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Avoidance of sulphuric acid solutions by groups of juvenile brook trout, *Salvelinus fontinalis* and the corresponding effect on plasma cation regulation.

Simon Calvert James Pedder

A Thesis

in

The Department

of

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ABSTRACT

Avoidance of sulphuric acid solutions by groups of juvenile brook trout, Salvelinus fontinalis and the corresponding effect on plasma cation regulation.

Simon Calvert James Pedder

The avoidance response by groups of twenty juvenile brook trout, Salvelinus fontinalis was studied, when they were given the choice between clean water (pH 7.4) and different levels of decarbonated sulphuric acid treated water (pH 6.0, 5.5, 5.0, and 4.0) over 96 hr. Behaviour of fish was examined by the avoidance of test individuals to acidic conditions; significant avoidance by test individuals was found below a pH of 5.5. A breakdown in social interactions (aggression, dominance and territoriality) was seen in the lower pH levels (4.0, 5.0 and 5.5) when compared to controls (pH 7.4). Plasma cation concentrations of test individuals indicate that lowest level without avoidance (pH 5.5) had greatest effect on ion regulation. Avoidance at the lowest acid level (pH 4.0) kept ion fluctuations minimal.

Ninety-six hr bioassays, where fish were only subjected to treated waters (pH 4.0, 5.0, 5.5, 6.0), showed

a decrease in plasma sodium levels, especially at pH 4.0. Plasma calcium concentrations increased, with no change between pH 5.0 and 4.0. Potassium levels decreased with increasing acid concentrations. Plasma magnesium was decreased but only slightly when compared to the fluctuations of the other ions. No mortality was recorded in any of the tests. Results suggest that brook trout have the ability to recognize depressed pH conditions below pH 5.5 as aversive and respond appropriately.

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Last and least the boys from "The Falls" along with Pat and James for their continual ability to keep me from work.

DEDICATION

To my parents, James Dension Pedder and Mary Francis Pedder, for all their love and support over the years and memories to last a life time.

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INTRODUCTION

Acid in the environment

All precipitation is slightly acidic due to the presence of carbon dioxide in the atmosphere, which when dissolved in water creates carbonic acid (H_2CO_3). However, when industrial gases such as sulphur dioxide (SO_2), sulphur trioxide (SO_3), nitrogen oxide (NO) and nitrogen dioxide (NO_2) are emitted in the atmosphere, they are oxidized and hydrolyzed and converted into sulphuric and nitric acid. The major reactions are as follows;

$$2SO_2 + O_2 = 2SO_3$$

$$SO_3 + H_2O = 2H^+ + SO_4^-$$

$$2NO + O_2 = 2NO_2$$

$$3NO_2 + H_2O = 2H^+ + 2NO_3^- + NO$$

$$4NO_2 + 2H_2O + O_2 = 4H^+ + 4NO_3^- \text{ (Vermeulen 1978)}$$

Sulphur and nitrogen oxides are released as gases from ore smelters, coal-fired generating stations, oil and gas refineries and automobiles. These atmospheric pollutants combine with water vapour in the air giving these

pollutants long range atmospheric transport. They return to the earth as "wet deposition" (acidified rain or snow) or as "dry deposition" (particulate matter or gases) on soil, forests, vegetation and water (Ministry of the Environment 1980).

Although acid precipitation has many destructive characteristics and targets, the reduction of fish populations in lakes with low buffering capacity (ability to maintain stable pH), due to low calcium concentrations, is the most obvious and alarming effect (Spry et al. 1981).

The acidification of freshwaters by acid precipitation and the corresponding detrimental effect on aquatic organisms has been documented as a Canadian (Beamish and Harvey 1972; Spry et al. 1981;) and international problem (Leivestad et al. 1976; Schofield 1976). Aquatic ecosystems under acidification show both reduced production and reduced decomposition. The accumulation of algae, often observed in acidified waters, is related to the reduced feeding activities by invertebrates. Due to increased sedimentation and reduced availability of nutrients, there is a shift in acidified lakes from bacteria to slow-acting fungi (Overrein et al. 1981). The invertebrate fauna (Zooplankton, crustaceans, insects, snails and bivalves, etc.) all show reduced diversity during acidification (NRCC

1981) The elimination of certain species may be caused by direct action of acid stress on a particular species or the occurrence of a disruption in an ecosystem's food web, causing elimination of a species due to its dependence on a acid-sensitive prey.

Reproductive and physiological responses of freshwater fish to acid

The effects of reduced environmental pH on freshwater fish has been extensively studied (see reviews by Fromm 1980; Haines 1981; Spry et al. 1981; Wood and McDonald 1982). Physiological disturbances caused by reduced pH have been reported to be modified by parameters such as type of acid (Packer and Dunson 1972) fish species (Spry et al. 1981) water ionic composition (McDonald et al., 1983) acclimation (McWilliams 1980) and season (Stuart and Morris 1985).

Heavy metals are frequently elevated in acid lakes (Beamish 1974a; Scheider et al. 1979) either from atmospheric deposition or by mobilization from the sediments due to increased solubility at low pH (Spry et al. 1981). However, work in this area has passed unnoticed by

investigators, because its effects are seen as secondary to acid (Beamish 1974b).

Recruitment failure due to egg and fry mortality has been regarded as a common cause of fish population losses (Rosseland et al. 1980). It has been shown that exposure of fish to depressed pH has a significant effect on reproduction and development (Mount 1973; Beamish 1976; Ruby et al. 1977). Reduction in reproductive processes due to lower pH levels have been shown to have the following order of sensitivity: egg production > fry survival > fry growth > egg fertility (Fromm 1980).

Many physiological studies have been performed with acute and chronic pH levels to determine the mechanisms of acid toxicity. Experiments at acute pH levels (< pH 4.0) point to a disturbance of the acid-base balance. Acidification of ambient water causes an acidosis in freshwater fish, the magnitude of which is proportional to the change in water pH (Booth et al. 1982). The origin of the acidosis observed during acid exposure is poorly understood, although it is probably at least partially due to increased inward diffusion of H^+ and (or) outward diffusion of HCO_3^- (Neville 1980). At lethal pH levels blood pH and $[HCO_3^-]$ decreases more rapidly as the ambient pH decreases (Ultsch et al. 1981). Neville (1979a) and

Booth et al. (1982) reported that compensation for the acid stress by fish is possible until water pH reaches pH 4.0, and that at higher chronic pH levels ionoregulation is the major concern.

Exposure to acidic water results in disturbance of the normal ionic balance of freshwater fish (Packer and Dunson 1970, 1972; Neville 1979b, 1980; McDonald et al. 1980; McWilliams 1980a; Ultsch et al. 1981; Fraser and Harvey 1984). Exposure to chronic pH levels (pH > 4.0) results in significant decreases in both plasma $[Na^+]$ and $[Cl^-]$ and muscle $[Na^+]$ and $[Cl^-]$.

The gills of freshwater fish are the major site for the diffusional loss of ions, with around 10 percent or less occurring in the urine (McDonald and Wood 1981). The major concern are the disturbances to both the active transport and the passive diffusion of Na^+ and Cl^-, with the similar undirectional fluxes of both dependent upon the severity and duration of the acid exposure (McDonald 1983).

Although the principle losses are believed to be sodium and chloride, due to their predominance in extracellular fluids, alterations in the amounts of potassium (Divery et al. 1977), calcium (Smith 1977; Fraser and Harvey 1984) and magnesium (Giles et al. 1984) have been reported. The importance of waterborne $[Ca^{2+}]$ on

fluctuations of plasma ion levels has been established by McDonald et al. (1980), with greater ion alterations associated with lower Ca^{2+} levels.

Effects of behaviour on toxicological response to contaminants

Behavioural responses to aquatic contaminants have not been extensively studied, although the attraction-avoidance response of aquatic organisms, such as fish, toward toxic discharges can alter survivorship rates (Geckler et al. 1976; Pedder and Maly 1985). At sublethal levels, behavioural changes could cause a decrease in normal predatory and spawning activity, thereby affecting the aquatic ecosystem. The dispersal and subsequent dilution of a pollutant from its source seems a reasonable argument for concentrating studies on low subacute levels rather than lethal. However, it has been reported that high doses of certain toxicants, such as copper, attract certain species (Giattina et al. 1982; Pedder and Maly 1985). If increasing attraction correlates with increasing toxicant concentration levels, fish may subject themselves to toxic levels at the source of the contamination.

Behavioural aspects have received little attention probably due to the difficulties in obtaining reliable and consistent data. Fish have been reported to avoid acidic solutions when swimming into a steep gradient in the laboratory (Ishio 1965), and in the selection of spawning sites in the field (Johnston and Webster 1977). More recently Jones et al. (1985) tested the short term behaviour of arctic char, Salvelinus alpinus to gradients of H^+ and CO_2 and found that above pH 5.5 fish avoided due to the presence of CO_2 while below pH 5.5 they avoided due to increased H^+ concentrations. Their study was similar to most behaviour tests, whereby visual observation of individual animals are recorded during daylight for less than 30 min (Cripe 1979).

An evaluation is required for the behavioural responses of groups of fish toward chronic pollutant levels for extrapolating laboratory results to field situations. Fish behaviour towards environmental pertubations, such as depressed pH levels caused by spring run-off, must be considered when evaluating the impact of aquatic contaminants on fishery resources.

The purpose of this study

The objectives of this research were to: (1) examine the behavioural responses of groups of trout given a choice of neutral or acidic waters over a long term period, (2) determine the lowest pH level whereby significant avoidance by the fish population would occur, (3) observe if the presence of lower pH levels would lead to increased avoidance and thus less physiological alterations, concerning ionoregulation, and (4) determine whether fish avoid acidic conditions at levels which could cause ecological alterations to fish populations.

Brook trout, Salvelinus fontinalis were selected for this study because of their known tolerance to acidic waters, with mortality threshold levels less than pH 4.0 (Packer and Dunson 1970), and their importance in the commercial and recreational fisheries in southern Quebec.

METHODS AND MATERIALS

Juvenile brook trout, Salvelinus fontinalis were purchased from Des Landes Pisciculture, Drummondville, Quebec and held in two aerated flow through holding tanks (a green fiberglass tank 210 x 60 x 55 cm high, and a white polyethylene tank 185 x 80 x 45 cm high). The fish were fed commerical (Martin's 83 G) food pellets for salmonids (Table 1) at a level of approximately 1% of their wet body weight per day. Fish were kept in dechlorinated Montreal city water (Table 2) under continuous flow conditions with a photoperiod of 12 hr light, 12 hr dark.

For both behavioural and physiological bioassays a two chambered artificial stream (250 x 60 x 30 cm high) constructed of polyvinyl chloride (PVC) was utilized (Fig. 1). White coloured vinyl covered the sides and bottom of the stream to aid in the observation of the fish. The experimental area was surrounded by black plastic sheeting to limit external disturbances. In all experiments, groups of 20 brook trout were added to the apparatus and allowed a two day orientation period. After the initial orientation period, the test procedure began, which in all cases lasted 96 hrs. In all tests, water flow rates in both chambers were regulated to 2 L/min for a calculated 99% replacement

Table 1. Analysis of trout food pellets (Martin's Feed Mills Ltd., Elmira, Ontario).

