STRATEGIES FOR ACID-BASE REGULATION IN FISHES

by

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ABSTRACT

Three sets of *in vivo* experiments were conducted to investigate several aspects of acid-base regulation in fishes. There are two possible ways that involve the gills of fishes in which the acid-base regulation of the extracellular fluid can be adjusted. First, CO_2 excretion can be adjusted by altering gill water flow to increase or decrease the Pco_2 tensions in the blood. The second mechanism would involve the exchange of ions across the gill epithelium to change the concentrations of H^+, HCO_3^- or NH_4^+ in the blood. The first two sets of experiments were, respectively, designed to investigate these two possibilites. The third set of experiments investigated the role that plasma catecholamines might play in regulating the pH of the extracellular fluid as well as the intracellular compartment of the red blood cell.

Experimental manipulation of ventilation in rainbow trout in steady state showed that gill water flow affected CO_2 excretion only at levels lower than about 100ml/min. Carbon dioxide excretion was retarded and blood Pco_2 pressures increased at these levels of gill ventilation. Increasing gill water flow above control levels effected neither O_2 or CO_2 exchange across the gill.

Dogfish, subjected to environmental hyperoxia and various levels of hypercapnia, showed the best correlation between gill

ventilation and plasma pH. There was a very weak correlation with plasma Pco_2 tension and plasma HCO_3^- concentrations did not affect ventilation at all. Gill ventilation increased exponentially as plasma pH declined.

Experiments that involved the fresh water trout and the sea water conger eel showed that water salinity had a direct effect on the acid-base regulation of the plasma. Recovery of plasma pH in both species, after an initial decline in response to exposure to environmental hypercapnia, was dependent on water salinity. The recovery was effected by an increase in plasma HCO_3^- concentration. There was also an associated decrease in plasma Cl^- concentration in both species, indicating the possible involvement of a Cl^-/HCO_3^- exchange process. When carp were exposed to environmental hypercapnia, a reduction in the active uptake of water Cl^-, while maintaining normal efflux rates, caused the reduction of the plasma concentration of this ion. Therefore, it seems that the modulation of this active Cl^-/HCO_3^- exchange process effected the HCO_3^- accumulation in the carp, and probably also in the trout and conger in fresh water.

Consistent with the data from the above carp experiment, further analyses of the electrochemical gradients for Cl^- in trout exposed to environmental hypercapnia at the three salinities showed that active exchange processes must have accumulated the plasma HCO_3^- by the proposed

Cl^-/HCO_3^- mechanism. These analyses also showed that the trout gill was about 2.5 times more permeable to Na^+ than to Cl^- in steady state control conditions. Furthermore, Na^+ is maintained out of electrochemical equilibrium more than Cl^- by a factor of about 1.5 - 2.0. This latter calculation was based on the comparison between the measured plasma concentrations of these ions and the expected concentrations based on a distribution according to the existing electrochemical gradents

Catecholamines are released in trout immediately after acid infusion. This release is proportional to the change in plasma pH relative to control values and functions to maintain the oxygen carrying capacity of the blood which would otherwise be compromised due to the Root shift. This data supports existing data showing that some of the effects which catecholamines have on the physiology of fishes include those which enhance the regulation of the acid-base status of the extracellular and red cell compartments. This data also suggests that the release of catecholamines during burst exercise is due, at least partially, to the excess proton load from the lactacidosis.

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GENERAL INTRODUCTION

INTRODUCTION

Fish have at least three major strategies for regulating the acid-base status of their body fluids that involve the environment. One is the adjustment of the blood Pco_2 tensions and the other two involve the transfer of ions between the fish and water. These are the ion exchange processes across the gill epithelium and those involving the renal system.

Studies on the role of the renal system in acid-base regulation in fishes are few by comparison with those on the roles of ventilation and ion transfers across the gill. The kidney has in some instances been seen as the major compensatory process in acidoses, in others it has been concluded that the kidney plays a negligible role. In summary, the available evidence suggests that the role of the kidney is generally minor relative to the potential of ion exchange processes across the gill epithelium for acid-base regulation (see Heisler 1985).

Data regarding the role of ventilation in correcting acid-base disturbances in fishes are also variable. Some trends are emerging, however, that point to a minor role in regulating acid-base balance in fishes. As suggested by Randall and Cameron (1973), there are several <u>a priori</u> reasons why the regulation of blood acid-base balance through

adjustments in blood Pco_2 would be inappropriate in fishes: 1) a very fine control of blood Pco_2 would be needed to regulate pH due to the log/linear relationship; 2) low Pco_2 tensions in fish blood limit the scope of adjustment; and 3) orientation of ventilation to adjustments of Pco_2 may compromise O_2 uptake, a disadvantageous condition in water where the O_2 content is lower than in air. There is evidence that the stimulation of ventilation during hypercapnia in fishes functions to offset the effects of the Bohr and Root shifts found in teleost blood, which lower the oxygen carrying capacity of the blood in acid conditions (Smith and Jones 1982). Exceptions to this trend are (1) elasmobranchs, which show a similar stimulation of ventilation in response to acid conditions but show no Bohr and Root shifts characteristics in the blood (Lenfant and Johansen 1966) and in the teleost family Cyprinidae in which there is very little stimulation of ventilation in hypercapnic conditions (Dejours 1973) but the blood exhibits both Bohr and Root shifts.

The experiments in Section 1. address two subjects regarding this broad area of ventilation and acid-base regulation in fishes. The first experiment examined the role of gill water flow in CO_2 excretion. Any limitation in this regard would define the scope of adjusting the acid-base status of the blood through changes in Pco_2 tensions by regulating gill water flow. The second experiment addressed

the question of which acid-base parameters might be influencing the stimulation of ventilation in the dogfish, in acid conditions.

A significant role of ion exchange processes in the regulation of acid-base balance in fishes has been demonstrated in most studies of this subject (see review by Heisler 1985). The movement of H^+, NH_4^+ and of HCO_3^- ions across the gill epithelium have been correlated to the movements of Na^+ (Maetz and Garcia-Romeu 1964, Kerstetter et al. 1970, Evans 1977, Payan 1978, Maetz 1973) and to Cl^- (Maetz and Garcia-Romeu 1960, De Renzis and Maetz 1973), respectively. The fact that pH compensation in fish takes longer and is less complete in fish in fresh waters than in waters of greater ionic strength such as the sea water, further implies the existence of a link between ionic groups.

The first two experiments in Section 2 were conducted to study the relationship between water salinity and acid-base regulatory performance. A freshwater teleost was acclimated to higher salinities and a sea water teleost was transferred to less saline waters. Acidotic conditions were induced in both species at the various salinities and changes in acid-base status were followed. The last experiment in Section 2 was carried out to determine unidirectional fluxes of Na^+ and Cl^- in carp, also exposed to environmental hypercapnia. This was carried out in an attempt to understand the mechanisms by which net changes in plasma ion

concentrations observed in fish under similar acid conditions were taking place.

An acidotic condition, like other stressful states in fish, elicits a release of catecholamines (Primmett et al. 1986, Boutilier et al. 1986, Perry 1986). While this increase in catecholamines has potent cardiovascular effects, some of the effects include the modulation of ion transport across epithelia and membranes. Some of those effects, such as the inhibition of Cl^-/HCO_3^- exchange and the stimulation of $Na^+/H^+(NH_4^+)$ exchange known in mammalian tissues have been demonstrated in the fish gill (Perry et al. 1984, Payan et al. 1975, Girard and Payan 1977, Payan 1978).

While a large body of data suggests that the role of ion exchange processes across the fish gill in the regulation of acid-base balance is a major one, most of the evidence is descriptive. Analyses of the trout-salinity-hypercapnia experiment of Section 2. and the experiments in Section 4. address the subject of the possible controlling factors for acid-base regulation in ion transfer processes between blood and water. The functional significance of catecholamines at the gill as well as the red cell membrane are also examined.

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

I. CANNULATIONS

All experimental animals were fitted with chronic indwelling cannulae under anaesthesia in short operations lasting about 10-15 minutes for dorsal aortic cannulations and about 25 minutes for cannulations of the abdominal dorsal aorta through an intestinal artery. All fish were anesthetized with Tricane methane sulphonate (MS222) at concentrations of 1:10,000 to initially render the animals unconscious and then maintained with a concentration of 1:20,000 on the operating table. Both solutions were buffered to about pH 7.5 with $NaHCO_3$. The operating table consisted of a water table with an adjustable netting which held the fish ventral side up. The gills of the fish were continuously irrigated with an anesthetic solution. The temperature of the operating table anesthetic was maintained at the same temperature as that used in the experiments and to which the fish had been acclimated. All syringes, catheters and cannulas were rinsed with heparinized saline prior to use.

Except for the experiments on the shark and conger eel, all fish were fitted with cannulae in the dorsal aorta by blind puncture in anesthetized fish. The technique of Smith and Bell (1964) or the technique of Soivio et.al. (1972) was

used. The size of the cannula (either PE50 or PE60) depended on the size of the fish and is specified in the Materials and Methods of each section.

In the first technique, a catheter (Sovereign Indwelling Canine Catheter 2 inch, 18 gauge) was used to make the blind puncture through the midline of the roof of the mouth, between the first and second gill arches and into the dorsal aorta. After the puncture was made, the metal needle in the catheter was removed and a polyethylene cannula was fed down the catheter into the aorta. The plastic catheter was then removed leaving the cannula chronically implanted in the vessel. The cannula was fed through a piece of PE200 cannula flanged on one end to anchor it in the mouth and which passed through the roof of the mouth via a hole in front of the nares. Ties with cotton or silk thread were made around the PE200 with the indwelling cannula just outside the fish to add another level of security to the cannula.

The second method involved the blind puncture of the dorsal aorta using a sharpened steel wire inserted into a length of cannula so that just the tip of the wire protruded from the cannula. With the same orientation for entry as the first method, the wire functioned to guide the cannula and to enter the wall of the dorsal aorta. The wire was removed and the cannula was advanced down the aorta between 5 and 7 centimeters depending on the size of the fish. This indwelling cannula was guided out of the fish in an identical

manner as above. However, since this cannula was necessarily short due to the inserted wire, a connector was used to attach it to a longer cannula to facilitate sampling.

In the experiments which involved the shark and the conger eel the dorsal aorta was cannulated through a gastric or intestinal artery. The body cavity was opened by a 3-4 cm long mid-ventral incision and a PE50 catheter was introduced into the gastric or intestinal artery and advanced through to the dorsal aorta. This cannula was securely tied to the vessel, was cut and fitted to a thick-walled PVC tubing (1mm i.d., 1.8mm o.d.) through which blood was sampled. The body wall was closed by two layers of sutures and the cannula was led out of the body cavity by another small incision caudal to the larger incision. Further details of this surgery are given by Toews et.al. (1983).

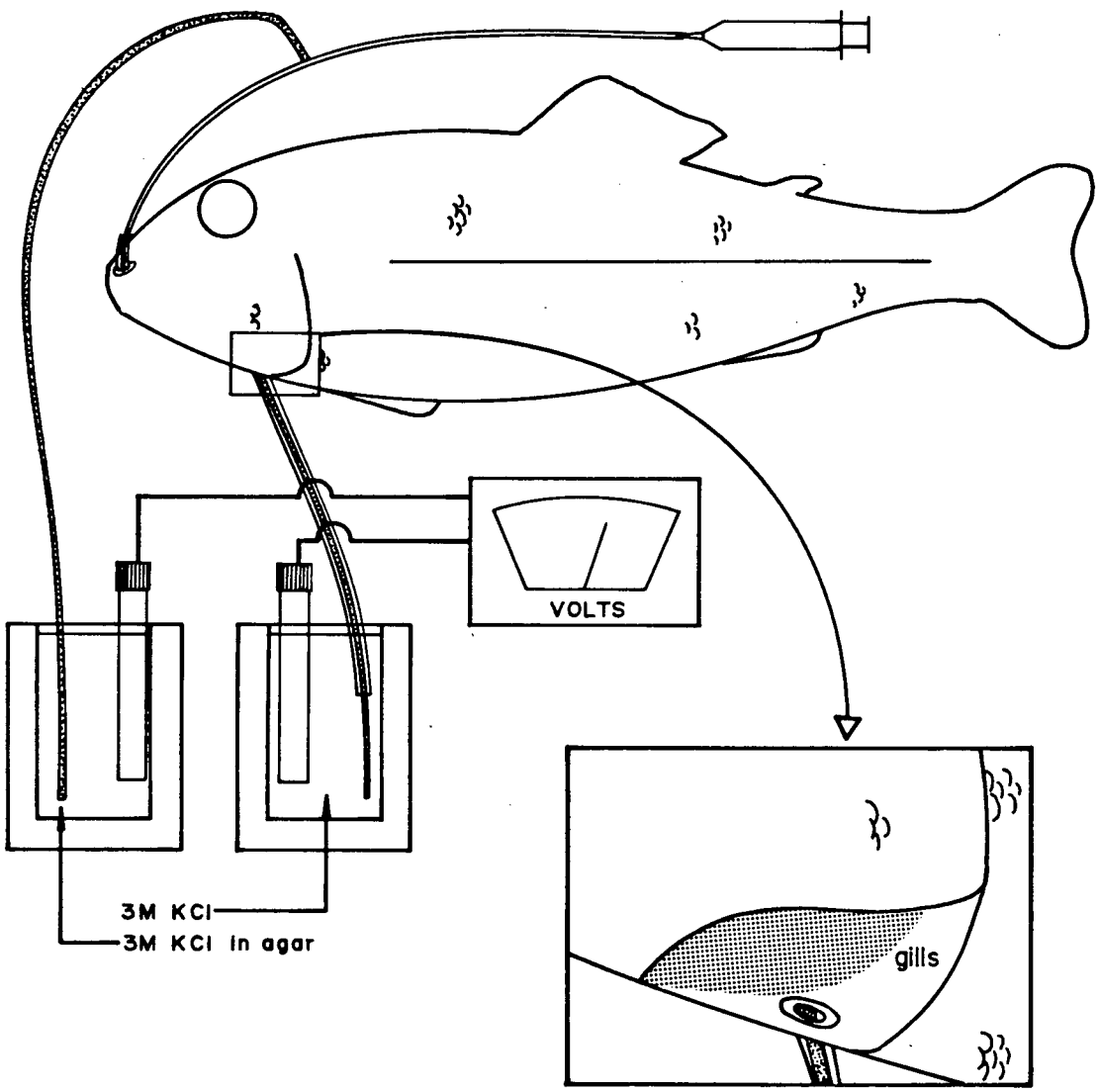
Following surgery, all animals recovered in perspex boxes with ample flow of aerated water. Cannulae were flushed daily with Cortland saline (Wolf 1963) containing 10,000 USP units/L sodium heparin. All animals were allowed to recover for at least 24h prior to starting experimental procedures.

II. MEASUREMENT OF TRANSEPITHELIAL POTENTIALS (TEP)

TEP was measured across the gill epithelium using pairs of calomel or silver-silver chloride electrodes. The reference

Figure G1. Apparatus for measurement of transepithelial potentials(TEP). Stipled 'bridge' cannulae contained 3M KCl set in agar. One was in contact with the cannulae in the dorsal aorta (clear) through a 'T' piece. The other reference was in contact with the water near the gills. The insert shows a close-up of the orientation of the reference bridge with part of the opercular cover cut away. The reference bridge was threaded through another cannula of larger diameter which was flared on one end to anchor it inside the opercular cavity.

APPARATUS FOR TEP MEASUREMENTS



and measuring electrodes were connected to the water and blood by PE50 cannulae filled with 3M KCL set in agar. The diagram below describes the apparatus. The reference electrode was placed near the gills by threading it through a larger diameter cannula which was sewn in place just inside the operculum. The measuring electrode was in contact with the blood through the saline which filled the indwelling cannula. There was no detectable leak of KCl into the blood. The "T" piece allowed the connection of the blood to either the measuring electrode or to the syringe which was used for blood collection or infusion procedures. The potential across the electrodes was measured using voltmeters which are specified in the Materials and Methods of indiviual experiments. The voltmeters were zeroed by shorting the circuit by connecting the electrodes with a short cannula containing the 3M KCL described above. The zero voltage used for the determination of the TEP value was that read when both reference and measuring electrode cannulae were placed in the water together. This offset voltage was smaller than 1 mV in all cases.

III. MEASUREMENT OF ACID-BASE PARAMETERS

A. Constants : The apparent first dissociation constant (pKapp) of carbonic acid and the solubility of carbon dioxide

(CO_2) determined by Boutilier et.al. (1985) were used.

B. pH : All measurements were made at the temperature of the animal. pH was measured on whole blood and red cell lysates using a Radiometer G279/G2 glass capillary electrode and K497 calomel electrode coupled to a PHM 84 pH meter. The red cell lysates were obtained by centrifuging whole blood to obtain the red cell fraction which was then twice frozen and thawed as described by Zeidler and Kim (1977). Minor deviations from this procedure are noted in the Materials and Methods sections of individual experiments. Radiometer precision phosphate buffers S1500 and S1510 were used in calibrations. Readings were referenced to the S1510 buffer and adjustments were made by adding or subtracting one half of the drift in the measured buffer value.

C. Total CO_2 : Total CO_2 was measured in water and blood samples using one of two methods which are specified in the Materials and Methods of each experiment. Standards for both techniques were made with dried $NaHCO_3$. The following procedure was employed when the Capni-Con III(Cameron Instruments Inc., Port Aransas, Texas) was used. This is a technique where HCO_3^- is converted to CO_2 by acidification and measurement is made of the change in Pco_2 (Cameron 1971). Plasma samples were taken from hematocrit tubes immediately after determination. When a CO_2-specific

gas chromatograph (Carle Model III, Carle Instruments Inc. U.S.A.) was used, the gaseous CO_2 evolved from the acidification of the sample was analysed by differentialthermal conductivity. Peak heights on the output recorder were referenced to standards (Lenfant and Aucutt 1966).

D. Pco_2 : Pco_2 values either were calculated using the Henderson/Hasselbach equation or measured with Radiometer electrodes and meters according to the recommendations of Boutilier et.al.(1978,1985).

E. HCO_3^- : HCO_3^- values for plasma samples were calculated by the equation,

$$HCO_3^- = TCO_2 - Pco_2 * (\text{solubility of } CO_2)$$

F. NH_4^+/NH_3 : Plasma total ammonia concentrations were determined by L-glutamic dehydrogenase/NAD enzymatic assays from either Sigma (Sigma Diagnostics U.S.A.) or Boehringer-Mannheim (Boehringer Mannheim Gmbh Diagnostica F.R.G.). Water total ammonia concentrations were determined by these methods or by an ammonia electrode as described below in V. for experiments that involved a Delta Bicarbonate System.

IV. ION CONCENTRATIONS

Plasma and water Na^+, K^+, Mg^{++} and Ca^{++} concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer Model 2380) as described byAnnio (1964). Chloride concentrations for water and plasma were determined by one of three methods which are specified in the description of individual experiments. In some experiments, plasma and water chloride concentrations were determined with a Buchler Cotlove chloride titrator according to the method of Cotlove (1963) using appropriate standards. In other experiments plasma and water (when concentrations were greater than 100mM) chloride concentrations were determined by titration using a Radiometer CMT10 Titrator. Water chloride concentrations were determined in some experiments with a system consisting of a solid state Cl^- sensitive electrode and reference connected to a microprocessor/ion analyser and was calibrated with NaCl standards and referenced to a particular standard between each measurement.

V. DELTA BICARBONATE SYSTEM

A closed water recirculation system such as that described

by Heisler et.al. (1976) was used for Salinity-Trout, Salinity-Conger and the Na^+-Cl^--Carp experiments. Variations of this system which were unique to those experiments are described in the Materials and Methods sections of the respective experiments. Such a system typically consisted of a fish box, an oxygenator and bubble trap system, and a water circulation pump. This system wasclosed for all non-volatile substances but open for gas exchange. The main advantage of such a system was that changes in ion concentrations due to exchanges between fish and water accumulate to levels that can be measured accurately . The potential problem of the build-up of toxic waste products such as ammonia was avoided by flushing the system with fresh water thermostatted to the temperature of the system every 24h.

This system allowed the continuous measurement of net HCO_3^- changes and an automated way to measure NH_4^+ concentrations in the water. Water from the fish chamber was continuously pumped to a pH glass electrode and a double electrolyte bridge (Ag/AgCl) reference electrode after equilibration with 1% CO_2 and pumped back to the fish chamber. All electrodes were acclimated to the particular salinity of the experiment for at least 3 weeks. The electrodes were connected to a high impedance isolation amplifier (Knick, Berlin F.R.G.) and the resulting signal was output to a recorder. At programmed intervals water was

pumped from the fish chamber along with a strong base from another reservoir to an ammonia electrode (Ingold Electrodes Inc., Lexington Mass. U.S.A.). The flow and base concentration were adjusted so that the resulting mixture had a pH of about 10. The base converted all NH_4^+ to physically dissolved ammonia which was sensed by the electrode. The signal from the electrode was amplified and filtered and the resulting peak heights on the output recorder were referenced to standards made from NH_4Cl.

VI. GASES

All gas mixtures were made up from pure gases or air mixed with Wostoff (Bokum, F.R.G.) gas mixing pumps.

SECTION 1.

VENTILATION AND ACID-BASE REGULATION IN FISHES

INTRODUCTION

Ventilation in fishes is stimulated by acid-base disturbances. This has been shown for fish exposed to acid waters (Hargis 1976; Hoglund and Persson 1971; Dively et al. 1977), for fish infused with acid or base (Cunningham 1974; Janssen and Randall 1975) and for hypercapnic fish (Van Dam 1938; Randall and Jones 1973; Janssen and Randall 1975; Eddy 1976; Smith and Jones 1982). This effect is also found in a wide range of species including elasmobranchs, cyclostomes and teleosts. Evidence in the literature suggests that the predominant cause in the responses by these species may be different.

The stimulation of ventilation in fish under acid conditions suggests that the stimulus is related to a need to alter either oxygen uptake or carbon dioxide excretion. There is a large body of data suggesting that the increase in ventilation in teleosts during acid conditions is due to a stimulus to increased oxygen extraction since Bohr and Root shifts would result in a lower oxygen carrying capacity of the blood by reducing the oxygen-hemoglobin affinity. Smith and Jones (1982) have shown that the increased ventilation in trout exposed to environmental hypercapnia was attentuated by increasing the oxygen content of the water. This strongly

suggests that in those species showing Bohr and Root shifts,the stimulation of ventilation is oriented to increasing oxygen uptake from the water. There are two exceptions to this, one the elasmobranch, the blood of which shows no Bohr or Root shift characteristics (Lenfant and Johansen 1966), but ventilation is stimulated in response to hypercapnic conditions (Randall et al. 1976; Heisler et al. 1976). The other is members of the family Cyprinidae which show little change in ventilation in spite of the blood showing evidence of Bohr and Root shifts (Dejours 1983).

Randall and Cameron (1973) have suggested three a priori reasons against the other possible role of increased ventilation in response to hypercapnia, that is the excretion of CO_2 and the consequent regulation of blood Pco_2 tension. First, the log/linear relationship of Pco_2 to pH would require very fine control of Pco_2 tensions to control pH. Second, the scope of adjustment is limited by the low CO_2 content of fish blood. Third, the orientation of ventilation to adjustments of Pco_2 may compromise O_2 uptake; a condition which is disadvantageous to life in water where the O_2 content is lower than air.

The two experiments described in this Section were designed to investigate two aspects of this broad subject of ventilation and its role in acid-base regulation in fishes. The first experiment investigated the actual scope of adjusting blood CO_2 tensions by altering gill water flow in

trout. Wood and Jackson (1980) estimated the capacity of Pco_2 adjustments via changes in gill water flow to be about 2 mmHg in that species. Gill water flow was experimentally manipulated and CO_2 excretion was measured directly in order to describe the relationship between these parameters over a range of ventilation volumes.

The second experiment in this Section investigated the control of ventilation in the larger spotted dogfish. The lack of Bohr and Root shifts in the blood of this elasmobranch emphasizes the possibility that the control of ventilation might be oriented towards some parameter or set of parameters other than the maintenance of O_2 saturation of the blood. A possibility is the regulation of pH, Pco_2 tensions or HCO_3^- levels in the blood by adjustments of blood Pco_2 via changes in gill water flow. Direct measurement of water flow over the gills of the dogfish during simultaneous exposure to environmental hyperoxia and varying levels of hypercapnia enabled the correlation of gill ventilation with the three acid-base parameters in the blood.

