiments, in November and December 1986, involved a younger population with fewer mature females. The day 0 serum parameters (Vg, SCa, TPP) were compared and no significant differences were found between the two experimental groups (t test, $p > 0.05$). The serum values for each pH group were pooled to increase the sample size.

Simple linear correlations were calculated for Vg and the indirect indicators SCa and TPP for fish at each pH condition. GSI and HSI data were compared non - parametrically, using either the Mann - Whitney two sample rank test (August experiment) or the Kruskal - Wallis single factor analysis of variance by ranks. All other data were compared with a t test (August experiment) or a single factor ANOVA.

Growth rates of the control and pH exposed groups were compared using a one way ANOVA. In August, growth was measured between day 10 and day 20; day 0 weights were considered unreliable due to a malfunctioning balance.

RESULTS

Induction experiment with immature fish

In June 1986, a preliminary experiment was performed using immature rainbow trout. These fish were injected with estradiol (E-2) and then exposed to 3 pH conditions (pH 4.9, pH 5.4 and control pH 7.7) for a period of 10 days. The aim of this experiment was to determine the pH conditions and exposure periods for the experiments with mature fish.

High levels of SCa and TPP were produced, indicating that vitellogenin was induced in the E-2 injected fish (Table 4). At the end of the experiment, on day 10, the mean SCa and mean TPP levels in induced fish were significantly higher than SCa and TPP in non - injected fish ($p < 0.05$; Table 4). These high levels of SCa and TPP on day 10 were not significantly different between the 3 groups of E-2 injected fish (control, pH 5.4 and pH 4.9 exposures; $p > 0.05$; Table 4). Since the pH 4.9 group had the lowest levels of induced SCa and TPP (Table 4), a lower pH (4.5) and a longer exposure period (20 days) were selected for the experiments with mature fish.

Table 4 : Serum total calcium and serum total phosphoprotein phosphorous levels in immature rainbow trout in June 1986. Estradiol (E-2) induced fish were sampled after 10 days of exposure to pH 7.7 (control), pH 5.4 and pH 4.9; non-induced fish were kept at control pH and sampled on day 0 of the experiment. (mean +/- S.E.M.; SCa: n = 10; TPP: n = 5).

	E-2 induced			non-induced
	pH 7.7 (a)	pH 5.4 (b)	pH 4.9 (c)	pH 7.7 (d)
SCa : (mg %)	18.5 +/- 2.3	19.3 +/- 1.6	15.4 +/- 1.5	5.4 +/- 0.4
TPP : (ug P/ml)	171.2 +/- 38.4	177.6 +/- 30.6	152.6 +/- 40.9	19.0 +/- 3.0

a = b = c > d; $p < 0.05$, ANOVA

Experiments with mature fish

The first experiment with mature fish was performed in August 1986, with the aim of examining the effect of acid exposure on Vg, SCa and TPP levels during early vitellogenesis.

Vg levels were not significantly different between the control and pH 5.5 groups during the experiment ($p > 0.05$; Fig.1). Vg did not significantly change in either control or pH 5.5 groups over the 20 days ($p > 0.05$; Fig.1), and it remained at low levels in both groups on day 20 (1.94 and 2.79 mg/ml in controls and pH 5.5 group respectively; Fig.1).

Vg decreased from day 0 to day 20 in the pH 4.5 group. The 2 mature females that survived a short term drop in pH, to pH 3.3, had Vg levels of 0.65 and 0.85 mg/ml on day 0; these fell to 0.25 and 0.65 mg/ml respectively, on day 20 (Fig.1).

SCa in the pH 5.5 group was significantly higher than the control SCa on day 20 ($p < 0.05$; Fig.2). This difference was evident in the different trends displayed by the two groups during the experiment. SCa in the pH 5.5 group showed a significant increase from day 0 to day 20, to reach 13.9 mg % ($p < 0.05$; Fig.2); this value was also significantly higher than the basal SCa levels in male fish ($p < 0.05$; Fig.2 and Table 5). In contrast, SCa in the controls remained at low levels (means ranging from 11.7 to 11.3 mg %) over the 20 day experiment and was not significantly

different than SCa levels in male fish (Fig.2 and Table 5).

Table 5 : Serum total calcium levels in male rainbow trout, exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days during experiments in 1986. (mean +/- S.E.M.; n = number of male fish).

Experiment	SCa (mg %)	n
August	10.4 +/- 0.6	7
September	10.4 +/- 0.9	10
November	8.7 +/- 1.3	12
December	9.0 +/- 0.5	14

TPP means in control and pH 5.5 groups were not significantly different during the 20 day experiment ($p > 0.05$; Fig.3). TPP did not significantly change in either group over the 20 days; the means ranged from 34.4 ug P/ml in the controls on day 0, to 56.2 ug P/ml in the pH 5.5 group on day 20 (Fig.3).

Figure 1: Serum vitellogenin levels in mature female trout exposed to pH 7.7 (control), pH 5.5, and pH 4.5 for 20 days in August 1986. Vg was not significantly different in control and pH 5.5 groups during the experiment. (t test, $p > 0.05$). Means +/- S.E.M. are shown for control and pH 5.5 sampling points.

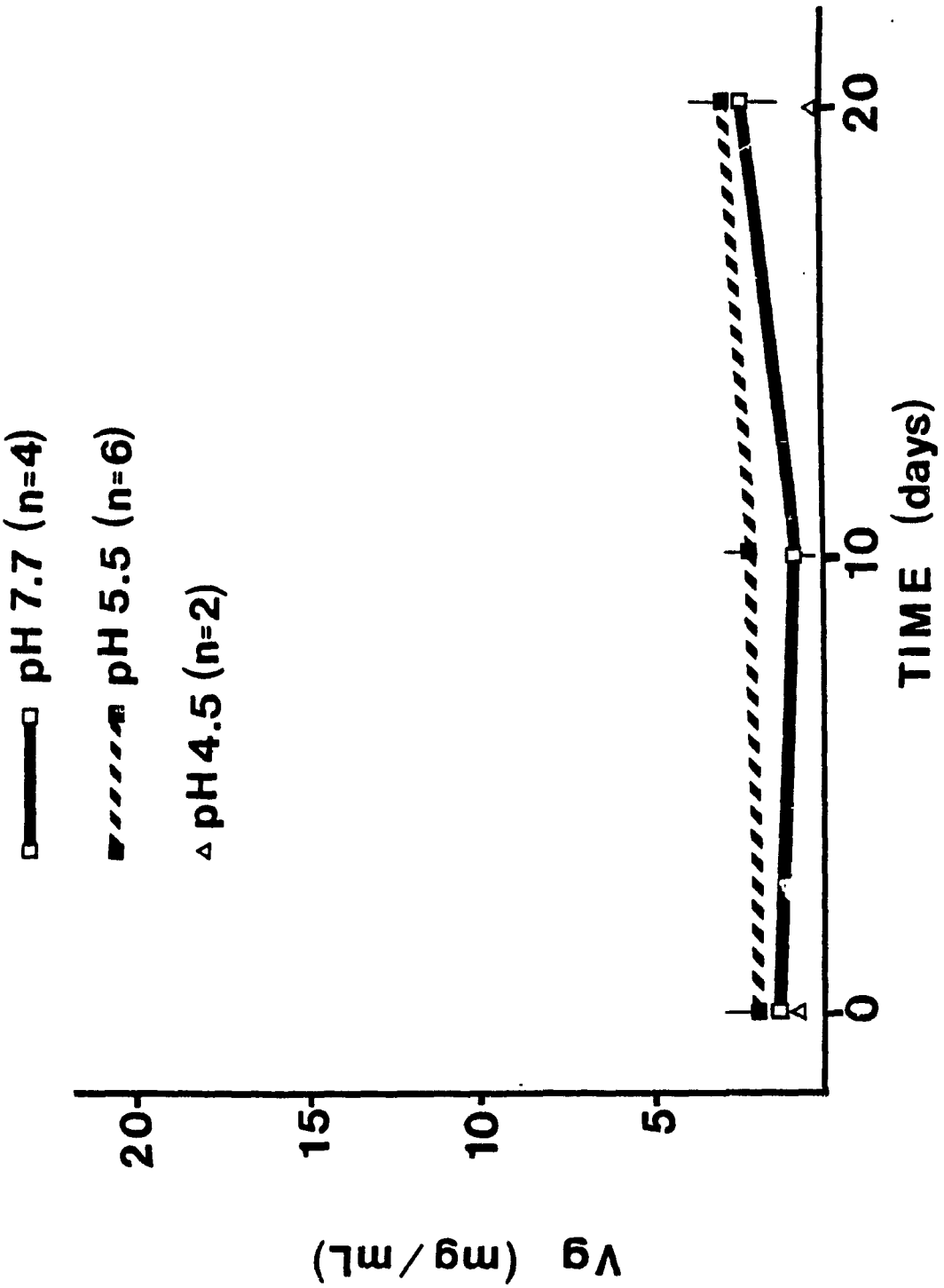

pH 7.7 (n=4)
pH 5.5 (n=6)
pH 4.5 (n=2)
Vg (mg/mL)
20
15
10
5
0
10
20
TIME (days)

Figure 2: Serum total calcium levels in mature female trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August 1986. The pH 5.5 group had significantly higher SCa levels than controls on day 20. (t test, $p < 0.05$). SCa in the pH 5.5 group significantly increased from day 0 to day 20. (correlated t test, p 0.05). Means +/- S.E.M. are shown for control and pH 5.5 sampling points.