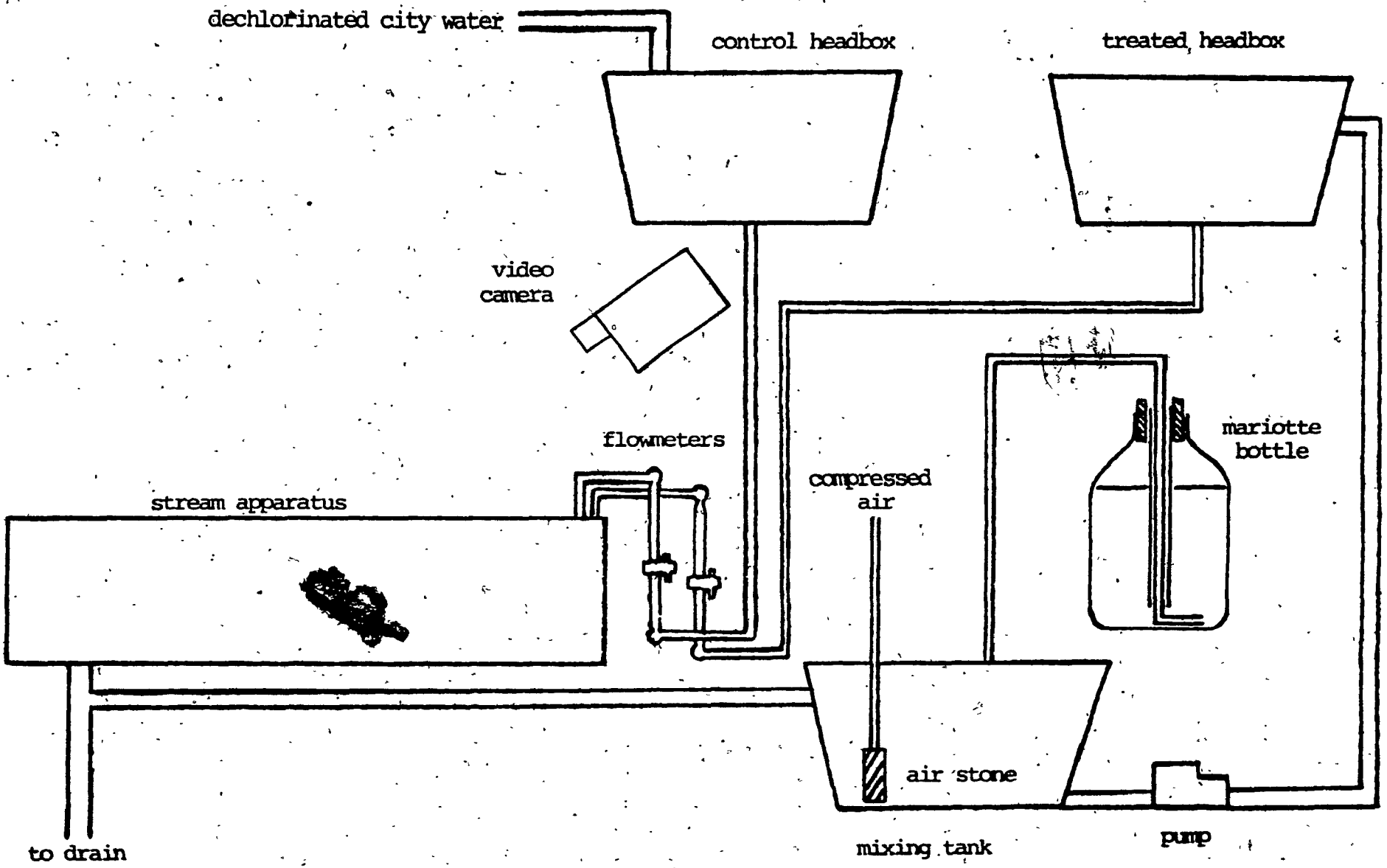
Crude Protein (min)	40.0%
Crude Fat (min)	10.0%
Crude Fibre (max)	3.0%
Vitamin A (min)	7500 i.u./kg
Vitamin D3 (min)	3000 i.u./kg
Vitamin E (min)	100 i.u./kg
Ascorbic Acid (min)	800 mg/kg

Table 2. Monthly (N=7) Montreal city water characteristics ± standard deviation (June to December 1985).

Characteristic	Value
Total hardness (mg/L as $CaCO_3$)	127.1 ± 2.8
pH	7.40 ± 0.1
Total alkalinity (mg/L as $CaCO_3$)	85.0 ± 2.5
Temperature (C)	12.0 ± 1.0
Dissolved Oxygen (mg/L)	10.6 ± 0.3

Figure 1. Schematic drawing of experimental stream apparatus showing the direction of water flow.

Figure 1.


dechlorinated city water
control headbox
treated headbox
video camera
flowmeters
mariotte bottle
compressed air
stream apparatus
air stone
to drain
mixing tank
pump

time of 4 hrs (Sprague 1973). On alternate days of all experiments, both chambers were analyzed for dissolved oxygen by an oxygen meter (Yellow Springs Instrument Co., model 54A). Saturation never fell below 80 percent throughout all experiments. Routine cleaning of the stream apparatus and repeated timing of flow rates was undertaken in between experiments. A clear plastic covered the stream apparatus during all testing. During all tests no mortality was observed.

Behavioural Bioassays

The artifical stream apparatus was modified such that one chamber would receive treated water and the other untreated (Fig. 2). Initial dye tests and earlier experiments with copper (Pedder and Maly 1985) showed a sharp gradient between sides. Initially dilute (0.1 N) sulphric acid ('Baker Analyzed' Reagent, Fisher Scientific Ltd.) was added to a mixing tank until the desired pH was achieved. The treated water was vigorously aerated with compressed air through air stones to remove excessive CO and then pumped to a head box from which water flowed by gravity to a flow meter (Manostat Corp., New York) which kept the flow rate constant. Control water passed into a

Figure 2. Top view of stream apparatus and separate chambers with the black boxes representing the sampling areas for determination of water characteristics of the bioassays.

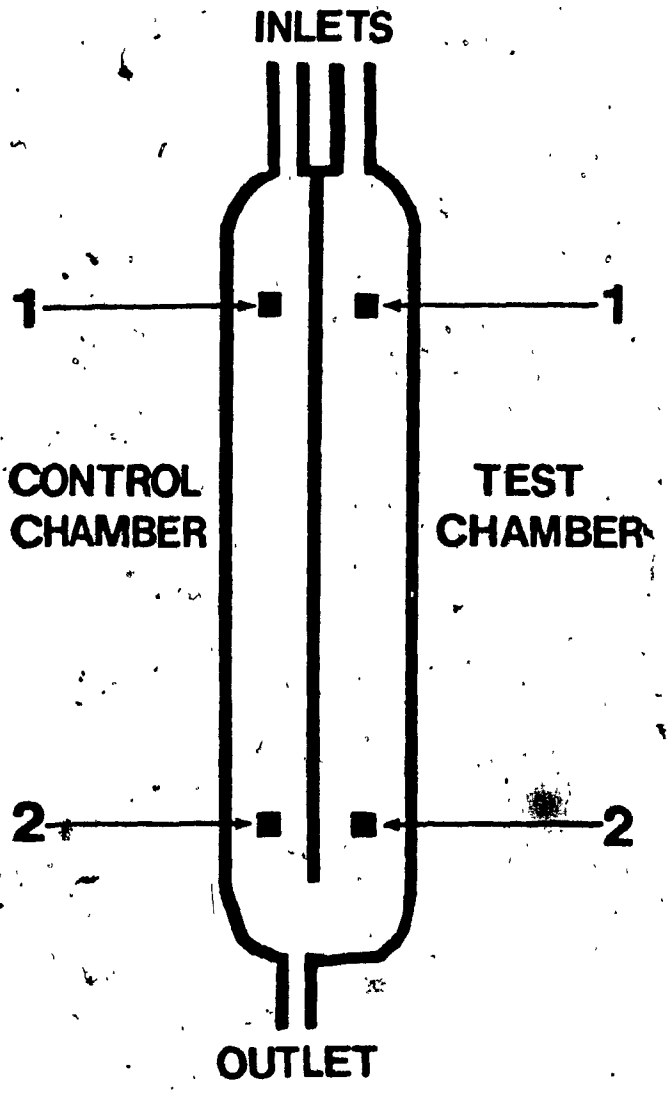


Figure 2.

separate head box and through a flowmeter to the control chamber of the stream. Drainage occurred at the undivided end of the stream where the test organisms had a choice of the waters.

Drainage water (a combination of treated and untreated water) was re-routed to the mixing chamber (Fig. 1) where dilute (0.1 N) sulphuric acid was added from a mariotte bottle (Leduc 1966) by 0.86 mm intramedic polyethylene tubing (Clay Adams) with a controlled flow rate until the desired pH was reached and the cycle was repeated. During testing a pH electrode (Radiometer, Copenhagen) recorded pH levels of the test water with a reference buffer standard. The drop rate of the sulphuric acid was regulated accordingly within an upper and lower control level ($\pm$ 0.2 of the desired pH) to keep a nearly constant pH level. The pH level of the control and treated water was taken at regular intervals at two separate areas of each chamber (Fig. 2) and are summarized in Table 3. Temperatures in both the control and treated chambers were recorded by standard mercury thermometers and found be 12.0 $\pm$ 1.0 C and 13.0 $\pm$ 1.0 C respectively.

All tests lasted 96 hr after the desired pH was obtained in the test chamber. Groups of 20 trout were added to the apparatus, 10 on each side, two days before the

Table 3. Regular (N=22) pH levels (± S.D.) in separate chambers.

Bioassay	Control		Test	
	#1	#2	#1	#2
Control #1	7.40 ± .01	7.40 ± .01	7.40 ± .01	7.40 ± .01
Control #2	7.40 ± .01	7.40 ± .01	7.40 ± .01	7.40 ± .01
Control #3	7.40 ± .01	7.40 ± .01	7.40 ± .01	7.40 ± .01
pH 6.0	7.35 ± .04	7.32 ± .06	6.04 ± .11	6.06 ± .08
pH 5.5	7.38 ± .03	7.34 ± .04	5.50 ± .08	5.54 ± .07
pH 5.0	7.36 ± .05	7.32 ± .05	4.97 ± .10	4.98 ± .07
pH 4.0	7.33 ± .06	7.31 ± .05	4.03 ± .10	4.03 ± .08

commencement of testing. Fish were fed on both sides of the apparatus during the orientation period, but were starved during the test period.

In both control and test conditions, the positions of the fish were recorded with a video camera (Hitachi HV-17AU) placed above the stream apparatus. Twelve 8 hr video tapes (Hitachi T-160) recorded the behaviour of the fish toward the contaminated water and the other test individuals throughout the 96 hr test period. During the darkness cycle of the photoperiod an illuminescent red light (Silvania, 15 W) and an ultra sensitive lens (Hitachi AV-125AI, 0.5 lux) was utilized.

The time fish spent in the separate chambers was determined by viewing the 8 hr video tapes on a video monitor (Hitachi VM-173) and counting the time period a given number of fish were in the contaminated chamber. Total amount of time fish spent in the treated water was calculated for each 24 hr period and analyzed by Chi square test (Zar 1984) to determine goodness of fit to a 50% - 50% distribution in each chamber for the 96 hr test period.

A second electrode (Radiometer, Copenhagen) was placed at the drainage end of the control chamber. The electrode was attached to a digital display board (Concordia University Science Technical Center, Montreal) from which pH

readings could be viewed on the video monitor to watch for any infiltration of treated water to the control chamber.

