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EFFECTS OF ACID EXPOSURE ON THE ION REGULATION AND SEAWATER ADAPTATION OF JUVENILE COHO SALMON (ONCORHYNCHUS KISUTCH).

by

James Frederick Francis Powell
B.Sc., Simon Fraser University

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The effects of acid-exposure on ion regulation and seawater adaptation in juvenile coho salmon (Oncorhynchus kisutch).

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James F. Powell

(name)

Sept 5, 1984

(date)

DEDICATION

I would like to dedicate this thesis to Mr. and Mrs. H.A. Simons, and to my parents, Mr. and Mrs. F.G. Powell. Without their help and support, this tesis may not have been possible.

ACKNOWLEDGEMENTS

I would like to thank Drs. B.A. McKeown and T.A. Watson for their patience and assistance in the preparation of this manuscript. I would also like to thank the laboratory technicians for their help: J.A. Johansen , D. Mitchell, K.C. Brown, M. Wheeler, K. Timewell and M. Dunlop with a special thanks to all my freinds in the lab.

It sorrows me that studies of a toxicological nature must be performed, however necessary. Of all that Science seeks to learn, we know of only one planet that is so complex and intricate that it contains life. Paradise only happens once.

ABSTRACT

The effects of acid precipitation on anadromous salmonids has led to concern over lifestage related effects and subsequent seawater adaptation after acid exposure in soft waters. Coho salmon smolts (Oncorhynchus kisutch) were exposed to a) a regimen of acidified water (pH 4.5, 5.1, 5.5) and control water (pH 6.2) for one week b) two pH regimens (pH 4.4 and 6.2) for one, two or three weeks, and subsequently subjected to a seawater challenge test after each week. Parrs were subjected to one week of acid exposure (pH 4.4 and 6.2) followed by a seawater challenge test.

Plasma [Na^+], gill Na^+-K^+-ATPase activity, hematocrit and plasma growth hormone concentrations were performed to determine the effects of acid exposure. Mortalities of acid-exposed and control fish were recorded.

Smolts exhibited decreases in plasma [Na^+] 7 days and Na^+-K^+-ATPase activity 14 days after onset of acid exposure. Parrs exhibited decreased plasma [Na^+] 24 h after onset of acid exposure and decreased Na^+-K^+-ATPase activity 3 days after onset of acid exposure. Plasma [Na^+] increased and Na^+-K^+-ATPase activity decreased in smolts after transfer to seawater. Parrs exhibited increased plasma [Na^+] and Na^+-K^+-ATPase activity immediately after transfer to seawater.

Plasma growth hormone was elevated in acid-exposed smolts but decreased shortly after exposure to seawater. Plasma growth hormone levels increased, however, above freshwater levels by 48

h of seawater exposure.

There were no observed increments of acid-induced effects among fish exposed to pH 4.5 to 5.5. Exposure to water of a low pH was found to have a greater effect on parrs compared with smolts in that plasma [Na^+] and Na^+-K^+-ATPase activity both decreased.

After 21 days of acid exposure, a size selective mortality developed which favoured the survival of larger smolts exposed to acid and salt water. Mortality was observed to be related to length of acid exposure. Transfer to 30 parts per thousand seawater after acid exposure was found to increase mortality.

It was concluded that acid exposure prior to entry into seawater was detrimental to coho salmon with regard to length of acid exposure, size and lifestage. The possible mechanism by which fish may be removed from populations is inhibition of gill Na^+-K^+-ATPase concomitant with decreases in plasma [Na^+].

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I. Introduction

Acid precipitation has been deemed responsible for the depletion of aquatic life in Scandinavia (particularly Norway) as well as North America (La Bastille 1981). Prevailing winds from industrial sources transport airborne particles and ions, particularly SO4 and NOx which are precursors to the formation of acid. They are subsequently deposited on soils and in waters that contain flora and fauna which may not tolerate a reduction in pH (Reed 1976, Haines 1981). The accumulation of precipitated acid in snow can lead to periods of relatively high acidic runoff from snow melt and result in episodic exposures of resident aquatic organisms (Johannessen and Hendriksen 1978).

Recently there has been controversy surrounding the effects of acid precipitation upon the watersheds of North America, such as witnessed in Scandinavia. Of particular interest is the potential impact of acid precipitation on soft water lakes and rivers of Ontario (La Bastille 1981). Studies have been undertaken by universities and government agencies to investigate the causes and effects of acidification in North American waterways.

According to such research, acidification of lakes and rivers of the Canadian Shield and Adirondack Mountains is caused by the absence of carbonate buffers, from limestone or sediments in glacial till, resulting in the low buffering capacity of

these waters. Hence, there is an increase in the susceptibility of these lakes to acidification via acid precipitation (La Bastille 1981). Effects have been found to include diminished primary (phytoplankton) and benthic invertebrate (zooplankton) production. Other effects of acidification of lakes and streams in Ontario include the reductions of fish population size and species diversity, and alterations of growth rates (Hendry et al. 1980, Harvey 1982). Harvey (1980) estimates that 200 Ontario lakes may have lost all resident fish populations as a result of acid precipitation.

Not only resident species of fish are affected by the acidification of lakes and rivers. Anadromous fishes, by nature of their biology, may also be affected as they return to freshwater for spawning. After hatching, juvenile fish remain in freshwater until natural cues induce downstream migration. Throughout their period of freshwater residency, anadromous salmonids are vulnerable to the effects of acidification of their environment. The period of freshwater residence of salmonid species varies from a few months in pink (Oncorhynchus gorbuscha) and chum salmon (O. keta), to one to three years in coho salmon (O. kisutch), chinook (O. tshawytscha), Atlantic salmon (Salmo salar) and steelhead trout (S. gairdneri).

Due to the economic, recreational and biological importance of the salmonids, various studies on lifestage and effect of acid exposure have been conducted. Some of these studies have included the effects of depressed pH on eggs and hatchability

(Carrick 1979, Daye and Garside 1979, Peterson et al. 1980), embryos and alevins (Daye 1980), fry (Carrick 1981) and fingerlings (Daye and Garside 1975). Recent work by Saunders et al. (1983) has sought to elucidate the effects of long-term exposure upon the growth and seawater adaptability of Atlantic salmon. To date, these studies represent the only investigations into the effects of depressed pH on the lifestage of an anadromous salmonid. Therefore, the ability of anadromous salmonids to adapt to seawater may be influenced by acid exposure during their freshwater residency period.

The recent introduction of Pacific salmon, particularly coho salmon to the Great Lakes (Donaldson and Joyner 1983) has placed this species in waters which are potentially sensitive to acid precipation (La Bastille 1981). The freshwater habitat of pacific salmon and in particular, coho salmon, along the west coast of British Columbia, Washington State and Oregon State is also sensitive to acid precipitation. This is due to the low buffering capacity of these water systems as they are underlain by a primarily graniferous substratum (La Bastille 1981). Having a freshwater residency of one year (Scott and Crossman 1973), juvenile coho salmon could therefore be endangered by acid exposure. The effects of acid-exposure may be manifest through population reduction and/or reduced growth rate.

Growth of anadromous salmonids includes the development of euryhalinity and is associated with morphological and behavioral characteristics involved in the progression from a freshwater

life form (parr) to a seawater viable form (smolt) (Folmar and Dickoff 1980, Wedemeyer et al. 1980). The effects of depressed pH on juvenile salmon as they develop euryhalinity (ie: parr-smolt transformation) is not well understood. However, measurements of physiological parameters associated with the parr-smolt transformation in salmonids has been implicated to be sensitive to changes in ambient pH (Fromm 1980, MacDonald 1983, Saunders et al. 1983). Studies of the physiological responses of freshwater salmonids to acid stress, have included ion regulation, and have been paralleled by studies of ion regulatory ability in the parr-smolt transformation of juvenile salmonids (Packer and Dunson 1970, Boeuf et al. 1978).

Studies on the exposure of freshwater salmonids to low pH have demonstrated that depressed plasma sodium (Na^+) concentration, hematological changes and mortality occur (Packer and Dunson 1972, Milligan and Wood 1983). Physiological factors involved in the parr-smolt transformation include the development of ionoregulatory ability to regulate internal ion concentrations, particularly Na^+, in freshwater and seawater (Folmar and Dickoff 1980).

The impact of acid exposure could be investigated in coho salmon by measuring physiological parameters which are employed to monitor and assess the parr-smolt transformation. Such measurements could include quantification of Na^+-K^+-ATPase activity, plasma Na^+ levels, and hematocrit changes. Gill Na^+-K^+-ATPase has been used in previous investigations as an

index of smoltification and has been implicated to be sensitive to acid stress (Zaugg 1980, Saunders et al. 1983). Concomitant with developmental changes in gill Na^+-K^+-ATPase (Zaugg and Wagner 1973), there are also increases in plasma Na^+ levels (Wedemeyer et al. 1980) which may be depressed by acid exposure (Packer and Dunson 1972). Analysis of mortality in acid-stressed juvenile coho salmon may further elucidate possible causes of death resulting from acid stress.

Endocrine involvement in whole body growth in the parr/smolt transformation and in ionoregulatory development has also been implicated (Folmar and Dickoff 1981, Johnston and Saunders 1981). Therefore, the change in plasma levels of growth hormone which plays a certain role in growth and ionoregulatory development, may yield further information to better evaluate the impacts of acid stress on coho salmon parrs and smolts. The significance of acid exposure on seawater adaptability of juvenile coho salmon can be obtained by observations of the above parameters in a seawater challenge test.

Coho salmon parrs and smolts were subjected to acid stress in soft water prior to seawater entry in order to determine possible deleterious effects of acid exposure on juvenile coho salmon. To aid in this evaluation gill Na^+-K^+-ATPase, plasma Na^+ concentration, plasma growth hormone concentraion, hematocrit and mortality occurrence in acid-stressed fish were investigated in coho salmon reared in soft water. Experiments were undertaken with the purpose of predicting the impacts of acid exposure upon

juvenile coho salmon.

The objectives of this research in investigating the effects of acid exposure on juvenile coho salmon are summarized below:

a) to determine the effects of a range of pH regimens on the physiology of coho salmon smolts;

b) to evaluate life stage dependant variables of acid exposure prior to and during seawater exposure;

c) to determine time dependant effects of acid exposure on coho smolts prior to and during seawater exposure.

This study was undertaken to determine whether or not, acid exposure in soft water prior to entry into seawater has deleterious effects on the iono(osmo)regulatory ability in freshwater and seawater which may lead to physiological disturbances and an impairment of seawater adaptability.

II. Materials and Methods

Experimental fish

Yearling coho salmon (Oncorhynchus kisutch) were obtained from Capilano Hatchery, North Vancouver, B.C. on January 28, 1982 and February 18, 1983. Immediately after transport, the fish were transferred to two 800-L fiberglass tanks serviced by flow through dechlorinated city water (Seymour watershed). The water supply had a temperature of 9.0 ± 0.3C (mean ± standard error of the mean), in February rising to 10.8 ± 0.4C in June of both years and had a pH of 6.2 ± 0.02 . Fish were fed commercial fish pellets (Moore and Clarke) once per day to satiation and the uneaten portion siphoned from the tank.

Each tank was supplied with a submersible pump to provide water movement required for normal fish growth and development (W.C. Clarke pers. comm.). Currents within the tank also helped to distribute food and mix administered acid. Portions of the tanks were covered with black plastic to provide a shadowed refuge. Photoperiod was adjusted to follow the natural spring regimen for 50° latitude (Vancouver) and was employed for all holding periods and experiments.

Dilution Apparatus

The acid dilution apparatus employed a Manostat variable speed peristaltic pump to deliver acid of a known concentration (0.5N H_2SO_4) from a 2 L reservoir at a constant rate (1.44 ml/min.) to the tank's incoming water supply (Figure 1).

Water flow to all experimental tanks was regulated by an adjustable valve (Roto-Flow). Hence, the pH of the delivery water could be varied at three different points of the apparatus: the acid concentration in the reservoir, the pump delivery rate and the water flow of the incoming water.

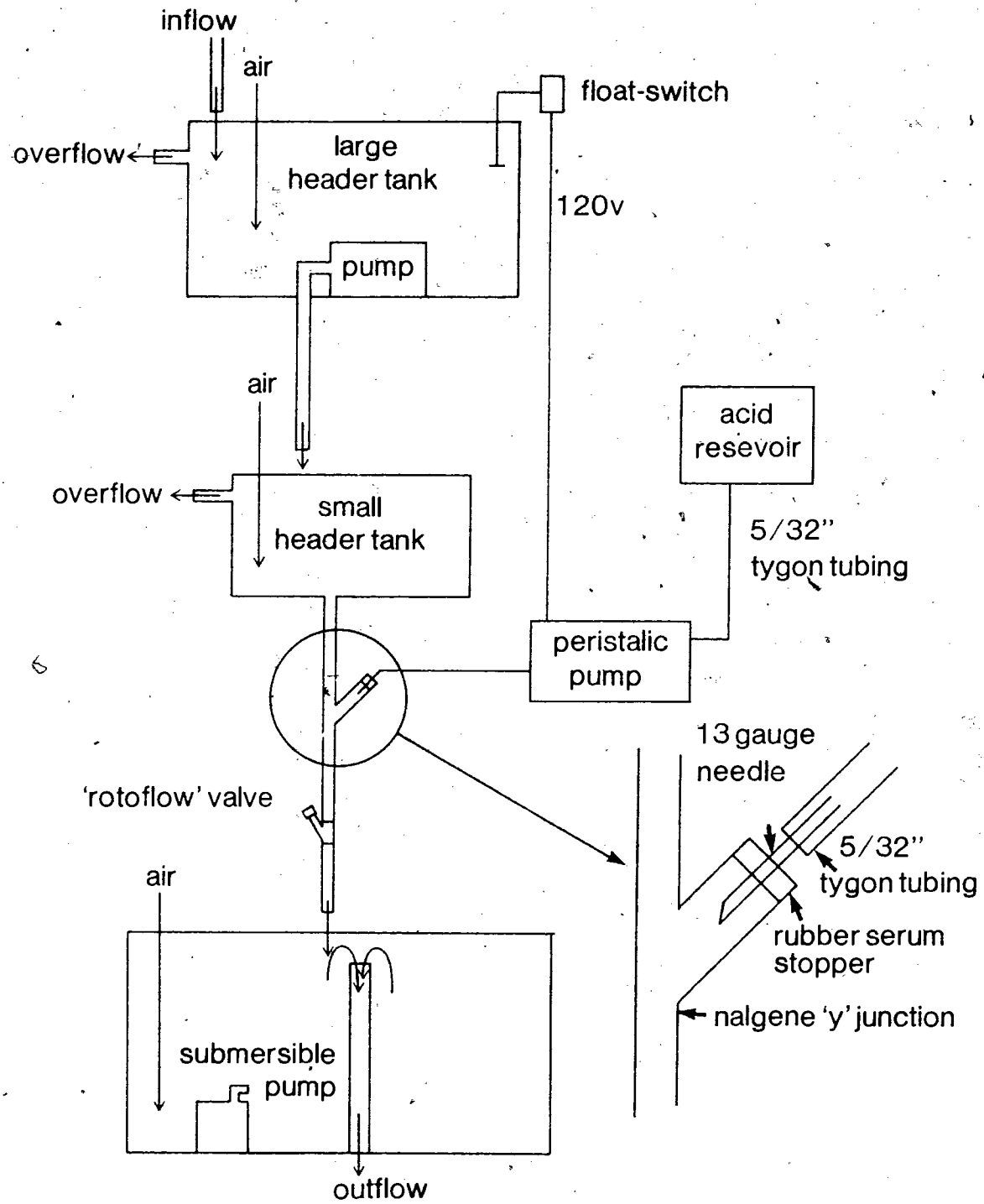
Constant water supply to experimental tanks was maintained by a two stage header tank system: a submersible pump from a large volume header tank supplied water at a constant rate to a smaller volume header tank. Both header tanks were supplied with flow rates that ensured a water delivery to the experimental tanks at a constant pressure, velocity and volume (Figure 1).