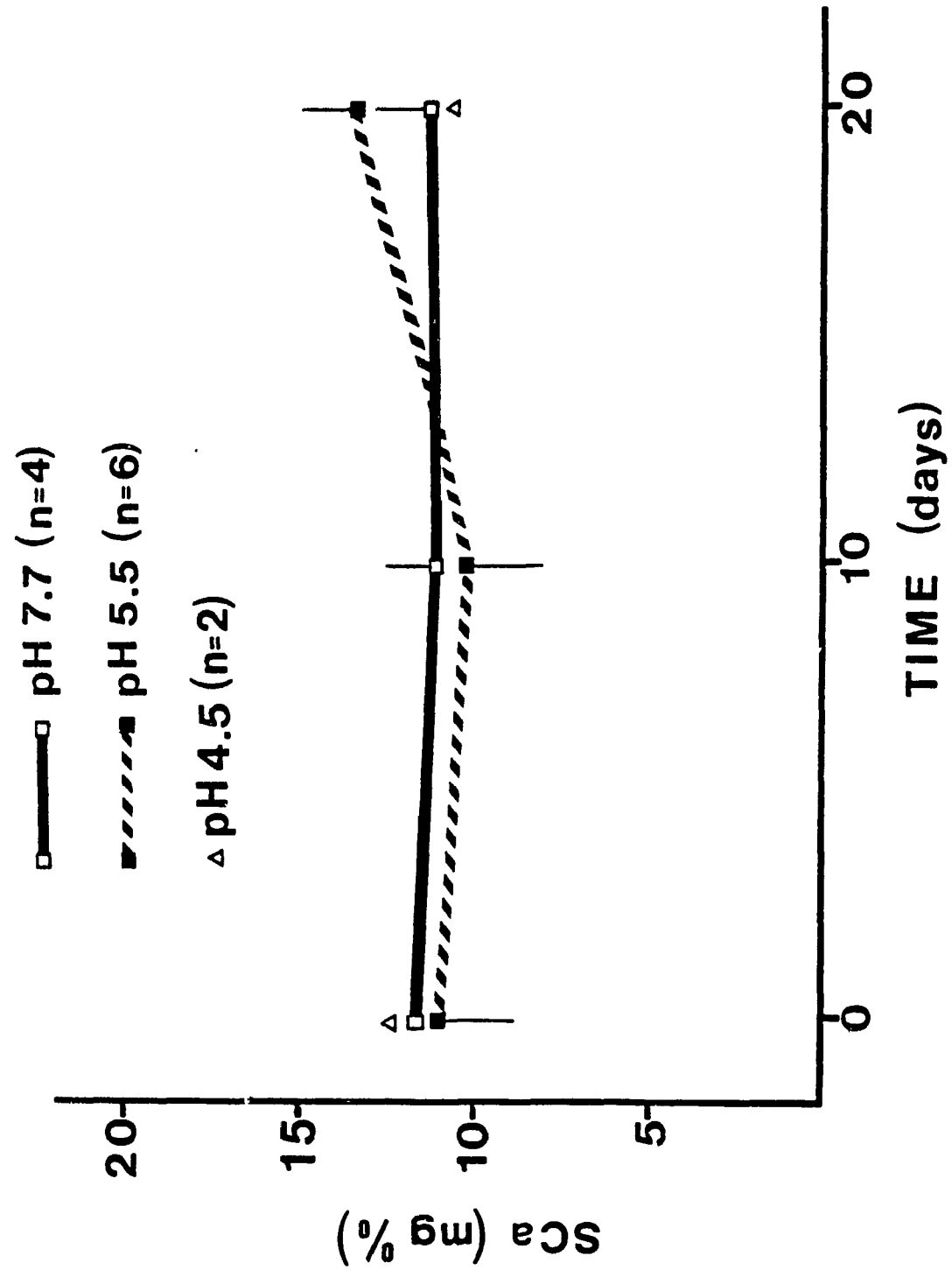
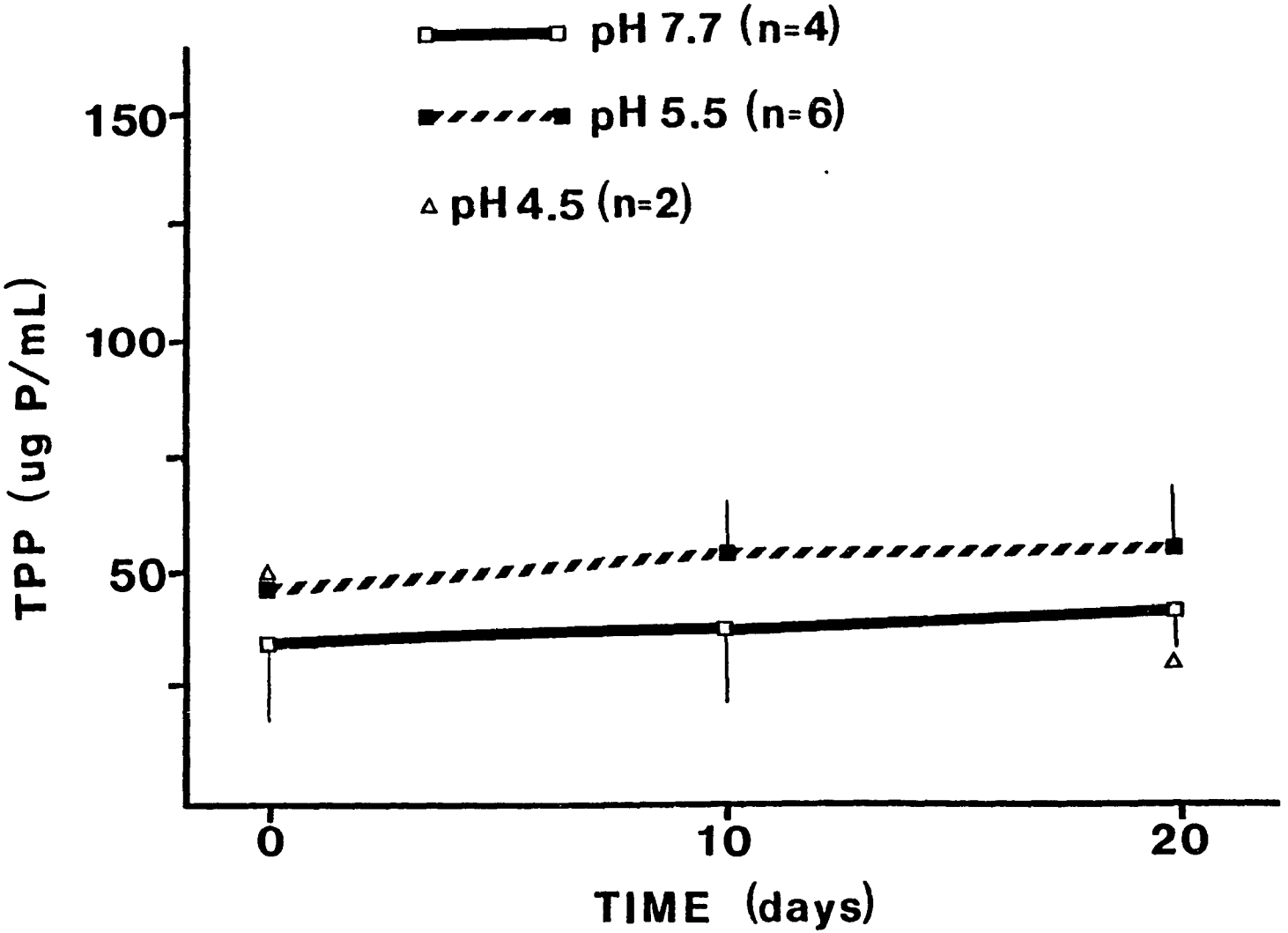

pH 7.7 (n=4)
pH 5.5 (n=6)
pH 4.5 (n=2)
SCa (mg %)
20
15
10
5
0
10
20
TIME (days)

Figure 3: Serum total phosphoprotein phosphorous levels,in mature female trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August 1986. There were no significant differences in TPP levels between pH 5.5 and control groups during the experiment. (t test,$p > 0.05$). Means +/- S.E.M. are shown for control and pH 5.5 sampling points.


pH 7.7 (n=4)
pH 5.5 (n=6)
pH 4.5 (n=2)
150
100
50
TPP (ug P/mL)
0
10
20
TIME (days)

The second experiment with mature fish began in September 1986, with the aim of examining the effect of acid exposure on Vg, SCa and TPP levels during late vitellogenesis.

Vg means were not significantly different between the control, pH 5.5 and pH 4.5 groups in the September experiment ($p > 0.05$; Fig.4). Both control and pH 5.5 groups showed a similar trend, as Vg significantly increased from day 0 to day 20 in the 2 groups ($p < 0.05$; Fig.4). The control Vg rapidly increased, from 4.38 mg/ml on day 0, to 11.38 mg/ml on day 20 (Fig.4). In the pH 5.5 group, serum Vg rose from 5.69 mg/ml on day 0 to 9.46 mg/ml on day 20 (Fig.4).

In contrast, the pH 4.5 group did not show an increase in Vg. At this pH, Vg did not significantly change from day 0 (6.35 mg/ml; Fig.4) to day 20 (8.03 mg/ml; Fig.4). While the day 20 Vg means were not significantly different amoung the groups (Fig.4), the trend in Vg at pH 4.5 was different than that seen in the control and pH 5.5 groups.

SCa means were also not significantly different between control, pH 5.5 and pH 4.5 groups on any sampling day (Fig.5). The pattern in SCa for all 3 groups was similar to that seen in the Vg results.

The control and pH 5.5 groups both had significant increases in SCa over the 20 days, to reach means of 19.8 and 19.6 mg %, respectively ($p < 0.05$; Fig.5). The pH 4.5 group showed no significant change in SCa during the experiment ($p > 0.05$; day 0 mean of 14.1 mg % compared to day 20 mean of 14.8 mg %; Fig.5). Thus, the trend in SCa at pH 4.5 was different than that shown by the control and pH 5.5 groups and is similar to the pattern found in serum Vg in this experiment.