MATERIALS AND METHODS

EXPERIMENT 1A. VENTILATION AND CO_2 EXCRETION IN TROUUT

FISH :

Rainbow trout, Salmo gairdneri, between 192 and 353 g were obtained from a commercial hatchery and maintained in outdoor fiberglass tanks supplied with aerated and dechlorinated Vancouver tap water (5-10°C; pH 6.9-7.1; $CaCO_3$ 4 ppm). They were maintained on a dry trout pellet fed ad libitum with a self feeder. Fish were starved for at least 48 h prior to surgical procedures and subsequent experimentation.

SURGERY, APPARATUS AND EXPERIMENTAL PROCEDURES :

Fish were fitted with chronic indwelling dorsal aortic cannulas by the method of Smith and Bell (1964)(see General Materials and Methods) and with rubber Van Dam masks according to the method described by Cameron and Davis (1970). After the operation each fish was inserted into a narrow black box in one part of a two chambered perspex box. The rubber mask, which was attached to the mouth and snout of the fish, was secured to the divider between the two chambers and acted as a dam that insured all water passing from the front chamber to the back chamber was via the mouth and gills of the fish.

Inspired and mixed expired waters were thus separated and ventilation volumes were influenced by adjusting the water height in the inspired water chamber anterior to the fish. This was done by adjusting the height of the front chamber overflow standpipe relative to that in the posterior chamber, which carried the overflow to waste. Positive, zero and

negative pressure heads from the mouth to opercular chambers were effected in this way.

A period of 24 h followed the setting of any pressure head before measurements were taken. Changes in head pressure were always from positive to negative although the magnitude of change and the number of changes imposed per fish were not consistent.

MEASUREMENTS :

Ventilation volume ($\dot{V}g$) was determined by collecting the outflow water from the standpipe in the chamber containing the trunk of the fish. Collections were made over 1 min periods and volumes were determined by weight. Concurrent measurements of ventilatory frequency (f) were made by counting opercular movements through a small clear section in the black box containing the fish. Several counts per minute were averaged. Stroke volume of the buccal pump ($\dot{V}sv$) was calculated as $\dot{V}g$ divided by f.

Inspired (Pi_{O2}) and mixed expired (Pe_{O_2}) water oxygen tensions as well as arterial oxygen tensions were measured with Radiometer oxygen electrodes thermostatted to the experimental water temperature. Total oxygen content (Ca_{O2}) of the blood was measured with a Lex-O_2-Cont apparatus (Lexington Instr.).

Plasma CO_2 tension (Pco_2) was measured with a

Radiometer electrode thermostatted to the experimental temperature according to the recommendations of Boutilier et al. (1978). Measurements of total CO_2 in the inspired ($Cico_2$) and mixed expired ($Ceco_2$) water samples as well in plasma samples were made by gas chromatography according to the method described in General Materials and Methods.

Hematocrit was measured according to the method of Snieszko (1960). Gas transfer rates ($\dot{M}o_2$ and $\dot{M}co_2$) were calculated by the Fick principle. pH of the extracellular (pHe) and red cell intracellular (pHi) fluids were measured as described in General Materials and Methods. Concentrations of the catecholamines, adrenaline and noradrenaline of 11 plasma samples taken from fish at several head pressures were determined by high pressure liquid chromatography (HPLC) according to Woodward (1982). Whole blood lactate concentrations were assayed enzymatically with Sigma reagents (Sigma bulletin no. 826-UV).

STATISTICS :

Correlation and regression analyses were used to describe the relationships between parameters in this experiment. Analysis of variance (ANOVA) was used to compare the statistical significance among means with a 5 % level of rejection.

EXPERIMENT 1B. SHARK - HYPEROXIA - HYPERCAPNIA

ANIMALS :

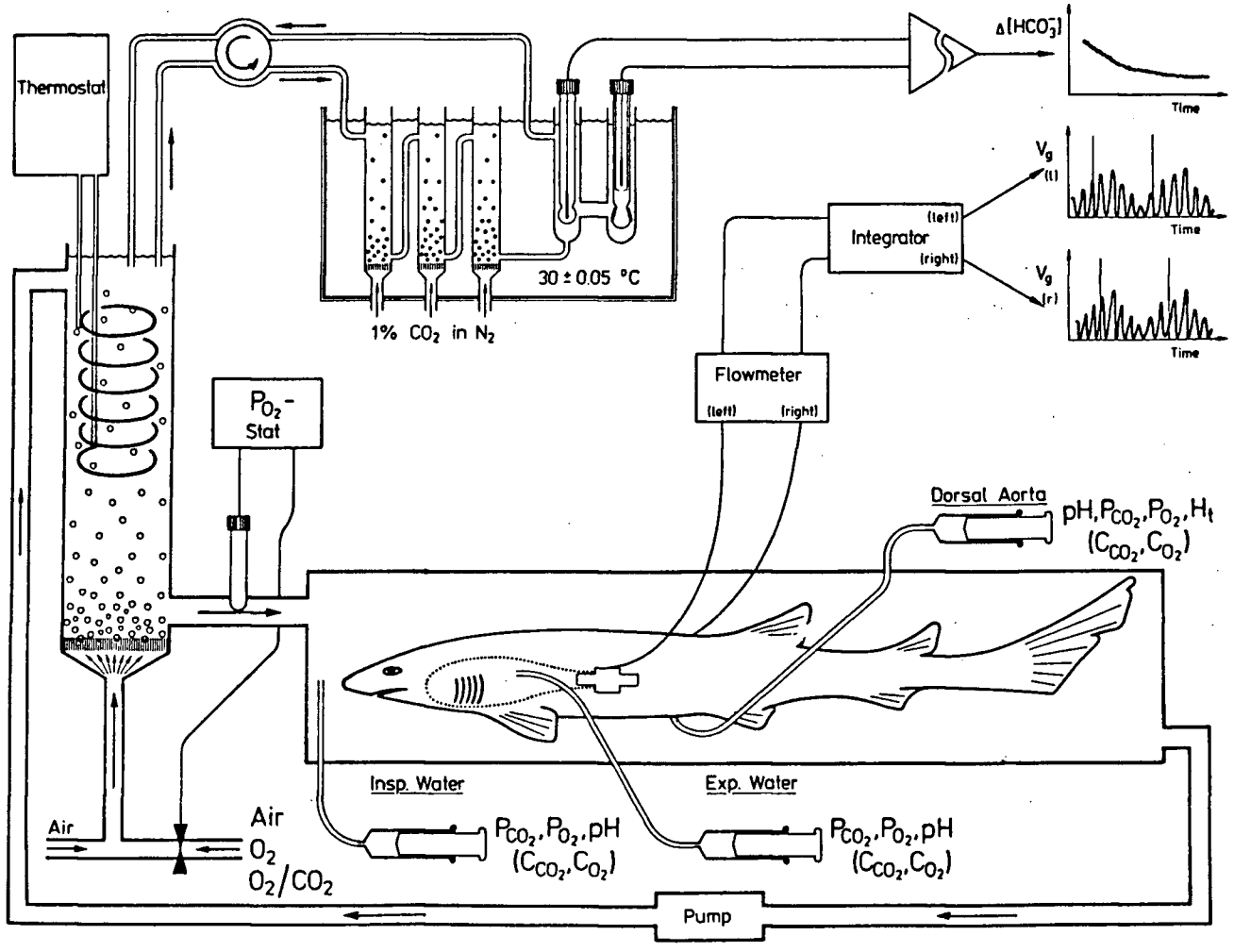
Larger spotted dogfish, Scyliorhinus stellaris, weighing between 1550 to 2820 g were caught in the Bay of Naples, Italy. They were held in 200 l fiberglass tanks in the laboratory without feed until they were used for experimentation. The tanks received a continuous flow of ambient sea water (pH 7.9; [HCO_3^-] 2.3 mM). Water temperature was maintained at 19°C.

SURGERY AND APPARATUS :

A chronic indwelling cannula was inserted into the dorsal aorta through an intestinal artery as described in General Materials and Methods. Latex rubber bags were fashioned from the thumbs of surgeons gloves and attached over the gill slits to collect the expired water. Electromagnetic flow probes were connected to the ends of these bags to measure the ventilation volume. The animals were recovered in a 'Delta Bicarbonate System' (Fig. 1) described in General Materials and Methods and in detail in Heisler (1978). There was a recovery period of 24 h experimental procedures were initiated.

PROTOCOL AND MEASUREMENTS :

Figure 1. Diagram of experimental apparatus used to conduct respiratory and acid-base regulatory experiments in the shark, Scyliorhinus stellaris. See text for details.


Thermostat
30 ± 0.05 °C
1% CO_2 in N_2
$\Delta[HCO_3^-]$
Time
V_g (l)
Time
V_g (r)
Time
Integrator
(left)
(right)
Flowmeter
(left)
(right)
P_{O_2}-Stat
Dorsal Aorta
pH, P_{CO_2}, P_{O_2}, H_t
(C_{CO_2}, C_{O_2})
Insp. Water
Exp. Water
Air
Air
O_2
O_2/CO_2
P_{CO_2}, P_{O_2}, pH
(C_{CO_2}, C_{O_2})
P_{CO_2}, P_{O_2}, pH
(C_{CO_2}, C_{O_2})
Pump

After 3 control samples were taken, the ambient Po_2 tension was elevated to 500 mmHg of atmospheric pressure. After a period where only the oxygen tension was increased, the Pco_2 tension of the ambient water was also raised to various levels for each fish to induce a range of acidoses and consequent range in blood pH, Pco_2 and HCO_3^- levels. The hypercapnia levels ranged from 7 to 44 mmHg. Approximately 700 ul of blood was removed throught the cannula at each sample time for the direct measurement of blood pH, Pco_2 and Tco_2. Blood HCO_3^- concentrations were calculated according to the methods described in General Materials and Methods. Blood samples were taken at 30 min, 1 h and 2 h after the onset of hyperoxia and every 30 min during the combined hyperoxia/ hypercapnia exposure

Two additional experiments were carried out. The protocol and sampling procedures were identical to that for the above experiment. In the first, 0.3 M $NaHCO_3$ or 0.6 M HCl was infused through the cannula with each elevation in Pco_2 in order to minimize the changes in blood pH. In the second additional protocol, the water Pco_2 was elevated to only one level. The natural accumulation of blood HCO_3^- was supplemented by infusion of $NaHCO_3$ through the cannula.

STATISTICS :

Correlation analyses were used to describe the

relationships between data sets.

RESULTS

EXPERIMENT 1A. : ROLE OF VENTILATION IN ACID-BASE REGULATION

All animals were quiescent and in steady state with respect to gas exchange. The mean plasma adrenaline concentration of 11 samples from 5 fish experiencing various pressure heads was 2.6 $\pm$ 0.42(mean $\pm$ 1S.E.) nanomoles/l, a value consistent with resting levels for fish. Blood lactate concentrations of 8 animals in Van Dam boxes were also consistently low, 0.58 $\pm$ 0.02(means $\pm$ 1S.E.) mM, and did not vary significantly with the changes in head pressure.

The imposed pressure heads effected significantly different ventilation volumes, although there was considerable overlap in the ranges of $\dot{V}g$ at negative, neutral and positive heads (Table 1). As shown by others, fish varied stroke volume of the buccal pump rather than frequency of breathing to adjust ventilation volume (Fig. 2) in the face of imposed pressure heads (Van Dam 1938; Davis and Cameron 1971; Randall and Jones 1973).

The relationship between $\dot{V}g$ and arterial Pco_2 was an inverse power function (Fig. 3). Vg and arterial Po_2 showed the reverse relationship.

Table 1. Gill ventilation volumes ($\dot{V}g$) of fish experiencing various head pressures in a Van Dam apparatus.

RANGE OF HEAD PRESSURES(mm)	RANGE OF $\dot{V}g$ (ml/min)	MEANS ± STD.ERR.		
-8 to 0	26.8 to 131.3	56.28 ± 6.95	n.s.	sig.
0	42.2 to 139.9	75.13 ± 12.27	n.s.	
0 to +21	48.4 to 342.4	145.92 ± 19.39		

n.s. not significantly different by ANOVA ; $P<.05$
sig. significantly different by same test

Figure 2. The relationship between breathing frequency (f), the volume of water pumped each breath or stroke volume ($\dot{V}_{sv}$) and the volume of water flowing over the gills ($\dot{V}g$) of trout with a Van Dam apparatus. Best fit linear regression lines :

$$\dot{V}sv = 0.0892 + 0.1456 * \dot{V}g;\ r^2 = 0.9211;\ N = 42$$
$$\dot{V}sv = 288.145 - 0.7093 * f;\ r^2 = 0.0025;\ N = 39$$

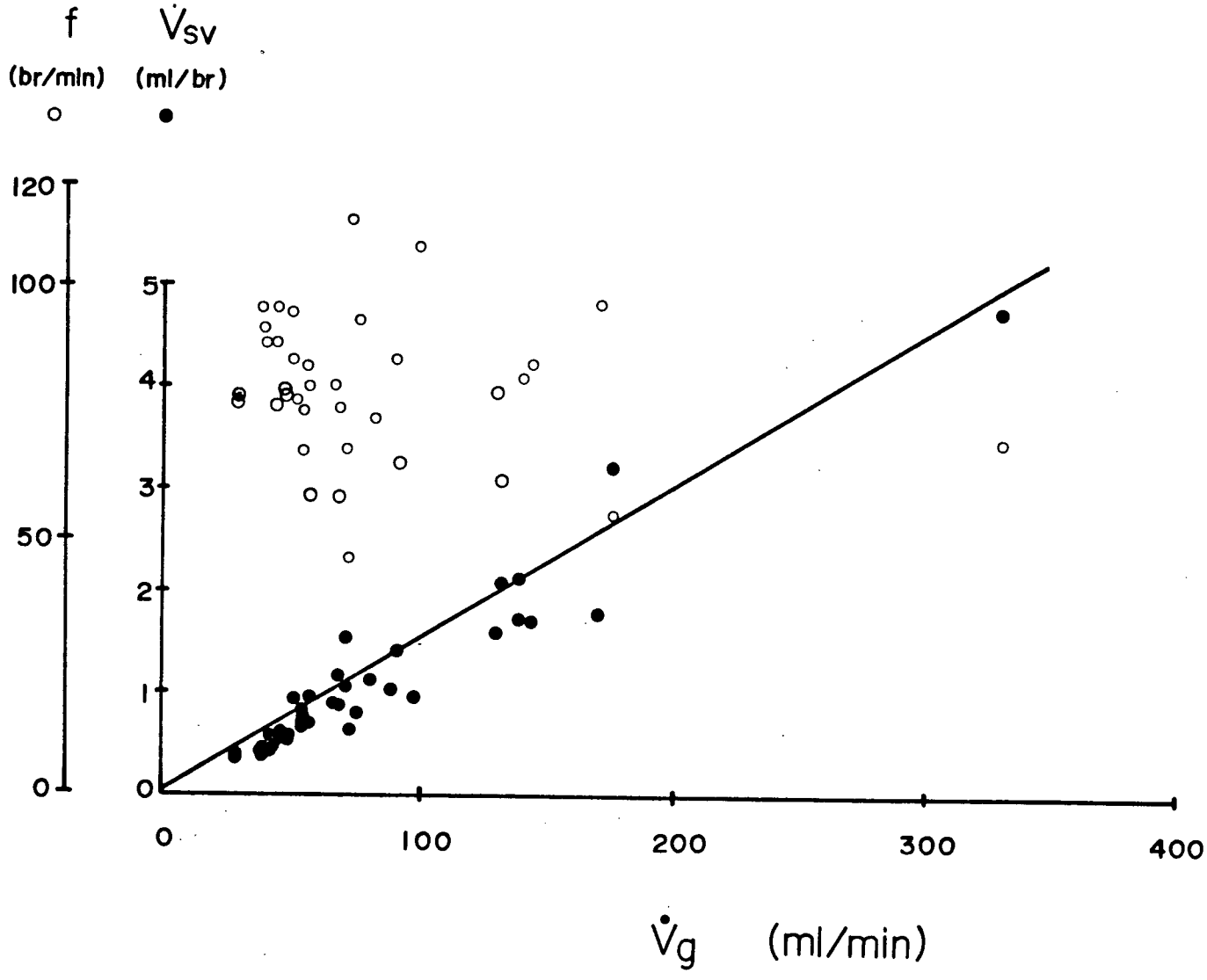
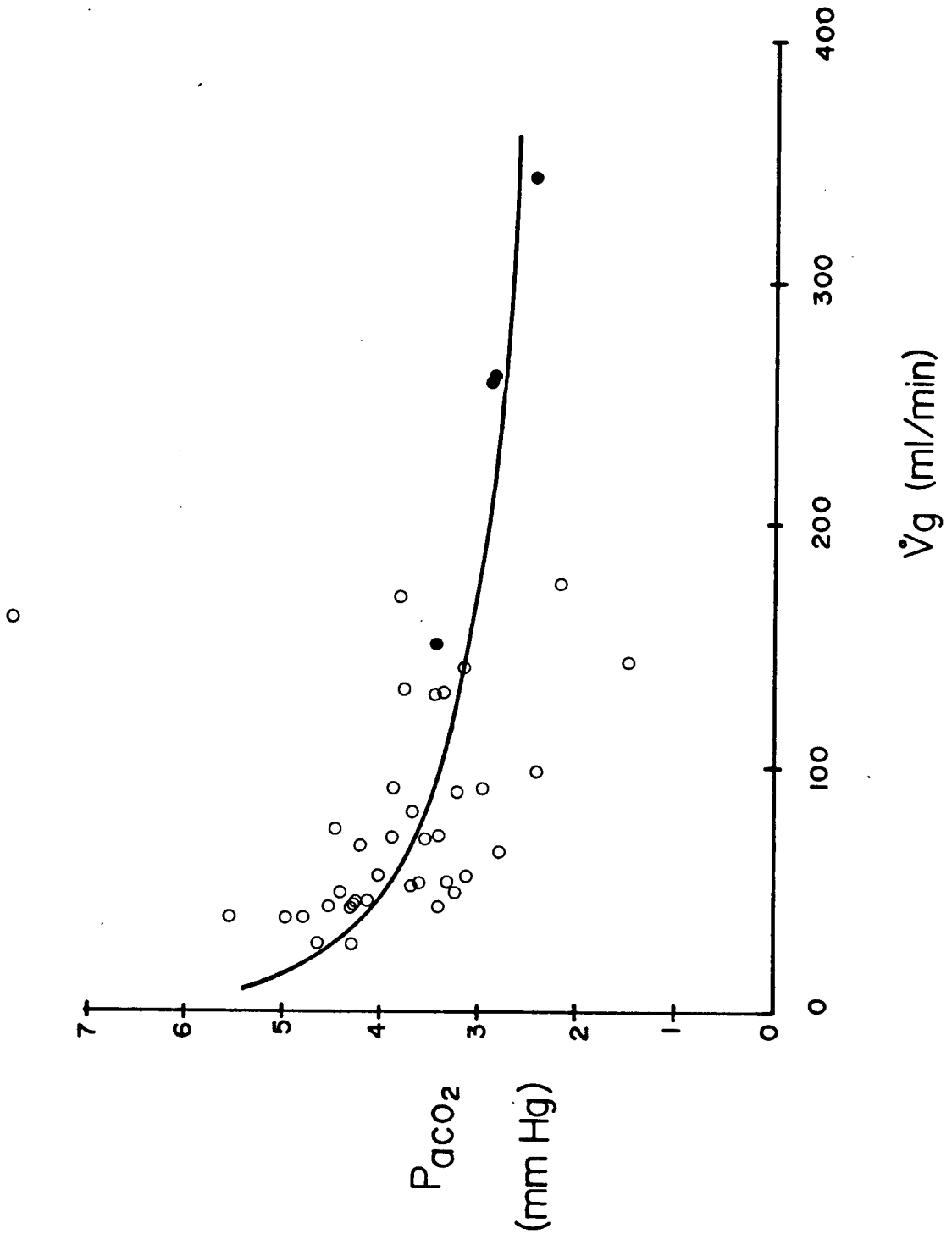

f
(br/min)
$\dot{V}_{SV}$
(ml/br)
120
100
50
0
5
4
3
2
1
0
0
100
200
300
400
$\dot{V}_g$ (ml/min)

Figure 3. The relationship between arterial Pco_2 and ventilation volume ($\dot{V}g$) over the range of ventilation volumes imposed on trout in this study. Black dots represent fish which ram ventilated. Best fit power curve : $Pco_2 = 8.95*\dot{V}g^{-0.209}$; $r = 0.484$; $N = 43$.


7
6
5
4
3
2
1
0
0
100
200
300
400
P_{aCO_2} (mm Hg)
$\dot{V}g$ (ml/min)

$$Pco_2 = 8.95 * \dot{V}g^{-0.209} \quad r = 0.484, N = 43$$

$$Po_2 = 27.66 * \dot{V}g^{0.292} \quad r = 0.469, N = 31$$

There was a significant negative correlation between Po_2 and Pco_2 : r = -0.784, N = 31 (Fig. 4).

CO_2 excretion, $\dot{M}co_2$, showed a weak but significant negative correlation with arterial Pco_2 : r = -0.333, N = 40 . Higher convection requirements , $\dot{V}g/\dot{M}co_2$, were associatd with lower Pco_2 values (Fig. 5). At low levels of $\dot{V}g$, both $\dot{M}o_2$ and $\dot{M}co_2$ decreased with gill ventilation volume. The mean respiratory gas exchange ratio, RQ, was 0.87 $\pm$ 0.04(means $\pm$ 1S.E.). Linear regression analysis of these parameters yielded a slope of 0.012 and r^2 = 0.637 (Fig. 6).

EXPERIMENT 1B. ROLE OF pH IN VENTILATION

Given sufficient oxygen, an elevation of environmental Pco_2 results in a plasma acidosis. Blood Pco_2 and HCO_3^- are elevated and as a consequence pH drops; gill ventilation increases as well.

The increase in ventilation is best correlated with changes in plasma pH (Fig. 7, 8 & 9). $\dot{V}g$ increases exponentially as plasma pH decreases (Fig. 10). The elimination of the pH change during this exposure to hypercapnia, causes a considerable decrease in the correlation between Pco_2 and $\dot{V}g$ (Fig. 11)as well as between $[HCO_3^-]$ and $\dot{V}g$ (Fig. 12).

Figure 4. The relationship between arterial Pco_2 and arterial Po_2 over the range of ventilation volumes imposed on trout in this study. $Pco_2 = 5.660 - 0.019*Po_2$; $r^2 = 0.6150$; N = 31.

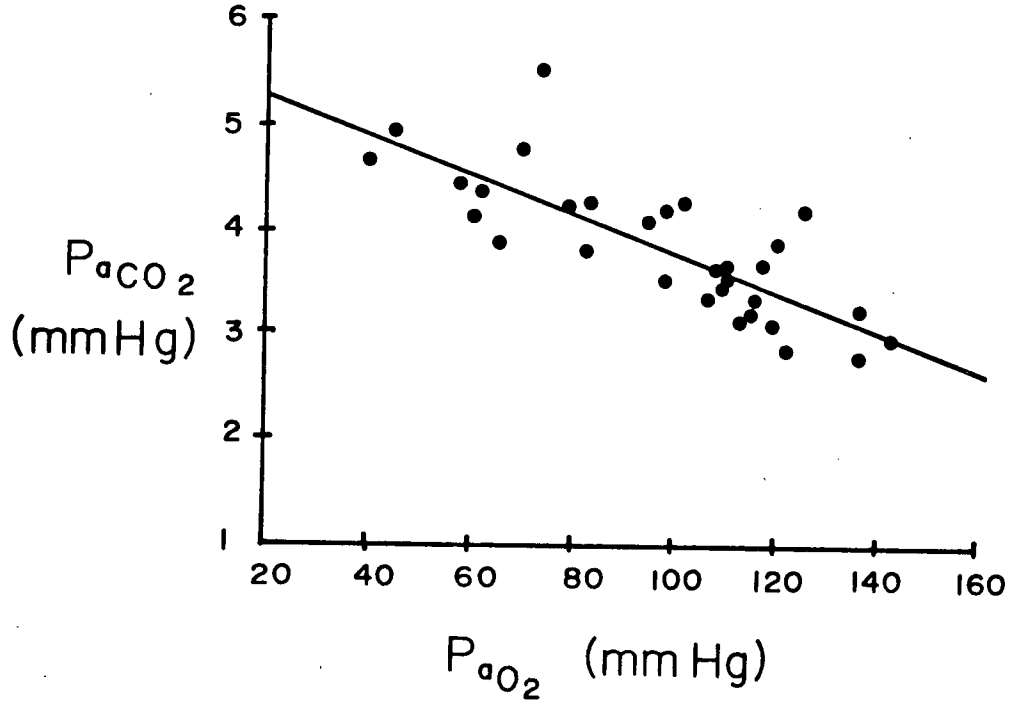

6
5
4
3
2
1
P_{aCO_2}
(mmHg)
20
40
60
80
100
120
140
160
P_{aO_2} (mm Hg)

Figure 5. The relationship between the convection requirement for CO_2 ($\dot{V}g/\dot{M}co_2$) and arterial Pco_2 over the range of ventilation volumes imposed in this study. ($\dot{V}g/\dot{M}co_2 = 3.165*Pco_2^{-0.477}$; r = 0.333; N = 40).