To prevent over acidification of the experimental tanks, the smaller header tank was equipped with a float switch that, when activated by reduced water levels, stopped acid delivery from the peristaltic pump.

All tanks, experimental and header, were equipped with at least one airstone to drive off excess CO_2 and saturate to at least 85% O_2 at 10C as measured by the modified Winkler method (American Public Health Association, APHA, 1980). Water flow

Figure 1:

Acid dilution apparatus employed in all experiments.


inflow
air
float-switch
overflow
large
header tank
120v
pump
air
acid
resevoir
overflow
small
header tank
5/32"
tygon tubing
peristalic
pump
13 gauge
needle
'rotoflow' valve
5/32"
tygon tubing
air
rubber serum
stopper
nalgene 'y' junction
submersible
pump
outflow

rates never fell below 1L/min in 120-L tanks and 2L/min in 800-1 tanks. For a biomass of 40g/L, such as used in all tanks, this water flow was deemed suitable for conditions of smolting in coho salmon (W.C. Clarke pers. comm.).

Water Quality

Measurement of water pH was performed three times daily in all experiments using a Corning Model 12 pH meter calibrated with three reference buffer solutions. Samples were temperature calibrated and stirred while determinations took place.

Water hardness as CaCO3 and Ca^{++} were below 5mg/L as determined by EDTA titration (APHA, 1980) and atomic absorption spectrophotometry respectively. Concentrations of sodium levels were determined by flame emmission spectrophotometry and was 24uEq/L .

Seawater was collected from Burrard Inlet, Vancouver, B.C. and had a pH range of 7.6 - 8.0 and salinity range of 29 - 30 parts per thousand as measured by an American Optical Refractometer.

Experiment I (1 Week Exposure, Smolts, 1982)

For this experiment, 200 coho salmon smolts (10.76 ± 0.3g, 10.9 ± 0.1cm), were randomly selected and equally distributed among four 120-L fiberglass tanks and acclimated for two weeks

prior to onset of the experiment. Tanks were equipped with flow-through dechlorinated city water at a rate of 1L/min. Fish were exposed for one week to water at pH 4.47 ± 0.07, 5.13 ± 0.07, 5.5 ± 0.06 and 6.21 ± 0.06 (control). Fish were sampled on days 0 (ie: no acid exposure) 1, 3 and 7. Details of fish sampling procedures are provided below. All sampling of experimental fish (n=5 per pH treatment) was performed at the same time of day to avoid circadian effects. Day 7 of the experiment was taken to be hour zero of a seawater challenge test which was performed on the remaining fish in each tank. The seawater challenge test, as described by Clarke and Blackburn (1977), is designed to assess the seawater viability and ionoregulatory ability of subject fish by measurement of plasma Na^+ levels after transfer to seawater from freshwater. Sampling procedues for the seawater challenge test were performed 7, 15, 25, and 48h after seawater exposure and followed the same regimens utilized for acid-exposed and control fish in freshwater. Hour zero of the test was 0900 on day 7 of acid exposure, therefore the sampling period at hour 15 was performed after sunset. Consequently, fish were removed from dark experimental tanks with the use of brief periods of illumination.

Sampling Procedures

Sampling procedures for all experiments included the weight and fork length measured, blood collection from a severed and swabbed caudal peduncle and the collection of gill tissue.

Unanaesthetized fish were held in a wet paper towel while blood samples were collected in an ammonium heparinized (2 USP units/tube) capillary tube held over the severed caudal artery. Hematocrit tubes were centrifuged for 3 minutes in a hemotocrit centrifuge (Clay Adams Autocrit II Model 0558) and hematocrits recorded. Plasma was decanted and stored at -20C. Plasma Na^+ levels were later determined by flame emission spectrophotometry (Pye Unicam Model SP191) on thawed and diluted (1:1000) samples. The spectrophotometer was calibrated over a linear range of 0.1 - 1.5 mEq/L with prepared standards.

Gill filaments were excised from gill bars and suspended in 1ml of homogenizing medium (300mM/L sucrose, 20mM/L Na2EDTA, and 100mM/L imidazole) adjusted to pH 7.1 with 1N HCl. Tubes containing suspended gills were quickly frozen by placement on dry ice and stored at -20C until assayed for Na^+-K^+-ATPase activity.

Dead fish were removed from all tanks without replacement. If mortalities occurred between observation periods (eg. pH measurement) of two to four hours, weights and fork lengths were still recorded.

Experiment II (1 Week Exposure, Parrs, 1983)

In order to elucidate the effects of acid exposure on lifestage dependent responses between parred and smolted coho salmon, two groups of 75 yearling parrs (11.43 ± 0.4g, 10.5 ± 0.2cm), were chosen at random and exposed to water at pH 4.37 ± 0.02 or pH 6.21 ± 0.06 (control) in two 800-L fiberglass tanks. The length of exposure, sampling procedures and sampling times corresponded to those of Experiment I with the exception of the sample size (n=8). Exposure to low pH was again followed by a seawater challenge test for all remaining fish.

Experiment III (Variable Time of Exposure, Smolts,1983)

This experiment investigated the possible effects of chronic acid exposure (21 days) in coho salmon smolts. Two groups of 200 smolts from the year-class as fish in Experiment II, (11.05 ± 0.2g, 11.05 ± 0.1cm), were maintained in two 800-L fiberglass tanks for a two week acclimation period. After acclimation, fish in one tank were acid stressed (pH 4.36 ± 0.07) and the other tank served as the control (pH 6.21 ± 0.02). Fish were maintained in the two tanks for up to three weeks. Sampling was performed on days 0, 1, 3, 7, 10, 14, 17 and 21. On days 7, 14 and 21, groups of 48 fish were removed at random from each experimental tank and transferred by net to one of two 800-L fiberglass tanks containing seawater for a seawater

challenge test. Sampling procedures for blood, tissue and physiological parameters were the same as outlined above, except that the sample size was n=8 fish per treatment (acid-stressed and control) for all sampling periods except control fish n=0 on day 17, n=5 for day 21, hour 7 and hour 48 of the final seawater challenge and n=0 for hours 15 and 25 of the final seawater challenge test.

Gill Na^+-K^+-ATPase Analysis

Gill tissue was analysed for Na^+-K^+-ATPase activity by the method of Zaugg (1982). This method was utilized rather than more refined enzyme extraction techniques due to the accomodation of an increased sample size not afforded by other methods. More rigorous methods of enzyme preparation, however, were shown not to be necessary to examine the relative effects of acid stress upon gill Na^+-K^+-ATPase in coho salmon.

Gill samples, frozen in homogenizing medium, were thawed on ice and homogenized at 4C in a glass homogenizer with a ground glass pestle powered by an electric drill for 7 - 8 strokes (approx. 1 stroke per second), until the filaments were disintegrated. The homogenate was transfered to a 12 X 75 mm borosilicate test tube. The homogenizer mortar was rinsed with 1 ml double distilled water, the rinse was then added to the test tube containing the homogenate.

The diluted homogenate was centrifuged for 7 minutes at 2 - 5C at 2,000g in a I.E.C. Centrifuge (Model PR-6). The supernatant was discarded from each tube and the tubes inverted to drain. The pellet in each tube was resuspended in 1 ml of homogenizing medium containing 0.1% (w/v) sodium deoxycholate (Sigma). This suspension was rehomogenized for 30 strokes (30 seconds) and centrifuged as before but for 6 minutes. The supernatant of this centrifugation was taken to contain a suspension of the microsomal fraction of the gill epithelial cell enzymes including Na^+-K^+-ATPase. This enzyme preparation was decanted and placed on ice.

A 100ul aliquot was pipetted into each of two 12 X 75 mm borosilicate test tubes one containing 0.7mL incubation medium, the other containing medium and the Na^+-K^+-ATPase specific inhibitor ouabain (0.42g/L, 1mM). Incubation medium contained 150mM NaCl, 25mM MgCl2-6H2O, 75mM KCl and 115mM imidazole brought to a final pH of 7.0 by the addition of 1N HCl. An aliquot of enzyme homogenate was saved for protein determination by the method of Albro (1975). Enzyme assays were conducted in duplicate. Na^+-K^+-ATPase activity was the difference in enzyme activity between medium containing no inhibitor and the medium containing inhibitor. Residual activity (ouabain insensitive) was taken to be Mg^{++}-ATPase. To each tube, and a blank containing no enzyme, 100ul of 30mM vanadium-free adenosine triphosphate (ATP) was added. ATP reagent was made by adding Na2ATP (Sigma) up to 10 ml with double distilled water including

100ul 5N NaOH to a final pH of 7.0. All tubes were shaken to mix the contents and placed in a 37C water bath (Fisher Versa-Bath) for 10 minutes and shaken for the first minute. The tubes were then immersed in ice water where they were shaken for one minute to stop the reaction. At this point, 250ul of 45% (w/v) trichloroacetic acid were added to each tube to precipitate larger proteins. All tubes were then centrifuged at 3,000g for 5 minutes to sediment precipitated debris. A 1 ml aliquot of the supernatant was used for inorganic phosphate determination by the method of Fiske and Subarrow (1925). Colourimetric determinations were performed at 650 nm using a Perkin-Elmer Coleman Model 55 spectrophotometer. Enzyme activity was expressed as the micromoles of inorganic phosphate liberated per milligram of protein per hour (uM Pi/mg protein/h).

Plasma Growth Hormone Determination

Plasma was taken from acid-exposed and control fish for both the first week and the first seawater challenge test of Experiment III for quantification of plasma growth hormone, according to the method described by Wagner (1984). Plasma was thawed on ice and 10ul were pipetted into a 12 X 75 mm borosilicate test tube. Duplicate or triplicate assays were performed on each fish sampled. To each tube, 100ul of 400 mM phosphate buffer (pH 7.0) were added along with coho salmon growth hormone-rabbit antibody and ^{125}I labled purified coho

salmon growth hormone. Ligands were incubated 24 hours at 5C at which time goat-anti-rabbit gamma globulin was added to remove excess unbound growth hormone-antibody fractions. Quantification of ^{125}I for bound growth hormone was performed on a Bechman Gamma 4,000 gamma counter. Units of quantification were expressed as nanograms growth hormone per ml of plasma (ng/ml).

Statistical Analyses

One way analysis of variance (ANOVA)[1] and the Student-Neuman-Keuls tests for comparisons of means were applied as appropriate to data from Experiment I. The Student t-test was used for data collected in Experiments II and III for comparisons between two treatments. Comparisons of critical values of correlation were tested for significance by values reported in Sellers (1977). Bartlett's test for homogeneity of variances was also applied. Significance was accepted at the 95 percent level ($P<0.05$), that is, "higher", "lower" and "different" designates significant differences ($P<0.05$).

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[1]ANOVA tables for Experiment I may be found in Appendix I

III. Results

Gill Na^+-K^+-ATPase Activity

There were no differences in smolt gill Na^+-K^+-ATPase in freshwater caused by acid exposure to low pH or during the seawater challenge test of Experiment I (Figure 2). Enzyme activities did not appear to increase upon transfer to seawater compared with the values obtained in freshwater. Gill enzyme activity values were multiplied by the plasma Na^+ concentration of each fish on an individual basis to illustrate the possible governing effects of Na^+ on enzyme activity. The result is a curve similar to that observed for enzyme activities (Figure 3) and a lack of significant difference.

Na^+-K^+-ATPase values for parrs in Experiment II (Figure 4) were generally one-half the magnitude of the previous experiment involving smolts. There were no differences in gill Na^+-K^+-ATPase activities in freshwater between acid-exposed fish and control fish, except day 3 of acid exposure where control levels were higher. Enzyme levels rose after seawater entry in both treatments as compared with freshwater levels. By hour 48 of the seawater challenge test, gill Na^+-K^+-ATPase activities were similar to freshwater levels.

Figure 2: Gill Na^+-K^+-ATPase activities (um Pi/mg protein/h) for coho salmon smolts undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.

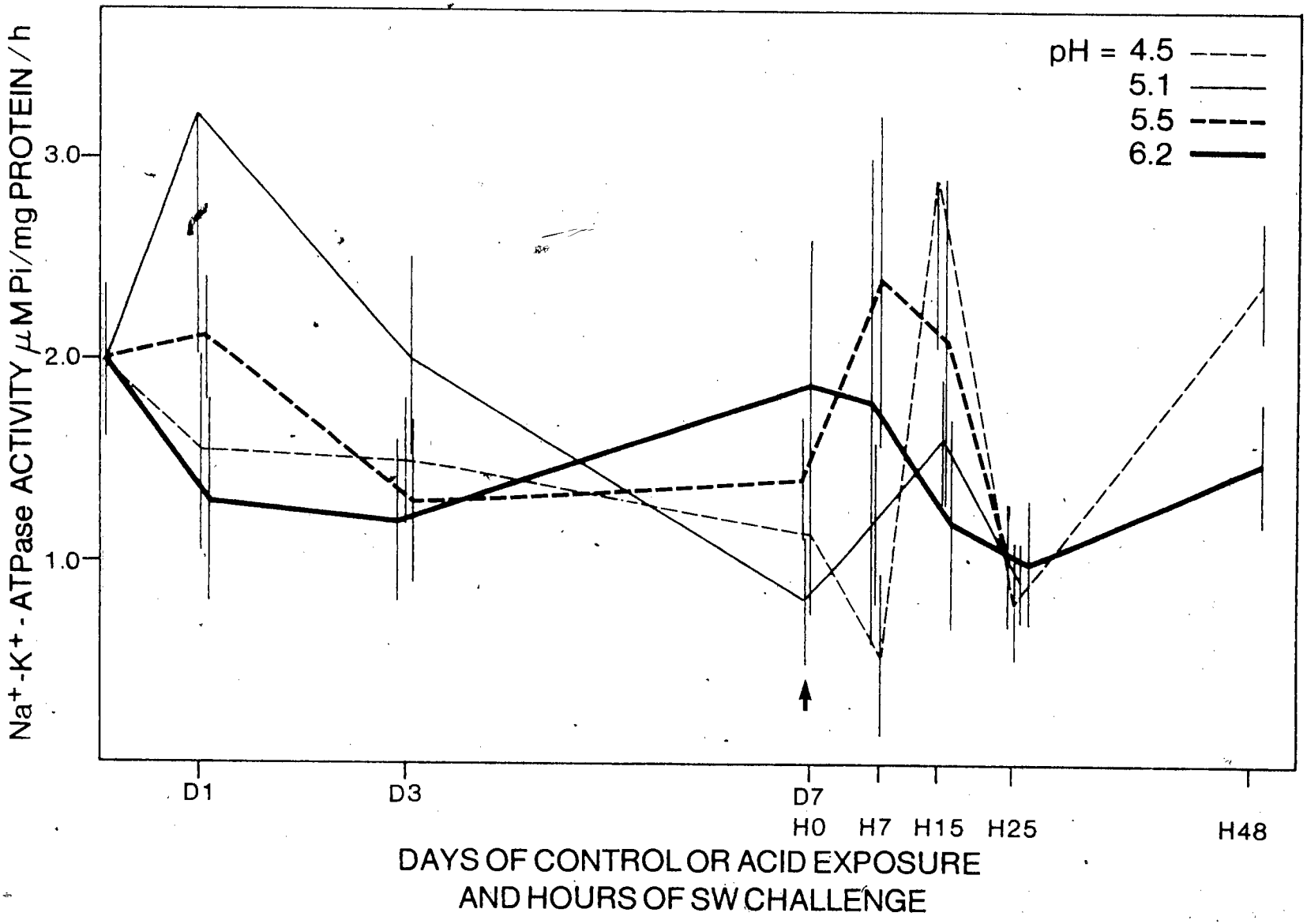

pH = 4.5
5.1
5.5
6.2
Na^+-K^+-ATPase ACTIVITY μM Pi/mg PROTEIN / h
3.0
2.0
1.0
D1
D3
D7
H0
H7
H15
H25
H48
DAYS OF CONTROL OR ACID EXPOSURE
AND HOURS OF SW CHALLENGE

Figure 3: Gill Na^+-K^+-ATPase activities normalized for plasma [Na^+] in coho salmon smolts undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.