There were no significant differences in mean TPP values between the 3 groups on any sampling day. On day 20, mean levels were 94.8 ug P/ml in the pH 5.5 group and 91.7 ug/ml in the pH 4.5 group.(Fig.6). The only significant increase in TPP over the experiment took place in the controls, which rose from 59.3 ug P/ml on day 0 to 89.5 ug P/ml on day 20 ($p < 0.05$; Fig.6).

The third and fourth experiments with mature fish were run between October and December, 1986. The aim of these experiments was to investigate the effect of acid exposure on Vg, SCa and TPP levels during late vitellogenesis, in this younger population. Results of these experiments were pooled to increase the

Figure 4: Serum vitellogenin levels in mature female trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days during September and October 1986. Vg was not significantly different in pH 7.7 (control), pH 5.5 and pH 4.5 groups during the experiment. (ANOVA, $p > 0.05$). Vg increased significantly in control and pH 5.5 groups from day 0 to day 20. (paired t test; $p < 0.05$). Each point represents a mean +/- S.E.M.

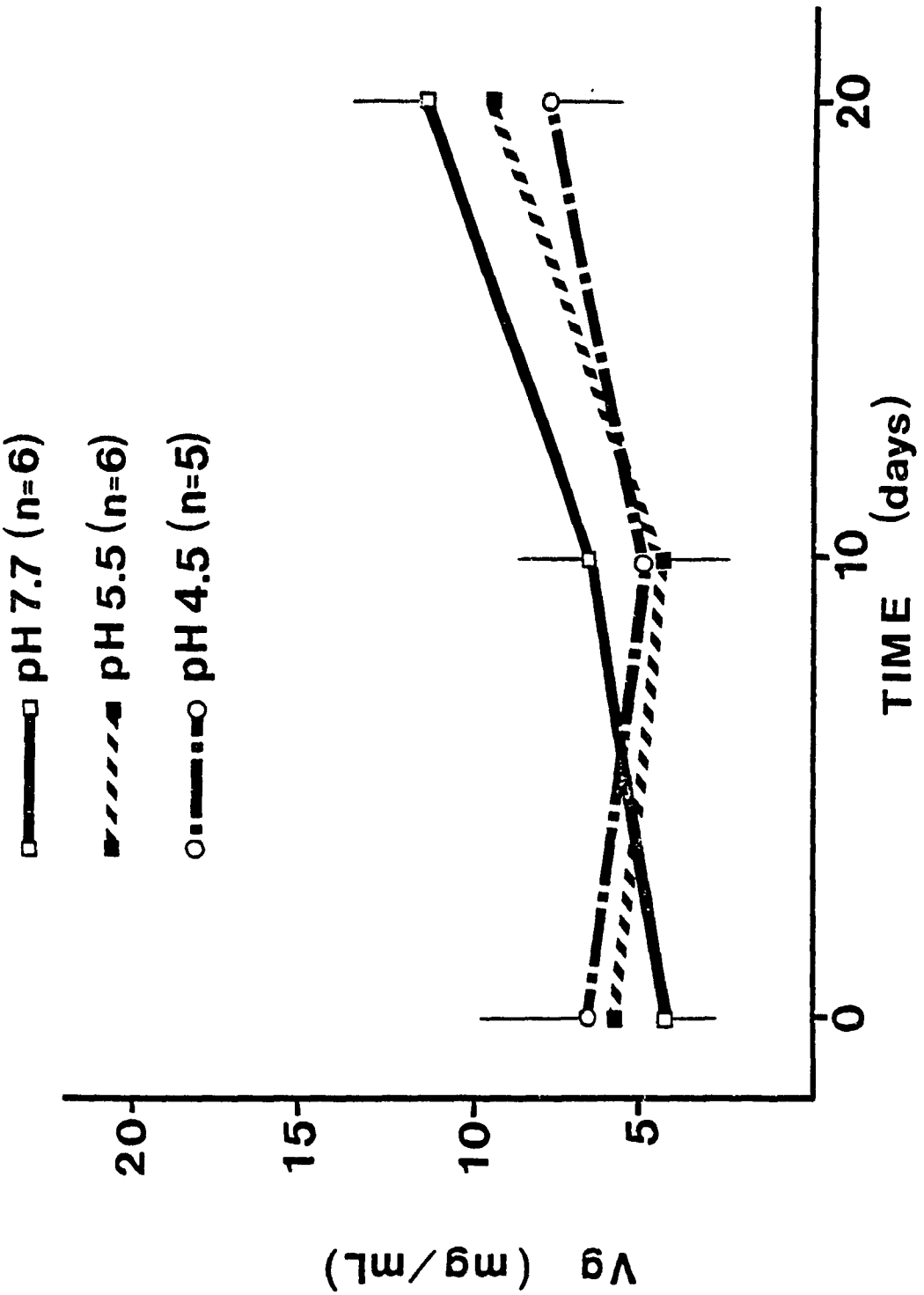

pH 7.7 (n=6)
pH 5.5 (n=6)
pH 4.5 (n=5)
Vg (mg/mL)
20
15
10
5
0
10
20
TIME (days)

Figure 5: Serum total calcium levels in mature female trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days during September and October 1986. SCa was not significantly different in control, pH 5.5 and pH 4.5 groups during the experiment. (ANOVA, $p > 0.05$). SCa increased significantly in control and pH 5.5 groups from day 0 to day 20. (paired t test; $p < 0.05$). Each point represents a mean +/- S.E.M.

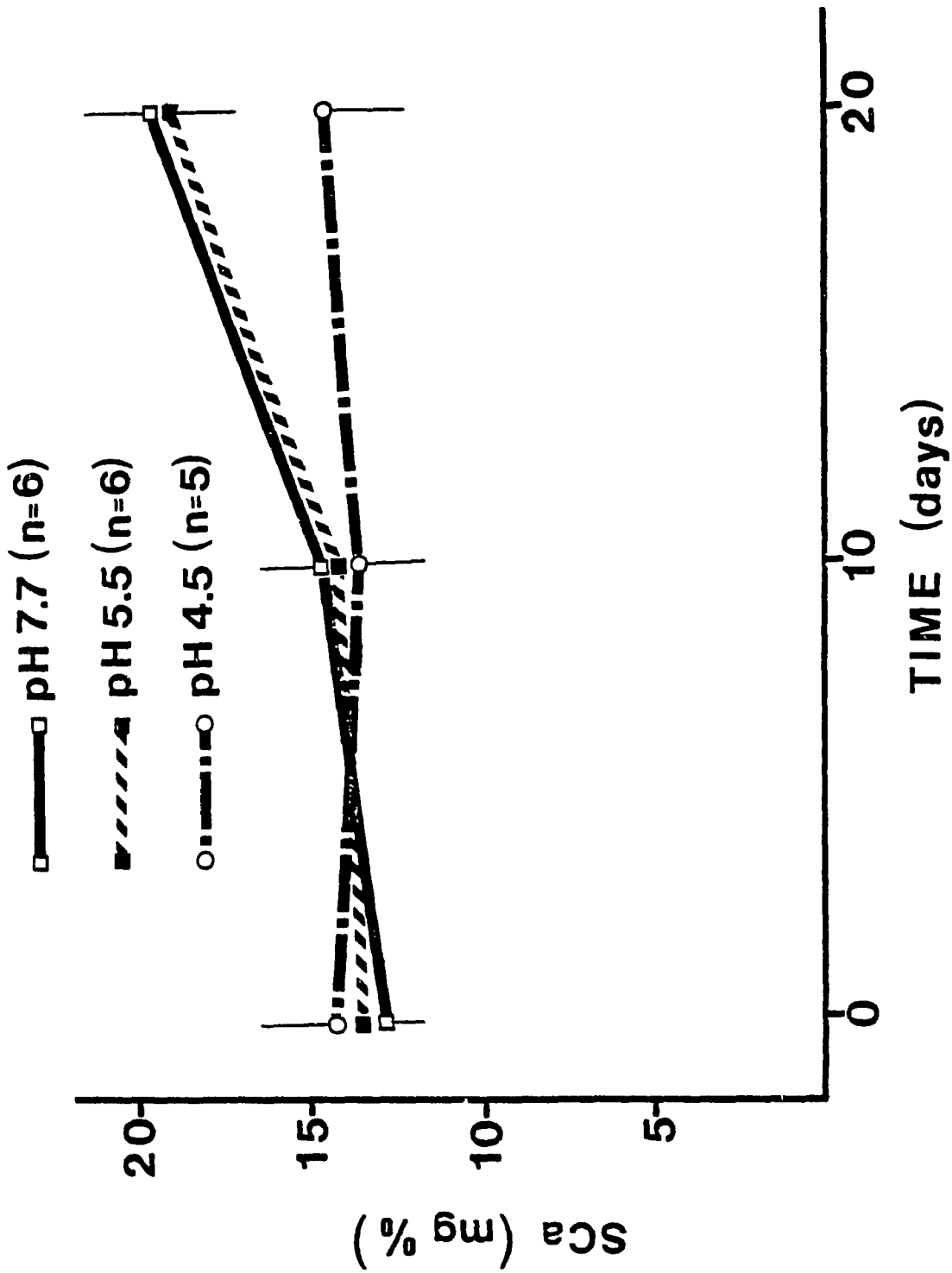

pH 7.7 (n=6)
pH 5.5 (n=6)
pH 4.5 (n=5)
20
15
10
5
S_{Ca} (mg %)
0
10
20
TIME (days)

Figure 6: Serum total phosphoprotein phosphorous levels in mature female trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days during September and October 1986. TPP was not significantly different in control, pH 5.5 and pH 4.5 groups during the experiment. (ANOVA, $p > 0.05$). TPP increased significantly in the control group from day 0 to day 20. (paired t test, $p < 0.05$). Each point represents a mean +/- S.E.M.