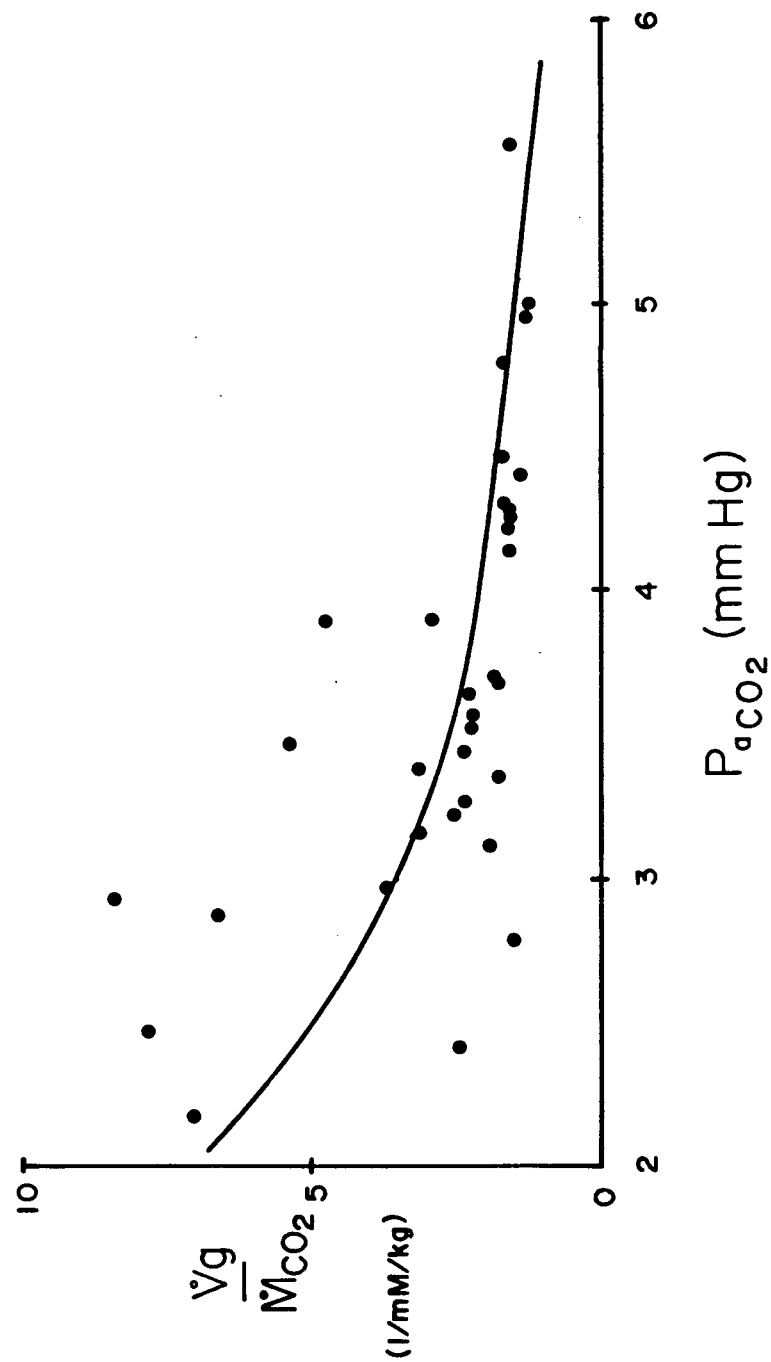

10
5
0
2
3
4
5
6
$\frac{\dot{V}g}{\dot{M}_{CO_2}}$
(l/mM/kg)
P_{aCO_2} (mm Hg)

Figure 6. The relationship between CO_2 excretion ($\dot{M}co_2$) and O_2 uptake ($\dot{M}o_2$) at different ventilation volumes. The gas exchange ratio was 0.87 $\pm$ 0.04. $\dot{M}o_2 = 0.298 + 1.012*\dot{M}co_2$; $r^2 = 0.637$; N = 42.

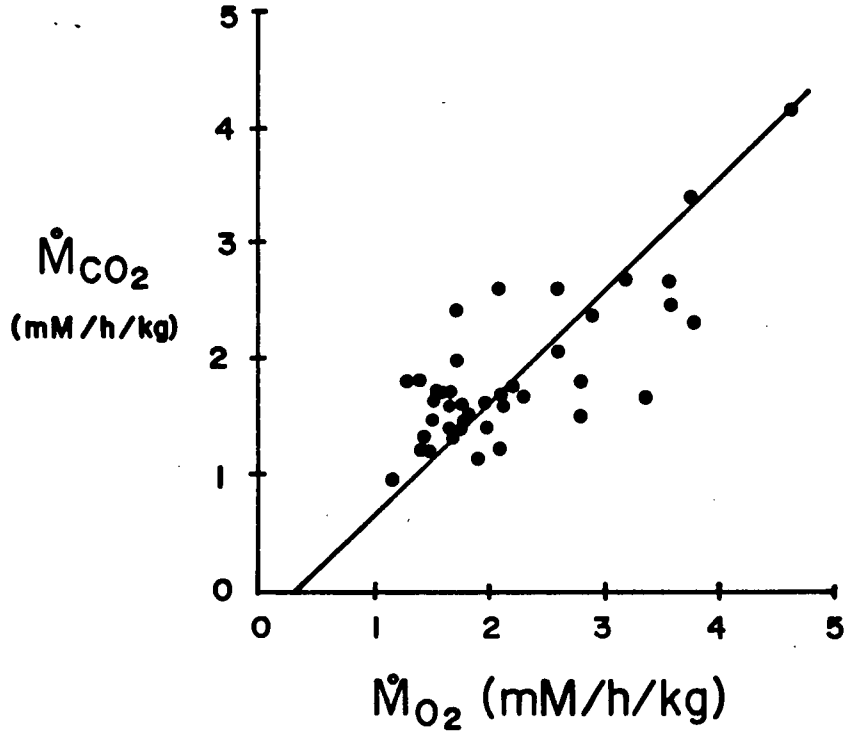

$\dot{M}_{CO_2}$
(mM/h/kg)
5
4
3
2
1
0
0
1
2
3
4
5
$\dot{M}_{O_2}$ (mM/h/kg)

Figure 7. The relationship between ventilatory volume and plasma pH (pHpl) in Scyliorhinus stellaris undergoing simultaneous exposure to environmental hyperoxia and hypercapnia. Ventilatory volume ($\dot{V}g$ act./$\dot{V}g$ contr.) is the ratio of the measured $\dot{V}g$ divided by the $\dot{V}g$ during hyperoxia. Each line represents the best fit line for all the experimental data for an individual fish. The data set shown includes two experiments. One is the combination of environmental hyperoxia and hypercapnia exposure alone. The other has the added treatment of $NaHCO_3$ or HCl infusion to keep the pH 'constant', or to minimize the change in pH.

Coefficients for each line :

$$\dot{V}g \text{ act.}/ \dot{V}g \text{ contr.} = a * e^{(b * pH)}$$

a	b	r^2
$5.3996 * 10^{15}$	-4.9322	0.950
$0.2600 * 10^{9}$	-2.4640	0.841
$5.7770 * 10^{6}$	-2.1344	0.792
$2.2580 * 10^{10}$	-3.2478	0.636
$4.7401 * 10^{10}$	-3.3712	0.696
$2.3955 * 10^{12}$	-3.8865	0.963
$1.3504 * 10^{21}$	-6.5133	0.934
$2.2187 * 10^{11}$	-3.4635	0.846
$1.1901 * 10^{11}$	-3.4600	0.830
$2.2668 * 10^{13}$	-4.2010	0.978
$2.8936 * 10^{11}$	-3.5686	0.833
$2.2743 * 10^{14}$	-4.4154	0.956
$3.1797 * 10^{11}$	-3.6072	0.860

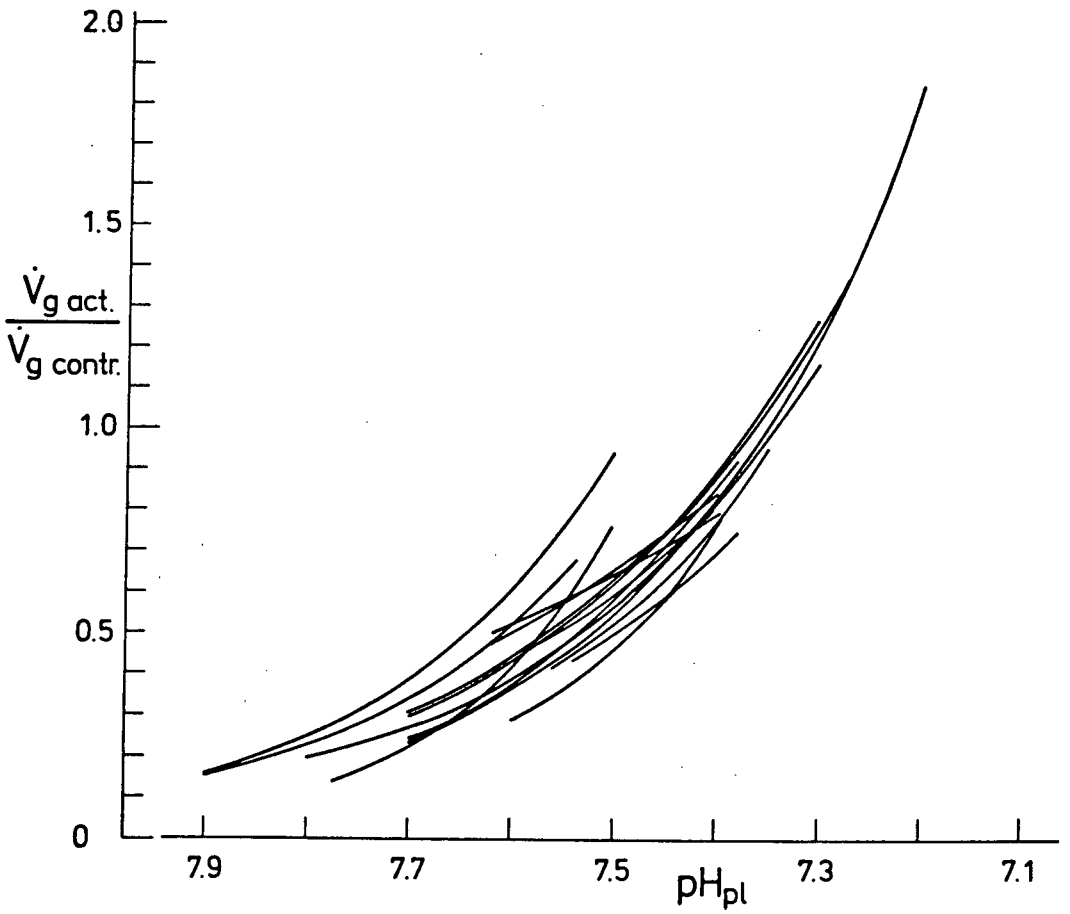

2.0
1.5
$\frac{\dot{V}_{g\ act.}}{\dot{V}_{g\ contr.}}$
1.0
0.5
0
7.9
7.7
7.5
pH_{pl}
7.3
7.1

Figure 8. The relationship between ventilatory volume and plasma pH (pHpl) in Scyliorhinus stellaris undergoing simultaneous exposure to environmental hyperoxia and hypercapnia. Ventilatory volume ($\dot{V}g$ act./$\dot{V}g$ contr.) is the ratio of the measured $\dot{V}g$ divided by the $\dot{V}g$ during hyperoxia. Each line represents the best fit line for all the experimental data for an individual fish. This graph shows only the data for the experiment which involved the exposure to environmental hyperoxia and hypercapnia.

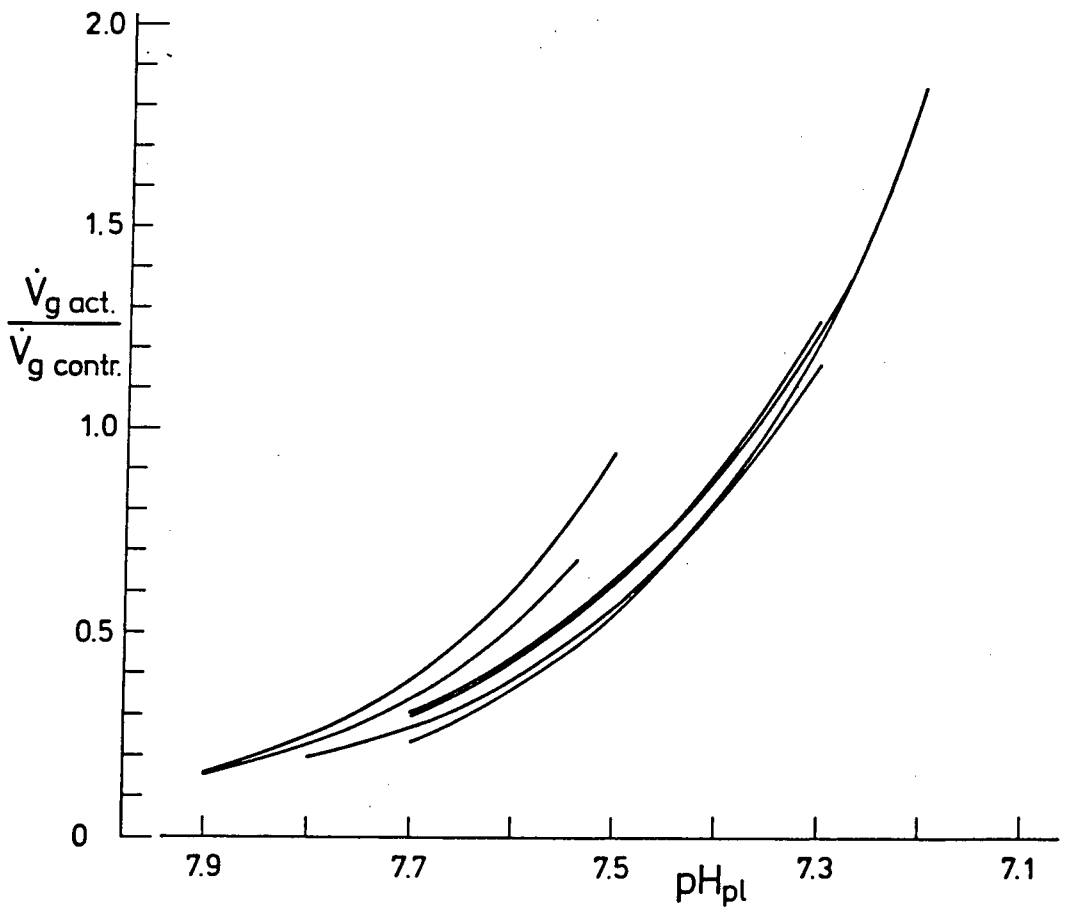

2.0
1.5
$\frac{\dot{V}_{g\ act.}}{\dot{V}_{g\ contr.}}$
1.0
0.5
0
7.9
7.7
7.5
pH_{pl}
7.3
7.1

Figure 9. The relationship between ventilatory volume and plasma pH (pHpl) in Scyliorhinus stellaris undergoing simultaneous exposure to environmental hyperoxia and hypercapnia. Ventilatory volume ($\dot{V}g$ act./$\dot{V}g$ contr.) is the ratio of the measured $\dot{V}g$ divided by the $\dot{V}g$ during hyperoxia. Each line represents the best fit line for all the experimental data for an individual fish. This graph shows only the data for the experiment which involved the exposure to environmental hyperoxia and hypercapnia and where $NaHCO_3$ or HCl was infused to minimize changes in pH due to the first treatment (Figure 7).

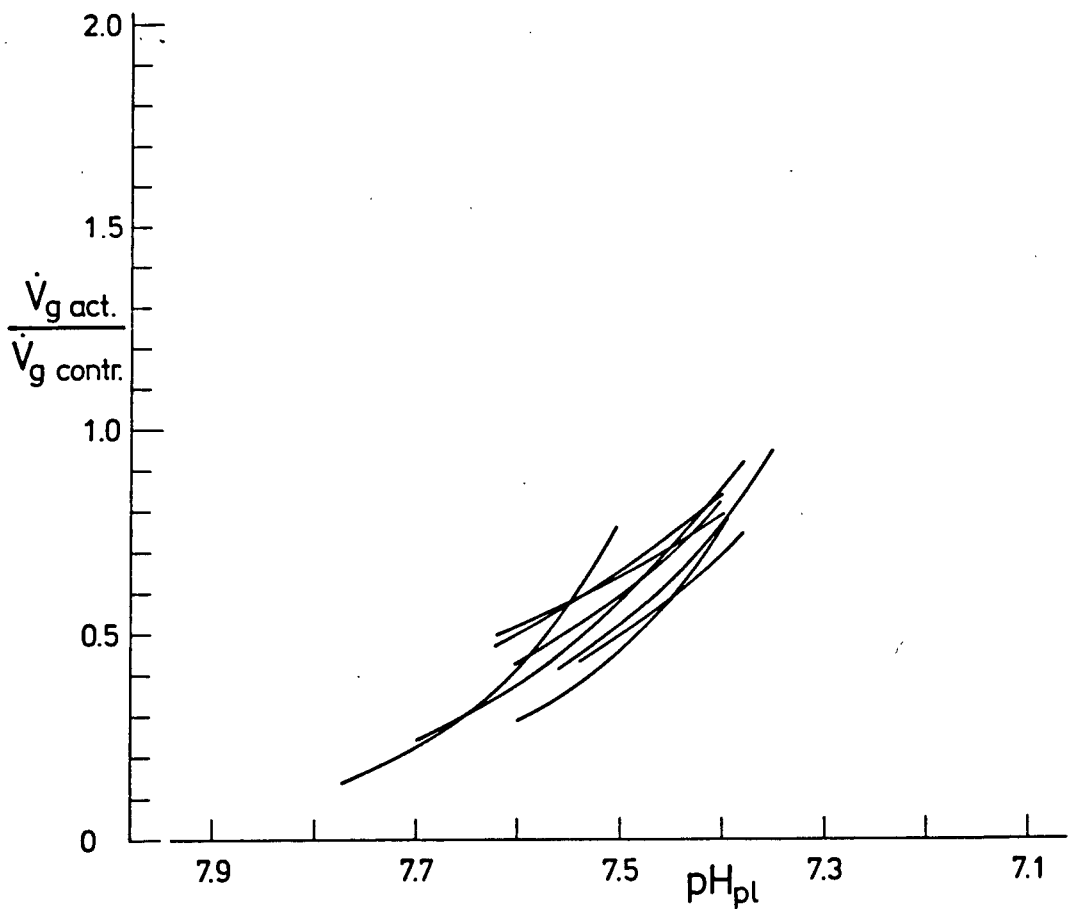

2.0
1.5
$\frac{\dot{V}_{g\ act.}}{\dot{V}_{g\ contr.}}$
1.0
0.5
0
7.9
7.7
7.5
pH_{pl}
7.3
7.1

Figure 10. The best fit line for the aggregate data set for both data sets described in Figure 7. above. Each symbol represents an individual fish.

Coefficients for the line :

$$\dot{V}g \text{ act.} / \dot{V}g \text{ contr.} = a * e^{(b * pH)}$$

a	b	r^2
$5.0476 * 10^{10}$	-3.353	0.875

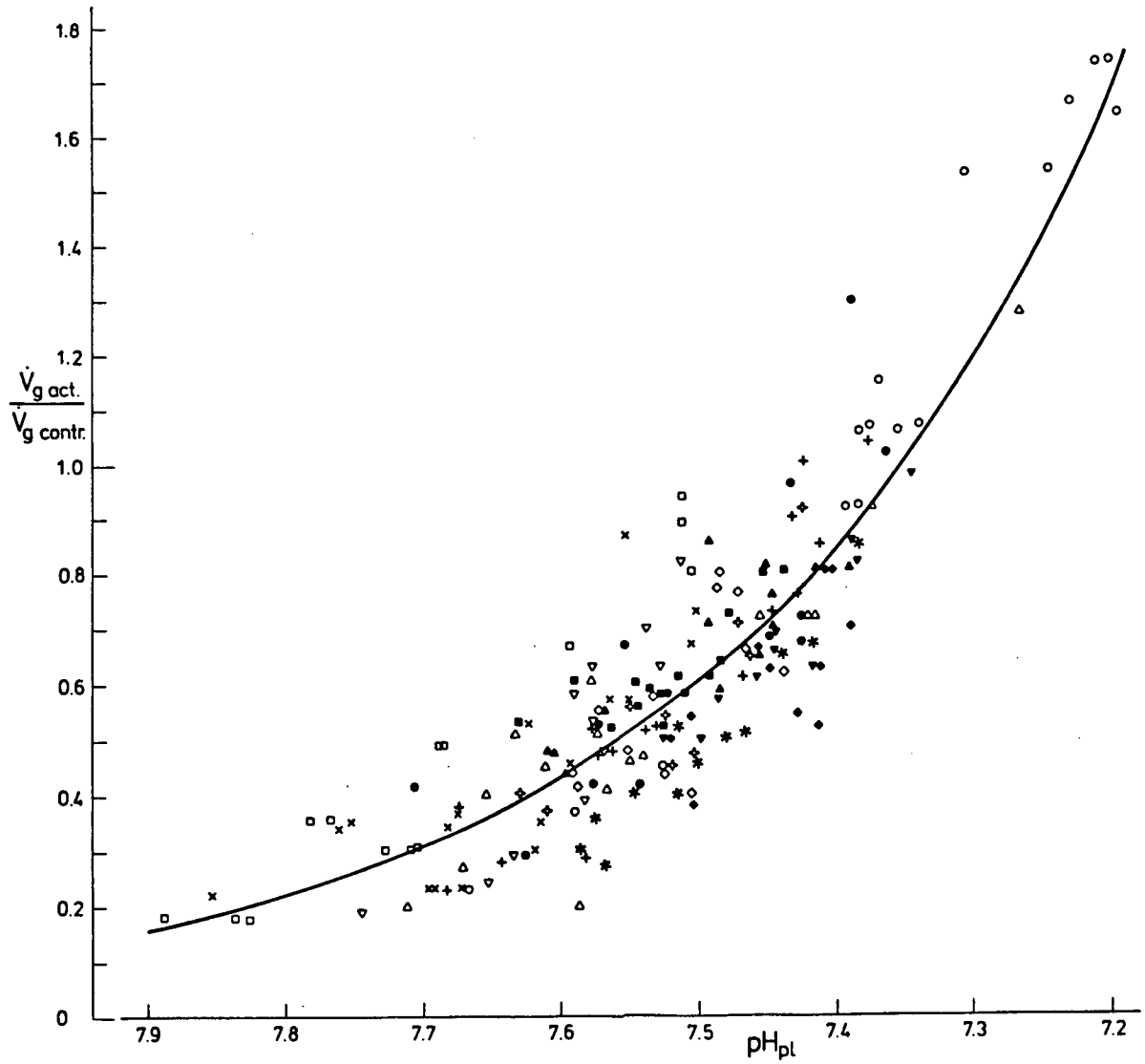

1.8
1.6
1.4
1.2
$\frac{\dot{V}_{g\,act.}}{\dot{V}_{g\,contr.}}$
1.0
0.8
0.6
0.4
0.2
0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
pH_{pl}

Figure 11. The relationship between ventilatory volume ($\dot{V}g$ act./$\dot{V}g$ contr.) and arterial Pco_2 in shark subjected to three experimental protocols : 1.exposure to environmental hyperoxia and hypercapnia (+); 2.exposure to environmental hyperoxia and hypercapnia and infused with $NaHCO_3$ or HCl to minimize changes in pH (o); 3.exposure to environmental hyperoxia and hypercapnia and infused with $NaHCO_3$ to levels beyond those accumulated by the animals (x).