Although not statistically recorded, observations of social interactions, such as aggression, dominance and terrioriality, were kept during the viewing of the separate control and test assays for comparison. Analysis of water chemistry was by standard methods (APHA 1971). Water characteristics for control and treated water were recorded daily and are summarized in Table 4.

Three control experiments took place; in June (control #1), July (#2) and December 1985 (#3), while behavioural tests concerning pH 6.0, 5.5, 5.0 and 4.0 were undertaken in August and September 1985.

Table 4. Daily (N=4) water chemistry levels (± S.D.) of both chambers during behavioural bioassays, in mmoles/L (except where noted).

Bioassay	Chamber	Hardness as $CaCO_3$	CO_2 (umoles/L)	Ammonia	Sulphate
Control #1	Control	1.20 ± .01	101.3 ± 7.8	1.48 ± .25	0.60 ± .06
	Treated	1.20 ± .01	100.5 ± 6.6	1.55 ± .19	0.61 ± .04
Control #2	Control	1.17 ± .01	96.5 ± 4.5	1.43 ± .34	0.62 ± .07
	Treated	1.18 ± .02	97.3 ± 6.4	1.38 ± .33	0.63 ± .04
Control #3	Control	1.22 ± .03	99.4 ± 7.0	1.48 ± .44	0.68 ± .07
	Treated	1.23 ± .04	98.7 ± 7.2	1.47 ± .54	0.66 ± .06
pH 6.0	Control	1.21 ± .01	94.8 ± 6.2	1.55 ± .24	0.60 ± .07
	Treated	1.22 ± .02	87.5 ± 3.7	1.53 ± .22	0.55 ± .07
pH 5.5	Control	1.20 ± .02	92.5 ± 5.3	1.88 ± .17	0.59 ± .05
	Treated	1.23 ± .02	82.5 ± 2.6	1.53 ± .19	0.72 ± .05
pH 5.0	Control	1.18 ± .02	87.5 ± 7.5	1.88 ± .28	0.56 ± .05
	Treated	1.22 ± .03	75.8 ± 5.3	1.58 ± .17	0.73 ± .04
pH 4.0	Control	1.17 ± .02	87.5 ± 5.1	1.93 ± .38	0.59 ± .07
	Treated	1.24 ± .02	65.8 ± 4.3	1.48 ± .22	0.78 ± .08

Physiological Bioassays

Test organisms used were housed under the same conditions as those utilized for the behavioural assays. Test water was delivered on both sides, through separate flowmeters, with the flow rate unchanged (2 L/min). Drainage was re-routed to the mixing tank, equipped with a pH electrode, where fresh water and dilute (0.1 N) sulphuric acid was added. The treated water was aerated as before, with the pH of the water adjusted within an upper and lower control level ($\pm$ 0.2 the desired pH) by increasing or decreasing the drop rate of the sulphuric acid in the mixing tank.

Regular water samples were taken and are summarized in Table 5. A thermometer on both sides showed average temperature to be the same at 13.0 $\pm$ 1.0 C. Water characteristics for the treated water are shown in Table 6. After the desired pH was reached in the test apparatus, the test period (96 hr) began. Tests at pH 6.0, 5.5, 5.0 and 4.0 took place during October, November and December of 1985.

Table 5. Regular (N=22) pH levels (± S.D.) in separate chamber during physiological tests.

Bioassay	Control		Test	
	#1	#2	#1	#2
pH 6.0	6.03 ± .10	6.04 ± .10	6.06 ± .09	6.04 ± .09
pH 5.5	5.54 ± .08	5.53 ± .09	5.52 ± .10	5.51 ± .07
pH 5.0	4.96 ± .10	4.99 ± .08	5.01 ± .16	5.05 ± .13
pH 4.0	4.05 ± .11	4.06 ± .09	4.02 ± .12	4.01 ± .10

Table 6. Daily (N=4) water chemistry levels (± S.D.) of treated water during physiological bioassays, in mmoles/L (except where noted).

Bioassay	Hardness as $CaCO_3$	CO_2 (umoles/L)	Ammonia	Sulphate
pH 6.0	1.23 ± .02	94.8 ± 5.6	1.48 ± .10	0.69 ± .08
pH 5.5	1.23 ± .02	83.3 ± 3.1	1.33 ± .22	0.69 ± .05
pH 5.0	1.23 ± .03	80.0 ± 3.4	1.45 ± .13	0.74 ± .05
pH 4.0	1.26 ± .01	75.0 ± 2.9	1.33 ± .17	0.79 ± .07

Plasma Ion Determination

After the conclusion of all the controls, behavioural, and physiological, experiments the fish were anesthetized with MS-222 (tricaine methane sulphonate, Sigma chemicals Co.) at 0.1 g/L. Then the fish were killed by a blow to the head and bled immediately from the caudal region into a 1.0 ml sterile syringe (Becton Dickinson Co.) and then weighed. The mean fish weights (Table 7) for each test were found not to differ significantly by a one-way ANOVA ($F = 2.106$, $DF = 10,209$, $P < 0.05$) (Zar 1984). The blood was transferred into a 1.5 ml Eppendorf micropipet and put on ice for 15 min. The blood was then centrifuged at 11,500 rmp for 15 min with an Eppendorf 5413 centrifuge. Plasma was removed with 50 ul micropipets (Fisher) and transferred into either 10 or 25 ml glass volumetric flasks (Kimex, USA).

Plasma ion (Na^+, Ca^{2+}, K^+ and Mg^{2+}) concentrations were determined, using the appropriate standards, by flame atomic absorption spectrophotometry (Perkin-Elmer Model 503) according to Bhattacharya (1977). To test for differences between the variances in plasma cation levels of the

Table 7. Mean fish weights (± S.D.) of test organisms for each bioassay (N=20).

Bioassay		Fish Weight (g)
Control #1		53.3 ± 10.2
Control #2		50.4 ± 13.8
Control #3		56.3 ± 10.7
Behavioural pH	6.0	52.9 ± 12.4
	5.5	53.0 ± 11.6
	5.0	51.2 ± 10.7
	4.0	53.1 ± 12.3
Physiological	6.0	58.2 ± 9.2
	5.5	53.0 ± 11.1
	5.0	49.6 ± 12.8
	4.0	58.1 ± 10.5

behavioural and physiological assays a two-way F-test (Zar 1984) for comparing variance was performed. A one-way ANOVA (Zar 1984) was utilized to test for whether a significant difference occurred between the mean plasma levels in summer and winter.

RESULTS

Behavioural Bioassays

In all controls and with the tests at pH 6.0 and 5.5, there was not a significant departure from a 50%-50% distribution. In both summer control experiments the mean percentages of time the fish spent in the test chamber were 49.0 ± 0.4 (X^2 = 0.021, P > 0.75) and 48.7 ± 3.1 (X^2 = 0.066, P > 0.75). The final control test was done during the winter, after the completion of all testing, which found the fish spending 53.5 ± 4.5 percent of the time in the test chamber with a X^2 value of 0.493 (P > 0.25).

In the control experiments social behaviour influenced spatial arrangement on both sides of the apparatus. Social interactions such as aggression, terrioriality and dominance were observed. Normally during the first few hours after the fish were put in the apparatus a dominance structure would develop. Quick establishment of a dominance hierarchy has been commonly reported from behavioural studies concerning brook trout (Fremeth 1973; Gibson et al. 1981). Dominant fish would protect the end area of the separate channels where the water inflow

occurred, while other test individuals would spend their time swimming in between these two areas. When individuals approached a dominant fish, acts of aggression occurred. This usually meant attempts at fin biting followed by a chase with the winner returning to claim his area. Such aggressive actions lasted the length of the test period (96 hr) and have been reported by other authors (Gibson 1978; Chiszar et al. 1975).

The mean percentages of time that the trout were present in the contaminated chamber during the test period are presented in Fig. 3. No avoidance was found at pH 6.0 (47.9% $\pm$ 0.8, X^2 = 0.172, P > 0.50) and preference changed little over time (Fig. 4). At pH 5.5 some avoidance occurred early in the test (Fig. 4) but it could not be considered significant with the total percentage of time spent in the treated chamber at 44.3 $\pm$ 8.2 (X^2 = 1,252, P > 0.25).

Avoidance of the treated water at pH 5.0 was evident within the first 24 hr where the fish spent only 10.0 percent of their time in the contaminated chamber. However as time progressed, avoidance of the test chamber decreased (Fig. 4), with the test individuals spending 45.4 percent of their time in the treated channel by the last day. The actual avoidance percentage for the 96 hr period was 25.0 $\pm$

Figure 3. Black dots represent percentage of fish located in the contaminated water at the different pH levels ($\pm$ S.D.) over the entire 96 hr period, with the letter "a" representing significant difference from the control (50%) distribution ($P < 0.05$).

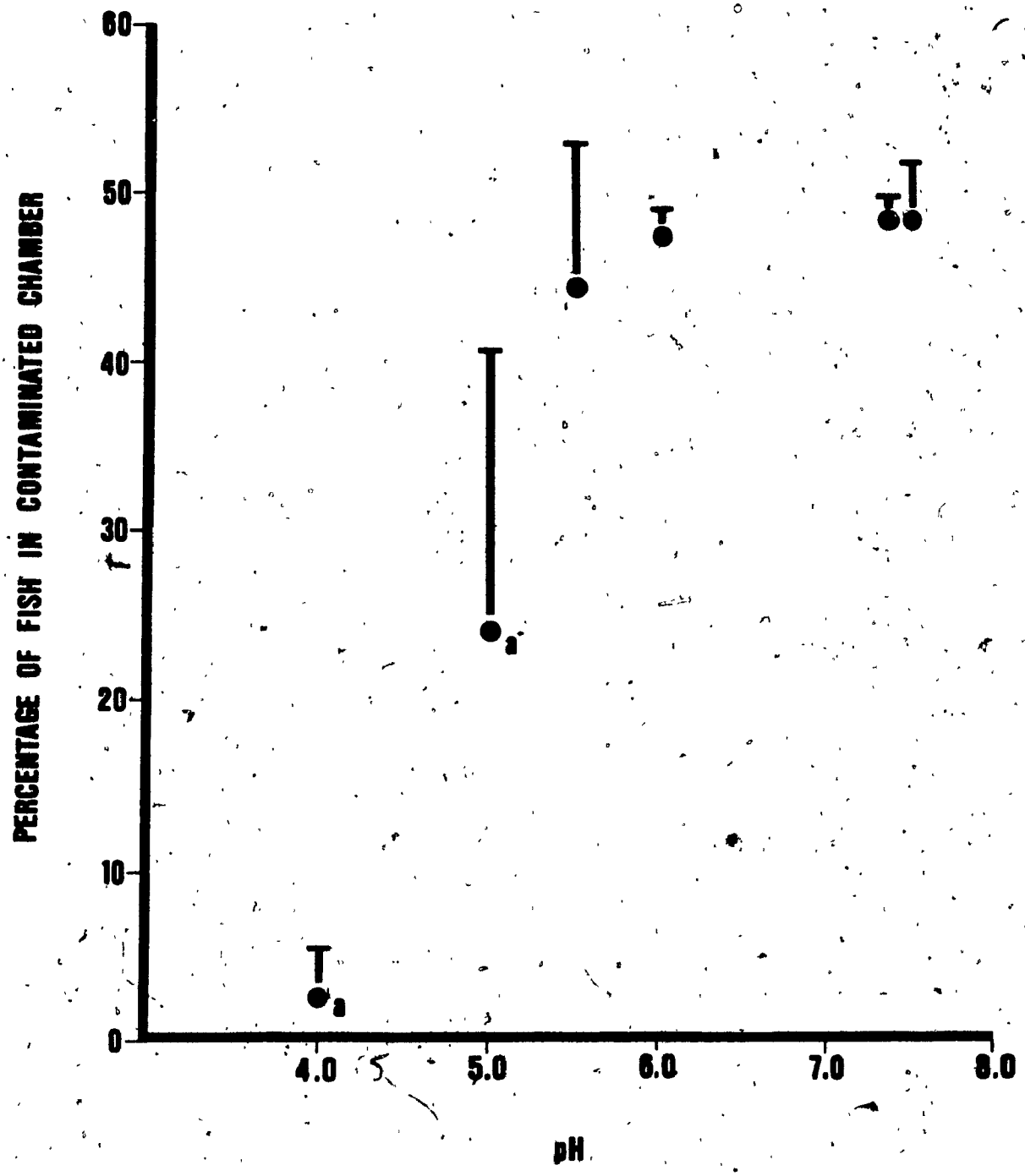

PERCENTAGE OF FISH IN CONTAMINATED CHAMBER
60
50
40
30
20
10
0
4.0
5.0
6.0
7.0
8.0
pH
a
a

Figure 3.