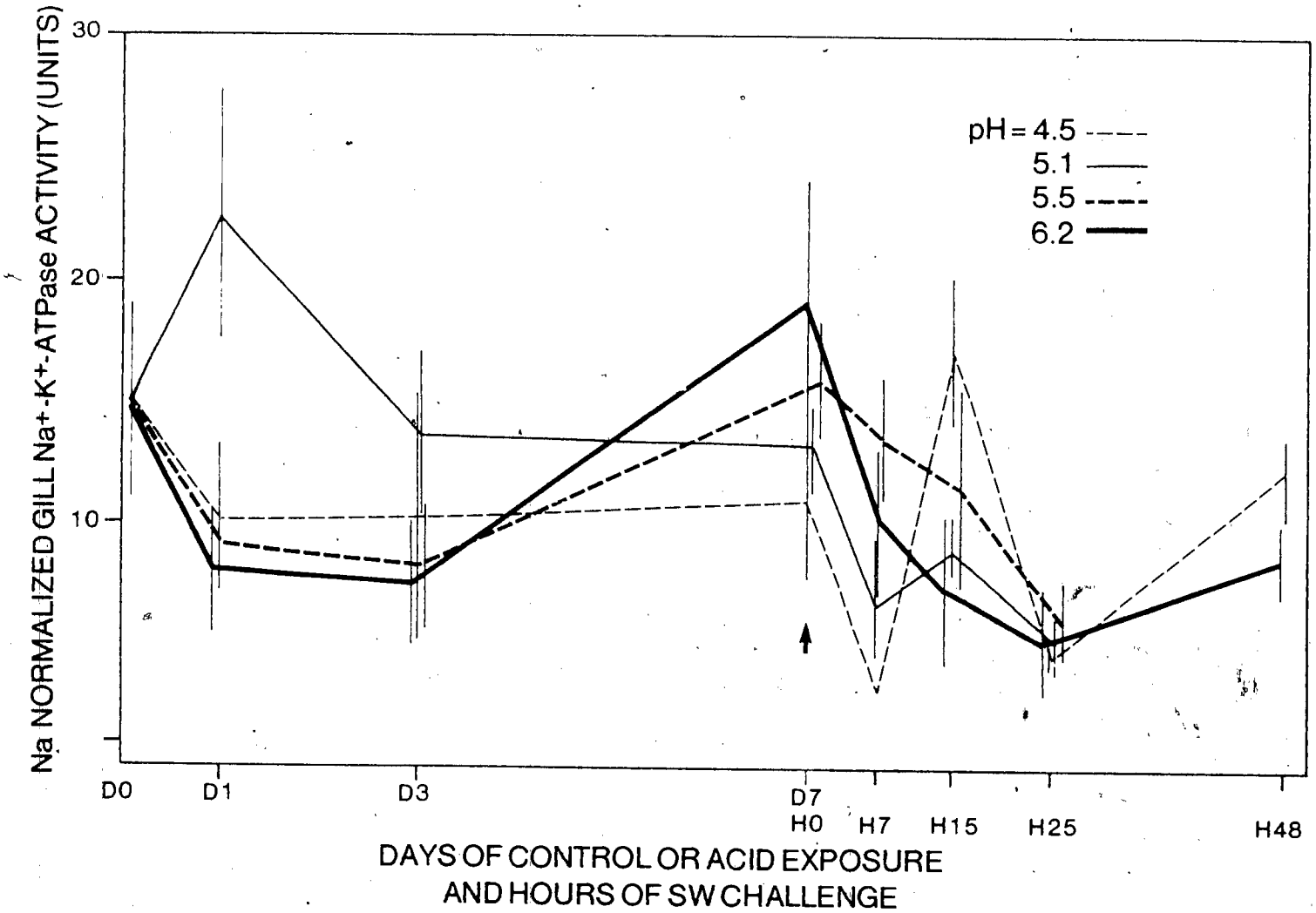
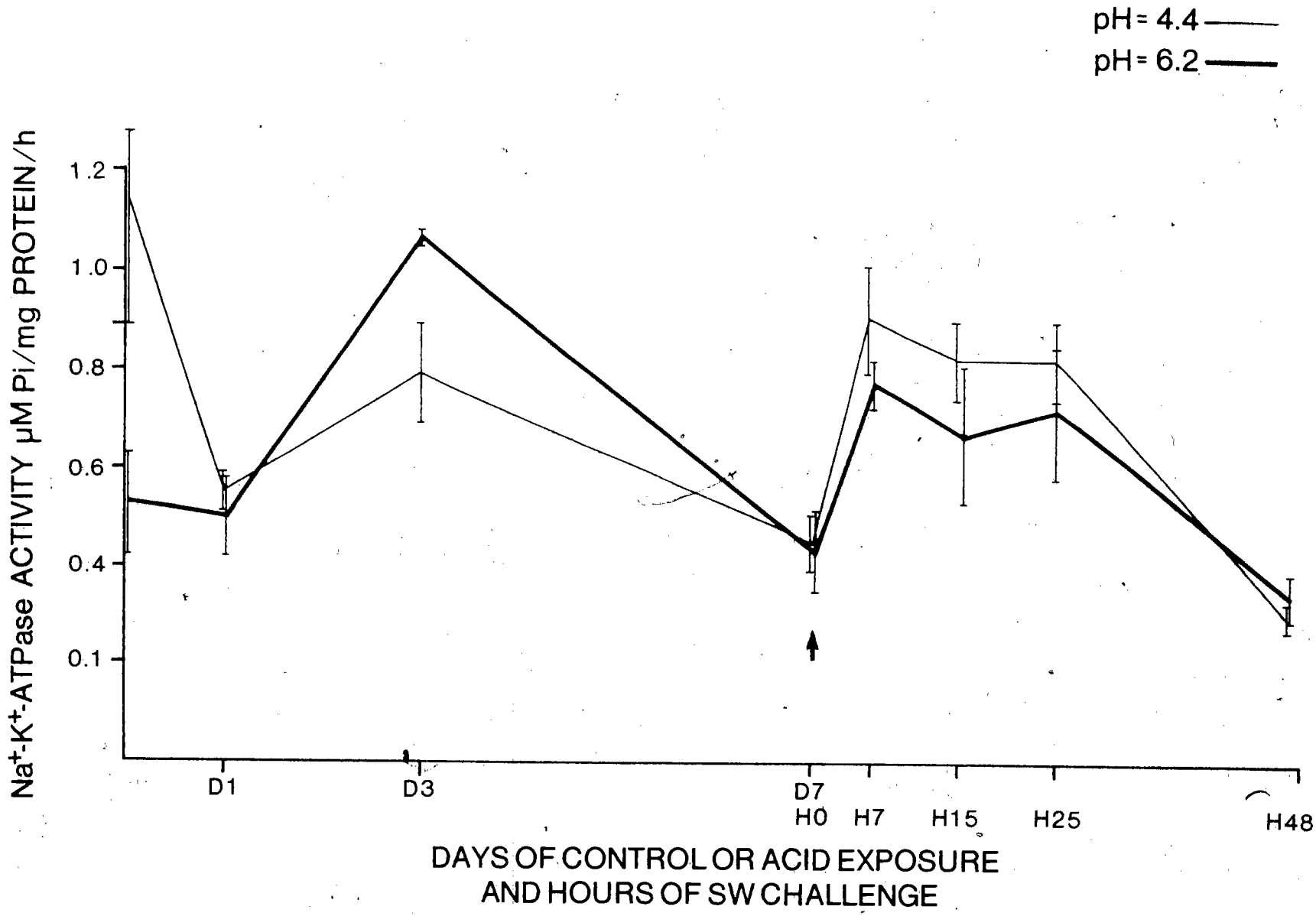

Na NORMALIZED GILL Na+-K+-ATPase ACTIVITY (UNITS)
30
20
10
pH = 4.5
5.1
5.5
6.2
D0
D1
D3
D7
H0
H7
H15
H25
H48
DAYS OF CONTROL OR ACID EXPOSURE
AND HOURS OF SW CHALLENGE

Figure 4: Gill Na^+-K^+-ATPase activities (um Pi/mg protein/h) for coho salmon parrs undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.


pH = 4.4
pH = 6.2
Na+-K+-ATPase ACTIVITY µM Pi/mg PROTEIN/h
1.2
1.0
0.8
0.6
0.4
0.1
D1
D3
D7
H0
H7
H15
H25
H48
DAYS OF CONTROL OR ACID EXPOSURE
AND HOURS OF SW CHALLENGE

In Experiment III controls had higher gill Na^+-K^+-ATPase activity compared with acid-stressed fish on day 14 of acid exposure (Figure 5). Enzyme activities were lower in all fish sampled during seawater challenge tests of Experiment III compared with data from the test in Experiment I. Controls sampled at hour 7 of the second seawater challenge test had lower gill Na^+-K^+-ATPase activity than freshwater controls sampled on day 14. There were increases in gill Na^+-K^+-ATPase activity from hour 25 to hour 48 in control fish in the second seawater challenge test. Acid-stressed fish in the third seawater challenge test underwent decreases in gill Na^+-K^+-ATPase activity by hour 25 of seawater exposure to the lowest level observed in this experiment. There was an increase in gill Na^+-K^+-ATPase activity to levels exhibited by control fish by 48h of seawater exposure in the third seawater challenge test, which may suggest the ion regulatory recovery of acid-stressed fish.

Plasma Sodium (Na^+)

After three days of acid-exposure (Experiment I), fish at pH 4.5 had lower plasma Na^+ levels than control fish (pH 6.2). There were also differences between fish of pH 5.1 and those of pH 5.5 and 6.2 (Figure 6).

Within 7 hours of seawater exposure, fish at all pH levels underwent significant increases in plasma Na^+ levels as compared

Figure 5: Gill Na^+-K^+-ATPase activities (um Pi/mg protein/hr) for coho salmon smolts undergoing 1, 2 or 3 weeks of acid exposure and a seawater challenge test after 1, 2 or 3 weeks of acid exposure.

Bars are ± SEM.

Arrows denote entry into seawater.

Thick lines indicate freshwater values, thin lines indicate seawater values.

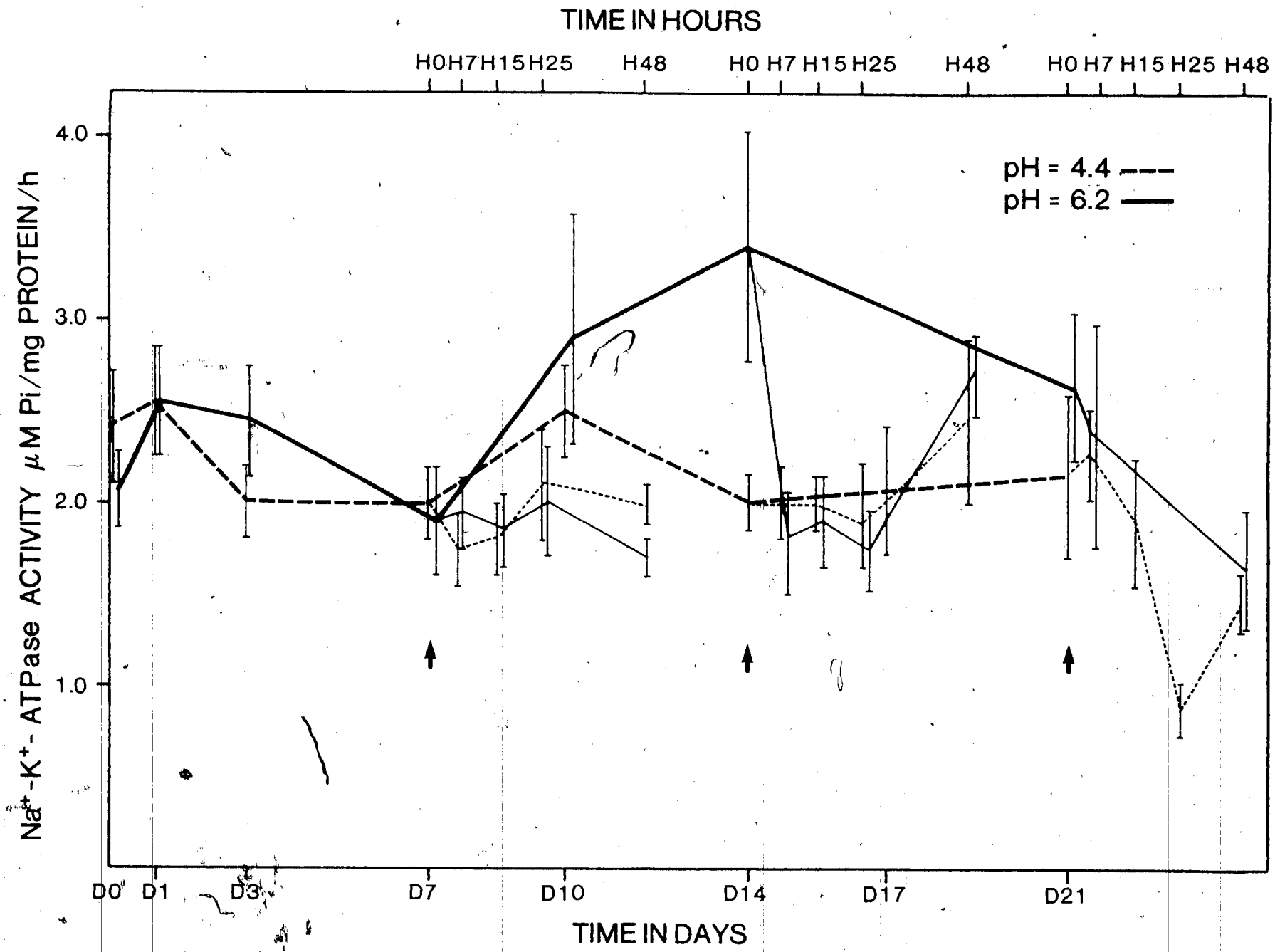
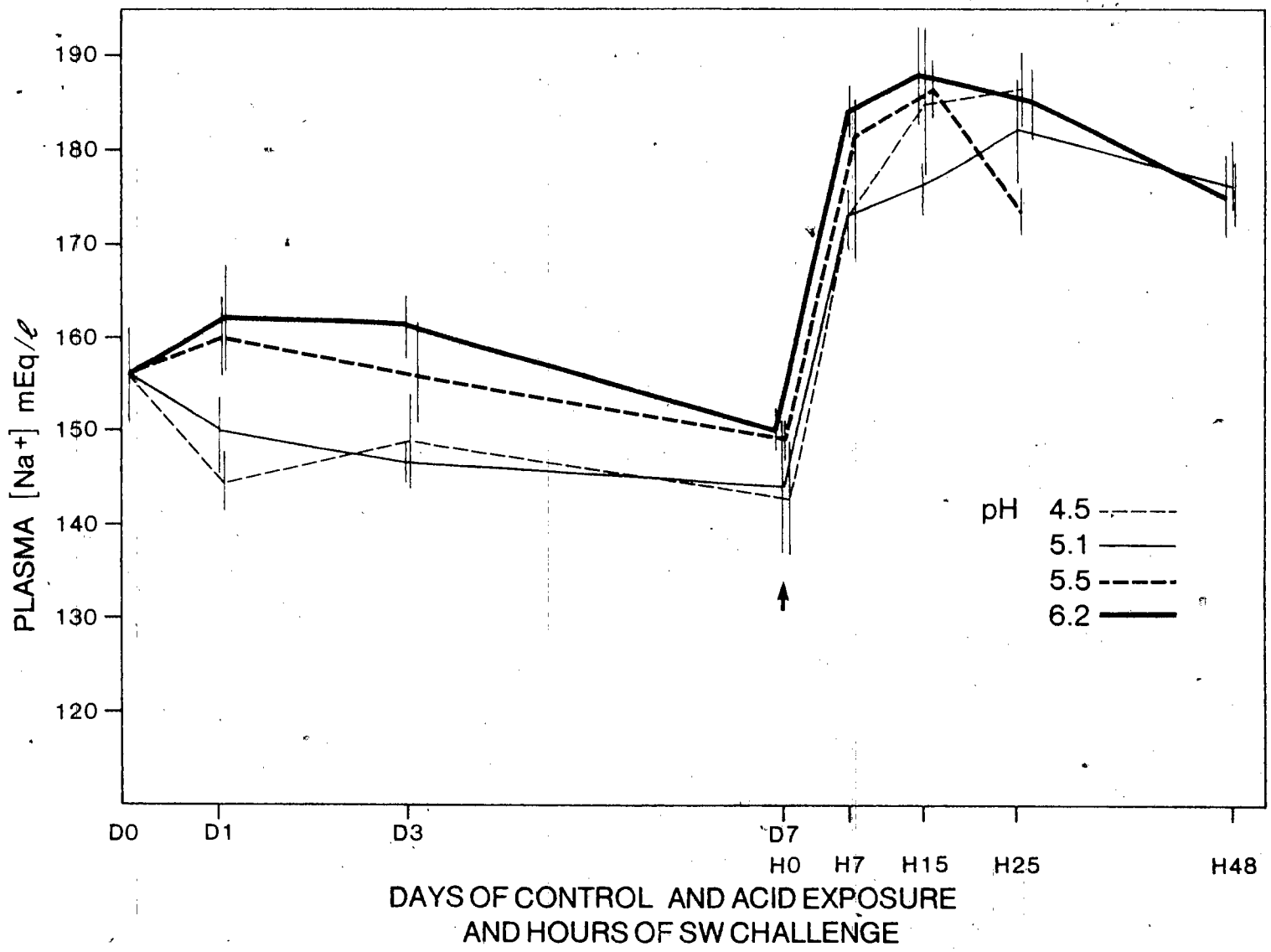