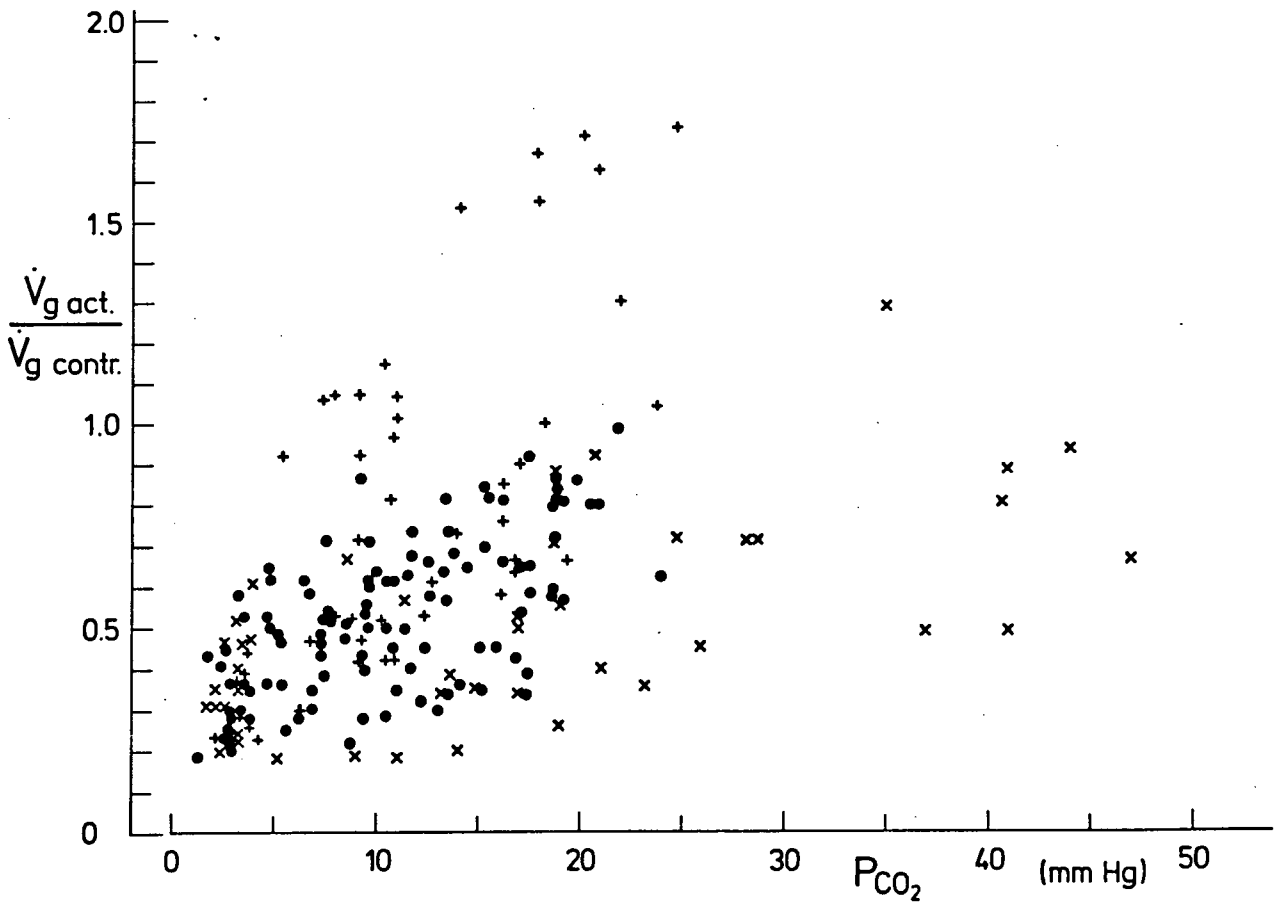
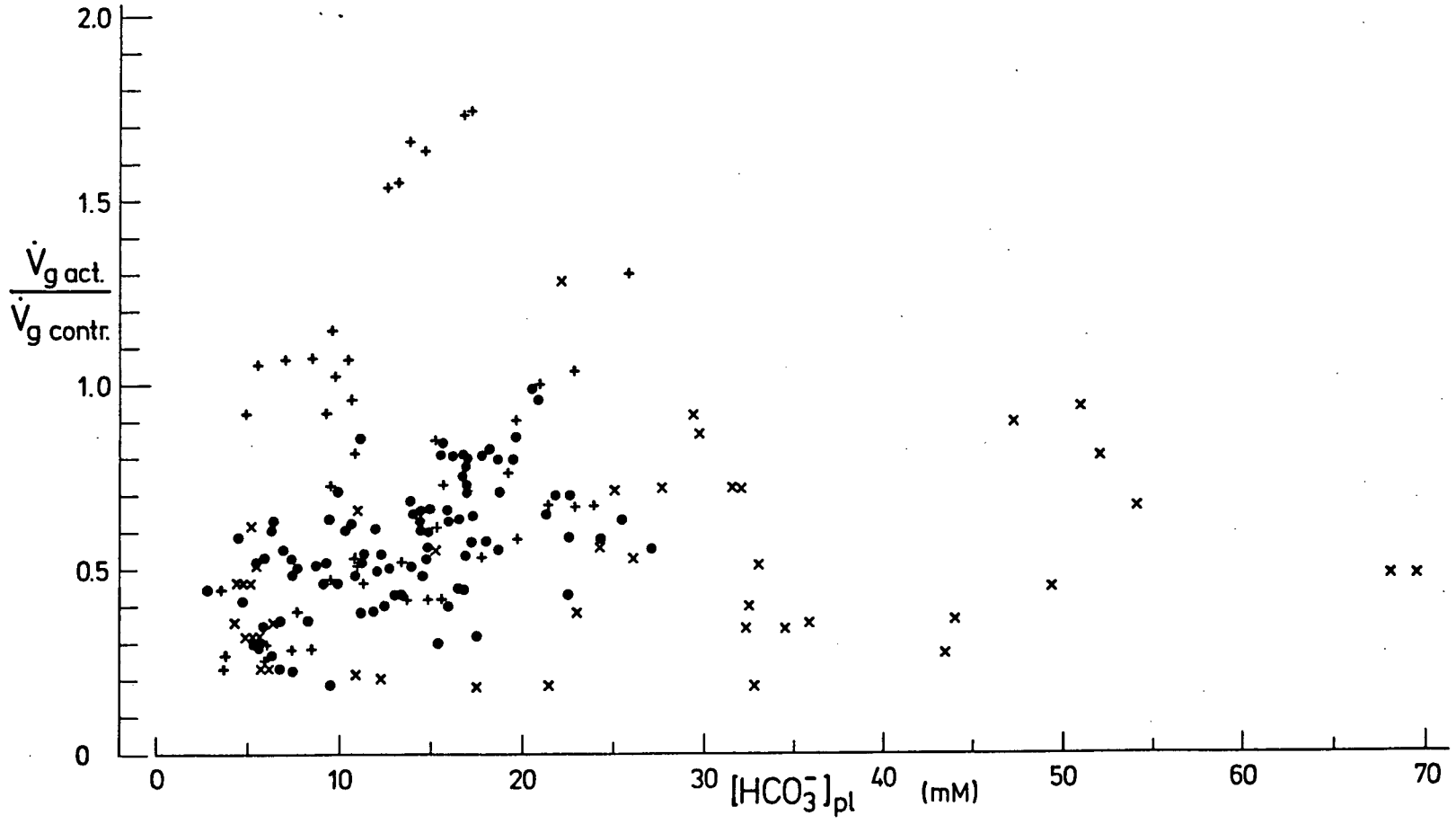

2.0
1.5
1.0
0.5
0
$\frac{\dot{V}_{g\ act.}}{\dot{V}_{g\ contr.}}$
0
10
20
30
40
50
P_{CO_2}
(mm Hg)

Figure 12. As in 11. except that the relationship between ventilatory volume ($\dot{V}g$ act./$\dot{V}g$ contr.) and arterial [HCO_3^-] in mM for the three experimental prolocols.


2.0
1.5
$\frac{\dot{V}_{g\,act.}}{\dot{V}_{g\,contr.}}$
1.0
0.5
0
0
10
20
30
$[HCO_3^-]_{pl}$
40
(mM)
50
60
70

Shark accumulate plasma HCO_3^- to compensate the fall in plasma pH due to environmental hypercapnia and there seems to be a maximum level to this accumulation. Infusion of $NaHCO_3$ beyond this maximum level produces no change in ventilation (Fig. 11 & 12).

DISCUSSION

The animals in experiment 1A were unstressed and in a steady state as far as all measured parameters were concerned. This is supported by the constant RQ and the agreement in the catecholamine and lactate concentrations to those reported by Mazeaud and Mazeaud (1981) and Heisler (1984) for resting fish.

$\dot{V}g$ levels below about 100ml/min result in an elevation of blood Pco_2 and a decrease in blood Po_2. At neutral heads, or in the absence of any imposed head pressures, the observed Vg levels were 42.2 to 139.9ml/min. The lower end of this range is in the region where Vg evidently limits gas exchange across the gills and affects blood gas tensions. Higher $\dot{V}g$ levels have no apparent effect on blood gas tensions. The diffusing capacity of the gill to these gases and / or the reaction velocities in blood and water with respect to these gases presumably have the major determing effect on Pco_2 and Po_2 at the high $\dot{V}g$ levels.

These observations are consistent with previous studies which have shown that hyperoxia, which reduces $\dot{V}g$, is associated with an increase in arterial Pco_2 (Wood and Jackson 1980) whereas hypercapnia, which results in an increase in $\dot{V}g$, has no effect on the Pco_2 differences between blood and water. Thus fish can increase gill ventilation during hypoxia, maintaining oxygen delivery, without causing increased CO_2 excretion and therefore a respiratory alkalosis. Wood and Jackson (1980) estimated that there could be a convective component to limiting CO_2 excretion which would result in an increase in blood Pco_2 by 2 mmHg. The data from this study confirms that estimate as the decrease in CO_2 excretion from the lowered $\dot{V}g$ resulted in comparable increases in blood Pco_2.

The reduction in gas transfer rates at the low ventilation levels was not expected since these animals were in steady state in this regard. Rather than reflecting unsteady states, the declining transfer rates with ventilation volumes may reflect tissue uptake and production of O_2 and CO_2, respectively. Burggren and Randall (1978) observed decreased O_2 uptake rates with ventilation and arterial Po_2 in the sturgeon and suggested that tissue oxygen utilization was determined by the blood : tissue oxygen gradient. There was no obvious correlation between arterial Po_2 and oxygen uptake in this experiment, however, both $\dot{M}o_2$ and arterial Po_2 decreased with $\dot{V}g$. The reduction in aerobic metabolism

could not be related to a reduction in respiratory effort because high $\dot{V}g$ was often associated with a positive pressure head and low $\dot{V}g$ with a negative head, such that there is no clear correlation between $\dot{V}g$ and the effort expended by the fish. The reduction in $\dot{M}o_2$ was not compensated by an increase in anaerobic metabolism as the lactate concentrations remained low in all fish.

These data agree with previous studies (Soivio 1981, Eddy 1974) that changes in gill water flow can only increase significantly the blood Pco_2 level in trout by decreasing ventilation volume. Carbon dioxide excretion cannot be increased by increasing gill water flow above normal levels. This implies that adjustments of gill ventilation could only be effective in correcting alkalotic conditions in the blood through increases in blood Pco_2.

Changes in gill water flow in fishes under a wide range of environmental conditions are reported in a large body of literature. The sensitivity of ventilation in fish to environmental oxygen content is well documented (Saunders 1962, Holeton and Randall 1967, Davis and Cameron 1971, Randall and Jones 1973, Dejours *et al.* 1977, Itazawa and Takeda 1978, Wood and Jackson 1980, Smith and Jones 1982). It is also known that ventilation is stimulated in fish stressed with exposure to acid waters (Hargis 1976; Hoglund and Persson 1971; Dively *et al.* 1977), infusion of acid or base (Cunningham 1974; Janssen and Randall 1975), and hypercapnia

(Van Dam 1938; Randall and Jones 1973; Janssen and Randall 1975; Eddy 1976; Smith and Jones 1982). It seems that an increase in blood Pco_2 tensions is important in the stimulation of ventilation. Exposure of fish to acid conditions externally or internally would cause some degree of hypercapnia through the dehydration of the HCO_3^- ion. Furthermore, Neville (1979a,b) has shown that ventilation in trout is not stimulated in acid conditions unless accompanied by hypercapnia.

A large portion of the reported data on the relationship between ventilation volumes and acid-base disturbances point to the orientation of the control of gill water flow to the maintenance of the oxygen uptake and carrying capacity of the blood (see review by Shelton et al. 1986). Oxygen seems to have a relatively greater influence on ventilation in fishes compared to CO_2 (Randall and Jones 1973; Dejours 1973). Acid conditions in the blood would reduce the carrying capacity for O_2 by the Bohr and Root effects which have been shown to exist in the blood of fishes (carp, Black and Irving 1937; trout, Cameron 1971, Eddy 1971, Boutilier et al. 1986; tench, Eddy 1973). Ventilation is reduced when fish are exposed to environmental hyperoxia (Smith and Jones 1982; Randall and Jones 1973) and there is only a minimal stimulation of ventilation with a superimposed condition of hypercapnia (Babak and Dedek 1907; Peyraud and Serfaty 1964; Dejours 1972). Smith and Jones (1982) showed that the

stimulation of ventilation in trout exposed to mild environmental hypercapnia could be abolished with hyperoxic conditions. There are, however, exceptions to this emerging trend in the published data. The blood of Cyprinid fishes exhibit Bohr and Root shifts but show little respiratory stimulus to hypercapnia (Dejours 1973). Furthermore, while the dogfish, Squalus suckleyi, shows a stimulation of ventilation with hypercapnia, Lenfant and Johansen (1966) showed that its blood showed no Bohr or Root shifts.

The possibility that factors other than O_2 carrying capacity may be driving ventilation in the elasmobranch is supported by the results of experiment 1B. The sensitivity of ventilation to blood Pco_2 and especially pH are more similar to the situation in other vertebrates than in the teleosts. The possibility of inputs from sensors of the acid-base status of the blood or some other closely related compartment in addition to inputs from the oxygen sensors cannot be ignored. A wide range of fishes, including those which exhibit Bohr and Root shifts in their blood, show this sensitivity to hypercapnia and while the response can be attenuated with oxygen, a residual response to CO_2 always remains.

The second reason given by Randall and Cameron (1973) against the regulation of Pco_2 in the blood to correct acidotic conditions is substantiated by the result of the first experiment. The scope of adjusting blood Pco_2 through changes in ventilation is low and only in the range of 2-3

mmHg. Further experiments that delineate the effects of O_2 and CO_2 as well as the acid-base parameters of pH and Pco_2 on the ventilatory response in fish are needed. Since it is not only those animals lacking Bohr and Root shifts that exhibit a sensitivity in ventilation to changes in the acid-base status of the blood, experiments of this nature on species such as those in the Cyprinidae would make valuable contributions to this field of fish physiology. Great gaps exist in the knowledge of the regulation of ventilation in fishes. While clear trends seem to be emerging among data sets, the exception are equally obvious.

SECTION 2.

TRANSEPITHELIAL ION FLUXES FOR ACID-BASE REGULATION IN FISHES

INTRODUCTION

Under steady state conditions, freshwater fish osmotically gain water through their gills and excrete excess water in the urine. Body electrolytes are also lost in this excretion. This loss, however, is counteracted by uptake of sodium (Na^+) and chloride (Cl^-) ions across the secondary lamellae (Girard and Payan 1977a). The chloride cells of the freshwater fish are small, poorly developed and few in number. The mitochondria and tubular systems are also poorly developed. All these characteristics are opposite to those of chloride cells in saltwater. In saltwater, fish diffusively gain Na^+ and Cl^- through the gill and also through the gut as they drink water to replace the osmotically driven water loss at the gill. The excess Na^+ and Cl^- is actively pumped out of the blood by the chloride cells (Karnaky et al. 1977, Foskett et al. 1979, Marshall and Nishioka 1980, Foskett and Scheffey 1982) located on the epithelium at the base of and between the secondary lamellae.

A major way in which fish regulate acid-base status is by transepithelial ion exchange across the gill (see Heisler 1980, 1982, 1984). Since Krogh (1939) first suggested that

the active uptake of Na^+ and Cl^- in the goldfish was coupled to the movement of the acid-base relevant ions H^+, NH_4^+ for Na^+ and HCO_3^- for Cl^-, many studies have confirmed the existence of these links. There is experimental evidence for both the $Na^+/H^+(NH_4^+)$ (Maetz and Romeu 1964; Kerstetter et al. 1970; Cameron 1976; Evans 1977; Payan 1978; Maetz 1973; Perry and Randall 1981; Wood et al. 1984) and the Cl^-/HCO_3^- (Maetz and Romeu 1964; DeRenzis and Maetz 1973; Perry and Randall 1981; Perry et al. 1981; Holeton et al. 1983) exchange processes in the fish gill.

There is circumstantial evidence that suggests that the ionic composition of the water affects the regulation of extracellular pH in response to acid-base disturbances (Table 2). Perry et al. (1981) showed that the recovery of plasma pH in trout exposed to environmental hypercapnia was greater in water with a higher concentration of Na^+ than in the ambient Vancouver tap water. One of the aims of this Section was to provide further experimental evidence for this relationship.

This Section reports on experiments where fish were subjected to acidotic conditions by exposure to environmental hypercapnia, a condition which can occur naturally from CO_2 production by surface plants such as hyacinth mats (Ultsch and Antony 1973) in fresh water as well as from the absence of photosynthesis combined with respiration and anaerobic glycolysis of animals and bacteria attached to falling particles of organic debris at depths of 50-100m in sea water

Table 2. Time course for pH compensation in fish stressed with environmental hypercapnia.

SPECIES	WATER Pco_2 mmHg	TIME h	REFERENCES
Conger conger	8	8-10[c]	Toews et al. 1983
Scyliorhinus stellaris	8	8-10[c]	Heisler et al. 1976
Ictalurus punctatus	10	24[p]	Cameron 1980
Salmo gairdneri	15	22[p]	Eddy et al. 1977
	5.2	72[c]	Janssen and Randall 1975
	7.5	24[p](52%)*	Perry et al. 1981
	7.8	24[p](88%)**	

c complete compensation; p partial compensation; * Percent recovery of H^+ ion concentration before hypercapnia was imposed, in dechlorinated Vancouver tap water; ** As * except Na^+ ion concentration in the water was raised to 3 mM.

(Harvey 1974). It was hoped that imposing this acid-base disturbance would stimulate ion fluxes across the gill epithelium and that the superimposition of additional treatments would result in a better understanding of the nature and mechanism of these ion exchange processes.

The acid-base regulatory performance of trout acclimated to waters of different ionic strength (Table 3) were compared on the hypothesis that altering the concentrations of Na^+ and Cl^- in the water should affect the regulation of blood pH if there is a dependence of the transepithelial movement of these ions to $H^+(NH_4^+)$ and HCO_3^-, respectively. Radioactive isotopes of Na^+ and Cl^- were also injected into carp to determine the directional fluxes of these ions during recovery from acidosis.

MATERIALS AND METHODS

EXPERIMENT 2A. SALINITY - TROUT - HYPERCAPNIA

ANIMALS :

Rainbow trout, Salmo gairdneri, weighing between 800 and 1535 g were obtained from a commercial hatchery and held indoors under ambient light conditions. They were held in large glass aquaria at a density of about 150 l/fish which

Table 3. Mean water Na^+ and Cl^- concentrations for the two experiments in this section. All concentrations in mM.

Trout - Hypercapnia - 3 Salinities

SAMPLING TIME	[Na^+]	[Cl^-]	[Na^+]	[Cl^-]	[Na^+]	[Cl^-]
CONTROL	2.32	3.29	97.72	113.82	320.72	333.82
HYPERCAPNIA						
+.25h	2.61	3.32	94.2	113.2	330.0	332.75
+.5h	2.45	3.25	93.8	113.1	323.5	331.17
+1h	2.19	3.33	98.2	114.2	314.0	332.58
+2h	2.42	3.42	100.6	113.9	318.17	332.25
+4h	2.54	3.38	94.4	111.4	318.67	327.42
+8h	2.28	3.58	97.8	113.0	323.5	324.92
+20h	2.41	3.58	96.6	110.5	319.0	332.33
+24h	2.38	3.46	96.0	110.5	333.0	335.4
RECOVERY						
+.25h	2.37	3.55	92.75	114.88	325.5	330.25
+.5h	2.18	3.58	95.6	110.9	325.8	332.2
+1h	2.17	3.45	94.2	108.8	315.0	331.7
+5h	2.45	3.58	97.4	113.9	338.4	334.1
+10h	2.47	3.25	99.4	114.6	330.6	333.8
+20h	2.21	3.41	95.4	114.8	313.8	336.0
+24h	2.67	3.2	96.75	117.0	335.25	337.1

Conger - Hypercapnia - 6 Salnities

SAMPLING TIME	[Cl^-]	[Cl^-]	[Cl^-]	[Cl^-]	[Cl^-]	[Cl^-]
initial [Cl^-] determ.	< 3	40	80	140	360	540

received decholrinated tap water at 10 ± 3°C (mean ± S.E.). The fish were fed to satiation several times a day. All fish were acclimated to 3 water salinities for at least 1 month prior to experimentation. Sea salts were added to ambient fresh dechlorinated water to achieve NaCl concentrations of about 3, 100 and 300 mM. The chemical composition of the salts used to make up the various salinities is given in Table A.1. of Appendix I.

SURGERY AND APPARATUS :

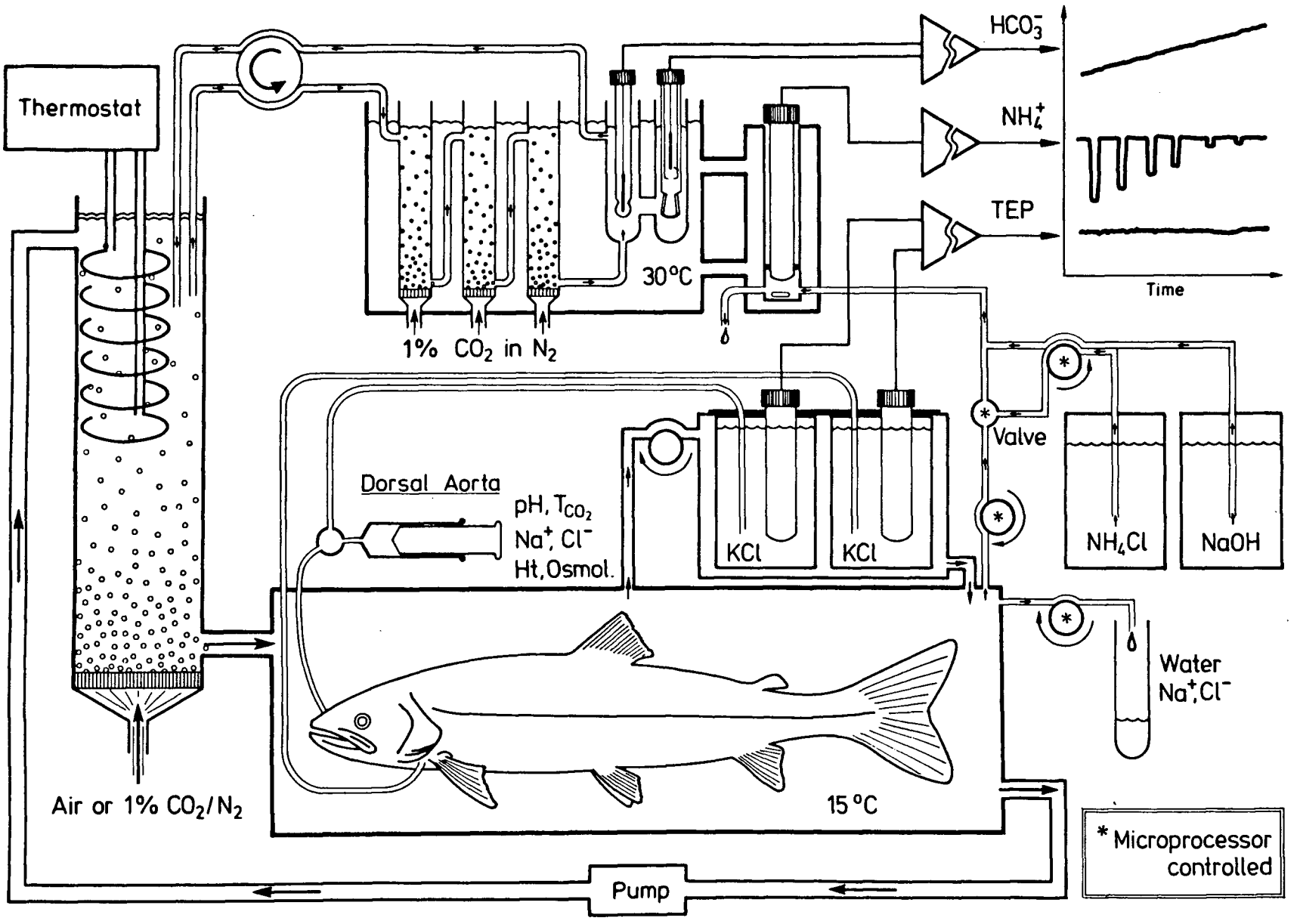
All animals were fitted with chronic indwelling cannulas in the dorsal aorta by the method of Soivio et al. (1972) as described in General Materials and Methods. The salinity to which the animal was acclimated was maintained in surgery and in subsequent experimental procedures.