Figure 4. Daily percentage of fish located in the contaminated chamber over the 96 hr test period. The open boxes, squares and triangles represent the controls, with the closed symbols the separate pH levels (diamonds representing the test at pH 6.0, boxes - pH 5.5, circles - pH 5.0, and triangles - pH 4.0).

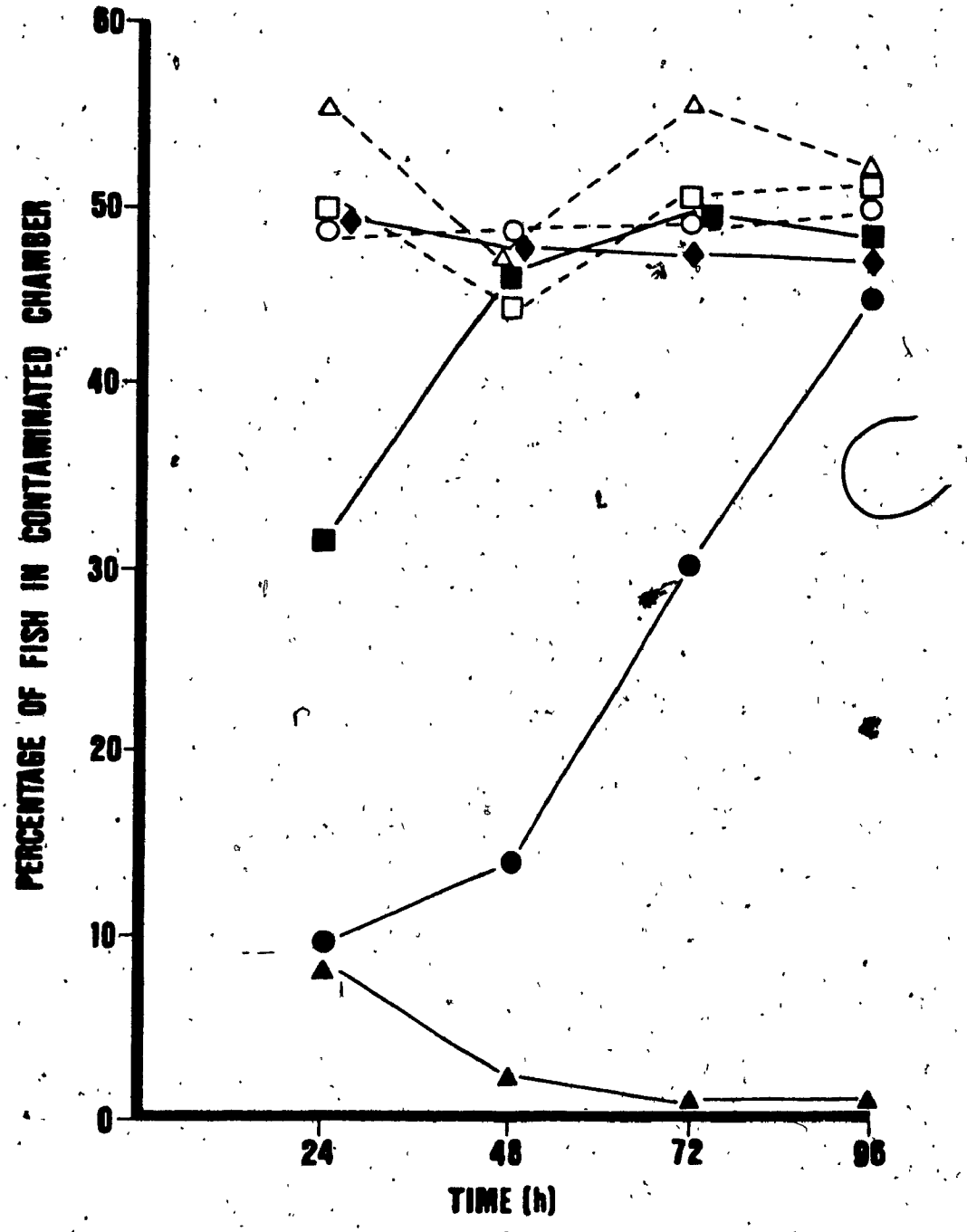

PERCENTAGE OF FISH IN CONTAMINATED CHAMBER
60
50
40
30
20
10
0
24
48
72
96
TIME (h)

Figure 4.

16.3 (X^2 = 24.080, P < .001), with the large standard deviation confirming the change in channel preference over time.

During the test with pH 4.0 test individuals were present in the treated channel for 8.5 percent during the first 24 hr, less than 1 percent by 48 hr, followed by almost complete avoidance. Total percentage test individuals spent in the contaminated chamber was 2.85 ± 3.8 (X^2 = 85.353, P < .001).

When early avoidance of the treated chamber occurred with the test at pH 5.5, crowding in the control chamber led to an increase in aggressive behaviour by some of the test individuals. This aggression resulted in some of the fish choosing to return to the contaminated chamber, creating an even dispersal throughout the apparatus. The highest pH level where the treated water changed spatial distribution was at the test at pH 5.0. During the first 48 hr of this test, dominant fish were not able to keep individual areas of the control channel to themselves. Subordinate fish would choose to subject themselves to aggressive behaviour rather than enter the pH 5.0 water. At the later stages of the test, preference for the control chamber decreased (Fig. 4) suggesting that certain fish later decided to choose an open, less desirable environment than crowded appropriate

water. With the test water at pH 4.0 all the test individuals congregated in the control channel by the end of the first day of testing. Fish only entered the contaminated area when physically harassed and chased by other individuals.

The taped recordings of the fish behaviour toward the contamination revealed changes between day and night periods. During the darkness cycle there was a marked reduction in movement but patterns of distribution and movement were similar in light and dark. The trout showed that they could acclimate to the test waters, as in the test at pH 5.0 where avoidance decreased with time, in both light and dark.

Plasma ion fluctuations

The concentrations of all ions (Na^+, K^+, Mg^{2+} and Ca^{2+}) in the three sets of control tests are listed in Table 8. The only significant difference ($P < 0.05$) seen between the seasons is the decrease in potassium levels during the winter. Under test conditions definite trends were seen with the varying pH levels. Sodium, potassium and magnesium

Table 8. Plasma ion levels (mmoles/L) of the three control tests (N=20).

	Control #1 (June 1985)	Control #2 (July 1985)	Control #3 (Dec. 1985)
Na^+	170.9 ± 7.10	169.9 ± 8.90	170.5 ± 16.7
K^+	3.59 ± 0.10	3.47 ± 0.15	3.18 ± 0.36
Mg^{2+}	1.34 ± 0.23	1.29 ± 0.15	1.29 ± 0.10
Ca^{2+}	2.11 ± 0.23	2.22 ± 0.17	2.17 ± 0.18

levels were found to decrease with increasing acid stress, whereas calcium ion levels increased. These trends were established by the plasma ion determinations of the fish which were subjected to acid conditions with no choice of waters (physiological assays).

The change in cation concentrations due to varying levels of acidity are presented in Figures 5 through 8. Sodium was found to be the most abundant ion during with Mg^{2+} having the lowest levels. In all cases control #1 represents control levels for the behavioural tests, with control #2 for the physiological tests.

Concerning the physiological assays, Na^+ (Fig. 5) showed the most obvious alteration over the different acid levels. Over a 96 hr period there was a reduction in plasma Na^+ of over 20% in pH 4.0 water, when compared to the control, with an average reduction of 37 mmoles/L from control levels.

With the behaviour tests the lowest levels of plasma Na was recorded with the test at pH 5.5, avoidance of the acidic water at the lower levels (pH 5.0 and 4.0) allowed the test individuals to maintain higher sodium levels. The higher Na^+ level at pH 4.0 represents the almost complete avoidance of the treated water with Na^+ ion concentrations similar to the control levels. The mean Na^+ ion level of

Figure 5. Mean plasma sodium concentrations (± S.D.) at the different pH levels with the open circles and dotted lines representing the behavioural bioassays and the closed circles and the black lines representing the physiological bioassays.

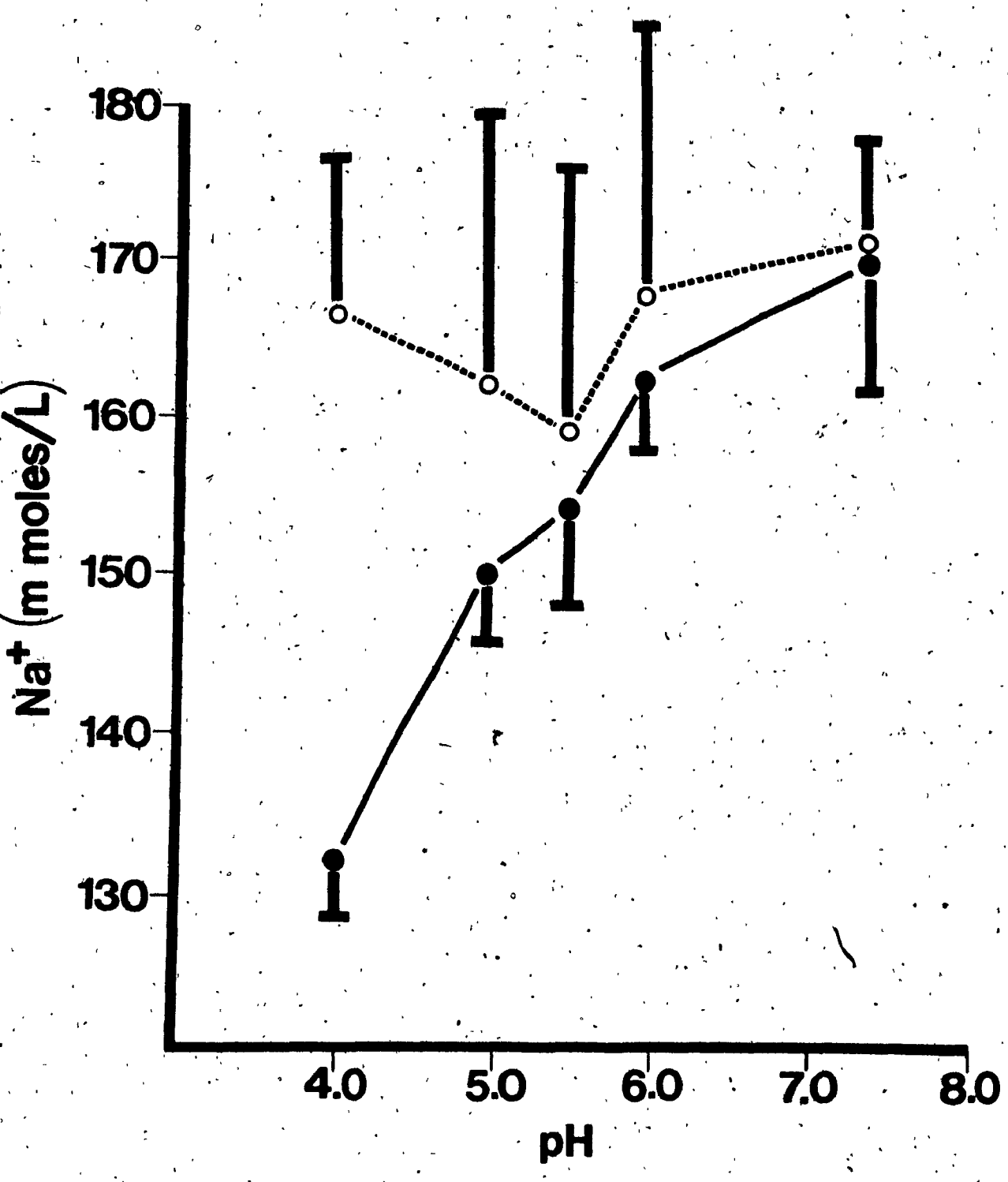


Figure 5.