TIME IN HOURS
H0 H7 H15 H25 H48 H0 H7 H15 H25 H48 H0 H7 H15 H25 H48
4.0
3.0
2.0
1.0
pH = 4.4
pH = 6.2
Na+-K+- ATPase ACTIVITY µM Pi/mg PROTEIN/h
D0 D1 D3 D7 D10 D14 D17 D21
TIME IN DAYS

Figure 6: Plasma [Na^+] (mEq/l) coho salmon smolts undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.


PLASMA [Na+] mEq/ℓ
190
180
170
160
150
140
130
120
pH 4.5
5.1
5.5
6.2
D0
D1
D3
D7
H0
H7
H15
H25
H48
DAYS OF CONTROL AND ACID EXPOSURE
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with freshwater values. No other differences in plasma Na^+ were observed.

Parrs in Experiment II exposed to pH 4.4 showed lower plasma Na^+ levels after 24h of acid exposure as compared with control fish (Figure 7). Acid-exposed fish continued to have lower plasma Na^+ levels throughout freshwater acid exposure. Plasma Na^+ in control fish remained unchanged from 160 ± 5 mEq/L throughout freshwater experimentation, whereas acid-exposed fish has a depressed plasma Na^+ of 133 ± 1.7 mEq/L on day 7.

Upon entry into seawater, plasma Na^+ levels in parrs increased above freshwater levels. Acid-exposed fish continued to exhibit lower plasma Na^+ than control fish 7 hours after entry into seawater , but were not different thereafter. The general trend of increased plasma Na^+ levels during the seawater challenge test follows the pattern of smolts in Experiment I (Figure 2).

In Experiment III (Figure 8), plasma Na^+ levels in smolts were higher in control fish on days 7, 10 and 14 of acid-exposure. By day 21, there was no difference in plasma Na^+ concentration between control and acid-stressed fish.

During seawater challenge tests in Experiment III, control fish had higher plasma Na^+ levels at hour 7 of the first seawater challenge test and hour 15 of the second seawater challenge test. Conversely, previously acid-exposed fish undergoing seawater challenge tests exhibited higher plasma Na^+

Figure 7: Plasma [Na^+] (mEq/l) of coho salmon parrs undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.

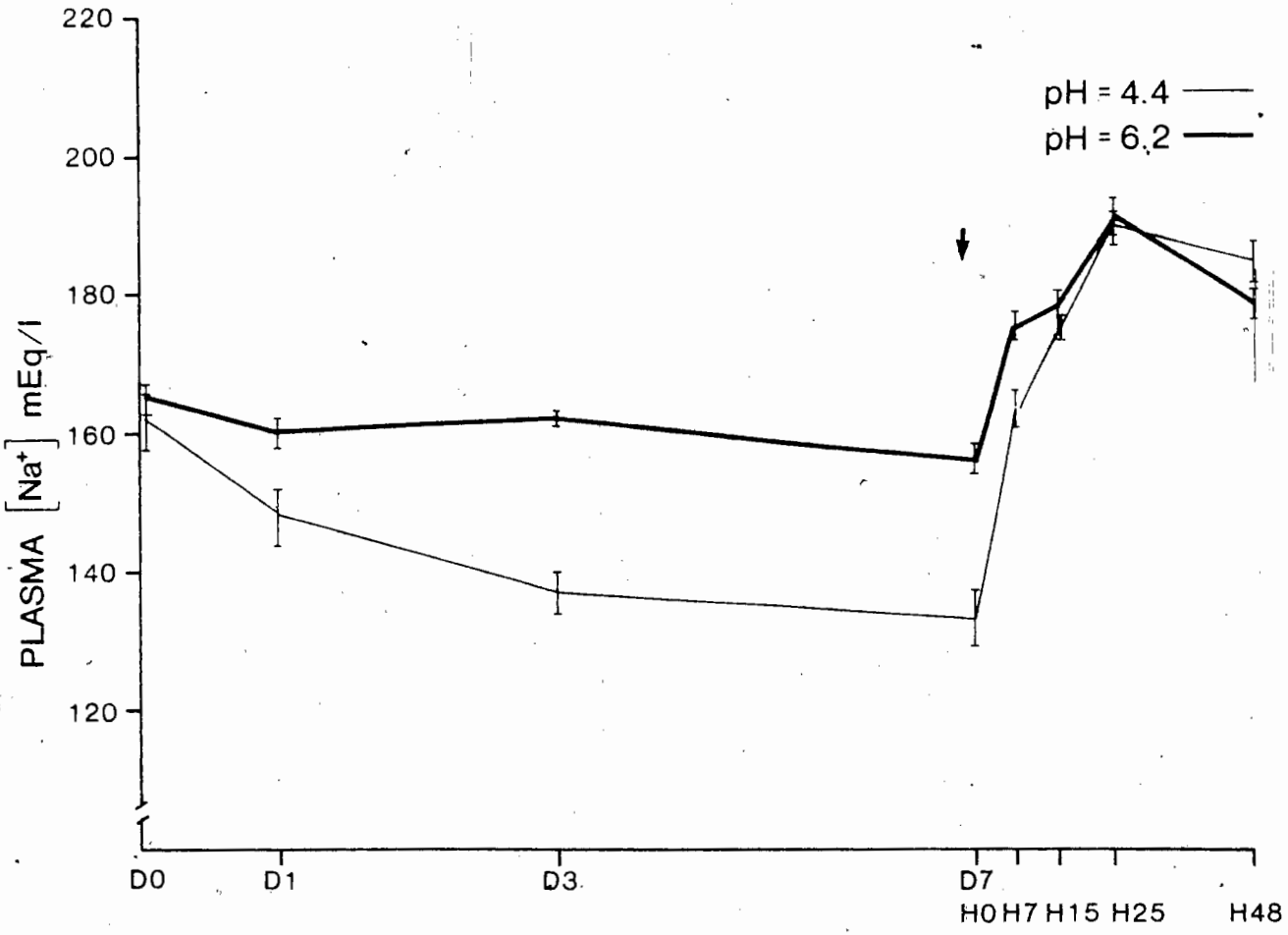
220
200
180
160
140
120
PLASMA [Na+] mEq/l
pH = 4.4
pH = 6.2
D0
D1
D3
D7
H0 H7 H15 H25
H48
DAYS OF CONTROL OR ACID EXPOSURE
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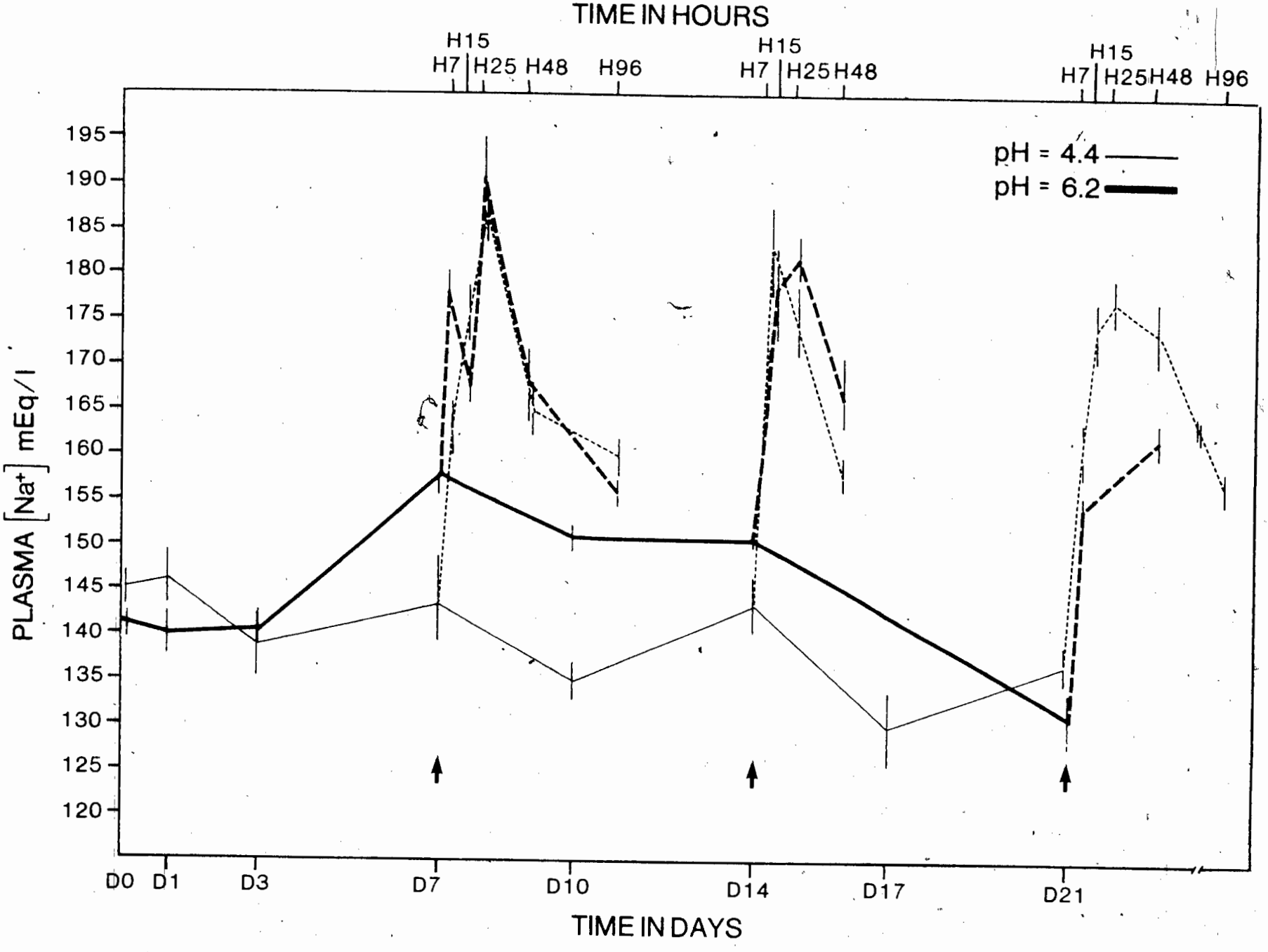
Figure 8: Plasma [Na^+] (mEq/l) of coho salmon smolts undergoing 1, 2 or 3 weeks of acid exposure and a seawater challenge test after 1, 2 or 3 weeks of acid exposure.

Bars are ± SEM.

Arrows denote entry into seawater.

Solid lines indicate freshwater values, broken lines indicate seawater values.


TIME IN HOURS
H15
H7
H25
H48
H96
H15
H7
H25
H48
H15
H7
H25
H48
H96
pH = 4.4
pH = 6.2
PLASMA [Na+] mEq/l
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
D0
D1
D3
D7
D10
D14
D17
D21
TIME IN DAYS

levels than controls at hour 15 of the first seawater challenge test and hours 7 and 48 of the third seawater challenge test. The phenomenon of elevated plasma Na^+ levels upon seawater entry in this experiment exhibited during all seawater challenge test of was similar to previous experimental results of this study.

Hematocrits

In Experiment I, one difference in hematocrits was observed between acid-exposed smolts (pH 4.5) and control fish (Figure 9). During hour 15 of the seawater challenge test, previously acid-exposed fish had higher hematocrits that control fish. No other differences in freshwater or seawater were recorded in this experiment.

During Experiment II, hematocrits of acid-exposed parrs were elevated above those of control fish 24h after the onset of acid-exposure (Figure 10). Hematocrits of acid-exposed fish remained higher than controls until they became lower than control fish hematocrits at hours 25 and 48 of seawater exposure.

In Experiment III, smolts exposed to acid had hematocrits lower than those of control fish during the second and third seawater challenge test at hours 48 and 7 respectively (Figure 11). No other differences in hematocrit were observed in Experiment III.

Figure 9: Hematocrits of coho salmon smolts undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.

PCV=packed cell volume.