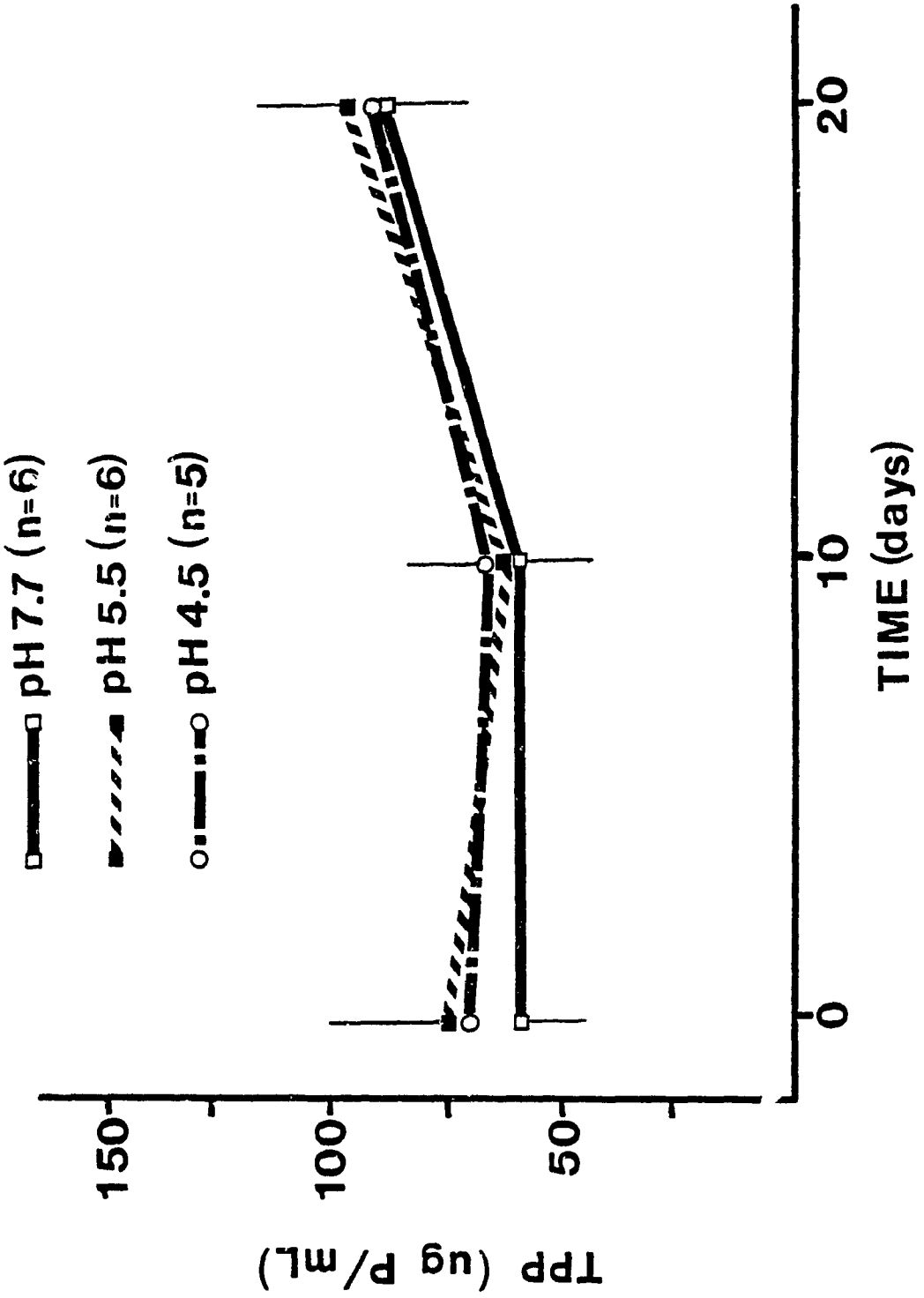
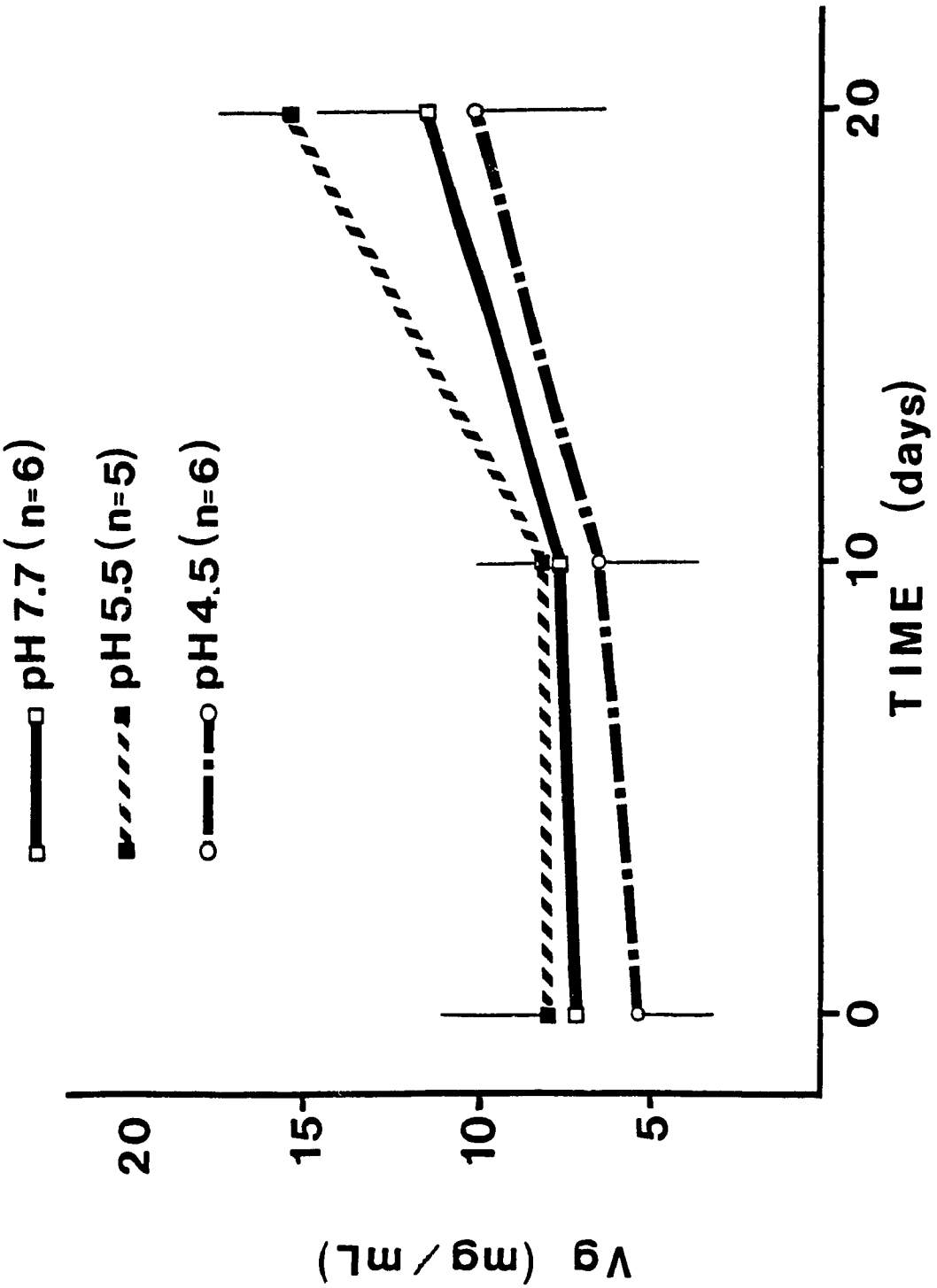
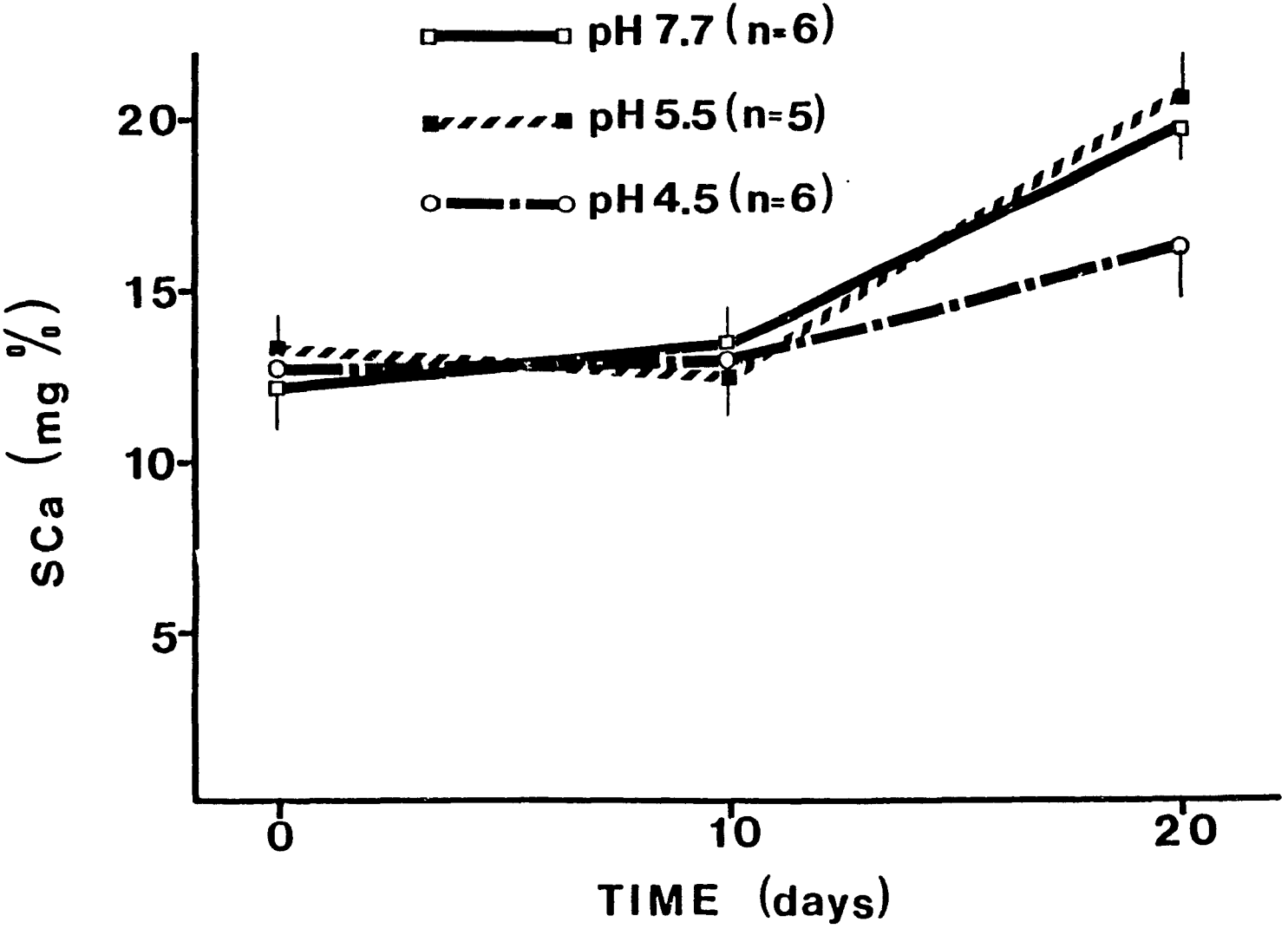
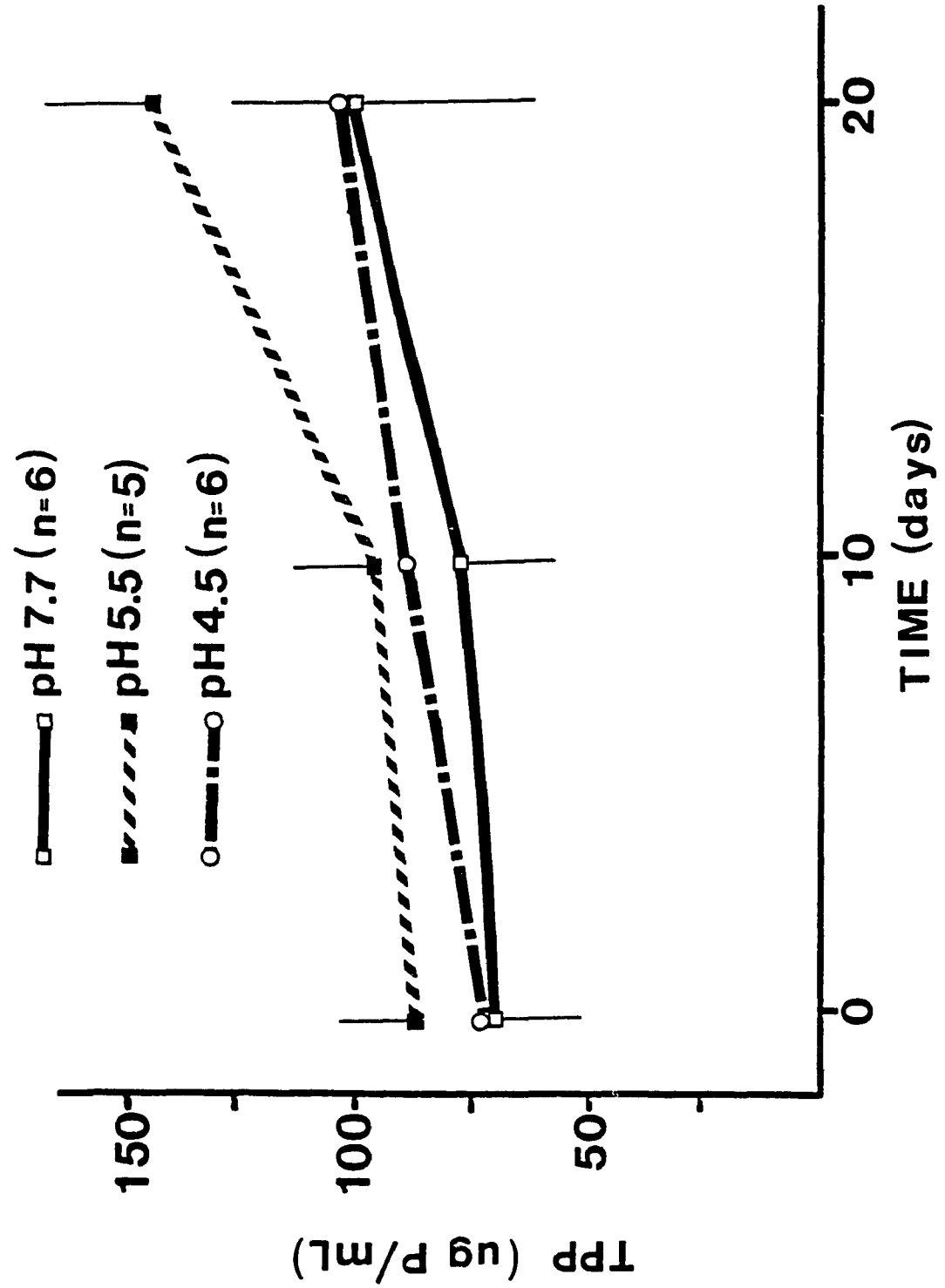