the test species involved with the behavioural test at pH 6.0, suggest little change from the control levels. Whereas the large standard deviation of the ion levels indicates greater movement between the two chambers due to the varying preferences of the test individuals.

Potassium ion concentrations (physiological) of the test individuals (Fig. 6) subjected to acid conditions declined with declining pH. When the fish where given a choice of waters, as with sodium, little difference occurred between pH levels 7.4 (control), pH 6.0, and pH 4.0, whereas a significant decline was seen with pH 5.5 and pH 5.0.

Plasma Mg^{2+} concentrations (Fig. 7) showed only a small decrease over increasing acid stress with the physiological assays. Similar results were seen between the control levels and with the test at pH 6.0. The initial decrease in Mg^{2+} levels is seen at pH 5.5 followed in order, at pH 5.0 and pH 4.0. With the behavioural tests, the same trend continued that was seen with the before mentioned ions (Na^{+} and K^{+}). Little variation occurred between the control, pH 6.0 and pH 4.0. The major ion fluctuations occurred at pH levels of 5.5 and 5.0. Again the large standard deviation was evidence of different preferences within the test population. Nevertheless, average plasma magnesium concentrations of the trout given a choice of

Figure 6. Mean plasma potassium concentrations (± S.D.) at the different pH levels of behavioural and physiological bioassays. With the open circles and dotted lines representing the behavioural bioassays and the closed circles and the black lines representing the physiological bioassays.

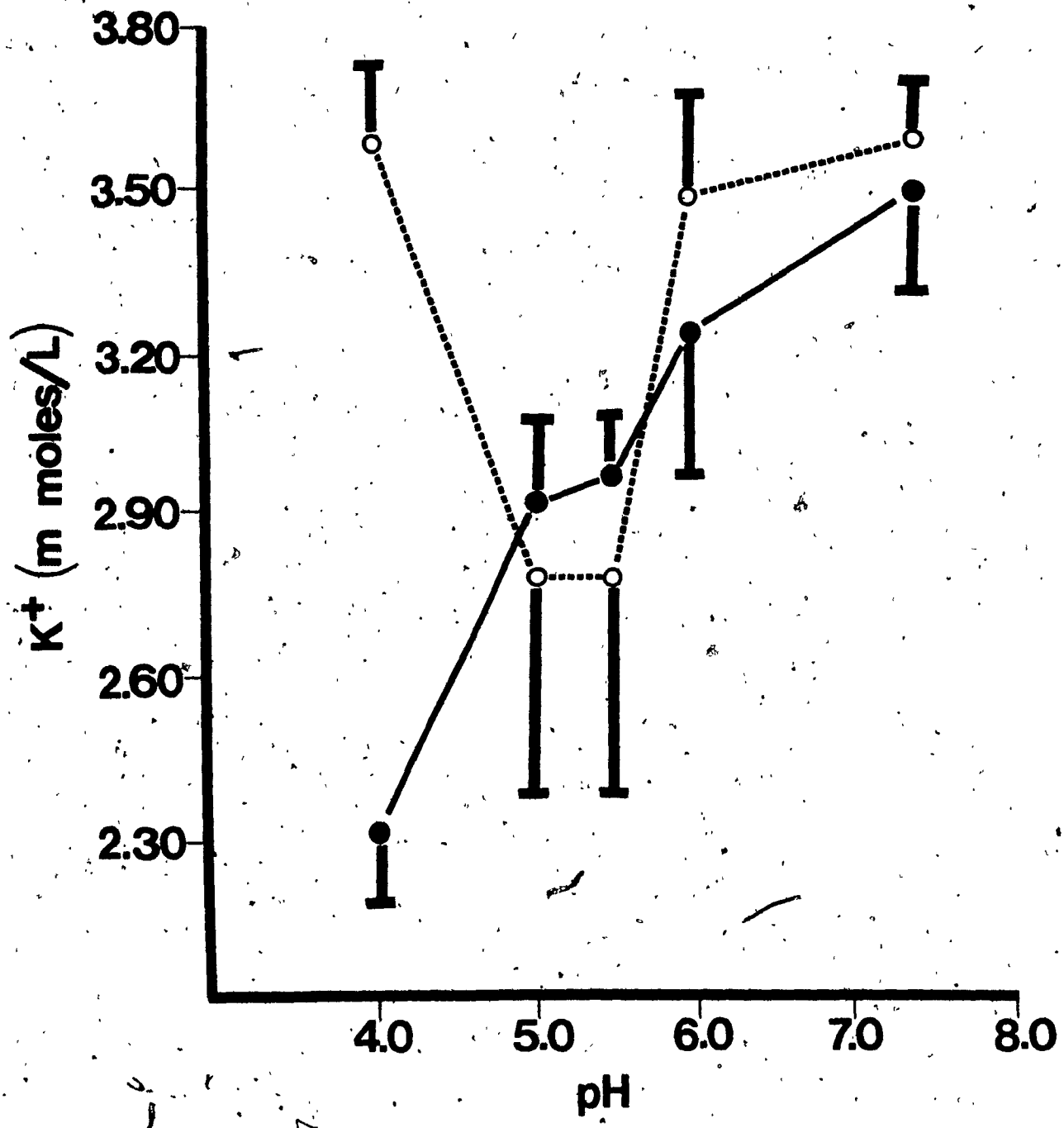


Figure 6.

Figure 7. Mean plasma magnesium concentrations (± S.D.) at the different pH levels of behavioural and physiological bioassays. With the open circles and dotted lines representing the behavioural bioassays and the closed circles and the black lines representing the physiological bioassays.

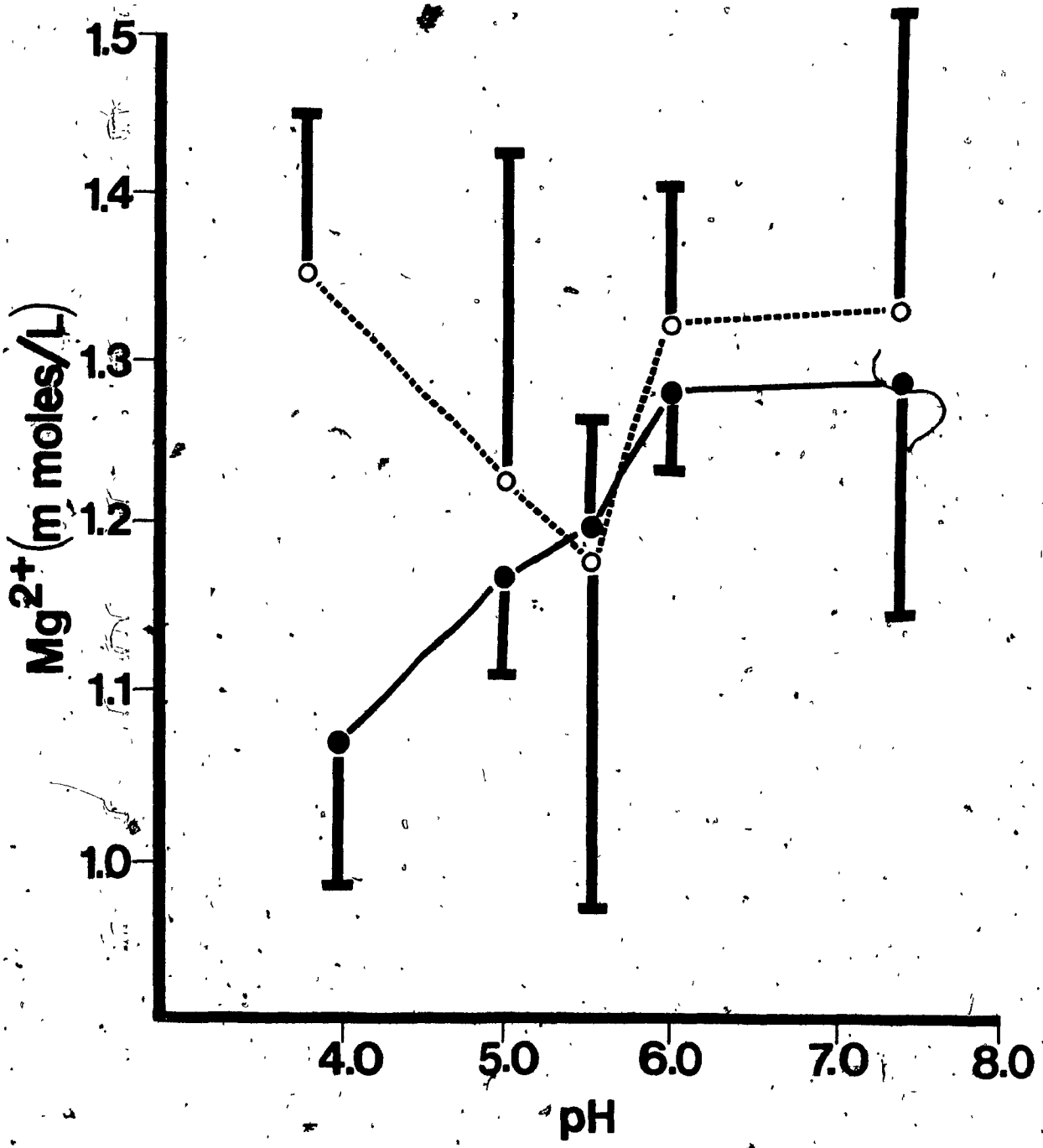


Figure 7.

waters at pH 5.5 were slightly lower than the fish allowed no preference.

Plasma calcium (Fig. 8) was the only ion tested to actually increase when subjected to increasing acid stress. Although little change occurred between control levels and pH 6.0, there were abrupt increases at pH 5.5 and pH 5.0 before levelling off at pH 4.0. The increase from control levels to pH 4.0 was the highest percent alteration of all ion fluctuations (over 25%).