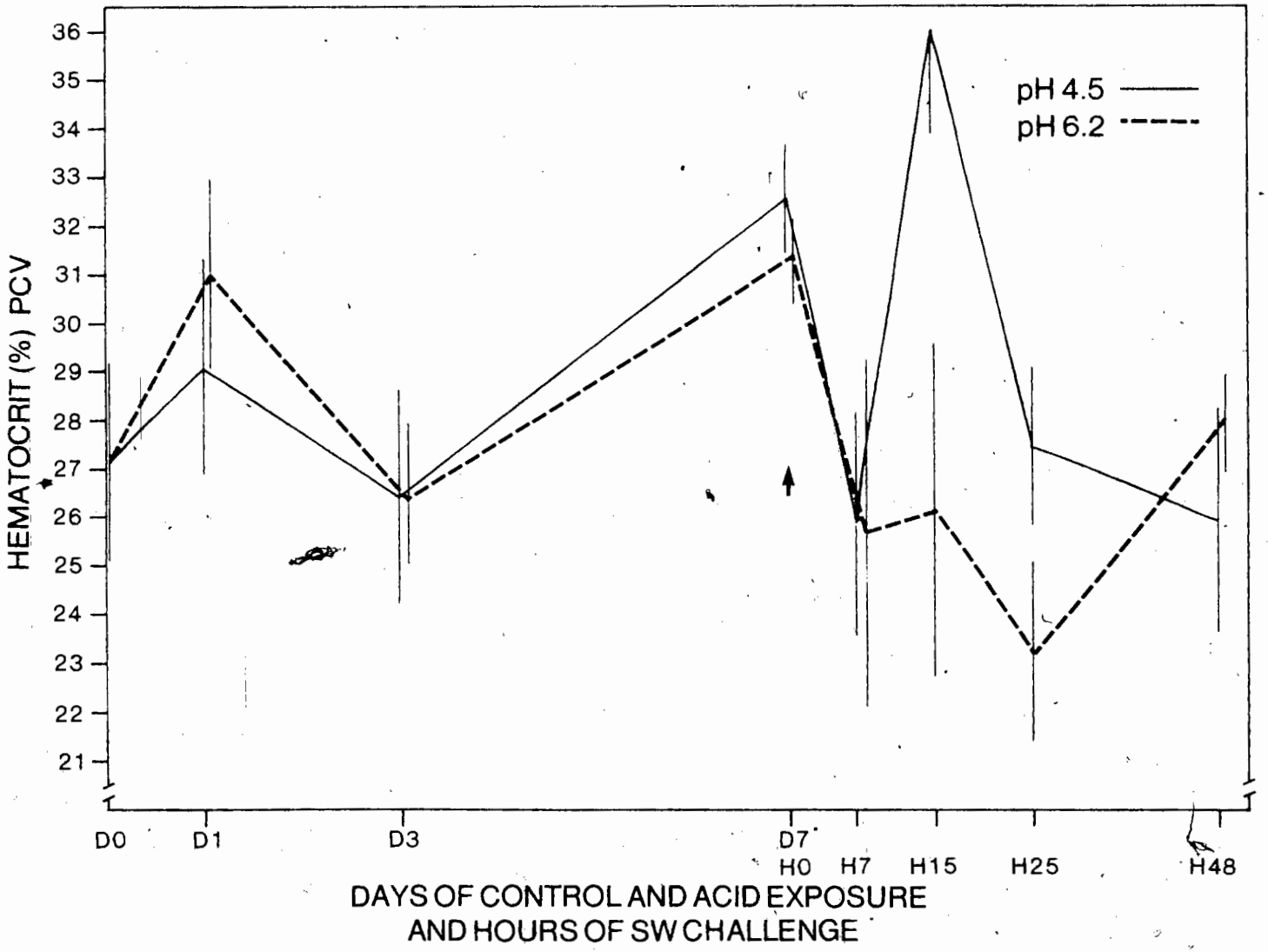

HEMATOCRIT (%) PCV
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
pH 4.5
pH 6.2
D0
D1
D3
D7
H0
H7
H15
H25
H48
DAYS OF CONTROL AND ACID EXPOSURE
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Figure 10. Hematocrits of coho salmon parrs undergoing 1 week of acid exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.

PCV=packed cell volume.

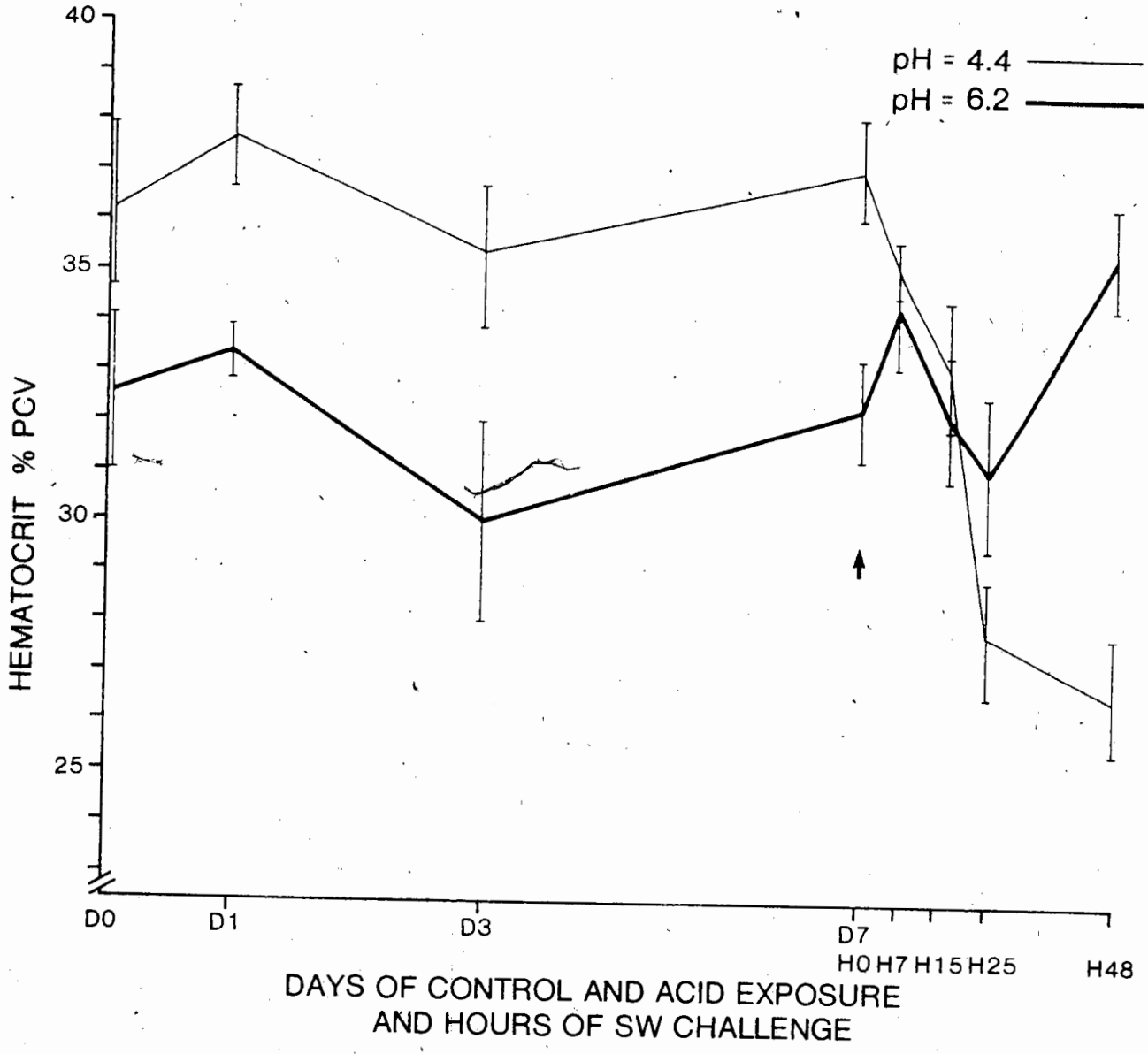

40
35
30
25
HEMATOCRIT % PCV
pH = 4.4
pH = 6.2
D0
D1
D3
D7
H0 H7 H15 H25
H48
DAYS OF CONTROL AND ACID EXPOSURE
AND HOURS OF SW CHALLENGE

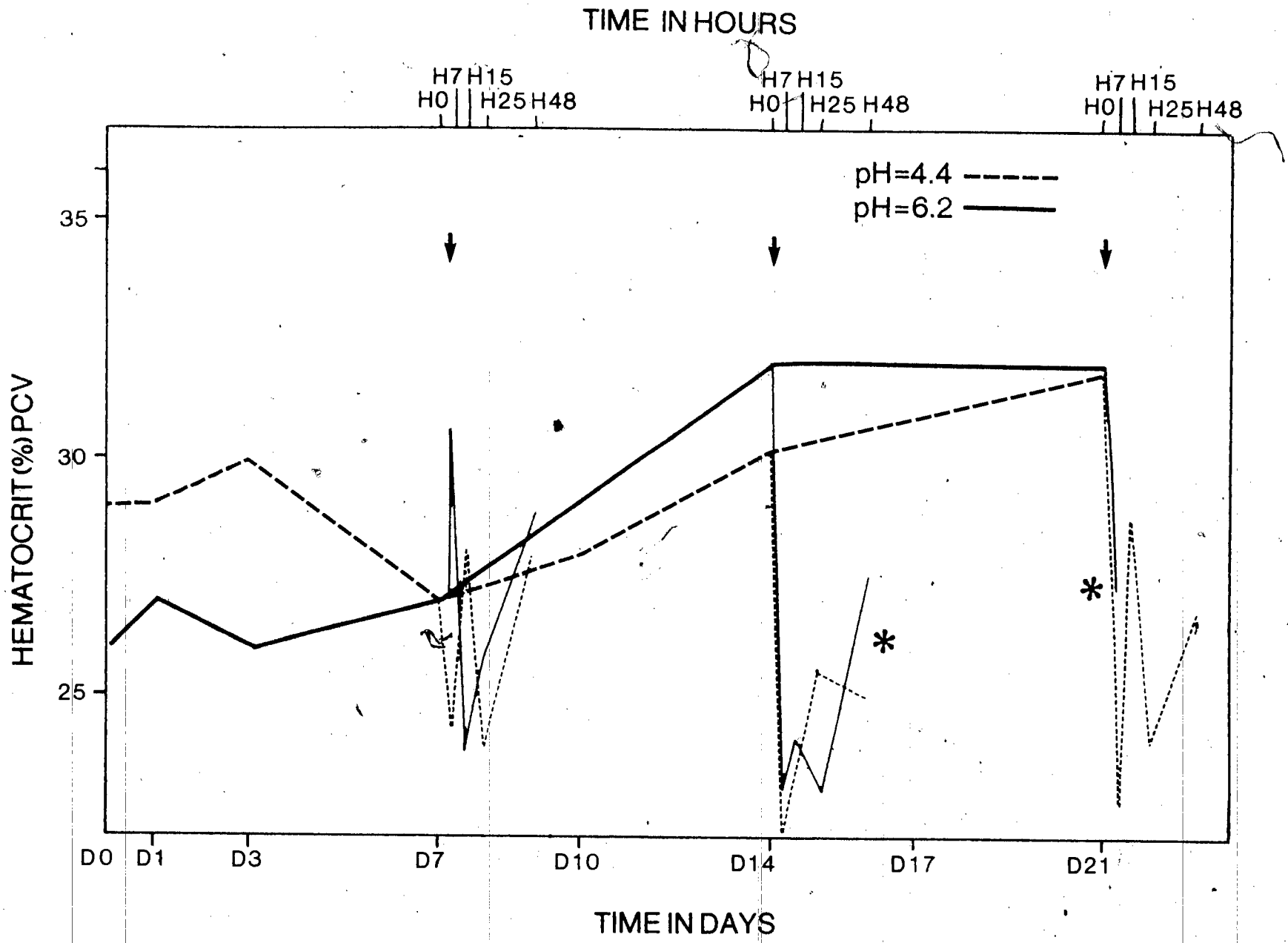
Figure 11: Hematocrits of coho salmon smolts undergoing 1, 2 or 3 weeks of acid exposure and a seawater challenge test after 1, 2 or 3 weeks of acid exposure.

Arrows denote entry into seawater.

PCV=packed cell volume.

Thick lines indicate freshwater values, thin lines indicate seawater values.

* indicates significant difference ($P \leq 0.05$).


TIME IN HOURS
H0
H7
H15
H25
H48
pH=4.4
pH=6.2
HEMATOCRIT (%) PCV
35
30
25
D0
D1
D3
D7
D10
D14
D17
D21
TIME IN DAYS

Mortalities

Fish deaths from Experiment I (smolts) and Experiment II (parrs) were too few in number for statistical analysis. Experiment I had no control fish deaths, four deaths of fish held at pH 5.1 , and one fish death at pH 4.5 exposed fish at hour 25 of the seawater challenge test. Experiment II had one mortality in acid-stressed fish at hour 25 of the seawater challenge test.

Percent cumulative mortalities for freshwater control and acid-exposed fish in Experiment III are shown in Table I. The number of deaths attributed to acid exposure was 14, compared with 4 deaths observed in control populations during the 21 day period of freshwater experimentation. The period of 7 - 14 days of acid exposure was observed to contain the highest number of acid-related mortalities.

In Experiment III, there were more acid-stressed fish deaths during the first seawater challenge test (16% vs. 6% of control fish) and the third seawater challenge test (49% of acid-stressed fish vs. 25% of control fish), (Table II). There were no apparent differences in mortalities on the bases of pH treatment during the second seawater challenge test. The observed deaths of acid-stressed fish in seawater appear to increase after 25h of seawater exposure. As length of acid exposure prior to seawater entry increased (7, 14 or 21 days),

Table I: Percent cumulative mortality of coho salmon smolts after 21 days of exposure to waters of different pH (Experiment III)

pH=4.4		pH=6.2	
Day of Mortality	Percent Cumulative Mortality	Day of Mortality	Percent Cumulative Mortality
Day 0 (6 hrs)	1.4 %	Day 7	3.9 %
2	4.2	10	5.9
3	5.6	17	7.8
7	6.9		
8	9.7		
11	16.7		
14	18.1		
19	19.4		
	n=72 fish exposed		n=51 fish exposed

Table II: Percent cumulative mortality of coho salmon smolts undergoing seawater challenge tests after 7, 14 and 21 days exposure to waters of different pH (Experiment III).

a first seawater challenge test

b second seawater challenge test

c third seawater challenge test

	pH=4.4			pH=6.3	
	Time of recorded mortality		% cum. mortality	Time of recorded mortality	% cum. mortality
SWIa	Hour	7	7	SWI Hour 7	3
		15	-	15	
		25	-	25	
		48	16	48	6
	n=32 fish sampled			n=32 fish sampled	
SWIIb	Hour	7	3	SWII Hour 7	
		15	9	15	11
		25	25	25	19
		48	-	48	25
	n=32 fish sampled			n=28 fish sampled	
SWIIIc	Hour	7	3	SWIII Hour 7	
		15	6	15	
		25		25	
		48		48	25
	n=65 fish sampled			n=12 fish sampled	

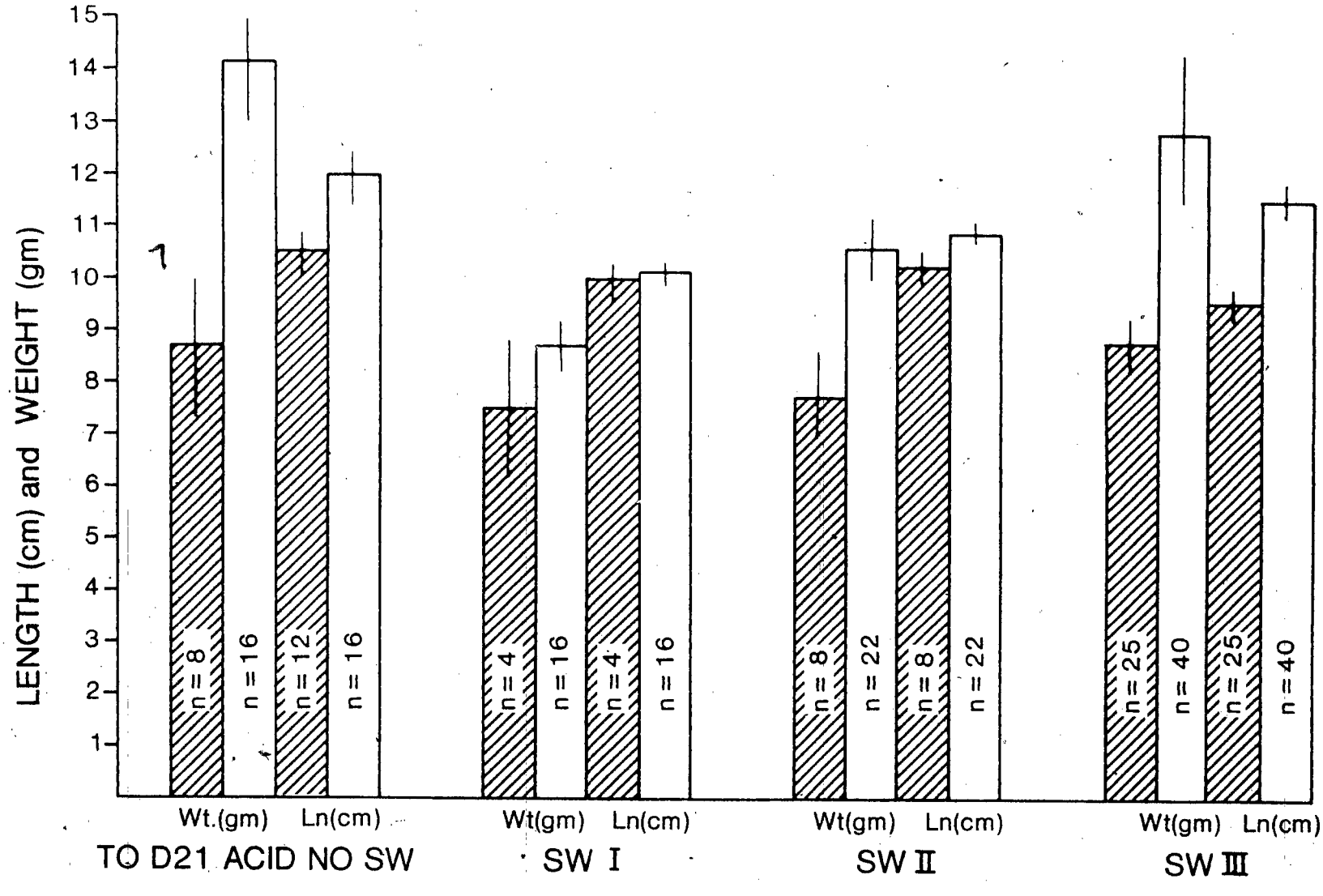
mortalities of acid-stressed fish appeared to increase, whereas control mortalities were not above 25%.