pH 7.7 (n=6)
pH 5.5 (n=6)
pH 4.5 (n=5)
TPP (ug P/mL)
150
100
50
0
10
20
TIME (days)

Figure 7: Serum vitellogenin levels in mature female trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in November and in December 1986. Vg was not significantly different in control, pH 5.5 and pH 4.5 groups during the experiment. (ANOVA, $p > 0.05$). Vg increased significantly in the pH 5.5 group from day 0 to day 20. (paired t test, $p < 0.05$). Each point represents a mean +/- S.E.M.


pH 7.7 (n=6)
pH 5.5 (n=5)
pH 4.5 (n=6)
Vg (mg/mL)
20
15
10
5
0
10
20
TIME (days)


pH 7.7 (n=6)
pH 5.5 (n=5)
pH 4.5 (n=6)
SCa (mg %)
20
15
10
5
0
10
20
TIME (days)


pH 7.7 (n=6)
pH 5.5 (n=5)
pH 4.5 (n=6)
150
100
50
TPP (ug P/mL)
0
10
20
TIME (days)

101.1 and 146.5 ug P/ml in the control, pH 4.5 and pH 5.5 groups respectively (Fig.9).

The trends in TPP during this experiment resembled those seen in the Vg reults. The day 20 TPP in the pH 5.5 exposure showed a highly significant increase over the day 0 mean ($p < 0.05$; Fig 9) and neither control or pH 4.5 groups had significant increases in TPP means from their day 0 values (Fig.9).

Correlations of Vg - SCa and Vg - TPP

The correlations between Vg and SCa values, and between Vg and TPP values, were calculated to describe the degree of association between measurements of the indirect indicators (SCa and TPP) with Vg under the different pH conditions. These correlations are presented in Tables 6 and 7.

In August, there was no significant correlation between SCa and Vg in the controls ($r = 0.11$, $p > 0.05$; Table 6). In the pH 5.5 group, Vg and SCa were correlated ($r = 0.56$, Table 6).

During late vitellogenesis, Vg and SCa had similar correlation coefficients in control, pH 5.5 and pH 4.5 treatments in both of the populations used in this study. In the September experiment, these ranged from

Ø.89 in the controls, to Ø.78 in the pH 5.5 and Ø.65 in the pH 4.5 groups (Table 6). With the younger population, in November - December, SCa and Vg were also correlated in the 3 pH groups (r = Ø.82, Ø.8Ø and Ø.58, in control, pH 5.5 and pH 4.5 respectively; Table 6).

Correlation coefficients of TPP and Vg for each of the experiments are presented in Table 7. During early vitellogenesis, in August, TPP was correlated with Vg in the control and the pH 5.5 groups (r = Ø.42 and Ø.66 respectively; Table 7). During late vitellogenesis, TPP and Vg were correlated in both of the populations of this study (r ranging from Ø.8Ø - Ø.89; Table 7). All pH groups had a similar range of correlations in both the September and November - December experiments.

Gonadosomatic and hepatosomatic indices

Neither GSI or HSI values were significantly different between acid and control groups in any of the experiments with mature fish ($p > Ø.Ø5$; Tables 8 and 9). The measurements of egg diameters also revealed no significant differences between the acid and control groups ($p > Ø.Ø5$; Table 1Ø).

Table 6: Linear correlation coefficients (r) between Vg and SCa levels, in mature female rainbow trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August, September and November - December 1986. (n.s. = not significant).

Experiment	Control	pH 5.5	pH 4.5
August	0.11 (n.s.) (n = 12)	0.56 (n = 18)	---
September	0.89 (n = 18)	0.78 (n = 15)	0.65 (n = 18)
Nov.- Dec.	0.82 (n = 15)	0.80 (n = 18)	0.58 (n = 18)

Table 7: Linear correlation coefficients (r) between Vg and TPP levels, in mature female rainbow trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August, September, and November - December 1986. All r values are significant ($p < 0.05$).

Experiment	Control	pH 5.5	pH 4.5
August	0.42 (n = 12)	0.66 (n = 18)	---
September	0.81 (n = 18)	0.86 (n = 15)	0.87 (n = 18)
Nov.- Dec.	0.80 (n = 15)	0.89 (n = 18)	0.83 (n = 18)

Table 8: Gonadosomatic indices (ovarian weight/body weight) x 100, in mature female rainbow trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August, September, and November - December 1986. (mean +/- S.E.M.)