Each animal was recovered in the experimental chamber shown in Figure 13. The system is described in General Materials and Methods as a 'Delta Bicarbonate System'. Experimental procedures were initiated at least 24 h after surgical procedures.

PROTOCOL :

The experimental protocol consisted of exposing fish acclimated at the above salinities to 24 h of 1 %

Figure 13. Experimental apparatus for experiment 2A.: Trout - Salinity - Hypercapnia . All components of this diagram are explained in General Materials and Methods under the descriptions of the 'Delta Bicarbonate System' and the apparatus for measuring transepithelial potentials (TEP).


Thermostat
HCO_3^-
NH_4^+
TEP
Time
30°C
1% CO_2 in N_2
Valve
Dorsal Aorta
pH, T_{CO_2}
Na^+, Cl^-
Ht, Osmol.
KCl
KCl
NH_4Cl
NaOH
Water
Na^+, Cl^-
Air or 1% CO_2/N_2
15 °C
Pump
* Microprocessor controlled

environmental hypercapnia and then observing the recovery from this exposure for 24 h after the high Pco_2 tensions were eliminated. Blood and water samples were collected 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 20 h, and 24 h after the beginning of the hypercapnia exposure and 15min, 30min, 1 h, 5 h, 10 h, 20 h, and 24 h after the end of the hypercapnia exposure.

The following procedure was carried out for each sampling time. 5 ml of water was removed from the system. The dorsal aortic cannula was connected to a 'T' piece which enabled contact with either a KCl/agar bridge for transepithelial potential (TEP) measurements (see General Materials and Methods) or a syringe for blood collection. One ml of blood was collected; the volume was replaced with heparinized physiological saline and then the valve on the 'T' piece was turned so that contact with the KCl/agar bridge was made. Contact with the KCl/agar bridge was maintained between most of the sampling times, allowing near continuous recording of TEP during the experimental periods.

Water HCO_3^- concentration was also recorded on a continuous basis with the apparatus described in General Materials and Methods. For this and TEP recordings, values were read off the chart at the sampling times listed above.

MEASUREMENTS :

Whole blood pH, total CO_2 (Tco_2 with the Capni-Con

III) and hematocrit were measured according the methods in General Materials and Methods. The remaining blood was centrifuged in Eppendorf vials to obtain separated plasma. An aliquot of plasma was acidified and frozen for total ammonia analysis and the remaining plasma was analyzed for Na^+ and Cl^- concentrations as well as for osmolarity. Sodium was measured by spectrophotometry and Cl^- was measured with the Radiometer CMT10 titrator (see General Materials and Methods). Total plasma osmolarity was measured with a micro-osmometer. Plasma Pco_2 tensions and HCO_3^- concentrations were calculated using the measured pH and Tco_2 values as described in General Materials and Methods.

Water samples were analysed for Na^+ and Cl^- concentrations. The method for Na^+ concentration determination was the same as for plasma but Cl^- concentrations were determined with the electrode method described in General Materials and Methods. Total ammonia concentrations were measured with an ammonia electrode in an automated way described in General Materials and Methods. That Section also describes the way in which continuous recordings of water HCO_3^- concentrations were determined. Net flux rates of ions were calculated in units of umol/Kg body weight/min by calculating rates of change in the contents (concentration * volume) of the particular ion.

STATISTICS :

Analysis of variance was used to discern statistical significance among the means of any parameter at three salinities. Paired Student's t test was used to test significance of differences between the mean control value and any subsequent experimental value at any one salinity. Linear regression analysis was used to describe the relationship between certain data sets. The level of rejection in all cases was 5 %.

EXPERIMENT 2B. CONGER - SALINITY - HYPERCAPNIA

ANIMALS :

Conger eel, Conger conger, weighing between 800 to 1500 g were caught in the Bay of Naples, Italy. They were brought to the laboratory where they were held at 19°C in 200 l fiberglass tanks supplied with ambient sea water before surgery and subsequent experimentation. The animals were not fed once they were in the laboratory, a time period which varied from 48 h to 2 weeks.

SURGERY AND APPARATUS :

All animals were fitted with chronic indwelling dorsal

aortic cannulas via a gastric or intestinal artery. The method of operation is described in General Materials and Methods. The animals were recovered in a recirculating water system similar to the 'Delta Bicarbonate System' described in General Materials and Methods in which the experiment was conducted. The chemistry of the water was identical to the acclimation water until the salinity changes described below were initiated. Water temperature was maintained constant at 20°C. An 8 to 24 h recovery period was allowed before initiating experimental procedures.

PROTOCOL :

There were three periods in the experiment; control, salinity change and hypercapnia. Each experiment lasted 8 hours excluding the control period. In the first three hours the salinity of the water was changed to one of six salinities which were all lower than sea water. In the following 5 h, the fish were exposed to 1 % environmental hypercapnia. Sampling periods were as follows : 2 control samples; 30 min, 1 h, 2 h and 3 h after the step change in salinity; and 30 min, 1 h, 2 h, 4 h and 5 h after the environmental hypercapnia was imposed.

At each sample period, 500 ul of whole blood was withdrawn through the cannula and analysed for the parameters described below.

MEASUREMENTS :

Whole blood pH, hematocrit and total CO_2 (Tco_2 with the Capni-Con III), plasma HCO_3^- concentration and Pco_2 tension and plasma Cl^- concentrations were calculated or determined by titration using the Radiometer CMT10 titrator as described in General Materials and Methods.

STATISTICS :

Correlation and linear regression analysis were used to describe relationships between data sets.

EXPERIMENT 2C. CARP-HYPERCAPNIA-ISOTOPE

ANIMALS :

Carp, Cyprinus carpio, weighing 1500 to 2000 g were obtained from a commercial hatchery and maintained in large glass indoors aquaria, at a density of about 100 l per fish, under natural light and receiving 15°C dechlorinated tap water. Fish were fed several times a day to satiation with a pelleted carp feed. Fish were acclimated for at least one month prior to use in experiments.

SUGERY AND APPARATUS :

All fish were fitted with chronic indwelling dorsal aortic cannulas using the method of Siovio et al. (1972) and recovered in a 'Delta Bicarbonate System' (Fig. 14) described in detail by Claiborne and Heisler (1984). The following protocol and measurements were carried out after a 24 h recovery period after surgery.

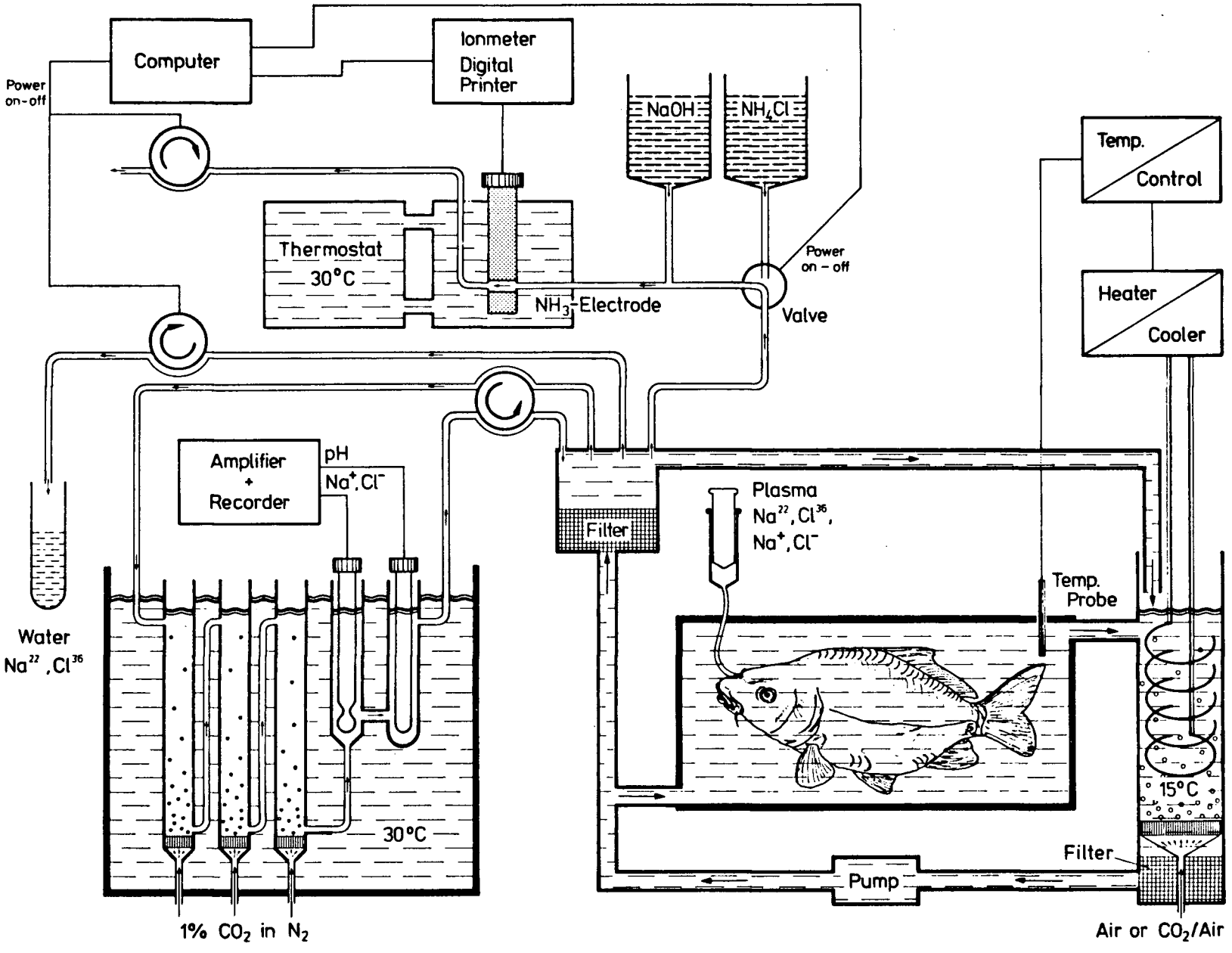
PROTOCOL :

One hundered uCi of the isotope Na^{+}_{24} and 20 uCi of Cl^{-}_{36} were injected through the cannula and the following procedures were carried out. After 3 pre-exposure control samples were taken, water and blood samples were taken during 48 h exposure to 5 % environmental hypercapnia and during a 21 h recovery period after the high CO_2 tension was turned off. Blood samples were taken at 0, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 25 h, 29 h, 33 h, 45 h, 46 h, 49 h, 53 h and 69 h after the onset of hypercapnia which lasted only 48 of the 69 h sampling regime. Water samples were collected at the same sampling times as for the blood except that additional samples were taken every 4 h whenever possible between the 53 and 69h sampling times.

MEASUREMENTS :

Water pH at a set Pco_2 as well as Na^{+} and Cl^{-}

Figure 14. Experimental apparatus for experiment 2C.: Carp - Isotope - Hypercapnia. All components of this diagram are explained in General Materials and Methods under the description of the 'Delta Bicarbonate System'. The experimental apparatus for experiment 2B. was similar to this apparatus.


Computer
Ionmeter
Digital
Printer
Power
on-off
NaOH
NH_4Cl
Temp.
Control
Thermostat
30°C
NH_3-Electrode
Power
on - off
Valve
Heater
Cooler
Amplifier
+
Recorder
pH
Na^+, Cl^-
Filter
Plasma
Na^{22}, Cl^{36},
Na^+, Cl^-
Temp.
Probe
Water
Na^{22}, Cl^{36}
15°C
30°C
Filter
Pump
1% CO_2 in N_2
Air or CO_2/Air

concentrations were measured by glass electrodes with appropriate reference electrodes within the 'Delta Bicarbonate System'. The Na^+ and Cl^- electrodes were calibrated with various NaCl solutions. Water HCO_3^- concentrations were calculated using the above measurements and methods. Plasma concentrations of Na^+ and Cl^- were determined by spectrophotometry and by titration using the Radiometer CMT10 titrator respectively.

Net fluxes of each ion between blood and water were determined by the system water volume and concentration changes of each ion. The unidirectional fluxes of each injected isotope were determined with the water and plasma activities for Na^+_{24} and Cl^-_{36} together with the 'cold' concentrations and specific activities of the ions. The activities of the isotopes were counted in Gamma and Beta counters. The Na^+_{24} activity of each sample was counted first. After a period of 3 weeks, a period exceeding the half-life of that isotope, the Cl^-_{36} activity was counted. Corrections for decay, background, counting time and efficiency were automated in the machines. Disintigrations per minute (dpm) were then calculated.

STATISTICS :

Student's t tests were used to compare the mean control fluxes of these ions to the mean fluxes after the treatments

were imposed. The 5 % level of rejection was applied in all cases.

RESULTS

EXPERIMENT 2A & 2B. EFFECTS OF WATER SALINITY ON ACID-BASE REGULATION IN FISHES

I. Acid - Base Regulation.

An elevation of water Pco_2 pressures caused plasma Pco_2 to rise 4-6 times the control values (Fig. 15 & 16). In trout and in the conger, the initial drop in pHe induced by exposure to environmental hypercapnia was compensated in varying degrees over the time course of the exposure period depending on the external water salinity (Fig. 17,18 & 19). There was a positive correlation between the degree of compensation and water salinity (Fig. 18 & 20).

In both species compensation of pHe was effected by accumulation of plasma HCO_3^- (Fig. 21 & 22). HCO_3^- accumulation in trout acclimated to 100 and 300mM NaCl was greater than those acclimated to 3mM (Fig. 21). There was a positive correlation between water salinity and HCO_3^- accumulation in the conger (Fig. 23).

Net H^+ flux changed from an influx during control

Figure 15. Means ± one standard error (S.E.) of plasma Pco_2 in rainbow trout acclimated to 3, 100 and 300 mM NaCl and exposed to environmental hypercapnia. Time course shown includes control, 1 % hypercapnia and recovery periods. All units in mmHg.

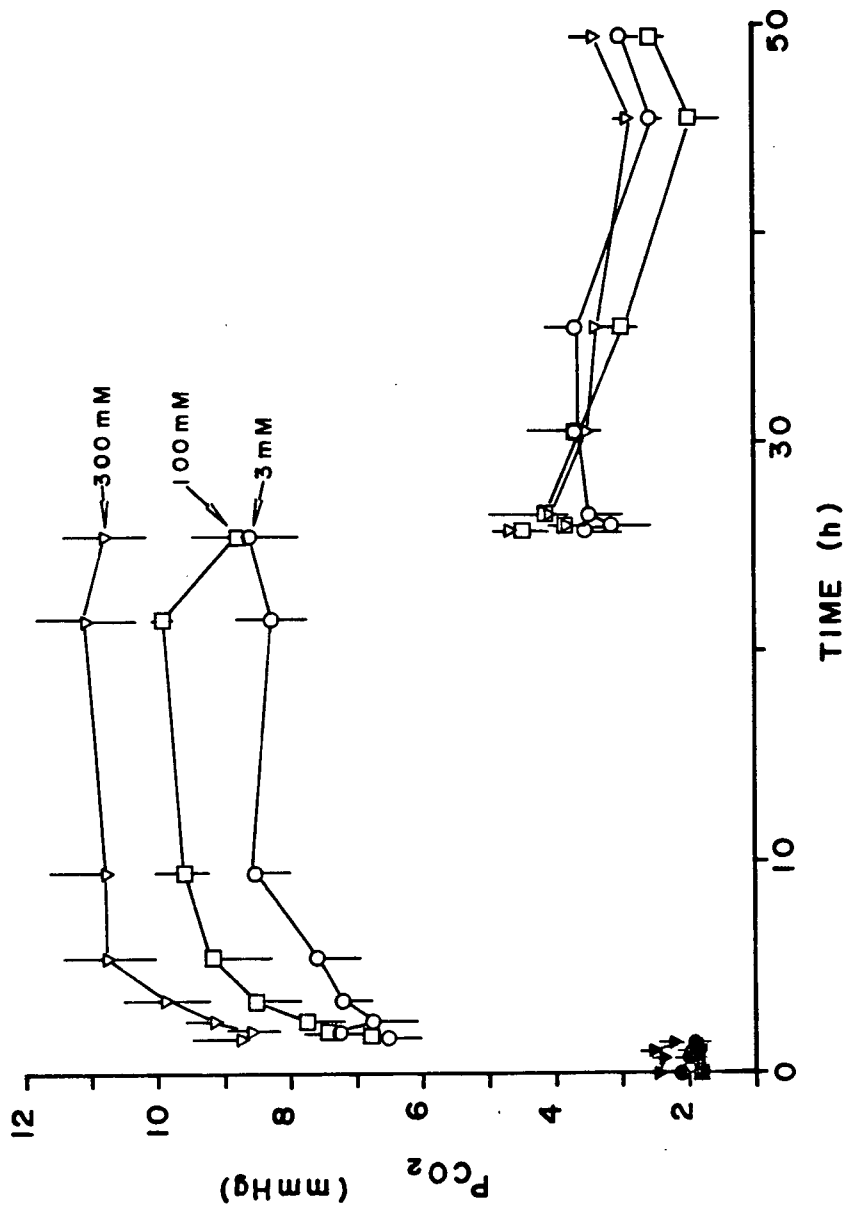

300 mM
100 mM
3 mM
12
10
8
6
4
2
0
10
30
50
TIME (h)
P_{CO_2} (mmHg)

Figure 16. Plasma Pco_2 in conger during control, salinity change and exposure to 1 % environmental hypercapnia. Each line represents an individual fish. Control period was in sea water. Salinities were changed in one step to those noted and fish were exposed to the hypercapnia at those salinities.

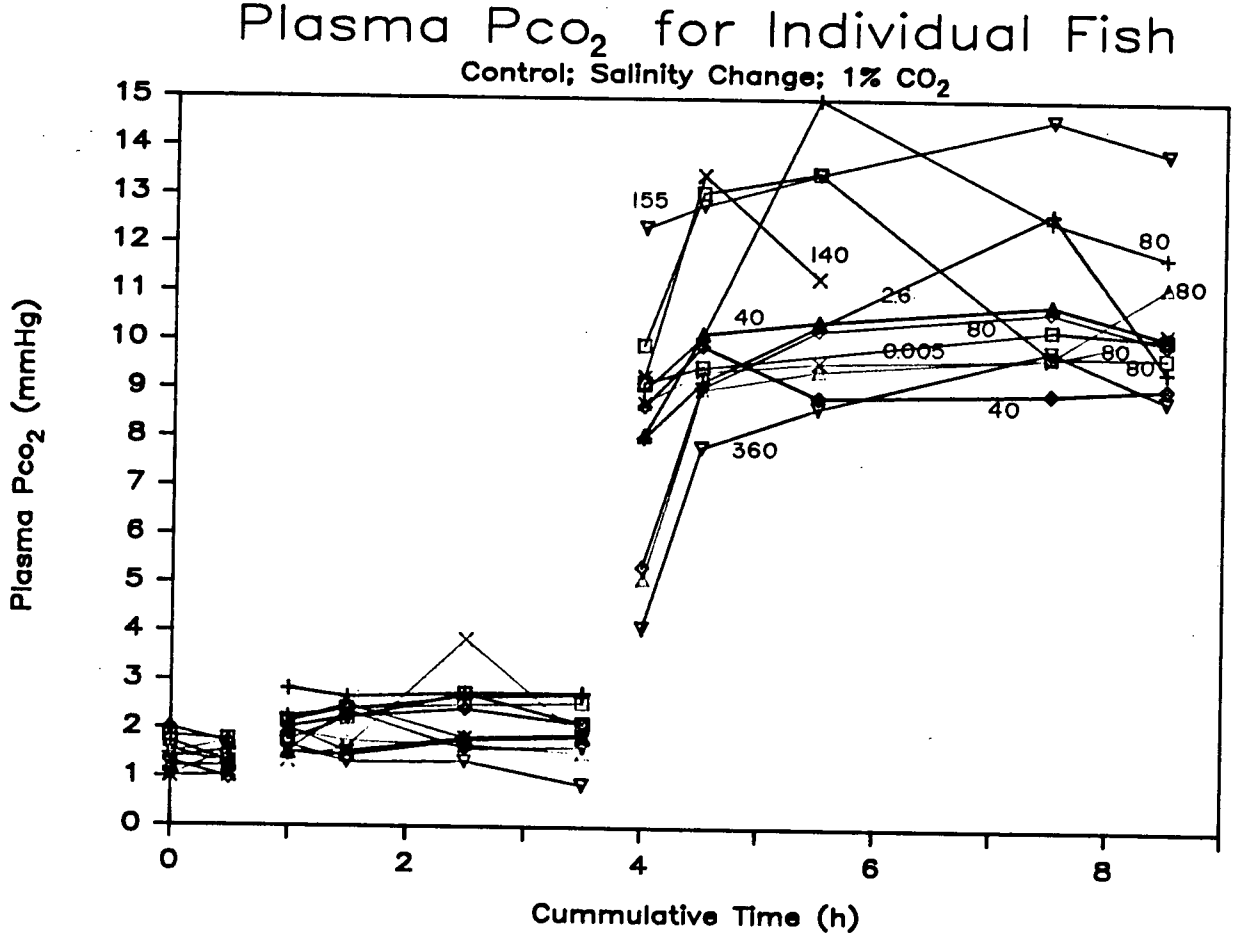

Plasma Pco2 for Individual Fish
Control; Salinity Change; 1% CO2
Plasma Pco2 (mmHg)
Cummulative Time (h)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0
2
4
6
8
155
140
80
80
40
2.6
80
0.005
80
80
40
360

Figure 17. Means ± S.E. of plasma pH in rainbow trout acclimated to 3, 100 and 300 mM NaCl and exposed to environmental hypercapnia. Time course shown includes control, 1 % hypercapnia and recovery periods.