Length and weight were examined and compared among dead fish and fish that survived 21 days of acid exposure (Figure 12), to determine if fish size was correlated to mortality among acid-exposed fish. Length and weight of acid-stressed mortalities in each seawater challenge test were likewise compared with acid-stressed fish that survived 25h of seawater exposure.

After 21 days of acid exposure, surviving fish were greater in length and weight than fish that died. There was no size difference between surviving and dead fish during the first seawater challenge test (Figure 12), which coincided with the least amount of mortalities observed in seawater (Table II). However, during the seawater challenge test after 14 days of acid exposure, surviving fish were greater in length and weight than fish that died (Figure 12). In the third seawater challenge test, lengths and weights were also greater in surviving fish than in fish that died. Thus, there was a size selective mortality that increased with increasing length of acid exposure prior to seawater entry.

The apparent size-dependent survival after acid exposure, was further investigated to determine if it was correlated with some of the other parameters measured. Correlation analyses were

Figure 12: Average weight and length (±SEM) of coho salmon smolts that died (hatched bars) or survived (solid bars) 21 days of acid exposure or seawater challenge tests after one (SWI), two (SWII) or three (SWIII) weeks of acid exposure. Sample size appears in parenthesis.


LENGTH (cm) and WEIGHT (gm)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
n = 8
n = 16
n = 12
n = 16
n = 4
n = 16
n = 4
n = 16
n = 8
n = 22
n = 8
n = 22
n = 25
n = 40
n = 25
n = 40
Wt.(gm) Ln(cm)
Wt(gm) Ln(cm)
Wt(gm) Ln(cm)
Wt(gm) Ln(cm)
TO D21 ACID NO SW
SW I
SW II
SW III

conducted for: weight vs. gill Na^+-K^+-ATPase activity, weight vs. plasma Na^+ and gill Na^+-K^+-ATPase activity vs. plasma Na^+. Values for 21 day acid exposure and each seawater challenge test were examined for surviving fish. Table III summarizes the regression equations and correlation coefficients of these tests.

During acid exposure, gill Na^+-K^+-ATPase activity was correlated with plasma Na^+ levels after 7 and 14 days of acid exposure (Table III). The only other relation between gill Na^+-K^+-ATPase activity and plasma Na^+ was during the second seawater challenge test.

Plasma Na^+ was found to be related to weight in freshwater after 21 days of acid exposure and again during the third seawater challenge test.

Gill Na^+-K^+-ATPase activity was not weight related in freshwater, although critical values of the correlation coefficent ($r<0.51$) approached significance. Gill Na^+-K^+-ATPase was weight related in the third seawater challenge test. Similar correlation analyses were performed for the same parameters for fish undergoing seawater challenge test in Experiment III (Table IV). Weight was observed to be related to gill Na^+-K^+-ATPase activity and plasma Na^+ concentration during the third seawater challenge test. Gill Na^+-K^+-ATPase activity was observed to be related to plasma Na^+ concentration during the second seawater challenge test.

Table III: Line equations for relations between gill Na^+-K^+-ATPase activity, plasma Na^+ concentration and body weight for coho smolts after 7, 14 and 21 days of acid-exposure (pH 4.4, Experiment III).

Day		Wt. vs Na⁺-K⁺-ATPase Activity	Wt. vs Plasma [Na⁺]	Na⁺-K⁺-ATPase Activity vs Plasma [Na⁺]
7	r=a	0.40	-0.31	0.72 *
	m=	0.50	-1.24	1.50
	b=	1.47	150.9	126.7
14	r=	-0.43	0.22	0.69 *
	m=	-0.33	0.30	1.27
	b=	2.94	140.2	119.3
21	r=	-0.35	0.63 *	0.03
	m=	-0.05	0.88	0.28
	b=	3.27	117.1	132.1

a r= correlation coefficient
m= slope
b= intercept

* denotes significant ($P \leq 0.05$) regression coefficient.

Table IV: Line equations for relations between gill Na^+-K^+-ATPase activity, plasma Na^+ concentration and body weight for coho smolts undergoing seawater challenge tests after 7, 14 and 21 days of acid-exposure (pH 4.4, Experiment III).

		Wt. vs Na^+-K^+-ATPase Activity	Wt. vs Plasma [Na^+]	Na^+-K^+-ATPase Activity vs Plasma [Na^+]
SWIa	r=d	-0.33	-0.44	0.07
	m=	-0.09	-1.10	1.41
	b=	2.70	193.5	181.9
SWIIb	r=	-0.04	-0.11	-0.76*
	m=	-0.01	-0.34	-13.4
	b=	1.99	179.3	200.8
SWIIIc	r=	-0.79*	-0.57*	0.49
	m=	-0.04	-0.32	5.73
	b=	i l 4>2.05	181.8	168.5

a first seawater challenge test

b second seawater challenge test

c third seawater challenge test

d r= correlation coefficient

m= slope

b= intercept

* denotes significant ($P \leq 0.05$) regression coefficient

Plasma Growth Hormone

Plasma growth hormone (GH) levels of acid-stressed coho smolts in Experiment III were higher than controls by day 3 of acid exposure (Figure 13). On day 7 of acid exposure, acid-stressed fish had higher plasma GH levels (146 ± 24.3 ng/ml) than controls, whose levels did not change in freshwater (range 85.2 ± 10.0 to 94 ± 10.8 ng/ml).

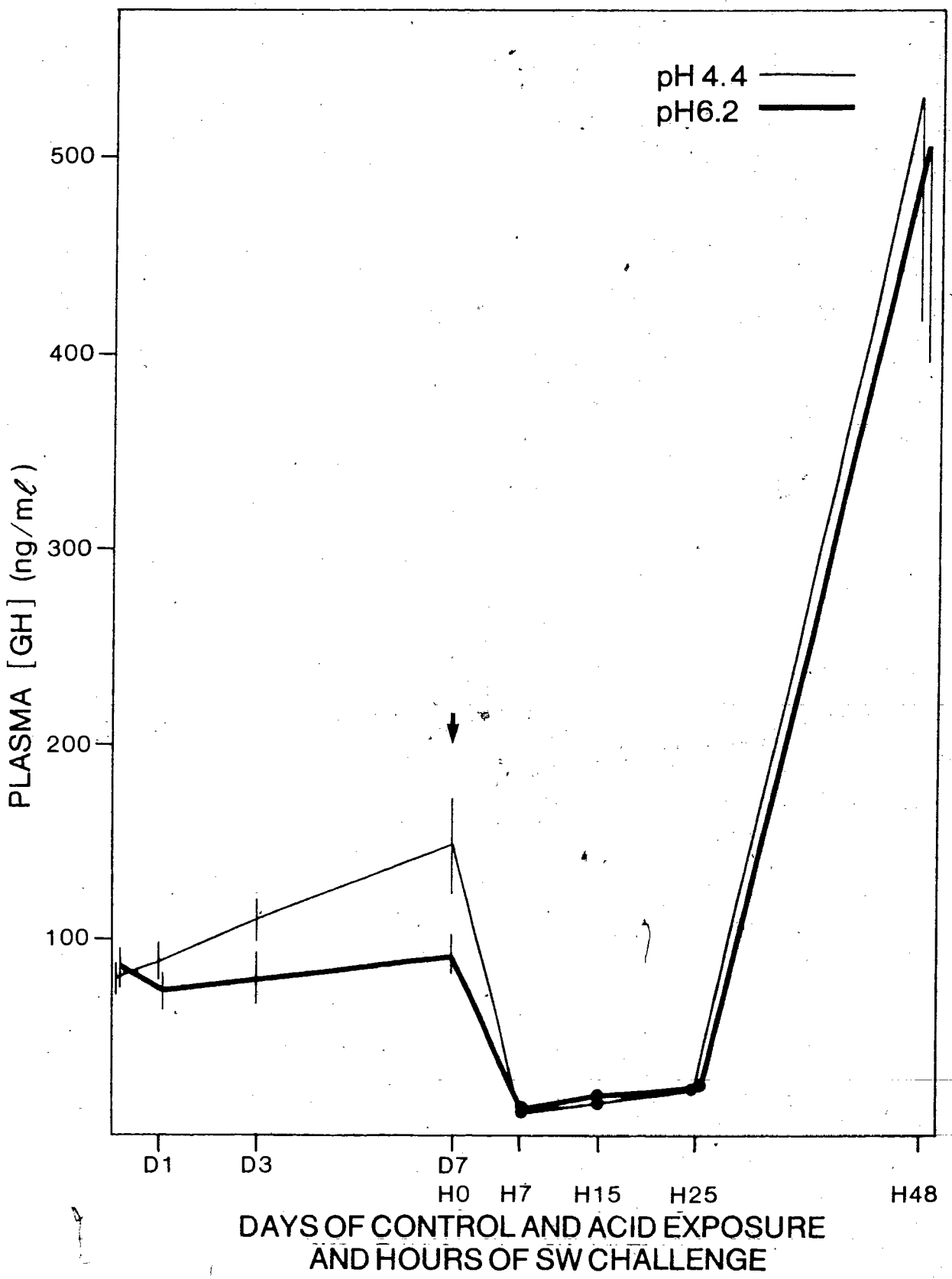
Upon entry into seawater, plasma GH levels in all fish, dropped to 10% of control levels. At hour 7 of the seawater challenge test, previously acid-exposed fish had lower plasma GH levels than control fish. No subsequent difference in plasma GH between acid-stressed fish and control fish in seawater were observed. Plasma GH levels in all fish increased during seawater exposure from hour 7 values, with an increase between hours 25 and 48 of seawater exposure. This increase in plasma GH was an approximately 5.6 fold increase from freshwater controls levels.

Purified coho salmon GH was applied to enzyme preparations to determine if direct stimulation of enzyme activity could be detected. Increased enzyme activity was not observed over the range of growth hormone concentrations tested (0, 5, 10, 20, 40, and 80 ng/ml).

Figure 13: Plasma growth hormone (ng/ml) of coho salmon smolts (Experiment III) undergoing one week of acid-exposure and a seawater challenge test.

Bars are ± SEM.

Arrow denotes entry into seawater.


pH 4.4
pH6.2
500
400
300
200
100
PLASMA [GH] (ng/mℓ)
D1
D3
D7
H0
H7
H15
H25
H48
DAYS OF CONTROL AND ACID EXPOSURE
AND HOURS OF SW CHALLENGE

IV. Discussion

Gill Na^+-K^+-ATPase Activity

Acid-exposure of smolts to a range of pH treatments (pH 4.5, 5.1, 5.5 and 6.2) did not show a direct effect of acid concentration in relation to enzyme activity. Consequently, subsequent experimentation (Experiment III) involved an increased sample size (n=8), prolonged acid exposure length (up to 21 days) and two pH treatments (pH 4.4 and 6.3) which represented the upper and lower limits tested in Experiment I.

Fish exposed to pH 4.4 (Experiment III) showed a depressed gill Na^+-K^+-ATPase activity 14 days after acid-exposure (Figure 5). Similar studies with Altantic salmon (Salmo salar) have demonstrated an inhibitory effect of acid exposure on gill enzymes (Saunders et al. 1983). This inhibitory effect was found to be related to length of exposure. In their investigations, Saunders el al. (1983) exposed Atlantic salmon (n=500) to acidified water (pH 4.3) from January to June. During this exposure period, acid-stressed fish were lower in gill Na^+-K^+-ATPase activity. It is possible that a maximum exposure period of 21 days was not long enough to elicit enzyme inhibition in all acid-stressed fish. However, enzyme activity levels on day 10 of acid exposure indicate that inhibition of

the enzyme occurred regardless of exposure length. Wide differences in enzyme activity between control and acid-exposed fish on day 10 of the experiment corresponded to the period of greastest mortality (Table II). This may imply that fish without high gill Na^+-K^+-ATPase activity may be selectively removed from the population.

In all seawater challenge tests of Experiment III, gill Na^+-K^+-ATPase activity was lower than freshwater control values for at least 25h of seawater exposure (Figure 5). If acid exposure causes reduced enzyme activity, and enzyme activities decrease after 25h of seawater exposure to levels not unlike acid-exposed levels, it may be implied that 25h of seawater exposure also inhibits enzyme function. This is supported by the observation that plasma Na^+ levels of fish undergoing seawater challenge tests in Experiment III were highest when gill enzyme activities were lowest. Other studies have indicated that full ionoregulatory ability is not obtained in smolts until 25h after entry into seawater, (Clarke and Blackburn 1977, Boeuf et al. 1976). This may indicate a period of enzyme response to external salinity. During the second and third seawater challenge tests (Experiment III), there were increases in enzyme activity in all fish which were concomittant with decreasing plasma Na^+ levels.

The period of relative enzyme inactivity and increased plasma Na^+ subsequent to entry into seawater has implications to changes in cell morphologhy and/or cell funtion (Philpott 1980). These changes appear to be independent of acid exposure prior to

entry into seawater. Examination of the principles involved in seawater adaptation of euryhaline fishes may elucidate the above observed phenomenon of enzymatic inactivity during changes in ambient salinity.

The gills of freshwater teleost have "chloride cells" which have as one of their ionoregulatory functions, the ability to acquire ambient Na^+. External ion acquisition is thought to be an enzyme mediated cotransport or codiffusion of intracellular H^+ or $NH4^+$ for extracellular Na^+ which takes place at the apical membrane of these cells (Clairborne et al. 1982). The Na^+/H^+ or $Na^+/NH4^+$ exchange thus conserves electrical charge while acquiring extracellular Na^+ in exchange for metabolic by-products. Concurrently, to prevent passive diffusion of Na^+ back into the external medium, Na^+-K^+-ATPase, located on the plasmalemma tubule system (TS) of the basolateral membrane, undergoes a counter ion exchange of extracellular K^+ to remove Na^+ from the cell to the plasma. If cellular K^+ becomes too high, K^+ readily diffusses back into the plasma or intracellular spaces (Maetz and García Romeu 1964, Evans et al. 1982, Kirschner 1983).