	GSI		
Expt.	Control	pH 5.5	pH 4.5
August	1.11 +/- 0.35 (n = 4)	2.04 +/- 0.94 (n = 6)	0.68, 0.48 (n = 2)
Sept.	6.84 +/- 2.14 (n = 6)	4.99 +/- 1.38 (n = 6)	6.55 +/- 2.41 (n = 5)
Nov.- Dec.	6.50 +/- 1.51 (n = 6)	9.33 +/- 1.42 (n = 5)	7.18 +/- 1.59 (n = 6)

Table 9: Hepatosomatic indices, (weight of liver/somatic weight) x 100, in mature female rainbow trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August, September, and November - December 1986. (mean +/- S.E.M.).

	HSI		
Expt.	Control	pH 5.5	pH 4.5
August	1.38 +/- 0.17 (n = 4)	1.56 +/- 0.08 (n = 6)	0.68,0.84 (n = 2)
Sept.	1.77 +/- 0.16 (n = 6)	1.61 +/- 0.09 (n = 6)	1.26 +/- 0.12 (n = 5)
Nov - Dec.	1.88 +/- 0.21 (n = 6)	1.99 +/- 0.04 (n = 5)	1.54 +/- 0.16 (n =6)

Table 10: Egg diameters of oocytes from mature female rainbow trout, exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August, September, and November - December 1986. (mean +/- S.E.M.)

	Egg Diameter (mm)		
Expt.	Control	pH 5.5	pH 4.5
August	1.25 +/- 0.31 (n = 4)	1.58 +/- 0.45 (n = 6)	----
Sept.	3.47 +/- 0.51 (n = 6)	3.11 +/- 0.62 (n = 6)	3.30 +/- 0.67 (n = 5)
Nov.- Dec.	3.13 +/- 0.30 (n = 6)	3.70 +/- 0.18 (n = 5)	3.50 +/- 0.44 (n = 6)

Hematocrits

Hematocrits were taken on day 20 in the experiments with mature fish to detect the effects of acid exposure on this blood parameter (Table 11). There were no significant differences between acid exposed and control groups ($p > 0.05$; Table 11).

Growth rates

Growth rates were calculated in the experiments with mature fish to ascertain if low pH would affect this parameter (Table 12). Fish exposed to pH 4.5 had negative growth rates, which were significantly different than the control growth rates ($p < 0.05$, Table 12). Fish growth at pH 5.5 was not different than controls, with the exception of the December experiment. These fish had negative growth rates, which were significantly different than the control value ($p < 0.05$, Table 12).

Water chemistry

The daily means of pH and dissolved carbon dioxide, and mean values for hardness and sulfate concentrations are presented in Tables 3, 13, 14 and

15. The control pH ranged from pH 7.53 in September to pH 7.80 in November (Table 3), an average of pH 7.7 for all experiments. The mean acid pH levels over all experiments with mature fish were pH 5.54 and pH 4.54.

Dissolved carbon dioxide (Table 13) was higher in acid exposure tanks than in the control water, but was lower than 20 mg/L, a level shown to be detrimental to fish exposed at pH 4.0 (Lloyd and Jordan, 1964; Spry et al, 1981).

Water hardness, as $CaCO_3$, was lower in the pH 4.5 tanks compared to the controls (Table 14). The water hardness increased in all test tanks from June to December experiments, due to differences in the ion concentrations (Ca^{2+} and Mg^{2+}) used to calculate hardness. Sulfate levels (Table 15) were higher in the acid pH tanks due to the use of sulphuric acid as the titrator. High sulfate levels are considered a characteristic of acidified lakes (Harvey et al, 1980).

Table 11: Hematocrit values of male and female rainbow trout exposed to pH 7.7 (control), pH 5.5 and pH 4.5 for 20 days in August, September and November - December 1986. (mean +/- S.E.M.).

	Hematocrit (% packed cell volume)		
Experiment	Control	pH 5.5	pH 4.5
August	39 +/- 2 (n = 10)	35 +/- 2 (n = 10)	40 +/- 2 (n = 4)
September	37 +/- 1 (n = 10)	41 +/- 1 (n = 10)	42 +/- 2 (n = 10)
November	37 +/- 2 (n = 12)	34 +/- 3 (n = 12)	36 +/- 2 (n = 12)
December	35 +/- 2 (n = 12)	37 +/- 3 (n = 12)	41 +/- 2 (n = 12)

Table 12: Instantaneous growth rates (mean +/- S.E.M.) of rainbow trout exposed to pH 7.7, pH 5.5 and pH 4.5 for 20 days in August, September, November and December 1986. (n = number of fish; * = significantly different than control, $p < 0.05$).

Experiment	pH	n	G ($\times 10^{-3}$)	
August	7.7	10	1.163 +/- 1.627	
	5.5	10	1.989 +/- 1.251	
September	7.7	10	4.293 +/- 0.763	
	5.5	10	5.702 +/- 0.669	
	4.5	10	- 1.966 +/- 1.660	*
November	7.7	12	5.326 +/- 1.460	
	5.5	12	2.600 +/- 1.633	
	4.5	12	- 3.272 +/- 1.607	*
December	7.7	12	4.397 +/- 0.471	
	5.5	12	- 3.946 +/- 1.751	*
	4.5	12	- 3.721 +/- 1.759	*

Table 13: Dissolved carbon dioxide levels in control and acid exposure tanks during experiments in June, August, September, November and December 1986. (mean +/- S.D.; n = number of analyses of each test tank)

		Dissolved CO_2 (mg/l)		
Expt.	n	Control	pH 5.4	pH 4.9
June	11	5.3 +/- 0.8	6.4 +/- 3.0	8.0 +/- 1.8
		Control	pH 5.5	pH 4.5
Aug.	20	4.9 +/- 1.3	6.1 +/- 0.7	8.2 +/- 1.9
Sept.	20	4.7 +/- 0.8	5.5 +/- 0.8	7.5 +/- 1.0
Nov.	20	3.9 +/- 1.1	6.3 +/- 0.8	7.5 +/- 1.4
Dec.	20	4.1 +/- 0.6	4.8 +/- 1.3	7.0 +/- 1.3

Table 14: Water hardness, calculated from calcium and magnesium ion concentrations, in control and acid exposure tanks during experiments in June, August, September, November and December 1986. (mean +/- S.D.; n = number of analyses of each test tank)

		Hardness (mg/L $CaCO_3$)		
Expt.	n	Control	pH 5.4	pH 4.9
June	7	77.7 +/- 2.1	59.6 +/- 7.5	60.7 +/- 7.2
	n	Control	pH 5.5	pH 4.5
Aug.	9	80.3 +/- 5.0	74.0 +/- 6.3	70.3 +/- 4.8
Sept.	7	99.3 +/- 4.3	94.7 +/- 3.9	96.2 +/- 2.9
Nov.	6	120.7 +/- 5.1	110.5 +/- 16.2	112.3 +/- 10.9
Dec.	7	113.7 +/- 2.6	101.1 +/- 6.8	94.5 +/- 17.6

Table 15 : Sulfate levels in control and acid exposure tanks during experiments in June, August, September, November and December 1986. (mean +/- S.D.; n = number of analyses of each test tank)

		SO_4 (mg/L)		
Expt.	n	Control	pH 5.4	pH 4.9
June	6	22.7 +/- 1.9	109.8 +/- 6.4	108.4 +/- 15.1
	n	Control	pH 5.5	pH 4.5
Aug.	7	23.5 +/- 2.9	109.3 +/- 12.4	111.3 +/- 11.7
Sept.	7	23.6 +/- 1.4	108.1 +/- 3.1	109.9 +/- 3.05
Nov.	7	21.1 +/- 1.1	97.7 +/- 5.7	111.8 +/- 24.5
Dec.	7	20.9 +/- 1.0	106.7 +/- 16.2	115.4 +/- 34.8

DISCUSSION

Effects of low pH on Vg levels:

This study was the first to examine the effect of low pH on Vg levels in mature rainbow trout, using a homologous radioimmunoassay.

Early vitellogenesis in rainbow trout was not affected by pH 5.5 exposure, under the conditions of the present work. In August, Vg levels in control and pH 5.5 groups did not significantly differ during the experiment (Fig.1).

This pH level may be tolerated by female trout during early vitellogenesis. Most fish can survive chronic exposure to pH levels $>$ 5.0 (EIFAC, 1969; Spry et al, 1981) and they may also be able to support the energetic cost of early vitellogenesis at these pH levels. In August, Vg in both control and pH 5.5 groups remained at low levels (Fig.1), similar to the Vg levels reported by Van Bohemen and Lambert (1981) for rainbow trout in August (1 mg/ml). Thus, during early vitellogenesis, production of low levels of Vg may continue during pH 5.5 exposure.

Extreme pH exposure does reduce Vg levels, even during early vitellogenesis. In August, two mature

females in the pH 4.5 test tank survived a short term exposure to pH 3.3, but their Vg levels decreased from day 0 to day 20 (Fig.1). These day 20 levels were also lower than the control Vg on day 20 (Fig.1). While this is a small sample size, it is possible that vitellogenesis may be interrupted or delayed in these fish. Such an effect would have important consequences for field populations, which can be subjected to short term but extreme drops in pH, due to input into the watershed from storms or snowmelt (Haines, 1981; Harvey et al, 1981). This type of episodic, but extreme pH exposure, merits further research to determine its long term effects on vitellogenesis.