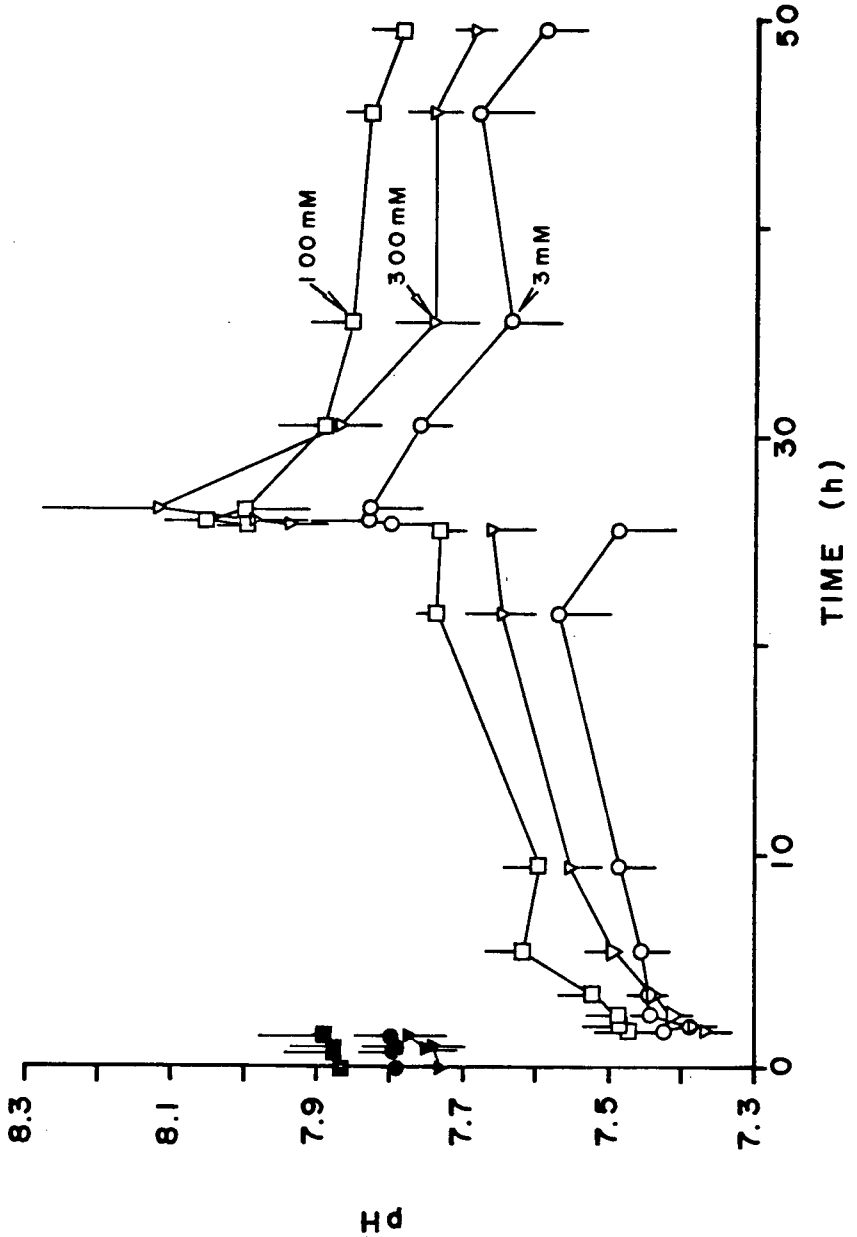

100 mM
300 mM
3 mM
8.3
8.1
7.9
7.7
7.5
7.3
pH
0
10
30
50
TIME (h)

Figure 18. As Fig. 17 except that all values were referenced to the average value during the control period. Means.

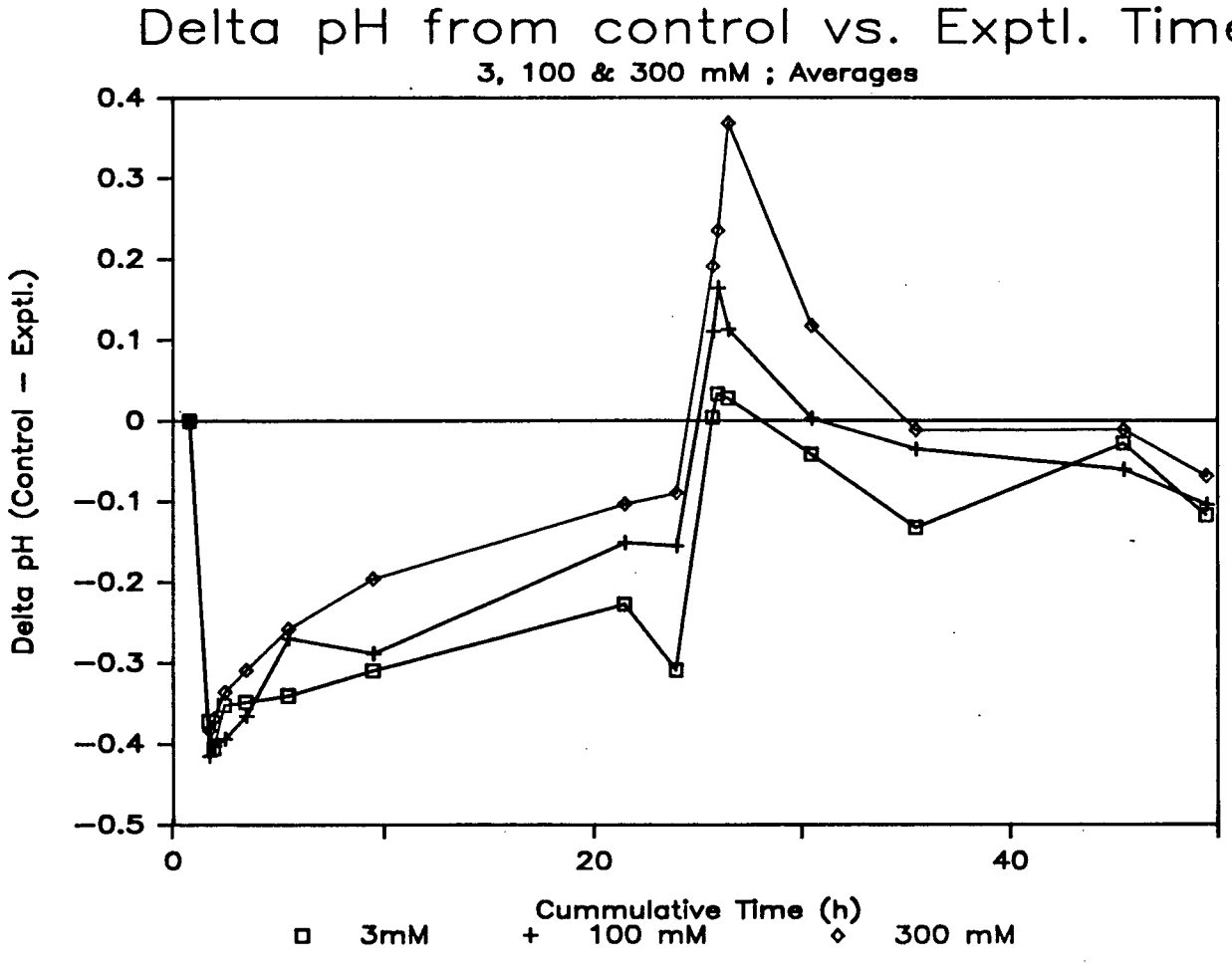

Delta pH from control vs. Exptl. Time
3, 100 & 300 mM ; Averages
Delta pH (Control – Exptl.)
0.4
0.3
0.2
0.1
0
−0.1
−0.2
−0.3
−0.4
−0.5
0
20
40
Cummulative Time (h)
3mM
100 mM
300 mM

Figure 19. Plasma pH changes from average control values in conger during salinity change and exposure to 1 % hypercapnia. Each line represents an individual fish. Actual average control values for pH were : 360mM=7.829, 140mM=7.736, 155mM=7.847, 0.005mM=8.843, 2.6mM=7.816, 80mM=7.783(mean of 6), 40mM=7.835(mean of 2).

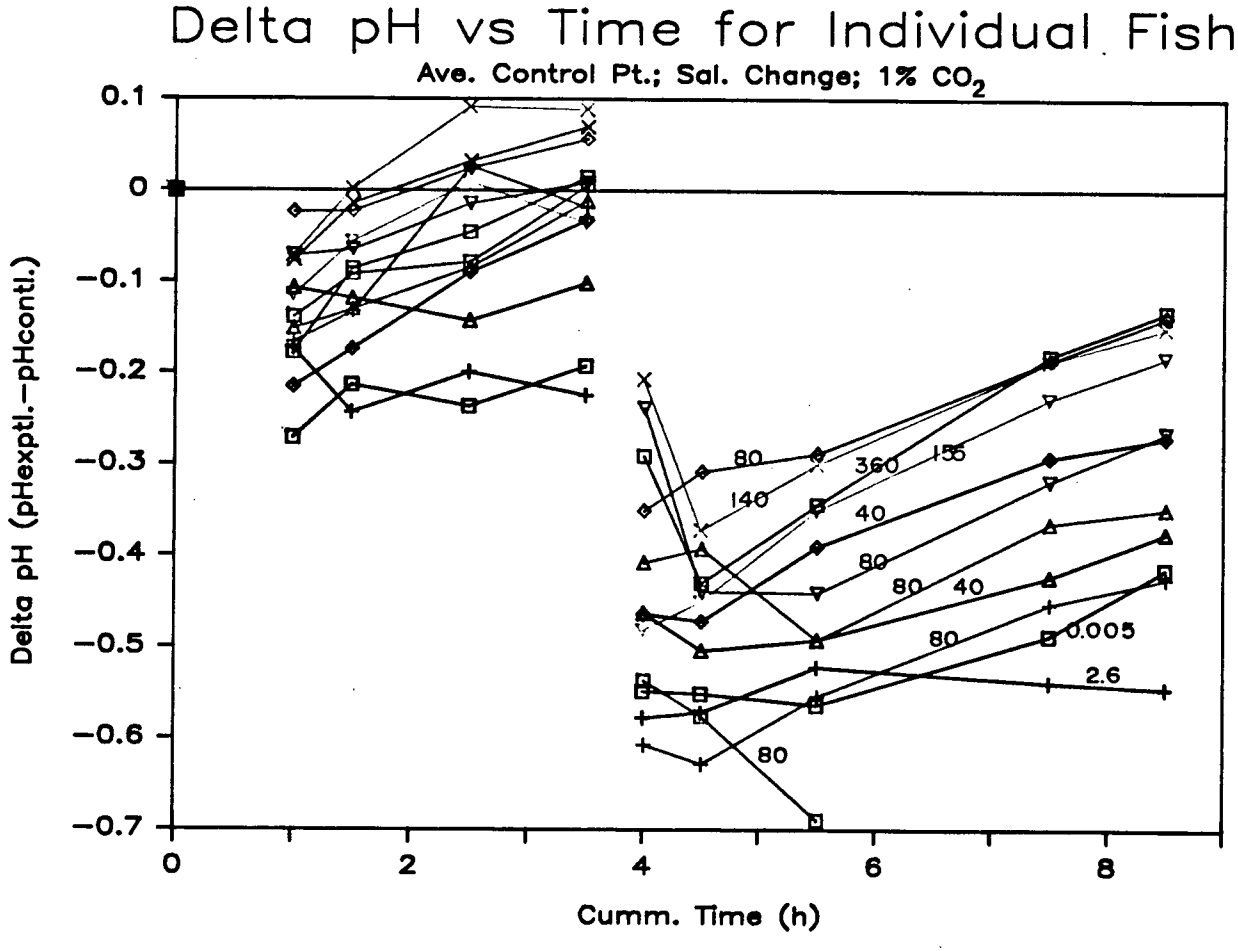

Delta pH vs Time for Individual Fish
Ave. Control Pt.; Sal. Change; 1% CO_2
Delta pH (pHexptl.−pHcontl.)
0.1
0
−0.1
−0.2
−0.3
−0.4
−0.5
−0.6
−0.7
0
2
4
6
8
Cumm. Time (h)
80
140
360
155
40
80
80
40
80
0.005
2.6
80

Figure 20. Effect of water salinity, represented by $[Cl^-]$, on pH recovery in conger. Percent recovery to the pH value measured prior to exposure to 1 % environmental hypercapnia was calculated by dividing the pH value at the end of the exposure period by the pre-exposure value and multiplying by 100. Results of linear regression analysis shown.

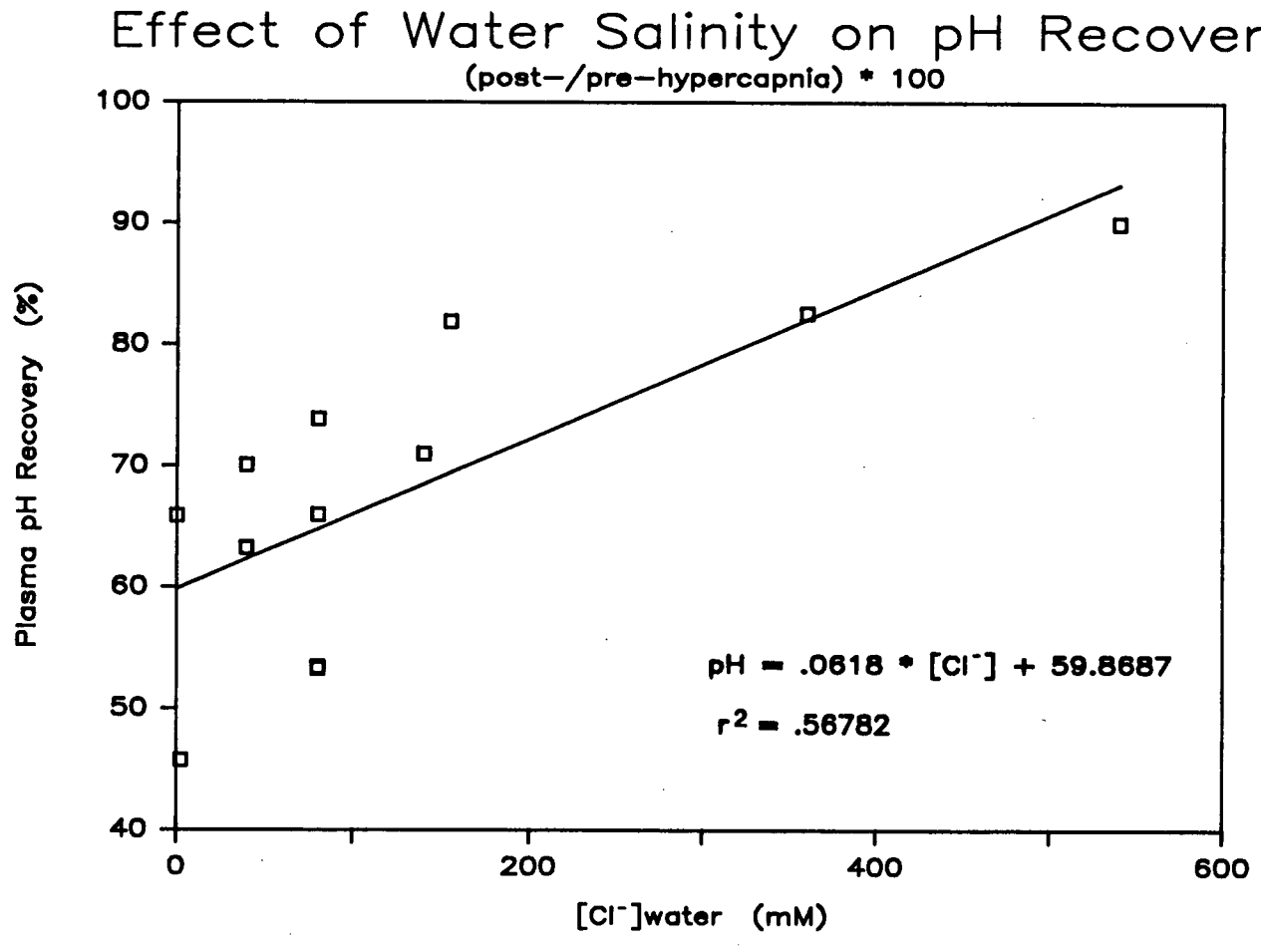

Effect of Water Salinity on pH Recovery
(post−/pre−hypercapnia) * 100
Plasma pH Recovery (%)
100
90
80
70
60
50
40
0
200
400
600
[Cl⁻]water (mM)
pH = .0618 * [Cl⁻] + 59.8687
r² = .56782

Figure 21. Means ± S.E. of plasma HCO_3^- concentrations in rainbow trout acclimated to 3, 100 and 300 mM NaCl and exposed to environmental hypercapnia. Time course shown includes control, 1 % hypercapnia and recovery periods. All units in mM.

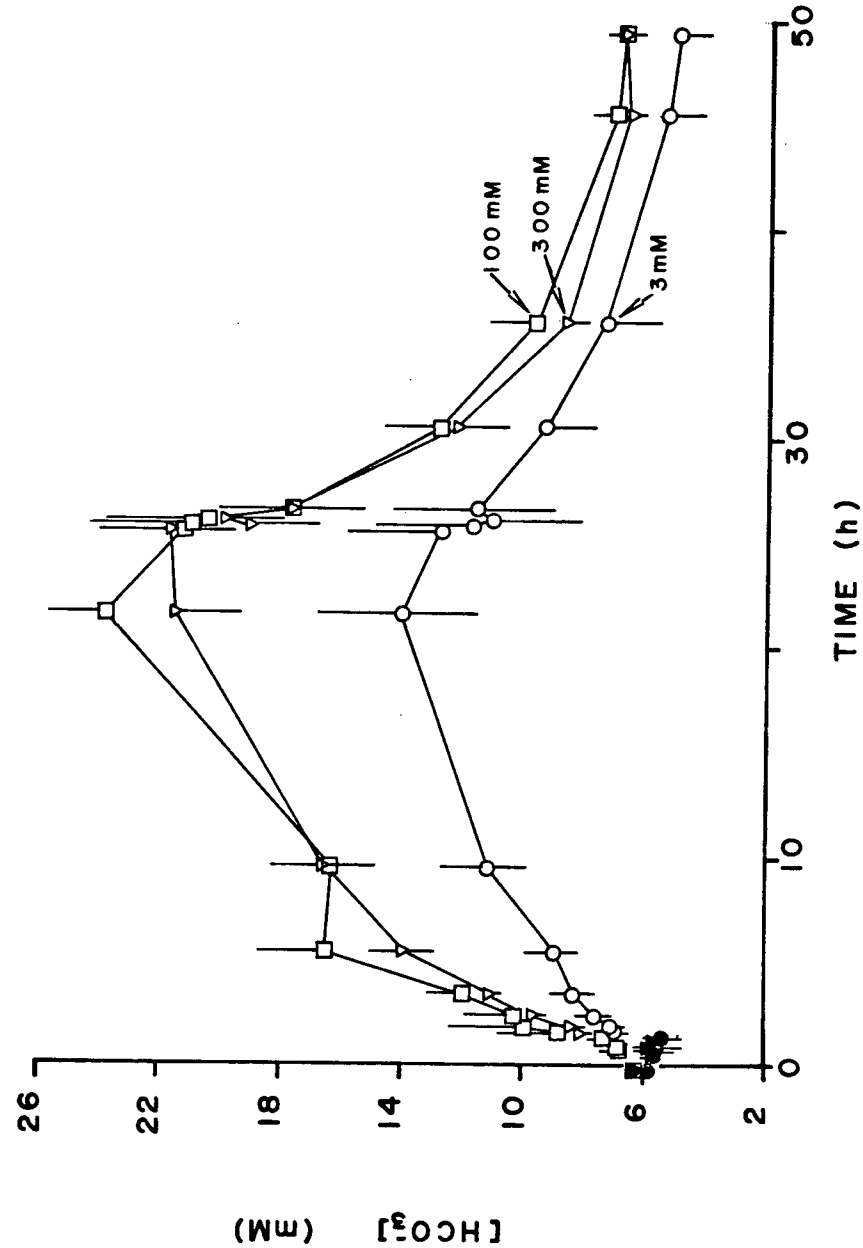

100 mM
300 mM
3mM
TIME (h)
[HCO3-] (mM)
0
10
30
50
2
6
10
14
18
22
26

Figure 22. Plasma HCO_3^- changes from mean control values in conger during salinity change and exposure to 1 % hypercapnia. Each line represents an individual fish. Actual average control values for HCO_3^- were : 0.005mM=3.68, 2.6mM=3.52, 40mM=2.92(mean of 2), 80mM=3.15(mean of 5), 140mM=3.07, 155=2.63, 360=3.40. All values in mM.

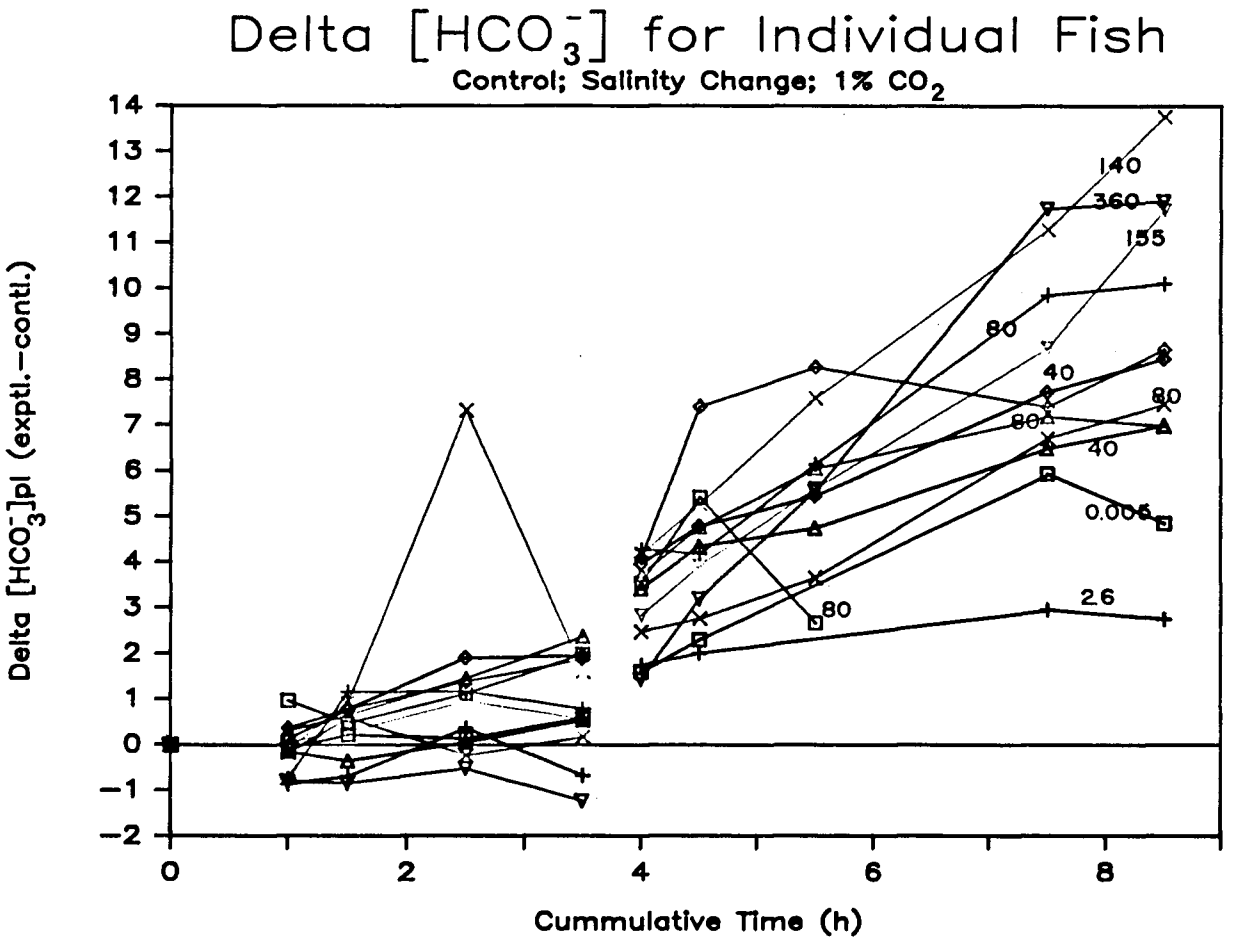
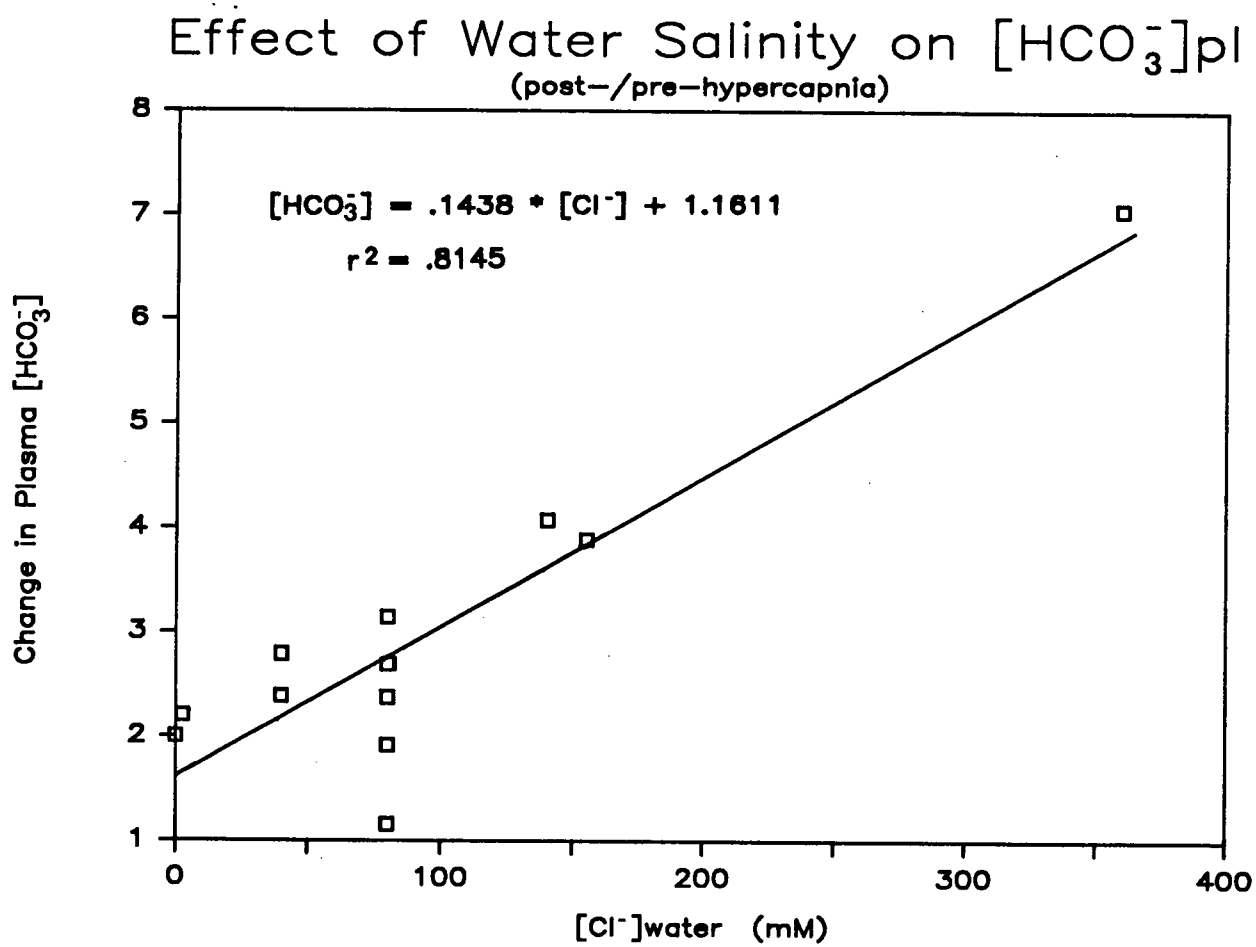

Delta [HCO3-] for Individual Fish
Control; Salinity Change; 1% CO2
Delta [HCO3-]pl (exptl.-contl.)
Cummulative Time (h)
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
0
2
4
6
8
140
360
155
80
40
80
80
40
0.006
80
2.6

Figure 23. Effect of water salinity, represented by $[Cl^-]$, on HCO_3^- accumulation in conger. Calculations as in Fig. 20 excluding multiplication by 100. Results of linear regression analysis shown.