With the behavioural tests the abrupt increase of calcium was again observed, from the control levels of to pH 6.0, up to a peak at pH 5.5. A slight drop occurred at pH 5.0 with avoidance at pH 4.0 bringing the calcium levels close to the control levels. Overall calcium had the largest ion level fluctuations, while having the smallest standard deviations.

Concerning the other ions it appears that the standard deviations were greater with the behaviour tests rather than the physiological test. Although this would seem reasonable, due to the presence of different water conditions in the separate chambers, this observation was only found to be significant with Na^+ values when tested by a two-way F-Test for variance.

Figure 8. Mean plasma calcium concentrations (± S.D.) at the different pH levels of behavioural and physiological bioassays. With the open circles and dotted lines representing the behavioural bioassays and the closed circles and the black lines representing the physiological bioassays.

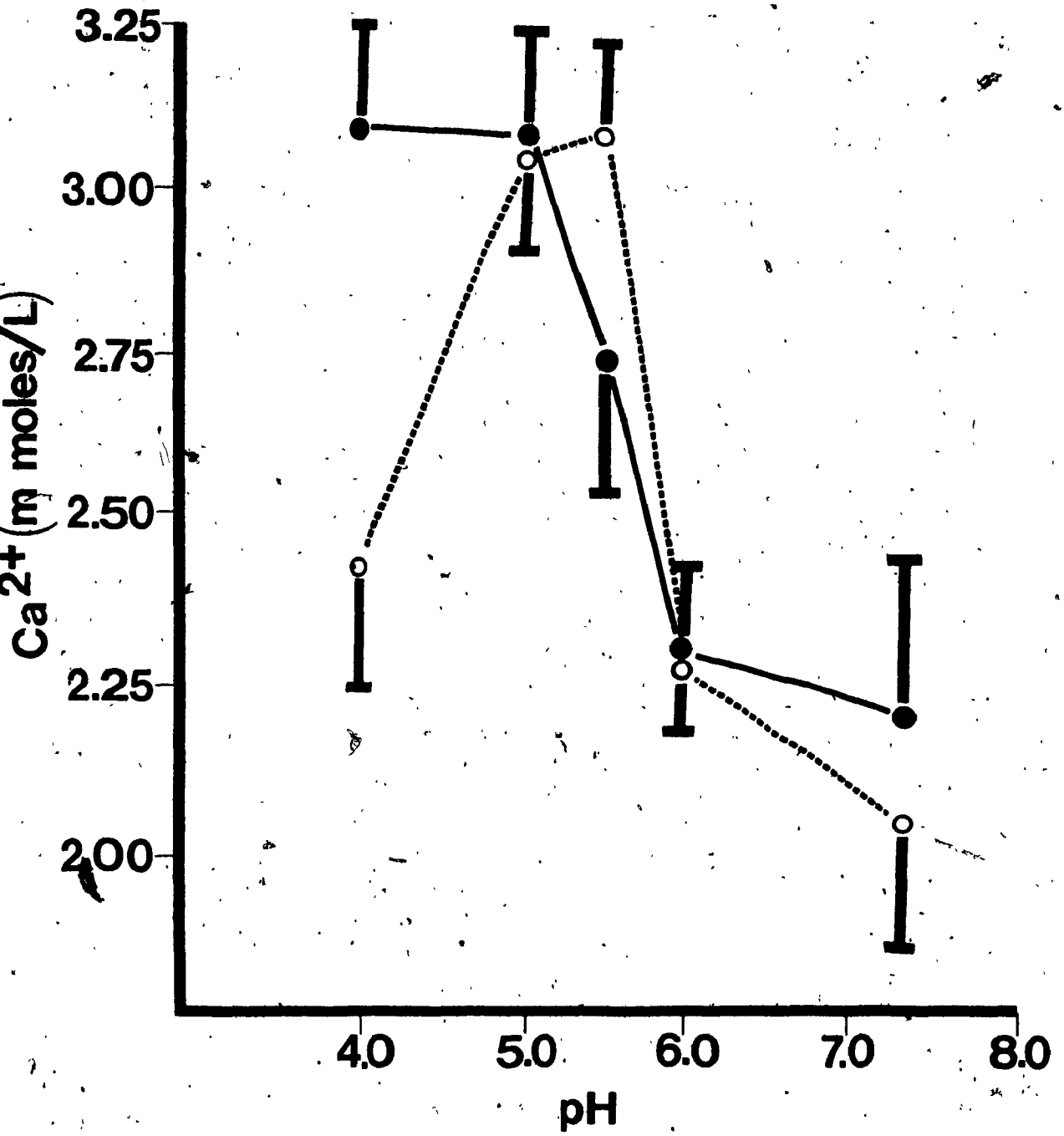


Figure 8.

DISCUSSION

Behaviour Studies

Behaviour, be it genetic, acquired or both, is an adaptive response to environmental variables of physical chemical or biological nature (Kleerekoper 1976). Modification of behaviour by toxicants may change the ability of the organism to deal with its environment. Changes in orientation and locomotion resulting from an environmental pertubation could cause alterations in an organisms ability to procure food, escape from predators, or reproduce (Kleerekoper 1976). Warner et al. (1966, in Johnson and Webster 1977) concluded that "quantitative behavioural change is the most sensitive indicator of toxicant induced change in living systems."

Attraction to the source of contamination will more likely bring on a physiological response which can be measured, such as death, in the short-term. However, observed short-term avoidance may decrease over time. Therefore organisms may return to a contaminated area causing detrimental physiological changes, which ultimately cause a reduction in a species population in an ecosystem.

My study investigated the behaviour of brook trout provoked by depressed pH contamination over 96 hr intervals. The behavioural response of fish toward this or any other contamination is crucial when attempting to assess and control the repercussions of a toxicant. The vast majority of behavioural tests (Jones 1948; Hoglund 1961; Ishio 1965; Kleerekoper et al. 1972; Westlake et al. 1974; Hara et al. 1976; Black and Birge 1980; Giattina et al. 1982; and Jones et al. 1985b) involve short-term (less than 3 hr) observations of single organisms subjected to sublethal toxic levels.

The artifical stream apparatus provided a sharp gradient between ordinary and modified waters, unlike that which one would usually encounter in nature. Ishio (1965) suggested that behaviour in toxic solutions depended on the gradient of the infiltration as well as concentration. Other authors (Kleerekoper et al. 1972; Westlake et al. 1974) have reported orientation differences with changing gradients, however, this has only been recognized with low levels of copper (less than 50 ug/L) and with certain species: goldfish (Carassius auratus) and channel catfish (Ictalurus punctatus).

However, Giattina et al. (1982) found no differences in avoidance threshold levels when exposing rainbow trout to

shallow and steep gradients of copper and nickel. Since Giattina and his co-workers (1982) showed that fish had no specific orientation behaviour toward shallow or steep gradients, I agree with their thought that, due to the ease of quantifying and analyzing the response, a steep gradient would seem more appropriate method for determining behavioral responses. Furthermore, the experimental design, including long-term monitoring, 12 hr photoperiod and usage of groups of test individuals, rather than single individuals, is more realistic than previous studies.

To date only limited research has determined behavioural affects of acid to fish. Hoglund (1961) reported that fish, upon contact with acidic water would back off, then move forward, repeating this process several times. Jones (1948) found that sticklebacks would avoid water more acidic than pH 5.6, Ishio (1965) reported that fish were able to detect and avoid high and low concentrations of hydrogen ions. Beamish et al. (1975) suggested that behaviour toward low pH may have negative effects on breeding fish. In the field, Johnson and Webster (1977) found that gravid females avoided breeding sites acidified to pH 4.0 and 4.5 however, discrimination was not evident at pH 5.0.

MacFarlane and Livingston (1983) studied the

behavioural response of Gulf killifish, Fundulus grandis, to continued exposure to sublethal levels of acid. They found that a threshold level of pH 5.5 was sufficient to disrupt the normal diurnal activity of the fish. Unfortunately the researchers did not reduce the increased CO_2 concentrations reported in the water during acidification, hence the cause of the disruption could not be pinpointed.

Investigators looking at the role of CO_2, (Hoglund 1961; Yoshii et al. 1980; and Jones et al. 1985b) have concluded that under acidic conditions at or above pH 5.5, fish avoid the treated water due to presence of higher levels of CO_2, which could be related to possible respiratory stress. My results show that the fish did not show avoidance behaviour above pH 5.5 in decarbonated water. Strong avoidance reactions at pH 5.0 and 4.0 (Fig. 3) suggest that fish can respond to gradients of high H^+ concentrations. My findings with brook trout support the findings of Jones et al. (1985b) who found that arctic char, Salvelinus alpinus, avoid water more acid than pH 5.5. Such results would point to a dual avoidance system (with CO_2 being the stimulus above pH 5.5 and H^+ at lower pH levels) as suggested by Jones et al. (1985b) thereby increasing the survival of fish populations in nature.

Avoidance thresholds did not differ between our study and similar tests with single individuals (Jones et al. 1985a; Jones et al. 1985b). However, observations of social behaviour demonstrate that dominance and territoriality can influence spatial arrangement in a contained area. Fish may be entering contaminated water to escape aggressive behaviour.

In control and with the higher pH tests, the dominance structure was quickly achieved and lasted the length of the test period. Quickly established and long lasting dominance within brook trout populations is a common occurrence (Fremeth 1973), however during the first day of the test at pH 5.5 and continually during the tests at pH 4.0 and 5.0 aggressive behaviour caused the dominance hierarchy to change often over the test period.

The change in preference over the test period (96 hr) seen with the test at pH 5.0 (Fig. 4) suggests that trout can recognize a pertubation but may acclimate to hazardous conditions over a long term study. Acclimation to contaminated water has not been examined in any detail during most behaviour tests. As mentioned by Cripe (1979), results based upon short-term monitoring do not take into consideration possible effects of long-term exposure such as acclimation and behaviour response to the toxicant in

darkness. Concerning the latter, we found no change in attitude toward the contamination during darkness, only reduced movement which was observed during all control testing.

Such results would suggest the importance of studying long-term behavioural response toward environmental contaminants which enter natural systems over a long period of time, such as depressed pH conditions during spring run-off. Species which spawn in the spring, such as rainbow trout, Salmo gairdneri and lake trout Salvelinus namaycush (Gunn and Keller 1984) may initially avoid acidic spawning streams between pH 5.0 and 5.5, only to spawn later in the same acidic conditions. Furthermore the preference-avoidance response to metals such as aluminum, known to be mobilized from watersheds under acidic conditions, should be quantified to assess the full impact of pH depression on fish populations.

Physiology studies

The gills of freshwater fish serve a variety of functions but first and foremost they are the respiratory organs (McDonald 1983). Since the gills are responsible for the exchange of respiratory gases, they are also the site for the diffusional loss of ions. Since freshwater fish are hyperosmotic, they extract sodium and chloride from the environment. To maintain electroneutrality sodium ions are exchanged for blood ammonium ions, while chloride ions are changed for blood bicarbonate ions (Evans 1975).

These essential ions (Na^+, Cl^- and to a lesser degree K^+) are transferred across the gill epithelium by (Na^+, K^+)-ATPase and Ca^{2+}-ATPase dependent ionic pumps (De Renzis and Bornancin 1984). They are also responsible for the extrusion of the metabolic by-products (HCO_3^-, H^+ and NH_4^+). Excretion of HCO_3^- allows for the loss of respiratory CO_2, while the excretion of H^+ and NH_4^+ provides for the loss of metabolic acid and nitrogenous waste respectively (Forster and Goldstein 1969). An increase of environmental H^+ concentrations causes an influx of H^+ across the gill, thereby elevating blood hydrogen ion

levels.