Late vitellogenesis in mature rainbow trout was not affected by sublethal pH exposure, under the conditions of this study. In the September experiment, Vg levels in control, pH 5.5 and pH 4.5 groups were not significantly different after 20 days (Fig.4). Different indicators of reproduction agree with the Vg findings of this study. Tam and Payson (1986) exposed adult brook trout to pH 5.56, pH 5.16 and pH 4.48 for 10 months, and found no effect of acid exposure on total egg counts, or on average egg

weight, after ovulation. These different indicators of reproduction support the conclusion that late vitellogenesis is not disrupted by pH 5.5 or pH 4.5 exposure.

While not affecting vitellogenesis, sublethal pH exposure may affect other aspects of oogenesis. In contrast to the results of this study, Weiner et al (1986) reported that oogenesis in rainbow trout was affected by exposure to pH 5.5 and to pH 4.5. Eggs from exposed parents, even when incubated in control water, had significantly less successful hatchability than controls.

There are two possiblities that may explain the different conclusions of this report and the study of Weiner et al (1986). One is that pH 5.5 and pH 4.5 exposure may affect other aspects of oogenesis not examined in the present study, such as maturation and ovulation of oocytes. The second explanation is that different experimental conditions may influence the affect of sublethal pH exposure on Vg levels, which would affect oogenesis. Weiner et al (1986) used softer water (9 mg/L) and a longer exposure period (42 days) than the conditions of this study (an average hardness of 97 mg/L, and 20 days exposure). In

addition to the effects of a longer exposure period, fish also experience greater stress at low pH in soft water than in hard water (Spry et al, 1981; McDonald, 1983a). Vitellogenesis and oogenesis may be affected by low pH under these conditions.

There is an indication from this study that late vitellogenesis in female rainbow trout may be delayed by pH 4.5 exposure. In the September experiment, Vg levels in the control and pH 4.5 groups were not statistically different, as the variation within each group was greater than the variation between the treatment groups. However, the trends in the control and pH 4.5 groups were different (Fig.4). It should be clarified that the correlated t test used to examine these trends is not a statistical test of the effect of pH exposure for this population. Yet this difference in trends between the control and pH 4.5 groups is biologically important, and may indicate that vitellogenesis at pH 4.5 was delayed.

The control fish showed a significant increase in Vg over the 20 days, more than doubling from day 0 to day 20 (4.23 - 11.38 mg/ml; Fig.4). This rapid increase in Vg has been observed in other studies during late vitellogenesis in rainbow trout. van

Bohemen and Lambert (1981) reported that Vg levels increased from 0.4 mg/ml in September to 7.2 mg/ml in October. Scott and Sumpter (1983) observed that Vg levels in winter spawning trout started a rapid increase in September and October, approximately doubling. Other salmonids show a similar pattern 3 - 5 months before spawning. Landlocked Atlantic salmon, Salmo salar ouananiche, spawning in mid-November to mid-December, showed a rapid increase in Vg levels between June and August (So et al, 1985).

This rapid increase in Vg did not occur in the pH 4.5 females in September. On day 20, their mean Vg was 8.08 mg/ml, not significantly different than their day 0 value of 6.35 mg/ml (Fig.4). This much slower increase in Vg at pH 4.5 implies that vitellogenesis at this pH may be delayed; this delay may have been more pronounced if the exposure period had been prolonged.

There are several mechanisms which may explain why Vg levels did not increase in the pH 4.5 group. One possibility is that low pH exposure directly affects estrogen levels, leading to decreased synthesis of Vg in the liver. Yet this appears unlikely, as estrogen levels in brook trout (Tam et al, 1987) and estradiol

levels in rainbow trout (Weiner et al, 1986) were not affected by acid exposure. Another possibility is related to the higher maintenance costs brought on by the stress of acid exposure. Trout chronically stressed by acid show increased plasma cortisol and increased plasma glucose levels (Lee et al, 1983; Brown et al, 1984, 1986; Tam et al, 1987); the general reaction to stress includes increased carbohydrate metabolism. In addition, trout are also subjected to higher costs of ionoregulation during acid exposure (Wood and MCDonald, 1982; McDonald, 1983a). Faced with these increased maintenance costs, the organism may redirect some energy from Vg production, when exposed to low pH. This would result in a plateau in Vg levels, which may delay vitellogenesis.

Such a delay in vitellogenesis may lead to fewer mature eggs, or perhaps to a delay in ovulation. In the field, Frenette and Dodson (1984) found that brook trout in an acid lake had apparently fewer mature eggs than those in an unstressed population. Laboratory studies indicate that ovulation in some individuals may be delayed by low pH. Weiner et al (1986) found that approximately 10 % of the eggs were retained by

the ovary, and thus had not ovulated, in one third of the females exposed to pH 4.5 (2 out of 6). Tam and Payson (1986) also reported that ovulation was delayed at pH 5.16 and pH 4.48 in their study with brook trout, though rapid oocyte development occured at all pHs. A delay in vitellogenesis due to low pH exposure may lead to delayed or partial ovulation and contribute to recruitment failure in acid stressed populations.

This possibility suggests the need for further research, since recruitment failure is considered the major threat to the survival of field populations stressed by acid pH. The sensitivity of the RIA for Vg allows an assessment of the reproductive status of a population at several times of the year; this would be a useful technique to determine if chronic low pH stress delays vitellogenesis in these fish.

During late vitellogenesis in a younger population, acid pH exposure did not decrease Vg levels. In the November - December experiment, there were no significant differences between control, pH 5.5 and pH 4.5 Vg levels on day 20 (Fig.7). The pH 5.5 exposure was the only group which had a

significant increase in Vg, rising from 7.86 mg/ml on day 0 to 15.58 mg/ml on day 20 (Fig.7).

The control group of this population did not show the increase in Vg that would be expected during late vitellogenesis (Fig.7). There were no differences in dissolved O_2, dissolved CO_2, SO_4 or temperature found between the treatment tanks in this experiment, or with tanks in the other experiments to explain the pattern found in control Vg in November - December.

One explanation may be that the acclimation period of this younger population was not sufficient for adaptation to laboratory conditions. These fish arrived in the laboratory in early October and may have been stressed by the change from the ambient temperature (8 - 10 C) to that of the laboratory water (12 C). The acclimation period was 3 weeks in November and 8 weeks in December; compared to approximately one year for the older population. This period, especially in the November experiment, may not have provided sufficient time for adaptation to our laboratory conditions. Unlike the control group, the pH 5.5 fish may have recovered from this stress during the experiment, which would explain why their Vg levels increased.

The effects of pH exposure on serum calcium

SCa is an important parameter for the general physiological status of an organism, and is associated with Vg in mature female teleosts. During vitellogenesis, SCa levels in females increase substantially above basal levels in non-vitellogenic fish. In Salmo gairdneri, reported basal SCa levels have ranged from 8.9 to 10.1 mg % (Bjornosson and Haux, 1985) to a range of 12 +/- 2 mg % (Bromage et al, 1981). Mature female trout have attained levels of 38.5 mg % during late vitellogenesis (Sumpter, 1985) and 58 mg % prior to spawning (Whitehead et al, 1978). Yet few studies have examined SCa levels in vitellogenic rainbow trout chronically exposed to sublethal pH levels. In this study, SCa levels in acid stressed rainbow trout were compared with control females, during early and late vitellogenesis.

During early vitellogenesis, pH 5.5 exposure did not affect SCa. In the August experiment, SCa in the pH 5.5 fish increased and was significantly higher than controls on day 20 (Fig.2). This increase is probably reflecting the higher Vg levels on day 20 in these fish (2.79 mg/ml, compared to 1.94 mg/ml in control fish; Fig.1), suggesting that the pH 5.5 fish

were at a later stage of vitellogenesis than the controls. It is likely that the higher SCa in pH 5.5 fish is not due to an effect of pH exposure.

During late vitellogenesis, SCa was not affected by sublethal pH exposure. SCa in controls, pH 5.5 and pH 4.5 groups were not significantly different during experiments with two populations (September: Fig.5; November - December:Fig.8). This agrees with results of Weiner et al (1986), who reported that SCa in vitellogenic female trout was not different in control, pH 5.5 or pH 4.5 fish after 42 days of exposure. This suggests that SCa in rainbow trout may not be a sensitive indicator of low pH stress

However, this present study indicates that trends in SCa during late vitellogenesis may be affected by pH 4.5 exposure. In the September experiment, SCa in both control and pH 5.5 groups significantly increased over the 20 day experiment, but SCa in the pH 4.5 group did not significantly change from day 0 to day 20 (Fig.5).

There are two possibilities, either or both of which may explain the pattern observed at pH 4.5 in September. One possibility is that SCa is reflecting the trend in Vg at this pH, as Vg also did not

increase significantly over the 20 days (Fig.4). The other possibility is that SCa in pH 4.5 exposed trout did not significantly increase because these fish lost calcium at low pH; SCa levels were then maintained, perhaps by mobilization of calcium reserves. A few studies have shown that losses of calcium occur in immature trout at low pH (4.0 - 4.5). In soft water, at pH 4.3, SCa decreased from day 0 levels and did not recover after 5 days of exposure (McDonald et al, 1980). In hard water, renal excretion of calcium increased, by a factor of 2 to 3, for a period of 4 days; normal calcium output was finally restored due to a decrease in urine output (McDonald and Wood, 1981). Calcium can also be lost from the gills. McWilliams (1982) found increased rate of loss of radiolabelled Ca^{2+} from isolated brown trout gills in acid water; gillbound calcium was also lost more quickly from fish acclimated in neutral environments than from an acid exposed population. Hobe et al (1984) also used radiolabelled Ca^{2+} to trace body calcium losses in acid stressed rainbow trout. At pH 4.0 - 4.2, there was a net loss of whole body calcium after 12 hours, mainly due to a temporary but drastic reduction in uptake (by 65 %) at the gill (Hobe et

al,1984). It is possible that the mature females exposed at pH 4.5 in September also suffered similar losses of calcium. These losses, even if temporary, would be expected to cause greater stress in a vitellogenic trout, due to their increased requirement for calcium.