During entry into seawater, the direction of Na^+ movement is reversed. As ambient salinity increases, Na^+ influx increases, whereby there is an increased movement of Na^+ to the plasma (Clarke and Blackburn 1977). During exposure of the teleost chloride cell to increasing salinity there is increased permeation of the cell by the tubular system. This increases the

cell surface area relative to its volume and presumably brings the membrane bound Na^+-K^+-ATPase in closer proximity to energy sources such as mitochondria (Philpott 1980). The time course of increasing number of chloride cells with the development of the tubular system corresponds to the time course of Na^+-K^+-ATPase activity elevation which is preceeded by increased proliferation of mitochondria (Philpott 1980). The end result is increased enzymatic activity to presumably regulate the increasing demands of ion transport in seawater. This may reflect the period of enzyme inactivity exhibited by fish in Experiment III.

Towle et al. (1977) found that enzymatic properties of Na^+-K^+-ATPase in Fundulus heteroclitus appeared to be identical, during exposure to either fresh or seawater, indicating that the same enzyme was used in Na^+ acquisition in freshwater and Na^+ excretion in seawater. However, the functional model of Na^+-K^+-ATPase as a regulator of plasma Na^+ concentration has changed. The model proposed by Silva et al. (1977) and Karnacky (1980), involves carrier mediated transport of Na^+ and Cl^- across the basolateral membrane from the plasma to the cell interior. A high intracellular Cl^- concentration favours diffusion of Cl^- to the less electronegative ambient seawater. Na^+ inside the chloride cell is then transported via Na^+-K^+-ATPase to extracellular spaces and into accessory cells in exchange for K^+. High Na^+ concentrations in intracellular spaces and in accessory cells would favour the passive diffusion of Na^+ to the outside seawater. Thus, responses to seawater

exposure of euryhaline fishes would be manifest in enzymatic and cell morphological changes that would aid in the establishment of the above in response to increasing ambient salinity. From observations of Experiment (III), it may be concluded that acid-exposure prior to seawater entry does not effect ion regulatory mechanisms that govern seawater survival. This would indicate that inhibition due to acid exposure of fish gill Na^+-K^+-ATPase is reversible or transient or that mechanisms for seawater adaptation indicative of smoltification are unaffected by acid exposure prior to seawater entry.

The period of 0. - 25 h post seawater entry where Na^+-K^+-ATPase levels are depressed has not been previously observed. This period could possibly represent the time for cellular changes associated with seawater adaptation such as elaboration of the chloride cell tubule system and associated Na^+-K^+-ATPase activity.

There are noteworthy observations upon comparing the Na^+-K^+-ATPase activities of parrs (Experiment II) and smolts (Experiment III) (Figures 4 and 5). In freshwater, the values for Na^+-K^+-ATPase activity in both acid-stressed and control parrs are one-half to one-third the Na^+-K^+-ATPase values for control smolts. This is in agreement with past investigations which descriminate between parrs and smolts on the basis of gill Na^+-K^+-ATPase activity (Zaugg and Wagner 1973). Because fish used in both Experiment II (parrs) and Experiment III (smolts) were from the same population of fish, further evidence is given

to the observation that Na^+-K^+-ATPase activity increases on a seasonal and developmental basis (Wagner 1974a, Boeuf et al. 1976, Johnston and Saunders 1981).

Exposure of parrs to seawater caused an increase in gill Na^+-K^+-ATPase activity (Figure 4). This increase is not unlike the highest levels recorded in freshwater. The increase in gill Na^+-K^+-ATPase may reflect an attempt of seawater exposed parrs to regulate plasma ion concentrations via gill Na^+-K^+-ATPase.

By comparison of gill Na^+-K^+-ATPase (Figure 4) with plasma Na^+ values of parrs (Figure 7) in seawater, it would appear that plasma Na^+ concentration increases independent of Na^+-K^+-ATPase activity. This would suggest that gill Na^+-K^+-ATPase and other ion regulating mechanisms available to the parr, by virtue of its age and morphology, are not sufficient to permit complete ion regulation of plasma ion levels.

Comparisions of gill Na^+-K^+-ATPase activity in parrs (Figure 4) with smolt enzyme activity (Figure 5), shows that levels increase upon exposure to seawater, whereas smolt values decrease upon exposure to seawater. Towle et al. (1977) found gill Na^+-K^+-ATPase activity in Fundulus heteroclitus to be related to external salinity as in salmon. Therefore, decreased Na^+-K^+-ATPase activity of the smolt gill in response to seawater exposure may be representative of the time involved to induce cellular, biochemical, behavioural and morphological changes to permit seawater survival. Conversely, parrs do not exhibit a period of depressed enzymatic activity because parrs must

develop these mechanisms to permit seawater survival. Hence, parrs may react to seawater exposure utilizing the ion regulatory mechanisms which they do possess, namely Na^+-K^+-ATPase. Hossler (1980) proposed that the increased production of Na^+-K^+-ATPase may be a response to long-lived messenger ribonucleic acids (mRNA). Conte and Lin (1967) observed increased deoxy-ribonucleic acid (DNA) turnover rates in the gills of chinook salmon transferred to seawater. These results indicate de novo synthesis of gill epithelial protein(s) required 4-6 days which corresponds to the period for development of complete ion regulatory ability observed in seawater transferred coho salmon (Folmar and Dickoff 1980). A more immediate response to external salinities may be elicited by hormone levels which have a direct effect on cell nuclei in minutes rather than days.

Plasma Sodium (Na^+)

Acid-exposed smolts in Experiment I had lower plasma Na^+ levels on day 3 of acid exposure. This was observed between fish of pH 6.2 and 5.5 and fish exposed to water of pH 5.1 as well as between fish of pH 6.2 and fish held at pH 4.5 (Figure 6). In a subsequent experiment involving smolts (Experiment III), lower plasma Na^+ levels were observed in acid-exposed compared with control fish from day 7 to day 21 of freshwater experimentation. These observations agree with past studies where exposure to low

pH in freshwater has been found to decrease plasma Na^+ levels in many species of fish including yellow perch (Perca flavescens) (Lyons 1982), brook trout (Salvelinus fontinalis) (Packer and Dunson 1970), rainbow trout (Salmo gairdneri) (Spry et al. 1981) and Atlantic salmon (Salmo salar) (Saunders et al. 1983).

Packer and Dunson (1972) confirmed that prior to death in acid-stressed fish, there is a massive loss of plasma Na^+. During exposure to extremely acidic conditions (pH 3.0 to 3.5), Na^+ loss may be of secondary importance to mortality, the primary cause of death being depressed pO2 of the blood (Packer and Dunson 1972, Dively et al. 1977, Fromm 1980, Milligan and Wood 1983). However, in conditions of moderate acid exposure (pH 4.0-4.5), blood pO2 levels of rainbow trout were not significantly reduced enough to cause fatal anoxia (Neville 1979). Therefore, it may have been possible that mortalities observed in this study during exposure to moderate acid conditions (pH 4.4) may have been due to failure to regulate plasma ion concentration. For example; during the period of highest mortality in Experiment III (Table II) and during the period of greatest differences in Na^+-K^+-ATPase activity between control and acid-exposed fish, plasma Na^+ differences are at their greatest (Figures 5 and 8). This would suggest that the failure to regulate plasma Na^+ levels via Na^+-K^+-ATPase resulted in death after at least 10 days of acid exposure. Further support for this hypothesis is obtained from the observations of Booth et al. (1982) who observed respiratory failure due to

lowered ambient pH is not likely to be experienced outside the laboratory. In such cases where moderate acid exposure exists, electrolyte loss, primarily Na^+ may be the underlying cause of death through failure to regulate internal ion concentration. Johnston et al. (1984) observed that during chronic acid exposure (pH 4.4), Atlantic salmon exhibited an increased ion permeability of the branchial epithelium and inhibition of active transport mechanisms which may have resulted in plasma electrolyte losses in freshwater.

Further evidence for plasma Na^+ loss as the primary cause of death at moderate pH is obtained by examination of respiratory failure. The commonly accepted model of respiratory failure at low pH exposure involves the accumulation of mucus on the gills of acid-exposed fish in response to damage caused by acid exposure. This mucus accumulation then reduces the capacity of the gill filaments' secondary lamellae to acquire oxygen and expel CO_2. This condition then causes a decrease in blood pH (as a build up of blood pCO_2 ultimately resulting in death (Fromm 1980). In observations of this study, no visible build up of mucus was detected on gill surfaces. The lack of mucus accumulation on the gills of acid-exposed fish in this study would suggest that the death of acid-exposed fish was not due to respiratory failure but may in part be due to the failure to regulate plasma ion concentration.

Parrs in Experiment I underwent greater losses in plasma Na^+ as a result of acid exposure than smolts in Experiment III

(Figures 7 and 8). Differences in plasma Na^+ concentration between control and acid-exposed parrs were observed 24 h after acid exposure. These differences were maintained throughout freshwater experimentation. Conversely, plasma Na^+ levels in smolts exposed to acidified water (pH 4.4) were unaffected by acid stress until 7 days of acid exposure. It would appear that acid stress has a more profound effect on plasma Na^+ levels in parrs than in smolts.

Net plasma Na^+ loss is reflective of the fishes' ion regulatory ability to acquire ions from the environment in order to replace ions lost via passive diffusion across the branchial epithelia (MacDonald 1983). Smolts having developed more efficient mechanisms of ion regulation in comparison with parrs, would tolerate Na^+ losses caused by acid stress, better than acid stressed parrs. Evidence to support this hypothesis is gained from examination of the effects of acid-stress on parrs' gill Na^+-K^+-ATPase activity. Three days of acid-exposure caused decreases in enzyme activity whereas smolts have depressed Na^+-K^+-ATPase activity only after 10 days of acid exposure. The effect of acid-exposure on plasma Na^+ levels in parrs occurs before smolts. Plasma Na^+ is depressed in parrs after 24h of acid exposure compared to 14 days of acid exposure in smolts.

The results of this study suggest that parrs exposed to acidified waters have a greater net efflux of Na^+ resulting in depressed plasma Na^+ levels. There may be other contributing factors to plasma Na^+ loss in parrs exposed to acidified waters,

such as greater surface area to body weight, different gill membrane permeability to H^+ and Na^+, different vascular shunting through the gills or a combination of the above as well as other factors yet undisclosed.

There was no effect of a range of pH exposure treatments (Experiment I) or a time dependant effect of acid-exposure (Experiment III) upon smolt plasma Na^+ values during seawater challenge tests (Figures 6 and 8). The external sodium concentration of seawater would appear to mask any effect that acid-stress may have on gill permeability. That is, although acid stress may cause damage to branchial epithelia (MacDonald 1983), the gradient of external Na^+ in seawater relative to plasma Na^+ concentration is great enough that the rate of Na^+ influx cannot be distinguished on the basis of pH exposure prior to entry into seawater. Differences were not observed in plasma Na^+ concentration of acid-exposed and control parrs and smolts (Experiments II and III) after 15 h of seawater exposure. This would indicate that the mechanism of Na^+ influx from seawater to fish plasma is passive (Folmar and Dickoff 1980) and may be independent of lifestage. All fish, both acid-exposed and control, underwent increases in plasma Na^+ levels to a maximum at 25 h of seawater exposure and subsequent decreases thereafter. This phenomenon is observed by Clarke and Blackburn (1977) who also note that the ability of anadromous salmonids to regulate plasma Na^+ in seawater has been related to smoltification. The transient increases in plasma Na^+ upon entry

into seawater is under the influence of regulatory mechanisms including Na^+-K^+-ATPase, as well as decreases in gill ion permeability both of which are indicators of seawater adaptability (Johnston and Saunders 1981). Salmon smolts should be able to regulate plasma Na^+ concentrations to 170 mEq/l 24 h after transfer to seawater (Clarke and Blackburn 1977). The mechanisms which govern plasma ion concentrations (including Na^+-K^+-ATPase) are unaffected by acid-exposure prior to entry into seawater on the basis of comparisons between acid-stressed and control fish utilized in this study.

Hematocrits

In this study, hematocrits were observed to change (Figure 9-11) with lifestage (parr vs smolt) and with ambient water conditions (freshwater, acidified freshwater and seawater). Miles and Smith (1968) and Saunders et al. (1983) observed higher hematocrits in parrs than in smolts of coho and Atlantic salmon respectively. Saunders et al. (1983) also observed elevated hematocrits in parrs and smolts undergoing chronic acid exposure. Saunders et al. (1983) and Johnston et al. (1984) have also observed that hematocrits were higher in parrs that in smolts. Declines in hematocrits were likewise observed between parrs and smolts in this study and acid-exposed parrs were found to have elevated hematocrits in freshwater.

The phenomenon of hematocrit elevation of non-anadromous species exposed to acidified water has been observed in previous studies. Neville (1979) and Milligan and Wood (1983) observed increases in hematocrits for rainbow trout exposed to pH 4.0-4.5 for 12 days and 3 days respectively. The proposed mechanism for elevation of hematocrits due to acid exposure is eurythrocyte swelling, a decrease in plasma volume and mobilization of eurythrocytes from the spleen and other hemopoietic sources (Millgan and Wood 1983). Neville (1979) proposes that increased hematocrit was unlikely due to redistribution of water from the plasma to the tissues because tissue moisture content remained unchanged. This is also indicated in the present study by the observation of plasma Na^+ depression while hematocrits increased. Had there been redistribution of water from the plasma to the tissue compartment (hence increasing hematocrits), plasma Na^+ levels would likely not have undergone decreases although water and Na^+ may be regulated independently.

The effect of acid exposure appears to have a greater effect on parrs than on smolts. Increased hematocrit was observed to be elevated above control fish 24 h after the onset of acid exposure and remained higher than control fish values throughout acidified freshwater exposure (Figure 9). Conversely, hematocrits of acid-exposed smolts of Experiment III were not observed to be different at any time in freshwater (Figure 11). The influence that acid-exposure upon parr hematocrits as compared with smolt hematocrit may be reflected in the smolts

ability to regulate plasma constituents more precisely. That is, mechanisms that control plasma Na^+ concentrations hence plasma osmolality, in parrs are not as developed as compared with smolts. Therefore, decreasing plasma Na^+ in acid-stressed parrs may influence plasma osmolality which may in turn cause increased eurythrocyte swelling thereby increasing hematocrits. Further analysis of plasma osmolality of acid-stressed parrs would be needed to further elucidate the phenomenon of increased hematocrits.

Elevated hematocrits caused by eurythrocyte swelling is thought to be related to possible impairment of $O2$ uptake in acid-stressed fish (Milligan and Wood 1983). Increased eurythrocyte swelling in acid-exposed fish, is also thought to be symptomatic of blood acidosis (Packer in Dunson 1970). Acidosis of blood can cause a decrease in the oxygen carrying capacity in the blood via Bohr and Root effects (Packer and Dunson 1972, Milligan and Wood 1983). Therefore, if acid exposure causes increased eurythrocyte swelling and decreased oxygen carrying capacity through blood pH effects, examination of hematocrits in acid-exposed fish may be diagnostic of the aforementioned symptoms. The usefulness, however, of hematocrit observations as a diagnostic tool for acid exposure may be limited.