When McDonald and Wood (1981) continually exposed fish to low pH they found that the kidney could only excrete a portion (roughly a third) of the blood hydrogen ions when under continued acid exposure. McDonald (1983) reported that over 90 percent of the ammonia produced endogenously is excreted by the gills, while McDonald et al. (1983) found that branchial ammonia excretion during acid exposure was not affected by changes in the H^+ influx. Such results would suggest that ammonia excretion does not play a major role in H^+ excretion during acid stress.

The increase of H^+ influx at the gills results in substantial plasma acidosis (McDonald and Wood 1981). This in turn causes a continuing intracellular accumulation of H^+ and a corresponding loss of other cations (Ca^{2+}, K^+, Mg^{2+}, and Na^+) from the body fluid compartments (Spry et al. 1981). These changes cause a reduction in plasma volume, as indicated by decreased blood volume and increased hematocrit and plasma protein concentrations (Milligan and Wood 1982) thus increased blood viscosity and pressure occur. Furthermore, since fish respond to stress by releasing catecholamines into the circulation (Mazeaud et al. 1977), adrenergic effects such as vasoconstriction and cardioacceleration could augment the problem. These changes

would result in a greater cardiac effort, decreased tissue perfusion, and tissue hypoemia, which when severe enough could lead to death (Milligan and Wood 1982) as illustrated in Fig. 9.

Waterborne Ca^{2+} concentrations seem to be correlated to plasma ion fluctuations with the lower environmental Ca^{2+} associated with higher ionic effluxes (McWilliams 1982). Calcium is believed to bind to at least two sites involved in ion regulation: to the tight junction and to the Na^+ transport channels (McDonald 1983). This allows an increased access of Na^+ to the transport channels, causing a greater efflux of sodium, which has been shown by a number of studies with Ca^{2+} antagonists (Cuthberth and Maetz 1972; Eddy 1975; Greenwald and Kirschner 1976; Eddy and Bath 1979; and Benos 1982).

My findings indicate the ability of increased H^+ to cause disruptions in ionoregulation with moderate calcium levels ($CaCO_3$ = 1.2 mmoles/L). In hard water, acid-base disturbances are believed to accompany ion loss (Spry et al. 1981). Such an interaction should be expected since it is known that ionoregulation and acid-base regulation are to some degree coupled to electroneutral exchanges at the gills (McDonald et al. 1983).

The high loss of Na^+ reported here (Fig. 5),

Figure 9. Proposed sequence of death due to ionic imbalance (from Milligan and Wood 1982).

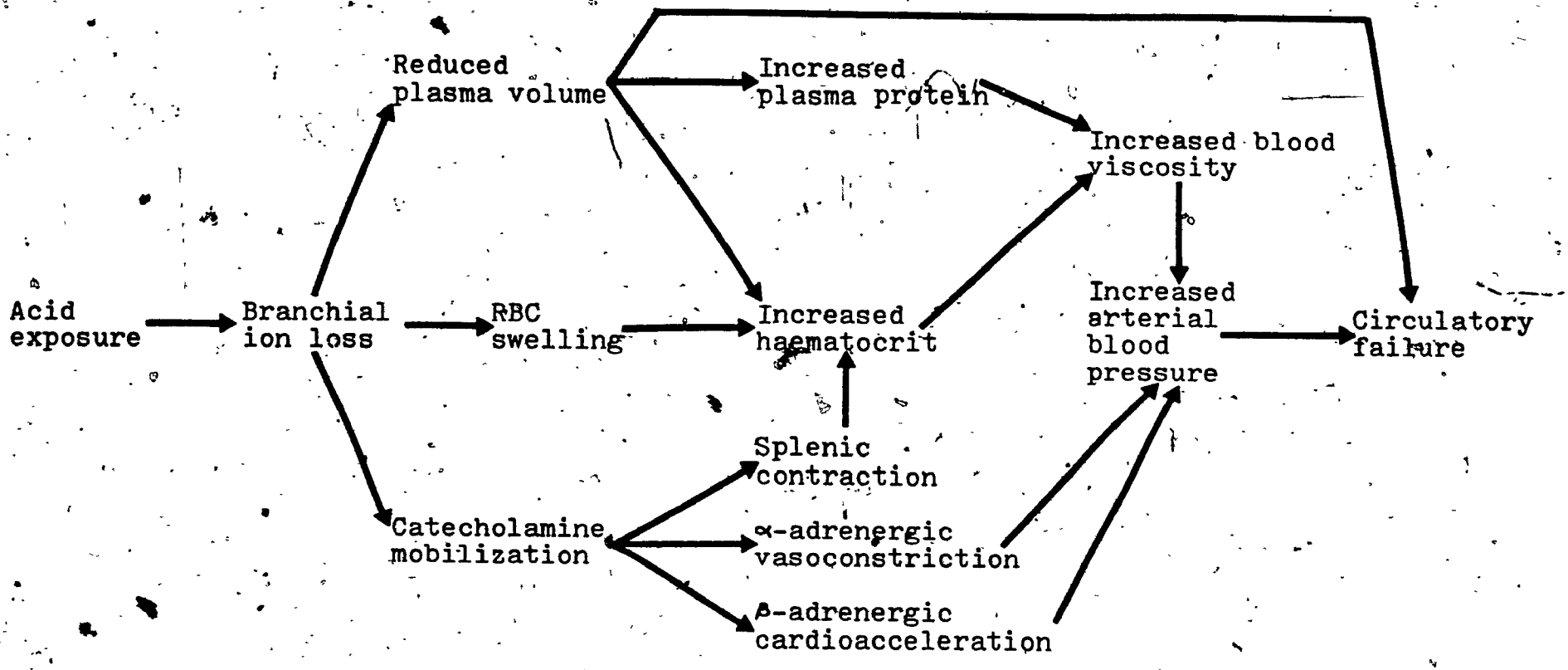

Reduced plasma volume
Increased plasma protein
Increased blood viscosity
Acid exposure
Branchial ion loss
RBC swelling
Increased haematocrit
Increased arterial blood pressure
Circulatory failure
Splenic contraction
Catecholamine mobilization
α-adrenergic vasoconstriction
β-adrenergic cardioacceleration

Figure 6.

correlates to a multitude of other studies (Packer and Dunson 1970; Maetz 1973; McWilliams and Potts 1978; McWilliams 1980b; and McDonald et al. 1983), all of which report major Na^+ reduction in plasma due to low pH conditions. The effect of low pH on Cl^- has not been as extensively studied, and was not examined in this study. Studies (McWilliams and Potts 1978; McWilliams 1980a; McWilliams 1982) have indicated that low pH has similar effects upon Na^+ and Cl^- efflux.

The similarity of these ion effluxes can be explained by the fact that any neutralization due to H^+ titration must be accompanied by either a reduction of cation concentration or an increase of alternative anion concentration for the maintenance of electroneutrality (Spry et al. 1981). Any slight difference between effluxes can be explained by the fact they work by different mechanisms, Na^+/H^+ or NH_4^+, Cl^-/HCO_3^- or OH^- (Evans 1975). The latest research comparing sodium and chloride reductions in plasma due to acid stress was completed by Giles et al. (1984) who reported identical alterations with both ions in rainbow trout, Salmo gairdneri (Fig. 10).

The other cations known to undergo alterations in plasma concentrations (K^+, Mg^{2+} and Ca^{2+}) have been reported to decrease concentration, increase concentration, or remain

Figure 10. Similar quantitiative sodium and chloride loss in rainbow trout exposed for 22 d at different pH levels and different times of the year. Open figures represent test period from Dec. to Jan., closed figures represent test period form Mar. to Apr. (from Giles et al. 1984).

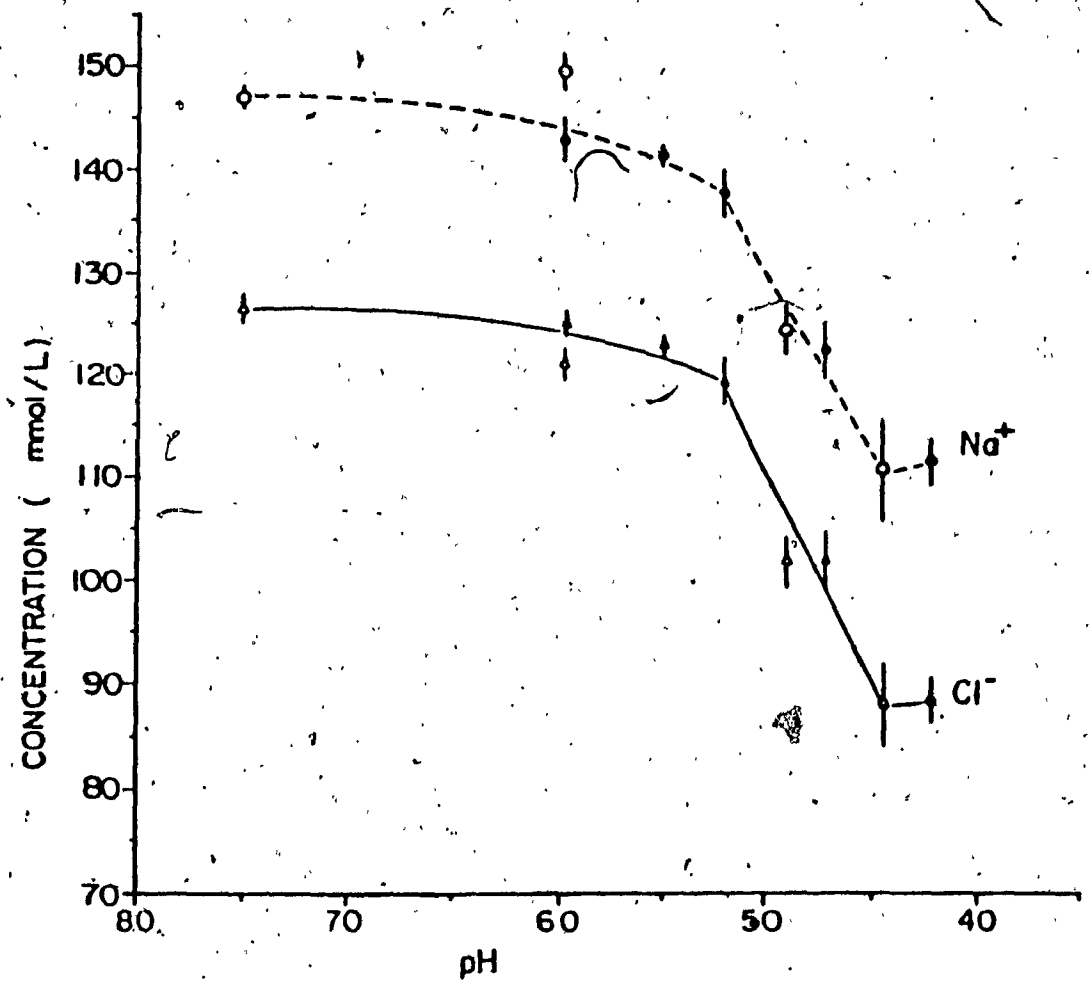

150
140
130
120
110
100
90
80
70
CONCENTRATION (mmol/L)
80
70
60
50
40
pH
Na+
Cl-

Figure 10.

unchanged. A comparison of ion fluctuations due to depressed pH is difficult, because the results of most lab and field studies vary according to water chemistry (i.e. $[Ca^{2+}]$), season, duration of exposure and species (Fraser and Harvey 1984).