Some workers have implied that mobilization of tissue calcium at low pH may compensate for these losses to maintain normal SCa levels, or to allow SCa to recover to normal levels. Neville (1979b) suggested that tissue calcium, in immature trout at pH 4.0, was consistently low because it was used to maintain SCa at control levels. Giles et al (1984) found that SCa was recovering to approach control levels at pH 4.2 - pH 4.5, perhaps due to mobilization of tissue calcium. Such tissue mobilization may have occurred in the vitellogenic trout exposed to pH 4.5 in September, and may account for the maintenance of SCa levels observed in these fish.

This trend observed in SCa at pH 4.5 may be of physiological importance for vitellogenic fish exposed to low pH. Low pH exposure may stress calcium regulation in these fish, compromising their successful reproduction. Furtner research is needed

to determine if calcium losses, as measured by changes in total body calcium (tissue, skin and skeleton) occur in vitellogenic trout exposed to low pH, and if these losses can affect SCa.

Correlations of SCa and Vg

Calcium has been used as an indicator for vitellogenin in many studies involving rainbow trout (Bailey, 1957; Whitehead et al, 1978; Bromage et al, 1984). However, few studies have examined the direct relationship between Vg and SCa in the same fish; none have examined both SCa and Vg in acid stressed fish. In this study, SCa and Vg values were compared, in control and in acid-exposed rainbow trout, during early and late vitellogenesis.

SCa is an unreliable indicator for Vg during early vitellogenesis. In August, there was no correlation between control SCa and Vg ($r = 0.11$; Table 6) and only a low degree of correlation in the pH 5.5 group ($r = 0.56$; Table 6). The control correlation agrees with a value reported by Sumpter (1985) for fish with low levels of Vg ($r = 0.20$; Vg = 2.42 mg/ml). In this present study, Vg was present at low levels in both groups (1.07 - 2.79 mg/ml, Fig 1),

and SCa did not increase above basal levels (11.3 mg % - 11.7 mg %) until day 20 in the pH 5.5 group (Fig.2; Table 5). Elliot et al (1984) also reported that SCa remained at basal levels when Vg was at low levels (2 - 5 mg/ml). Thus, during early vitellogenesis,SCa and Vg do not have a high degree of correlation and SCa is not a reliable indicator of Vg.

Later in the cycle, SCa and Vg have higher degrees of correlation. The r values were similar in the control and pH 5.5 exposed groups in the September and November - December experiments (Table 6). These values compare with the SCa - Vg correlation (r = 0.78) reported by Nagler et al (1987), for rainbow trout during late vitellogenesis. The pH 4.5 groups had the lowest correlation coefficients in both of the experiments (Table 6). However, none of the r values express a close enough relationship for the prediction of actual Vg levels from SCa measurements. The explanation for this low degree of correlation may be that SCa measures total serum calcium and includes both protein bound and ionic calcium (Bailey, 1957; Dacke, 1979; Bjornosson and Haux, 1985); thus it is not a precise indicator of Vg.

While SCa and Vg are only weakly correlated during late vitellogenesis, they do show similar trends at this stage of the cycle. Thus, SCa can be useful for differentiating vitellogenic and non-vitellogenic fish during late vitellogenesis. In this study, significant trends in Vg during late vitellogenesis were generally matched by similar trends in SCa (September: control and pH 5.5 groups, Figs.4 and 5; November - December: pH 5.5 group, Figs.7 and 8). SCa in control and pH 4.5 groups in the latter experiment also showed significant increases but these were matched by more modest increases in Vg (Figs.7 and 8). Elliot et al (1984) also found parallel changes in Vg and SCa during late vitellogenesis, in agreement with this study.

Effects of pH on TPP

Vg levels have been indirectly measured using phosphoprotein phosphorous in many fish, including flounder, Platichthys flesus, (Emmersen and Petersen, 1976), catfish, Heteroneustes fossilis (Nath and Sandararaj, 1981), and goldfish, Carrassius auratus (De Valming et al, 1980). In rainbow trout, it has been determined during the annual cycle by Whitehead

et al (1978), van Bohemen et al (1981) and by Da Costa (1986). This present work is the first to examine the effects of acid exposure on TPP in vitellogenic rainbow trout, during early and late vitellogenesis.

Under the conditions of this study, exposure to pH 5.5 or 4.5 does not affect TPP in vitellogenic rainbow trout. During early vitellogenesis, in August, TPP in pH 5.5 exposed rainbow trout was not different than controls (Fig.3). During late vitellogenesis, TPP in pH 5.5 and pH 4.5 groups was not different than controls in two populations (September and November - December; Figs.6 and 9).

One previous study has examined the effect of pH exposure on phosphoprotein levels in salmonids. Tam and Payson (1986) exposed brook trout to pH 4.48 for a period of one year, in water of similar hardness to that used in this study. Alkali-labile phosphoprotein levels (ALPP) were not different in control and pH 4.48 groups, which agrees with the TPP results of this study. Yet, brook trout are considered more resistant to low pH than rainbow trout (Daye and Garside, 1975); thus, TPP or ALPP in rainbow trout may be affected after a similar exposure period (one year) to pH 4.5.

Correlations of TPP and Vg

Some workers have examined the relationship between Vg and TPP or ALPP, but none have measured these parameters in acid stressed fish. In this study, Vg and TPP levels were compared in control and acid exposed rainbow trout, during early and late vitellogenesis.

During early vitellogenesis, TPP is not a reliable indicator of Vg. In August, there was no correlation between control Vg and TPP, and only a low correlation in the pH 5.5 group (Table 7). Vg in control and pH 5.5 groups was at low levels in August (Fig.7:means ranging from 1.07 - 2.74 mg/ml). van Bohemen et al (1981) also reported that ALPP was not correlated with Vg during early vitellogenesis, when Vg was at low levels (0.4 - 3.0 mg/ml). Thus, TPP cannot be a reliable indicator of Vg when Vg is low, during early vitellogenesis.

Later in the cycle, TPP and Vg do show a consistent, but still low, degree of correlation. The r values are similar in control and acid exposed groups, as well as being similar in the two populations (September and November - December; Table 7). They are also in the range reported by Nagler et al (1987) for ALPP - Vg ($r = 0.87$) and TPP - Vg ($r =$

Ø.97) for rainbow trout in December. However, the relationship reported in the present study is not close enough for the prediction of actual amounts of Vg from TPP levels. This is illustrated by the September experiment; the control TPP means on day Ø and day 1Ø were identical (59.3 and 59.4 ug P/ml; Fig.6), but the corresponding Vg levels differed (4.23 and 6.33 mg/ml; Fig.4).

The low degree of correlation between TPP and Vg has several explanations. One is the fact that rainbow trout Vg contains little phosphorous (Ø.6 %; Campbell and Idler, 198Ø; Ng and Idler, 1983). Thus, neither TPP nor ALPP can be sensitive indicators of Vg (So et al, 1985); especially during early vitellogenesis, when Vg is low. In addition, similar "basal levels" (15 - 25 ug P/ml) of TPP and ALPP have been detected in male rainbow trout (TPP:Whitehead et al, 1978 and Bromage et al, 1981; ALPP:van Bohemen et al, 1981). Thus neither TPP nor ALPP are specific for Vg because both measurements include other phosphoproteins in the serum. van Bohemen et al (1981) suggested that Vg may bind different amounts of phosphorous, depending on the stage of vitellogenesis. They measured Vg and Vg

bound phosphate in rainbow trout, over a complete cycle of one year, and found that similar levels of TPP or ALPP over one vitellogenic cycle may not correspond to the same quantity of Vg. For these reasons, measurements of phosphoprotein phosphorous (TPP or ALPP) are not precise indicators of Vg. While TPP cannot be used to predict exact values of Vg during late vitellogenesis, it can be useful for separating vitellogenic from non-vitellogenic fish. During late vitellogenesis, increases in Vg are matched by a trend in TPP. In the September experiment, control TPP and Vg increased significantly over the 20 days (Figs.4 and 6); in the November - December experiment the pH 5.5 TPP and Vg means also increased significantly from day 0 to day 20 (Figs.7 and 9). Based on these trends, TPP can indicate vitellogenic individuals during late vitellogenesis, which is in agreement with the conclusions of van Bohemen et al, 1981.

Growth rates and pH exposure

In this study, the growth rate of rainbow trout exposed to pH 4.5 was significantly lower than controls in all experiments. Fish exposed to pH 5.5

had a lower growth rate in one experiment (Table 12). The metabolic costs and stress of acid exposure may result in a curtailment of growth, similar to the effects on Vg production discussed previously.

Acid exposure has been reported to have variable effects on fish growth (increased growth, decreased growth, no effect of pH) in the laboratory. Jacobsen (1977) and Sadler and Lynam (1986) found that acid exposed brown trout, Salmo trutta, grew at the same rate as the controls. Other studies have shown decreased growth rates with acid exposure, in white sucker (Beamish, 1972), and in brook trout (Dively et al, 1977; Swarts et al, 1978). Reports of field studies have also shown variable effects of acid exposure on growth, and it has been suggested that the effects of acid on growth may depend on the population and habitat (Harvey, 1979).

There is no clear relationship between acid exposure, growth and reproduction. This study found no effect of pH 4.5 or pH 5.5 exposure on Vg levels, yet the growth rates of acid exposed fish were lower than controls in some experiments (Table 12), suggesting that growth may not be directly related to the reproduction of acid exposed fish.

This finding agrees with studies by Menendez (1976) and Tam and Payson (1986). They reported that growth rates of acid exposed brook trout were initially lower than controls, but that the total number of eggs produced was not affected by pH exposure. Mount (1963) reported the opposite finding, as acid exposure affected the reproduction of fathead minnows, but had no effect on growth. More chronic exposure studies are required to determine if the effects of acid exposure on growth and on reproduction are related.

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