Effect of Water Salinity on [HCO3-]pl
(post-/pre-hypercapnia)
[HCO3-] = .1438 * [Cl-] + 1.1611
r2 = .8145
Change in Plasma [HCO3-]
8
7
6
5
4
3
2
1
0
100
200
300
400
[Cl-]water (mM)

conditions to an efflux during the period of exposure to water hypercapnia in all groups of trout (Fig. 24.). Differences among groups of fish were not different. Net H^+ influx or HCO_3^- efflux was resumed at the end of the recovery period.

II. Plasma Ions.

Plasma Cl^- concentrations in both the trout (Fig. 25) and conger (Fig. 26) decreased during the exposure period compared to control concentrations. These changes were marked after 10h of exposure in the trout. Plasma Na^+ concentrations were increased in the trout acclimated to 100 and 300mM NaCl during the first 10h of exposure in the trout (Fig. 27). Plasma Na^+ concentrations were not measured in the conger experiment.

Plasma osmolarity in trout acclimated to 3mM was reduced compared to control values during exposure to hypercapnia. There were no significant trends in the 100 and 300mM groups (Fig. 28)

III. Acid-Base and Ion Correlations :

There were weak but significant correlations between plasma (Na^+ - Cl^-) and plasma HCO_3^- concentrations in trout at all three salinities (Fig. 29 a.b.c.). The reason

for using the parameter (Na^+ - Cl^-) was that the transepithelial flux of both ions could have resulted in the accumulation of plasma HCO_3^- via Na^+/H^+ and Cl^-/HCO_3^- exchanges. The ions were subtracted since the fluxes of Na^+ and Cl^- would have to be in opposite directions to accumulate the HCO_3^-. Therefore (Na^+ - Cl^-) represents the net effect of these combined fluxes. Further analysis showed that the correlation above was largely due to the Cl^-/HCO_3^- effect rather than the Na^+/H^+. Increases in plasma HCO_3^- were correlated with changes in plasma Cl^- (Fig. 30). There was no change in plasma Na^+ associated with the changes in plasma HCO_3^- (Fig. 31). There was also a negative correlation between changes in plasma Cl^- and plasma HCO_3^- in conger (Fig. 32).

IV. Transepithelial Potentials (TEP)

TEP values were negative, near zero and positive in trout acclimated to 3, 100 and 300 mM (Fig. 33 a.b.c.), respectively. There was a depolarizing trend in the initial sampling periods in fish in all salinities. TEP values generally increased during the exposure to hypercapnia in all groups as well. There was a trend towards control values during the recovery periods although there was considerable variability.

Figure 24. Means ± S.E. of net H^+ flux in rainbow trout acclimated to 3, 100 and 300mM NaCl and exposed to 1 % environmental hypercapnia. Time course shown includes control, 1 % hypercapnia and recovery periods. All values in umol/Kg/min.

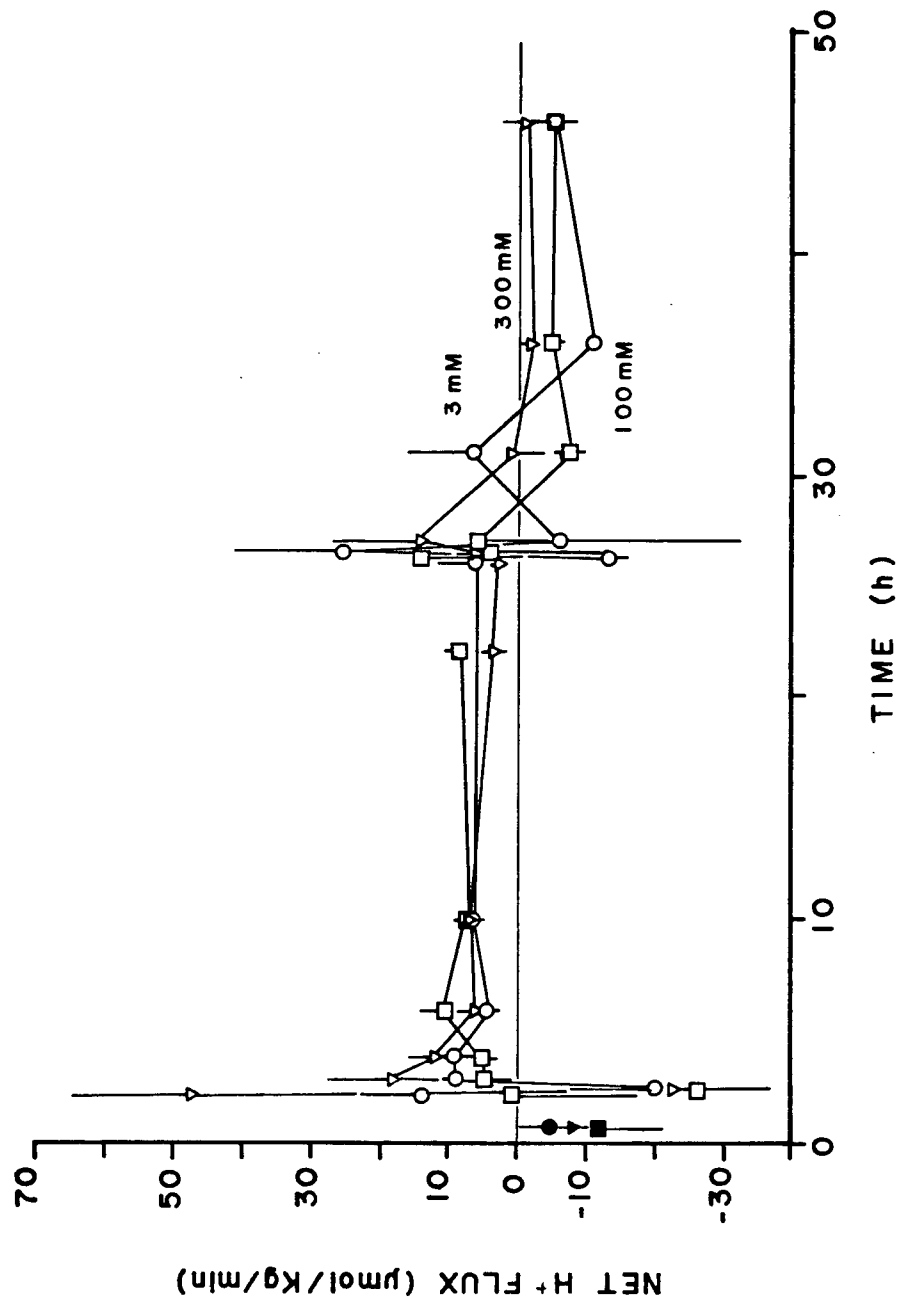

NET H+ FLUX (μmol/kg/min)
70
50
30
10
0
-10
-30
0
10
30
50
TIME (h)
3 mM
300 mM
100 mM

Figure 25. Plasma Cl^- concentrations in rainbow trout acclimated to 3, 100 and 300mM NaCl and exposed to 1 % environmental hypercapnia. Time course shown includes control, 1 % hypercapnia and recovery periods. All values in mM. Means $\pm$ S.E.

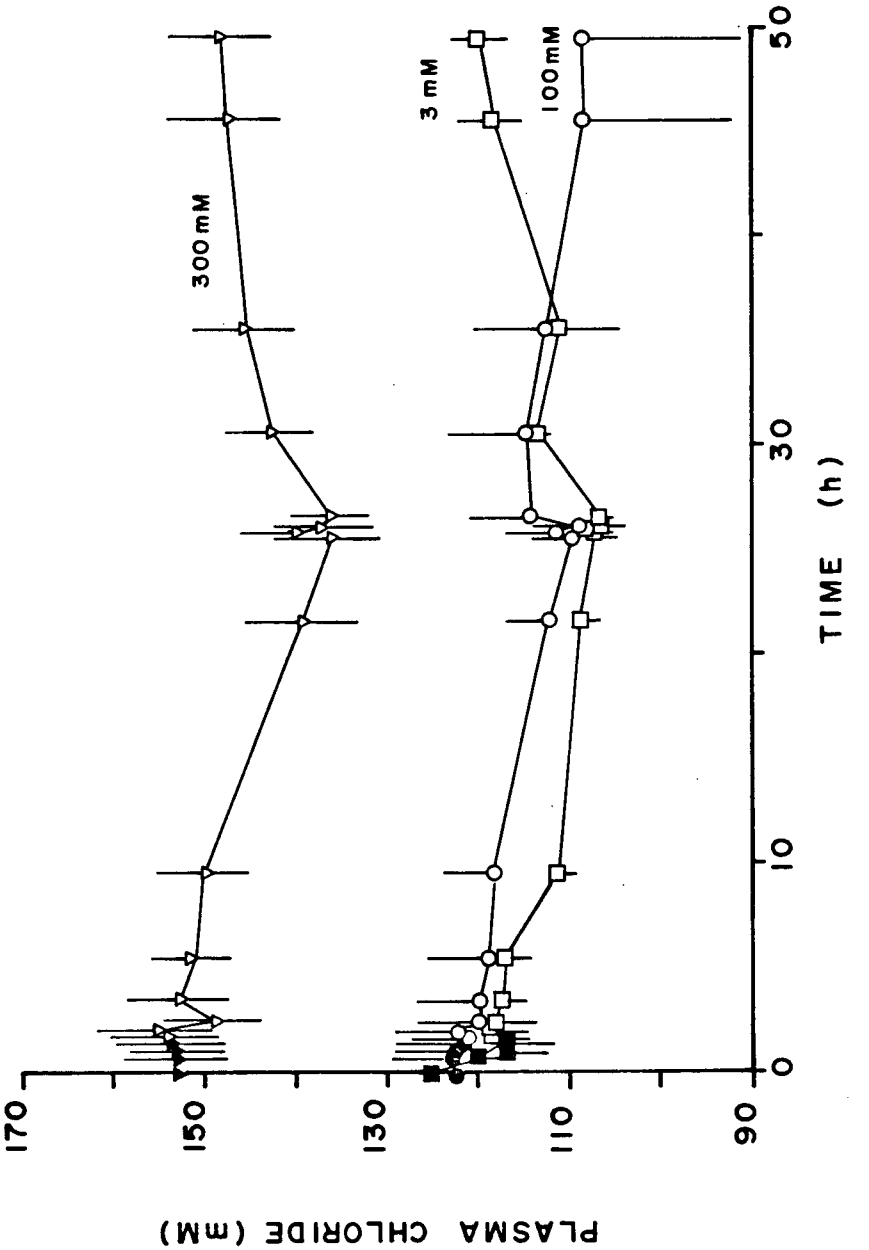

300mM
3mM
100mM
170
150
130
110
90
0
10
30
50
TIME (h)
PLASMA CHLORIDE (mM)

Figure 26. Changes in plasma Cl^- concentrations from average control values in conger during salinity change and exposure to 1 % environmental hypercapnia. Each line represents an individual fish. Salinity levels shown next to each line as Cl^- concentration.Actual average control [Cl^-] were : 0.005=150.25, 2.6=148.75, 80=151.88(mean of 2), 140=165.25, 155=155.25, 360=153.75. All values in mM.

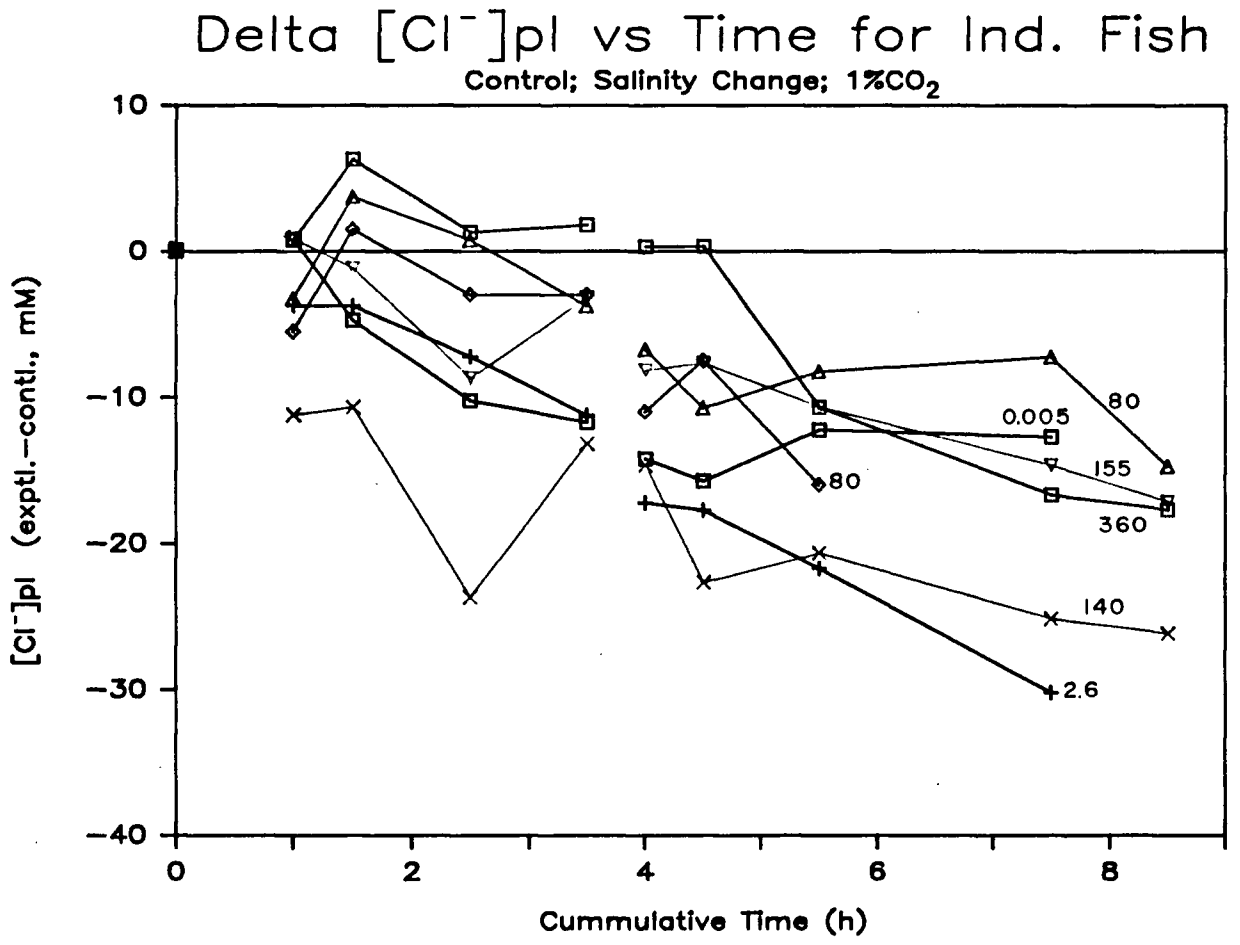

Delta [Cl⁻]pl vs Time for Ind. Fish
Control; Salinity Change; 1%CO2
[Cl⁻]pl (exptl.−contl., mM)
10
0
−10
−20
−30
−40
0
2
4
6
8
Cummulative Time (h)
0.005
80
155
360
80
140
2.6

Figure 27. Means ± S.E. of plasma Na^+ concentrations in rainbow trout acclimated to 3, 100 and 300mM NaCl and exposed to 1 % environmental hypercapnia. Time course shown includes control, 1 % hypercapnia and recovery periods. All values in mM.

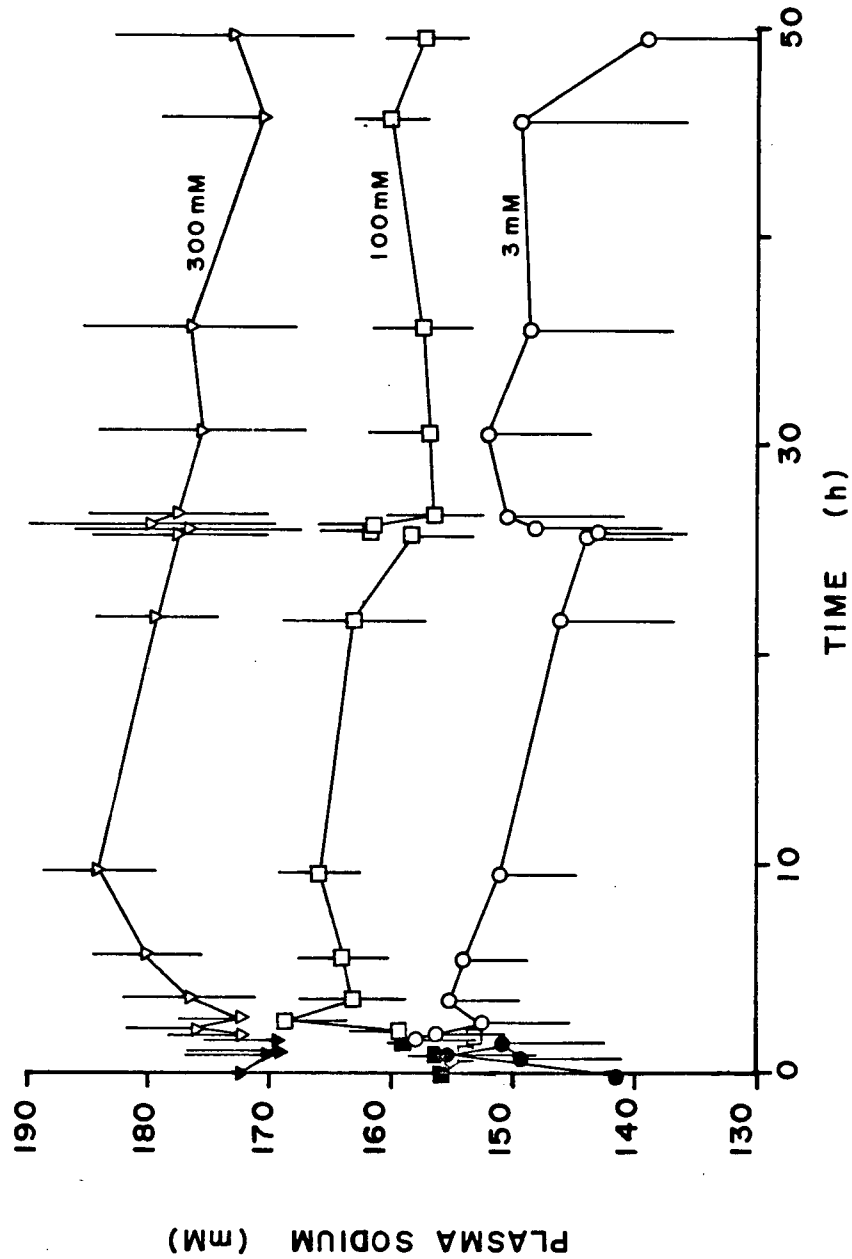

300 mM
100mM
3 mM
190
180
170
160
150
140
130
0
10
30
50
TIME (h)
PLASMA SODIUM (mM)

Figure 28. Means ± S.E. of plasma Osmolarity in rainbow trout acclimated to 3, 100 and 300mM NaCl and exposed to 1% environmental hypercapnia for 24h and recovered for 24h. All values in mOsmol.

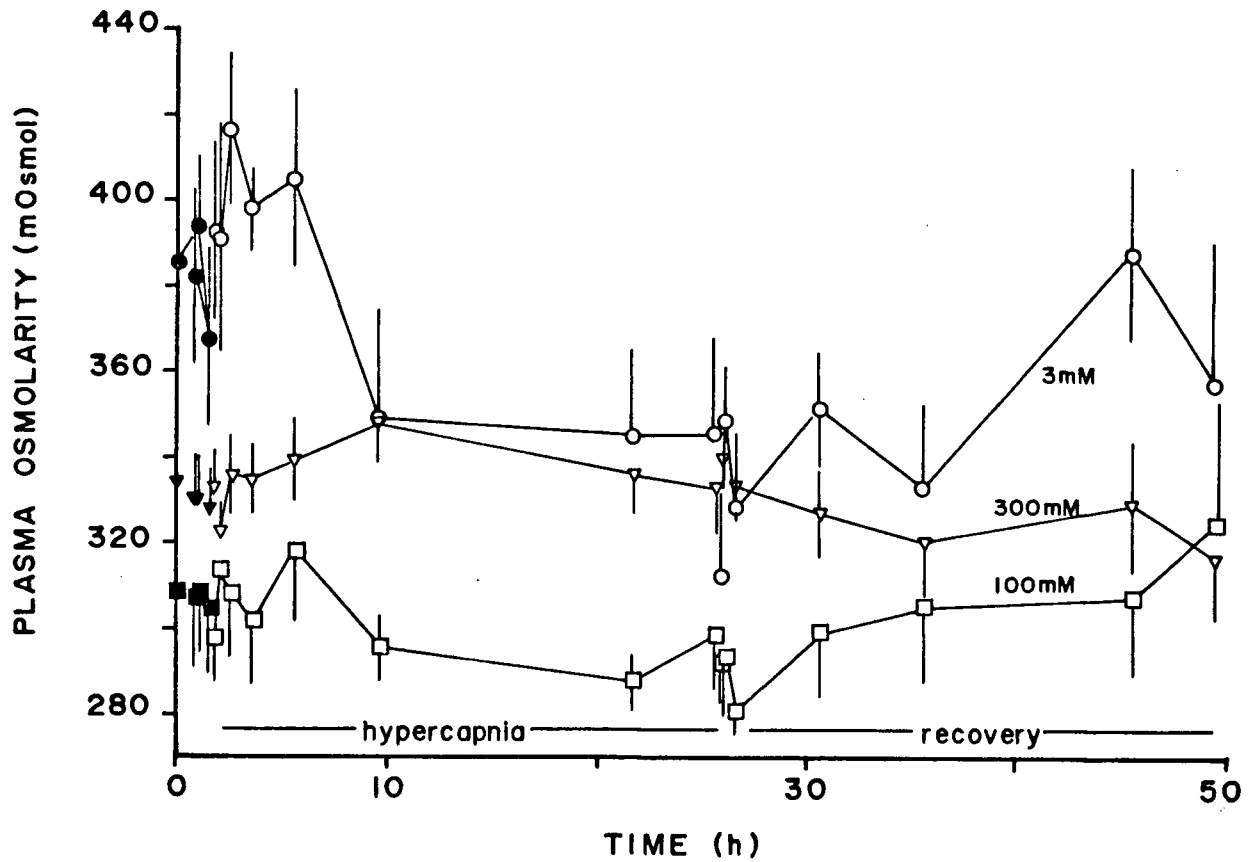

PLASMA OSMOLARITY (mOsmol)
440
400
360
320
280
3mM
300mM
100mM
hypercapnia
recovery
0
10
30
50
TIME (h)

Figure 29.a.b.c. Relationship of plasma ($[Na^+]-[Cl^-]$) to plasma $[HCO_3^-]$ during exposure and recovery from 1 % hypercapnia in rainbow trout acclimated to 3, 100 and 300mM NaCl, respectively. All values in mM. Best fit regression lines shown.