During entry into seawater, regulation of plasma volume appears to be less well defined in acid-exposed parrs than in control parrs (Figure 10). Hematocrits of acid exposed parrs in

seawater decreased steadily upon entry into seawater, whereas control fish exhibit increased hematocrits by hour 48 of seawater exposure. This may indicate that observed increases in plasma Na^+ upon seawater exposure in acid-stressed parrs may decrease eurythrocyte cell size by changing plasma osmolality. An increase in plasma osmolality hence a change in eurythrocyte size would therefore decrease hematocrits. Therefore, if gill permeability to ions is disrupted by acid-exposure, and ion regulatory enzymes are effected in their function (Johnston et al. 1984), hematocrits may respond as observed in acid-exposed parrs upon entry into seawater. Control parrs in seawater have not undergone eurythrocyte swelling or possible damage to gill epithelia that acid-exposure causes, hence do not have the changes in hematocrit that is observed in acid-exposed parrs.

Hematocrits of smolts (Experiments I and III) undergoing seawater challenge tests showed no differences between acid-exposed fish and control fish by 48h of seawater exposure, with the exception of the second seawater challenge test of Experiment III (Figures 9 and 11). There would appear to be little effect of acid-exposure length prior to seawater exposure upon hematocrits. This may be in part explained by mechanisms which govern plasma ion concentrations, hence plasma osmolality therefore eurythrocyte swelling, that are more developed in the smolt than in the parr.

Plasma Growth Hormone

A number of endocrine tissues have recently been reported to be involved with salmonid smoltification and osmoregulation (Folmar and Dickoff 1981, Foskett et al. 1983). Recent research by Folmar and Dickoff (1979) and Gallis et al. (1979) reported that gill Na^+-K^+-ATPase activity was influenced by plasma thyroxine and cortisol levels. Further investigations by Folmar and Dickoff (1981) indicate that thyroxine levels are useful indicators of smolt gill Na^+-K^+-ATPase activity.

Growth hormone (GH) may be involved in smoltification and osmoregulation due to its known growth promoting effects and its ability to influence seawater adaptability (Donaldson et al. 1979). Both mammalian and teleost growth hormone injections have been shown to facilitate survival and osmoregulation of salmonids in seawater (Komourdjian et al. 1976, Clarke et al. 1977). Changes associated with increased activity within the pituitary somototrophs have also been noted upon entry into seawater, indicating a role in seawater adaptation (Clarke and Nagahama 1977). Therefore, by investigations into plasma growth hormone concentrations during acid-exposure and seawater adaptation in coho salmon, further information may be obtained concerning the hormones involvement in seawater adaptation.

Smolts exposed to acidified water (Experiment III, Figure 13) underwent increases in plasma GH above control values after three days of acid exposure. These levels continued to increase

to 140% above control plasma GH levels by 7 days of acid exposure. To investigate if GH had a direct effect on gill Na^+-K^+-ATPase activity, in vitro application of the hormone was performed on the enzyme homogenate. This application of the hormone in vitro failed to stimulate increases in Na^+-K^+-ATPase activity. Therefore, on the basis of the above investigation, increased GH observed in acid-stressed fish was not likely involved with gill mediated ion transport. Possible reasons for GH increase in acid-stressed fish may have been to cause mobilization of energy reserves or other metabolic factors.

When coho smolts were transferred to seawater, their plasma GH levels decreased in both control and acid-exposed fish. This may be an indication that GH is no longer secreted from the pituitary somatotrophs but it may also be an indication that target tissues are utilizing GH at a higher rate than it is secreted into the blood.

The gradual rise in plasma GH levels after 25h in seawater and a larger increase after 48h in seawater may indicate that somatotrophs are increasing GH synthesis and secretion relative target tissue utilization, or not being utilized by target tissues.

Mortality

The development of a size selective mortality during acid exposure (Figure 12) may be attributed to plasma Na^+ loss and internal ion concentrations as regulated by Na^+-K^+-ATPase.

This study has demonstrated that Na^+ loss is a major cause of mortality in acid-stressed juvenile coho salmon. To reiterate, during conditions of moderate acid exposure, fish undergo changes in the permeability of gill epithelia and a concomittant inhibition of Na^+-K^+-ATPase which leads to a net plasma Na^+ efflux across the gill (MacDonald 1983, Johnston et al. 1984). If mortality results from plasma Na^+ decreases, and size selective mortality is apparent after 21 days of acid exposure, size should be related to plasma Na^+ concentration. Johnston and Saunders (1981) observed that smaller salmon may lose ions more readily to ambient freshwater due to a larger surface to volume ratio. The surface which is presumably most effected is the gill branchial epithelia. Hence any damage that acid-exposure may incur would affect gill mediated electrolyte loss more severely in small fish than larger fish. This hypothesis is supported by the observation of a weight-dependent plasma Na^+ concentration that developed after 21 days of acid exposure (Table III). The establishment of a correlation between weight and plasma Na^+ concentrations during a period in which the survival of larger fish is favoured, would indicate that smaller fish may be removed from the acid-exposed population by

losses of plasma Na^+.

Based on the above hypothesis, it would be anticipated that parrs, which are normally smaller than smolts, would experience greater losses in plasma Na^+ more readily upon exposure to acidified water. The present study supports this deduction by demonstrating a greater depression of plasma Na^+ in parrs than in smolts. In addition an initial depression of plasma Na^+ occurred after 3 days of acid exposure whereas smolts underwent 7 days of acid exposure before depressed plasma Na^+ levels were observed (Figures 6 and 8). However, parrs have less capability to acquire Na^+ via Na^+-K^+-ATPase compared with smolts (Boeuf et al. 1978).

During freshwater acid exposure, smolts did not develop a relation between weight and gill Na^+-K^+-ATPase activity (Table III) even though there was a concomittant establishment of a size selective mortality which favoured larger fish. This failure to establish a size relation to enzyme activity conflicts with previous investigations which have observed that the development of euryhalinity is size related (Elson 1957, Conte et al. 1966, Wagner 1974b, Landless and Jackson 1976, Johnston and Saunders 1981, Saunders et al. 1983, Johnston et al. 1984). Clarke and Blackburn (1977) note that the approximate minimum size for smolting to occur in chinook (Oncorhynchus tshawytscha) and sockeye salmon (O. nerka) is 4-5 g. The minimum size for rainbow trout (Salmo gairdneri) to survive in saltwater (50-60 g) increases as salinity rises above 25 parts per

thousand (Landless and Jackson 1976). Likewise, Atlantic salmon (Salmo salar) must reach a critical size to tolerate high salinities (Elson 1957, Farmer et al. 1978). Johnston and Saunders (1981) explain this phenomenon by citing higher surface to volume ratios in parrs as compared with smolts and/or slower growth rates in parrs. However, the greater survivability of smolts in seawater as compared to parrs may be accounted for by higher Na^+-K^+-ATPase activities in freshwater prior to entry into seawater (Boeuf et al. 1978). Therefore it would be expected that larger fish would have high Na^+-K^+-ATPase activity, and this activity would be related to fish size.

Examination of the correlation between gill Na^+-K^+-ATPase and weight (Experiment III) may elucidate reasons for the absence of a weight related Na^+-K^+-ATPase activity (Table III). During the first two weeks of acid exposure, gill Na^+-K^+-ATPase activity is correlated to plasma Na^+ concentration. By 21 days of acid exposure, there is no relation between gill Na^+-K^+-ATPase activity and plasma Na^+ concentration, at the time when size selective mortality favours large fish. Therefore, large acid-exposed fish, which have plasma Na^+ not unlike control fish values (Figure 8) survive after 21 days of acid exposure. This observation would suggest that larger fish have undergone inhibition of gill Na^+-K^+-ATPase by exposure to acid stressed water such as described by Saunders et al. (1983) and Johnston et al. (1984). Larger fish with plasma Na^+ concentrations equal to control fish either a) maintain plasma

Na^+ by mechanisms other than gill Na^+-K^+-ATPase b) undergo less damage to the gill epithelia hence retain Na^+ in acid-stressed water c) have lower surface to volume ratios or d) utilize a yet undisclosed ability to regulate and maintain plasma Na^+ selective to larger fish.

The probable mechanism for Na^+ regulation in acid-stressed smolts of this experiment may be lower surface to volume ratios. The evidence which supports this hypothesis is the observation of Na^+-K^+-ATPase activities which are not unlike control values after 21 days of acid exposure. It would appear from Table III that gill mediated Na^+ acquisition decreases as time increases, however, larger fish which remain after 21 days of acid exposure would experience less loss of plasma Na^+ due to lower surface to volume ratios. Therefore, any inhibitory effects of acid-exposure on gill Na^+-K^+-ATPase would be less detrimental to a larger fish than a small fish undergoing the same treatment.

Survival in seawater after acid exposure was observed in this study to be related to length of acid exposure prior to entry to seawater (Table II). There was also the development of size selective mortality that became apparent during the second seawater challenge test and increased in size selectiveness during the third seawater challenge test favouring the survival of large fish (Figure 2). After 21 days of acid-exposure, fish that survived a seawater challenge test were larger than fish that underwent 21 days of acid exposure and died during the seawater challenge test. Atlantic salmon reared at moderate pH

(4.4) did not develop levels of Na^+-K^+-ATPase activity typical of smolts (Saunders et al. 1983, Jonston et al. 1984). Saunders et al. (1983) observed little salinity tolerance after prolonged exposure to acidified water (pH 4.4), leading to the conclusion that depressed Na^+-K^+-ATPase levels would decrease seawater survivability by failure to ion regulate. They also concluded that the depression of enzyme activity was time related, that is, the longer the acid exposure, the greater the inhibition of Na^+-K^+-ATPase, hence the greater the observed mortality. The inhibitory capacity of decreased environmental pH must have a profound effect upon Na^+-K^+-ATPase activities in anadromous fishes, which is important to seawater adaptation.

The inability of acid-exposed coho smolts to adapt to seawater is dependent upon the duration of acid-exposure. This is reflected by the observations of increased mortality in acid-stressed smolts undergoing seawater challenge tests with increasing acid exposure prior to entry into seawater (Table II). After three weeks of acid exposure, mortalities of acid-exposed fish undergoing seawater challenge tests were almost twice those observed in control fish in seawater (49% vs 25%). Johnston et al. (1984) and Saunders et al. (1983) also recorded higher mortalities in acid-stressed fish than control fish during seawater challenge tests.

The fish that survived a seawater challenge test after 21 days of acid-exposure established a weight dependent relationship with plasma Na^+ levels and gill Na^+-K^+-ATPase

activity. This would imply that larger fish that survive 21 days of acid-exposure and a subsequent seawater challenge test may exhibit initial Na^+-K^+-ATPase inhibition in freshwater acid-exposure but the effect of inhibition on Na^+-K^+-ATPase is transitory. These larger fish were able to recover from any inhibitory effects on Na^+-K^+-ATPase that acid-exposure may entail which then enabled survival in seawater. The mechanism by which a difference in tolerance between smaller fish that died and larger fish that survived in seawater may have been low surface to volume ratios in larger fish. Evidence to support this hypothesis is the establishment of a weight dependent plasma Na^+ concentration whereby fish that survived 21 days of acid exposure and a seawater challenge test had lower plasma Na^+ concentrations on a per weight basis.

V. Summary and Conclusion

It has been demonstrated in this study that episodic acid-exposure of anadromous juvenile coho salmon in soft water may have adverse effects upon ion regulatory mechanisms in fresh and salt water adapted fish. These effects are manifest by changes in gill Na^+-K^+-ATPase activity, plasma Na^+ concentration, hematocrit, plasma GH levels and size selective mortality favouring the survival of larger fish.

The exposure of smolts to a gradient of pH regimens was not found to elicit incremental effects with increasing ambient acidity.

The effects of acid-exposure were more severe upon parrs than smolts and, these effects (Na^+-K^+-ATPase activity plasma Na^+ levels and hematocrit) were observed promptly upon acid-exposure in parrs. These effects remained undetected in smolts until ten to fourteen days of acid exposure.

The mortalities which arose as a result of the acid-exposure of smolts indicated that smaller fish would be removed from existing populations as duration of acid exposure increases. Entry into seawater will accentuate the effects of this size selective mortality. Therefore, seawater exposure after acid exposure increases the severity of size selective mortality whereby only large fish survive extended periods of acid-exposure and entry into seawater.

Larger fish may survive acid-exposure as a result of smaller surface to volume ratios which aid in the regulation of ion loss in freshwater and ion influx in seawater.

Acid exposure of coho salmon in freshwater was found to effect ionoregulation in freshwater and during seawater adaptation. It was concluded that changes in plasma Na^+ resulting from acid exposure were the result of failure in ionoregulatory mechanisms. These mechanisms, including gill Na^+-K^+-ATPase were influenced by fish size, lifestage and length of acid-exposure.

APPENDIX IA

Anova of the effect of pH on plasma [Na^+] for smolts held at pH 4.5, 5.1, 5.5 and 6.2 (Experiment I)

Day 1

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	950.5500	316.8500	5.386	0.0094
Within Groups	16	941.2000	58.8250		
Total	19	1891.7500			

pH 4.5 5.1 5.5 6.3

\- denotes pairs of groups (pH) significantly different at $p<0.05$

Day 3

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	670.0000	223.3333	2.634	0.0854
Within Groups	16	1356.8000	84.8000		
Total	19	2026.8000			

Not significant at P>0.05

Day 7

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	162.1500	54.0500	0.571	0.6422
Within Groups	16	1514.8000	94.6750		
Total	19	1676.9500			

Not significant at P>0.05

Hour 7 (seawater challenge test)

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	492.2797	164.0932	2.348	0.1049
Within Groups	19	1327.6333	69.8754		
Total	22	1819.9130			

Not significant at P>0.05

Hour 15 (seawater challenge test)

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	406.2303	135.4101	1.652	0.2129
Within Groups	18	1475.6333	81.9796		
Total	21	1881.8636			

Not significant at P>0.05

Hour 25 (seawater challenge test)

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	1050.8594	350.2865	1.868	0.1693
Within Groups	19	3562.9667	187.5246		
Total	22	4613.8261			

Not significant at P>0.05

Hour 48 (seawater challenge test)

Variable Sodium

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	12.7286	6.3643	0.073	0.9304
Within Groups	11	964.7000	87.7000		
Total	13	977.4286			

Not significant at P>0.05

APPENDIX IB

Anova of the effect of pH on smolt gill Na^+-K^+-ATPase activity of fish held at pH 4.5, 5.1, 5.5 and 6.3 (Experiment I).

Day 1

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	11.5845	3.8615	2.934	0.0614
Within Groups	18	23.6900	1.3161		
Total	21	35.2745			

Not significant at P>0.05

Day 3

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	1.8525	0.6175	0.773	0.5271
Within Groups	15	11.9875	0.7992		
Total	18	13.8400			

Not significant at P>0.05

Day 7

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	3.4500	1.1500	1.778	0.1918
Within Groups	16	10.3480	0.6467		
Total	19	13.7980			

Not significant at $P>0.05$

Hour 7 (seawater challenge test)

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	7.1383	2.3794	1.749	0.1893
Within Groups	20	27.2067	1.3603		
Total	23	34.3450			

Not significant at $P>0.05$

Hour 15 (seawater challenge test)

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	8.8704	2.9568	1.785	0.1861
Within Groups	18	29.8173	1.6565		
Total	21	38.6877			

Not significant at P>0.05

Hour 25 (seawater challenge test)

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	0.1842	0.0614	0.308	0.8196
Within Groups	16	3.1933	0.1996		
Total	19	3.3775			

Not significant at P>0.05

Hour 48 (seawater challenge test)

Variable Na^+-K^+-ATPase

By Variable PH Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	1	1.6875	1.6875	3.534	0.0895
Within Groups	10	4.7750	0.4775		
Total	11	6.4625			

Not significant at P>0.05

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