In our study we found that potassium levels dropped steadily from approximately 3.50 mmoles/L in the summer controls to 2.30 mmoles/L at pH 4.0. Mudge and Neff (1971) exposed brook trout to pH 4.0 for 72 hours and found an increase in serum potassium for the first 12 hours, but then found that plasma K^+ levels showed a marked decrease over the rest of the test period. Dively et al. (1977) reported higher potassium levels of brook trout subjected to pH 4.2 water than control fish, however only in fish subjected to acid water for less than a day. Fish exposed for 5 days were found to have similar potassium levels to controls, with considerable variation reported due to seasonal differences.

Other studies have reported increases in K^+ concentrations in plasma, working with other species such as rainbow trout (McDonald and Wood 1981), white suckers (Fraser and Harvey 1984) and brown trout (Stuart and Morris 1984). On the other hand Barton et al. (1984) found an average drop in plasma K^+ from 4-5 mmoles/L to 1-2 mmoles/L

when subjecting juvenile rainbow trout for 5 days in waters with pH levels of 5.7, 5.2 and 4.7.

Stuart and Morris (1984) investigated seasonal effects on ionoregulation and found that potassium levels were significantly higher in summer than in winter. My own data showed similar findings with summer control levels of 3.59 and 3.47 with a winter level of 3.18 mmoles/L (Table 8).

The most complete study undertaken concerning ionic imbalance of brook trout due to depressed pH levels was undertaken by Smith (1977). He subjected trout to pH values of 4.0 to 6.0 at 0.5 unit increments in low calcium water (0.3 mmoles/L) for a nine month period. At pH 6.0 the average potassium level was 3.0 mmoles/L, while all lower levels showed a drop in plasma K^+ concentrations (pH 5.5, 2.1; 5.0, 2.3; 4.5, 2.2; and 4.0, 2.5). Although not consistent with previous studies with other species, this long term study supports the findings of my study, and points to a reduction of plasma potassium in brook trout when subjected to low pH conditions.

All previous studies involving magnesium (Smith 1977; Fraser and Harvey 1984; and Giles et al. 1984) have reported a decrease in magnesium levels with increasing acid stress. Fraser and Harvey (1984) reported a decrease of

white sucker plasma magnesium from 2.12 mmoles/L at pH 6.6 to 1.86 mmoles/L at pH 4.0 when exposed for 19 days. Giles et al. (1984) subjected rainbow trout for 22 days and reported an average decrease of 28% in plasma magnesium concentrations at pH 4.2 from control (pH 7.5) levels. Smith (1977) found a drop in magnesium levels of brook trout from 1.7 mmoles/L at pH 6.0 to 1.4 mmoles/L at pH 4.5. My results follow the same trend with magnesium concentrations decreasing from control levels of 1.3 to 1.07 mmoles/L at pH 4.0.

Plasma calcium levels, unlike magnesium have long been investigated in regard to acid stress. Beamish et al. (1972) suggested that the long-term failure of calcium regulation may be the primary factor for reproduction failure in acidic lakes. In this study a steady increase in calcium was found in the plasma of acid exposed brook trout, as was reported by Smith (1977). Findings with other species show no specific trend with decreasing pH levels. Fraser and Harvey (1984) found an increase in Ca^{2+} in white sucker plasma when subjected to acid conditions down to pH 4.0, while Giles et al. (1984) found a decrease in Ca^{2+} in the plasma of rainbow trout exposed for 22 days, as did McDonald et al. (1980) who exposed rainbows for 5 days.

Beamish (1972) had previously suggested that bone

minerals could be involved as buffering sources as well as intracellular fluid buffers. Titration of Ca^{2+} and possibly Mg^{2+} could alter the concentration of these ions in the plasma, having a separate effect from the H^+ influx on ionic composition of body fluid compartments. Since the test individuals in this research were juveniles, titration from developing bones should be considered. Unfortunately for all the work done in this area, conflicting data has prevented any conclusions regarding mineral titration and calcium regulation.

Electrolyte and behaviour alterations

The mechanism for avoidance of detrimental pH levels has not yet been recognized. Our findings and the work done by Jones and his co-workers (1985a;b) reject Hoglund's (1961) thought that high concentrations of H^+ will not elicit an avoidance response when not accompanied with an increase in CO_2. Avoidance of high levels of CO_2 is explained by respiratory stress. This stress, however, cannot explain avoidance of high H^+ concentrations in decarbonated water.

Hara (1975) studied the olfactory responses to different amino acids in rainbow trout at varying pH levels. Although the responses were highly pH dependent, responses were only inhibited below pH 3.0 and above pH 10.0. In fact, peak responses were reported between pH 5.0 and 6.0. Thus it would seem that avoidance of pH 4.0 to 5.5 is unrelated to olfactory response.

Jones et al. (1985b) noticed that the threshold levels for changes in behaviour and blood electrolyte concentrations were similar. Species differences in threshold levels for ionic imbalance (Lee et al. 1983; Brown et al. 1984) are not unlike the variance seen with threshold levels of species behaviour. The findings of this study support the suggestion that electrolyte imbalance is correlated with behavioural alteration provoked by acid stress.

Of all the bioassays undertaken in this study, the most relevance to this idea are the behavioural tests at pH 5.5 and 5.0 and the corresponding plasma ion levels of the test species. The major ionic fluctuations were seen with the behavioural tests at pH 5.5. In fact with all of the ions except sodium the average ion alteration in the behavioural test was similar to the physiological test at pH 5.5. Such changes in ionic content can only be explained by

the fact that only initial avoidance occurred (Fig. 4) and that the movement between the clean and treated chamber accelerated the ion imbalance.

Strong initial avoidance during the test at pH 5.0 diminished over the test period until the fish showed virtually no preference by the last 24 hr of the test. As the test period continued social interactions led to aggressive behaviour by the more dominant fish which caused test individuals to enter the test chamber. Since the test water, although not desirable, was not life threatening more of the test species remained in the test chamber to avoid the aggressive behaviour of the dominant fish in the control chamber. This resulted in ion levels similar to the test at pH 5.5. In spite of the fact the fish did not spend as much time in the test chamber as with the test at pH 5.5, the higher H^+ level caused a similar plasma ion imbalance.

The behavioural test at pH 4.0 indicated that once the acid stress reached chronic levels avoidance could not be reduced over time such as was observed in the tests at pH 5.0 and 5.5. The fish showed abrupt avoidance during the first 24 hr which decreased throughout the rest of the test. No amount of aggressive behaviour could force test individuals into the test chamber for any relative amount of time. Comparable plasma electrolyte levels to control

concentrations indicate the lack of preference for this level of acidity.

CONCLUSIONS

In this study it has been shown that brook trout behaviourally and physiologically respond to the presence of depressed pH water. Findings with the behavioural tests suggest a change in normal social behaviour at pH levels of 5.5 and lower. Trout were found to avoid water acidified to pH 5.0 and 4.0 over the 96 hr test period. Early avoidance at pH 5.5 and 5.0 decreased over the test period. Increased aggressive behaviour by dominant fish lead to an increase over time of fish in the test chamber. However aggressive behaviour in the control chamber could not persuade fish to enter test water acidified to pH 4.0. Significant avoidance of the test chamber was determined at pH 5.0 thus the avoidance threshold lies between pH 5.0 and 5.5.

Plasma ion determinations indicate that depressed pH causes a correlated ion alteration according to the degree of acidity. Cationic fluctuations in past research yield conflicting results except for Na^+ loss which is the major physiological concern. Decreased ion fluctuation with test individuals given a choice of untreated and treated (pH 4.0 and 5.0) waters indicates that increased avoidance leads to less physiological damage, while decreasing avoidance at pH 5.5 led to highest plasma ion imbalance.

Since the chronic threshold for fish survival is considered lower than pH 5.5 for the acid tolerant brook trout (Smith 1977), avoidance of acid water less than pH 5.5 would suggest the fish are capable of recognizing and avoiding aversive waters. Similar avoidance threshold limits with less tolerant freshwater species such as rainbow and lake trout would lead to chronic and acute physiological imbalances with ionic and acid-base regulation and should be investigated.

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APPENDIX I

Daily amount of time (min) spent in each chamber during behavioural bioassays.

Control #1

Hr	Control	Test
0-24	59,212.8	55,987.2
24-48	58,944.0	56,256.0
48-72	59,020.8	56,179.2
72-96	58,060.8	57,139.2

Control #2

Hr	Control	Test
0-24	57,926.0	57,274.0
24-48	64,453.4	50,746.6
48-72	57,186.5	58,013.5
72-96	56,877.0	58,523.0

Control #3

Hr	Control	Test
0-24	49,424.6	65,775.4
24-48	58,944.0	56,256.0
48-72	48,702.7	66,497.3
72-96	57,100.8	58,099.2

pH 6.0

Hr	Control	Test
0-24	58,949.6	56,250.4
24-48	59,689.0	55,511.0
48-72	60,482.9	54,717.1
72-96	61,033.3	54,166.7

pH 5.5

Hr	Control	Test
0-24	78,438.5	36,761.5
24-48	60,779.2	54,420.8
48-72	58,325.5	56,874.5
72-96	59,204.6	55,995.4

pH 5.0

Hr	Control	Test
0-24	103,659.3	11,540.7
24-48	99,015.9	16,184.1
48-72	80,210.1	34,989.9
72-96	62,775.9	52,424.1

pH 4.0

Hr	Control	Test
0-24	105,421.3	9,778.7
24-48	113,230.0	1,970.0
48-72	114,516.5	683.5
72-96	114,500.4	699.6

APPENDIX II

Brook trout ion concentrations (mmoles/L) after the behavioural and physiological bioassays.

Na^+

	Behavioural	Physiological
Control	170.9 ± 7.1	169.9 ± 8.9
pH 6.0	167.8 ± 17.1	163.3 ± 4.6
pH 5.5	159.8 ± 15.7	154.3 ± 6.4
pH 5.0	162.3 ± 17.2	150.5 ± 4.6
pH 4.0	167.0 ± 10.4	132.3 ± 5.8

Mg^{2+}

Control	1.34 ± 0.23	1.29 ± 0.04
pH 6.0	1.33 ± 0.06	1.29 ± 0.04
pH 5.5	1.17 ± 0.22	1.19 ± 0.05
pH 5.0	1.22 ± 0.20	1.16 ± 0.05
pH 4.0	1.35 ± 0.10	1.07 ± 0.08

Ca^{2+}

	Behavioural	Physiological
Control	2.11 ± 0.23	2.22 ± 0.17
pH 6.0	2.28 ± 0.18	2.31 ± 0.08
pH 5.5	3.08 ± 0.13	2.74 ± 0.18
pH 5.0	3.05 ± 0.13	3.08 ± 0.14
pH 4.0	2.40 ± 0.14	3.09 ± 0.15

K^{+}

Controls	3.59 ± 0.10	3.47 ± 0.15
pH 6.0	3.47 ± 0.19	3.23 ± 0.23
pH 5.5	2.73 ± 0.39	2.92 ± 0.15
pH 5.0	2.73 ± 0.38	2.90 ± 0.16
pH 4.0	3.59 ± 0.11	2.31 ± 0.11