	Correlation coefficient		Coefficients for best-fit line $([Na^+]-[Cl^-])=a + b\ ([HCO_3^-])$	
	r	n	a	b
3mM	0.5281	64	0.9430	26.62
100mM	0.3676	57	0.7222	34.66
300mM	0.3503	85	1.4437	11.27

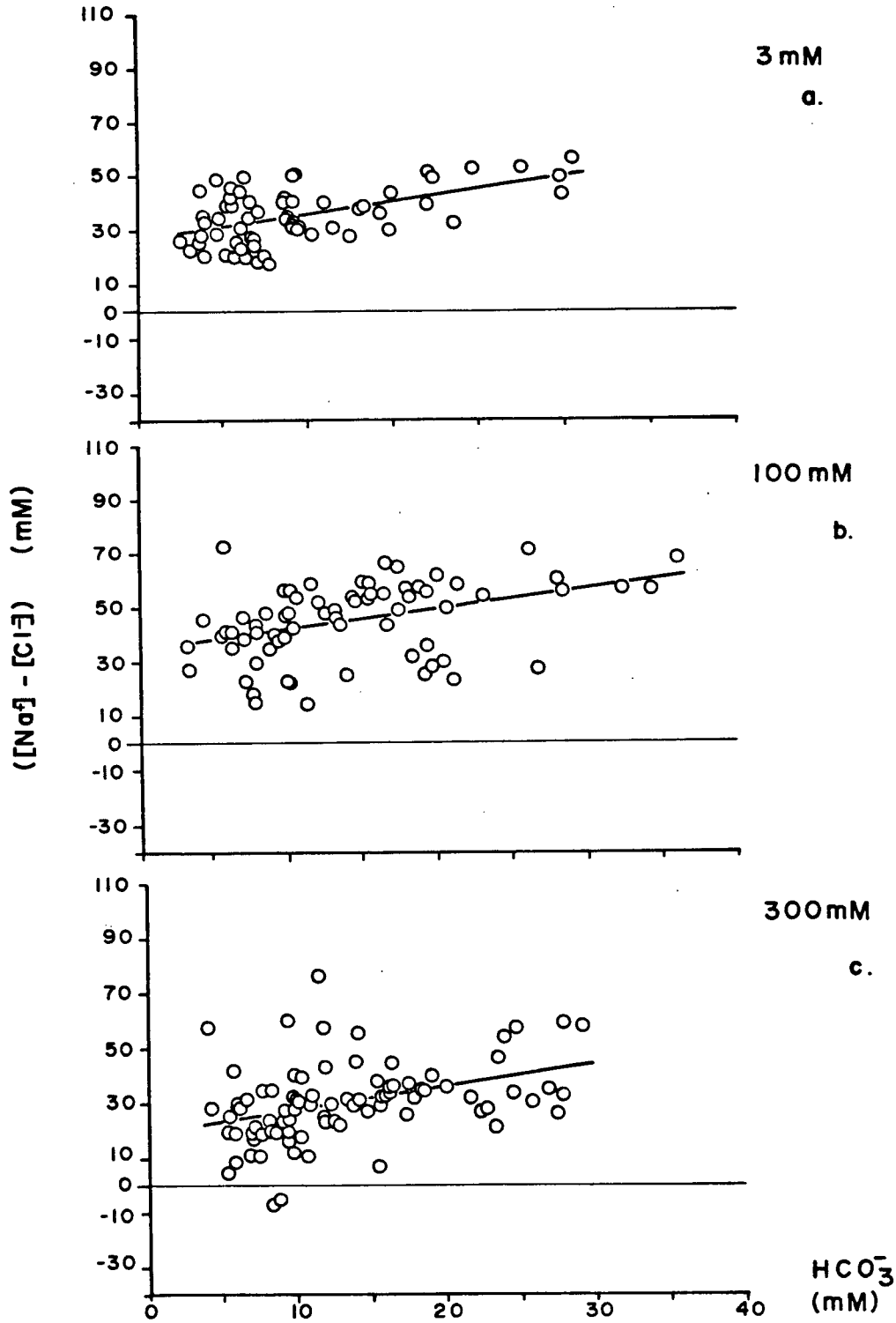

3mM
a.
100mM
b.
300mM
c.
([Na+] - [Cl-]) (mM)
HCO3-
(mM)
110
90
70
50
30
10
0
-10
-30
0
10
20
30
40

Figure 30.a.b.c. Relationship of changes in plasma Cl^- concentrations to corresponding changes in plasma HCO_3^- concentrations during exposure and recovery from 1 % hypercapnia in rainbow trout acclimated to 3, 100 and 300 mM NaCl, respectively. All values in mM. Best fit regression line shown.

	Correlation coefficient		Coefficients for best-fit line $([Na^+]-[Cl^-])=a + b([HCO_3^-])$	
	r	n	a	b
3mM	0.5084	58	-0.8470	-1.0584
100mM	0.3986	48	-0.5439	-0.0905
300mM	0.4139	74	-0.7463	-0.4684

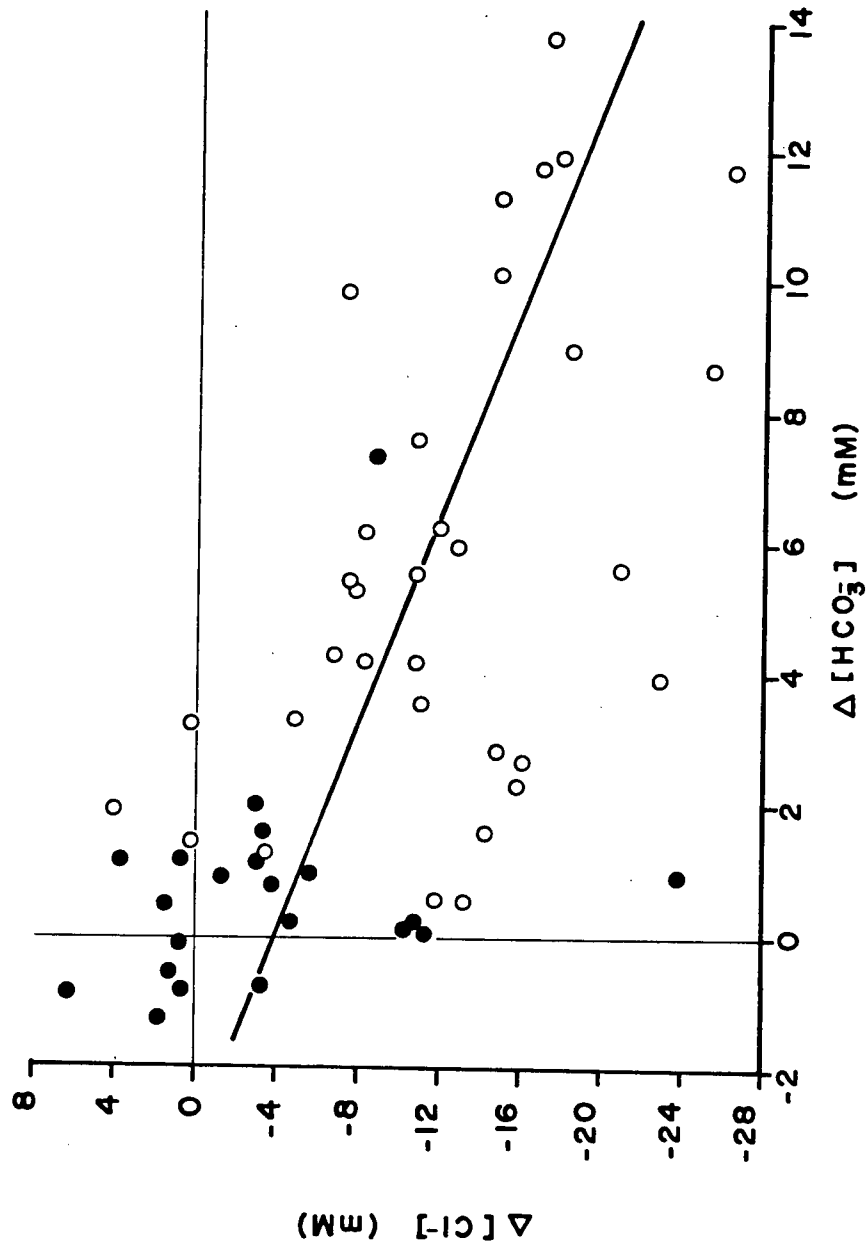

Δ [Cl-] (mM)
8
4
0
-4
-8
-12
-16
-20
-24
-28
Δ [HCO3-] (mM)
14
12
10
8
6
4
2
0
-2

Figure 31.a.b.c. Relationship of changes in plasma Na^+ concentrations to corresponding changes in plasma HCO_3^- concentrations during exposure and recovery from 1 % hypercapnia in rainbow trout acclimated to 3, 100 and 300mM NaCl, respectively. All values in mM. Best fit regression line shown.

	Correlation coefficient r	n	Coefficients for best-fit line $([Na^+]-[Cl^-])=a + b([HCO_3^-])$ a	b
3mM	0.0541	61	-0.2275	-1.0536
100mM	0.0255	59	-0.0424	0.1704
300mM	0.0361	74	0.0960	-0.3126

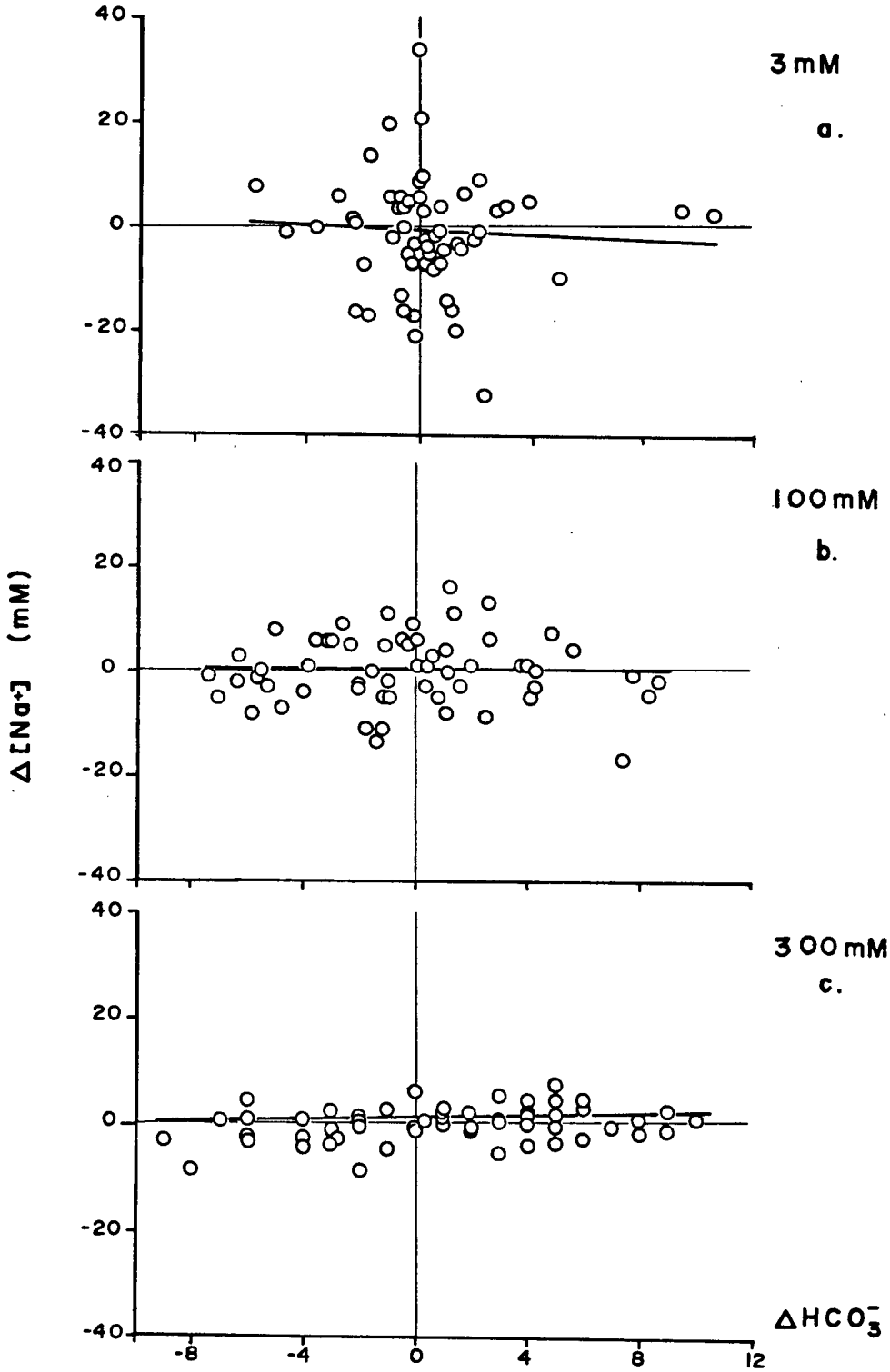

3 mM
a.
100 mM
b.
300 mM
c.
Δ [Na+] (mM)
ΔHCO3-
40
20
0
-20
-40
-8
-4
0
4
8
12

Figure 32. Relationship of changes in plasma Cl^- concentrations to corresponding changes in plasma HCO_3^- concentrations in conger exposed to salinity changes (filled circles)and exposure to 1 % environmental hypercapnia. All values in mM.

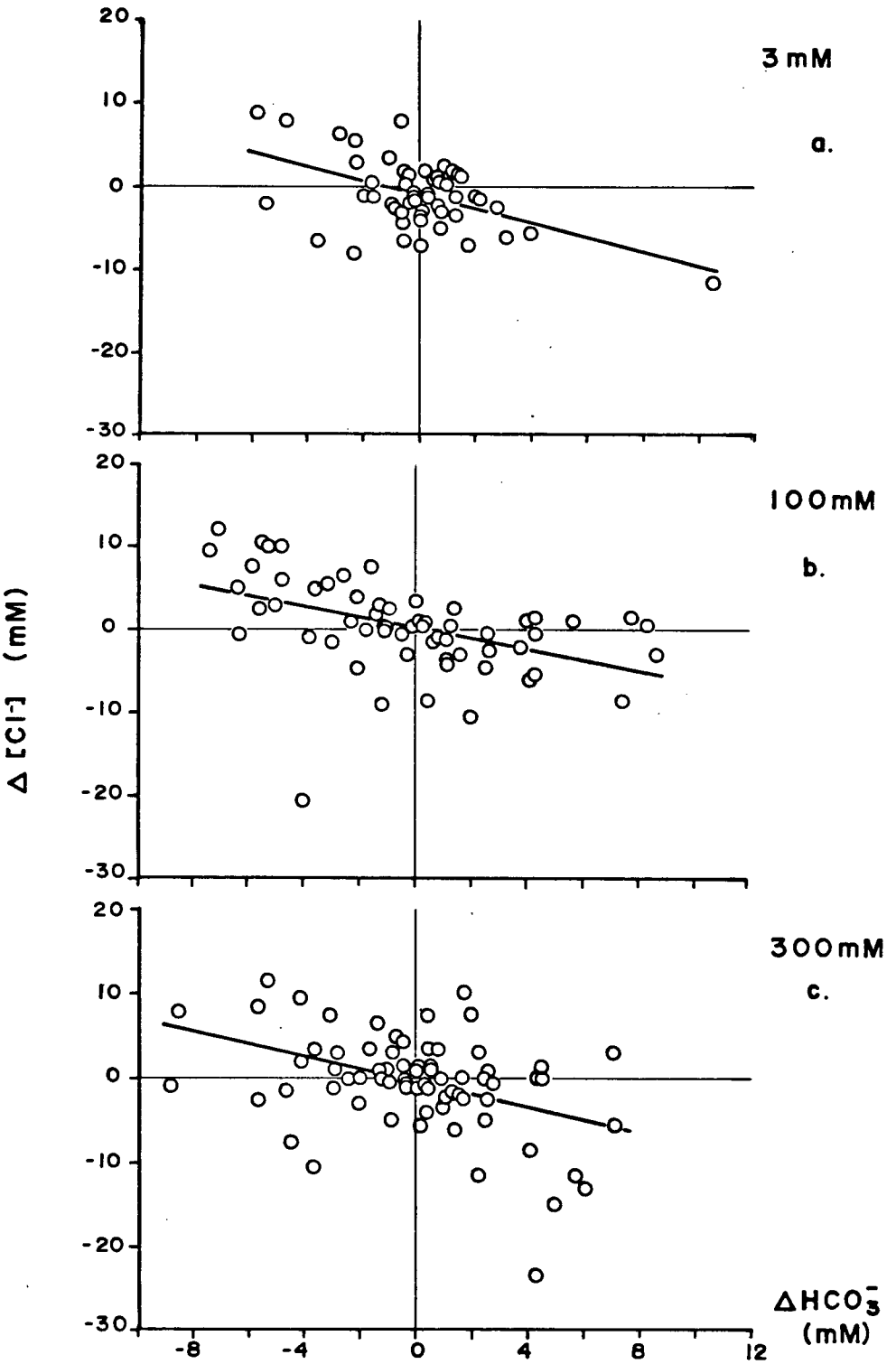
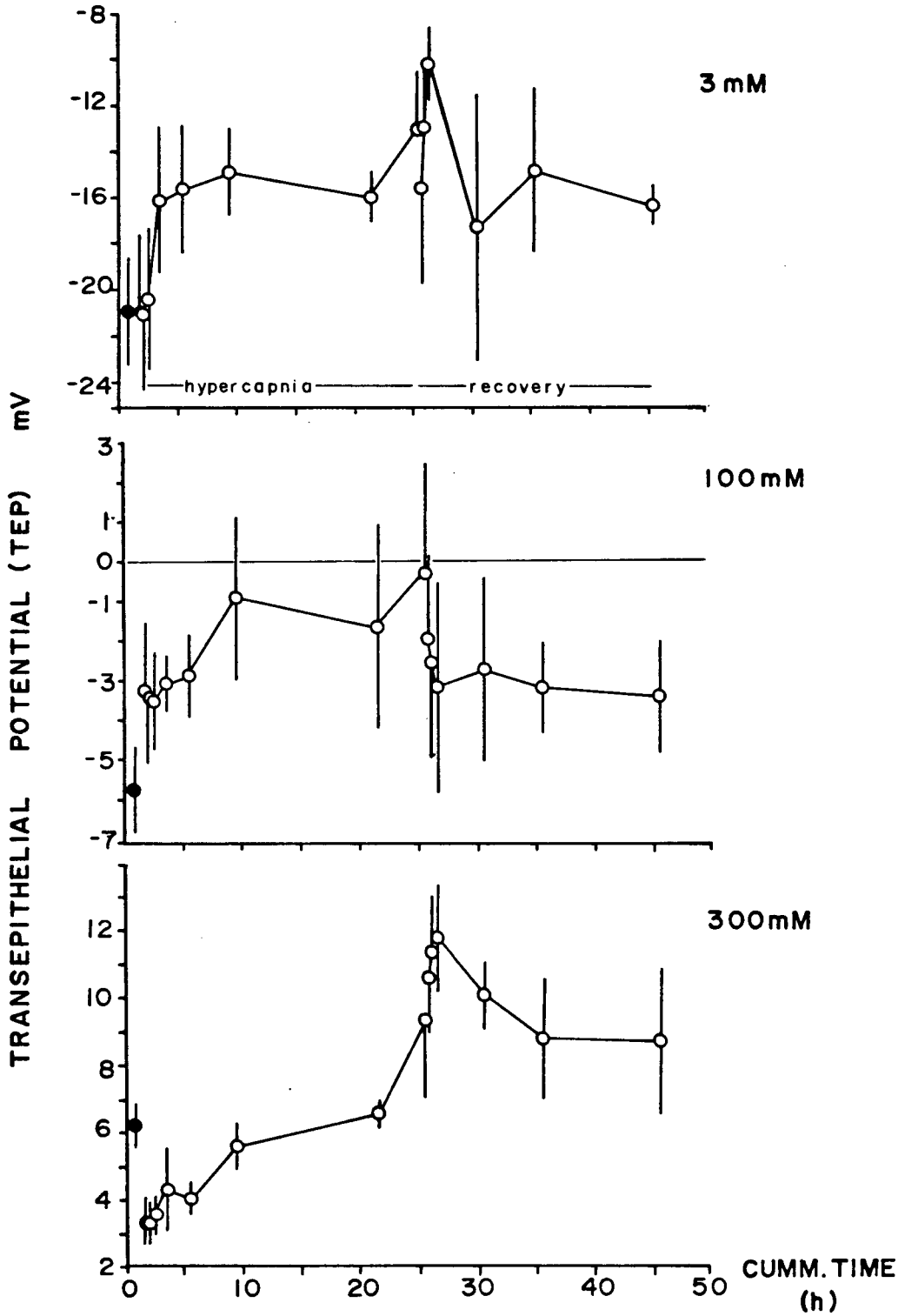

3 mM
a.
100mM
b.
300mM
c.
Δ [Cl-] (mM)
ΔHCO3- (mM)
20
10
0
-10
-20
-30
-8
-4
0
4
8
12

Figure 33.a.b.c. Means ± S.E. of trans-epithelial potential (TEP) values during control, exposure and recovery from 1% environmental hypercapnia in rainbow trout acclimated to 3, 100 and 300mM NaCl, respectively. All values in mV.


-8
-12
-16
-20
-24
3 mM
hypercapnia
recovery
3
1
0
-1
-3
-5
-7
100mM
12
10
8
6
4
2
300mM
TRANSEPITHELIAL POTENTIAL (TEP) mV
0
10
20
30
40
50
CUMM. TIME (h)

Hematocrit increased initially with the onset of hypercapnia and then declined thereafter with sampling in all groups of fish. Hematocrit values were higher and more variable in the group of fish acclimated in 3mM (Fig. 34).

EXPERIMENT 2C. CARP ISOTOPE EXPERIMENT

There was a net flux of Cl^- from the blood to the water during the exposure of the fish to 5% environmental hypercapnia. This trend was reversed after the CO_2 was turned off and aeration with air was resumed (Fig. 35). There was no change in the net flux of Na^+ over the course of the experiment relative to the trend for control fish (Fig. 35).

There was no change in the unidirectional efflux of Cl^- relative to the control trend over the entire course of the experiments (Fig. 36). Unidirectional Na^+ efflux was lower than the control trend from about the 24h mark during exposure to environmental hypercapnia. This trend continued througout the hypercapnia period as well as through the recovery period (Fig. 36).

The net flux results can only be explained by reduction of Cl^- uptake from the water in response to the exposure to environmental hypercapnia and the acid-base disturbance. Once the recovery period started, the uptake rate of Cl^- from the water returned to control levels. With the yet unchanged

Figure 34. Means ± S.E. of plasma hematocrit values (Hct in %) of trout acclimated to 3, 100 and 300 mM NaCl and exposed to 1% environmental hypercapnia for 24h and then recovered for 24h.

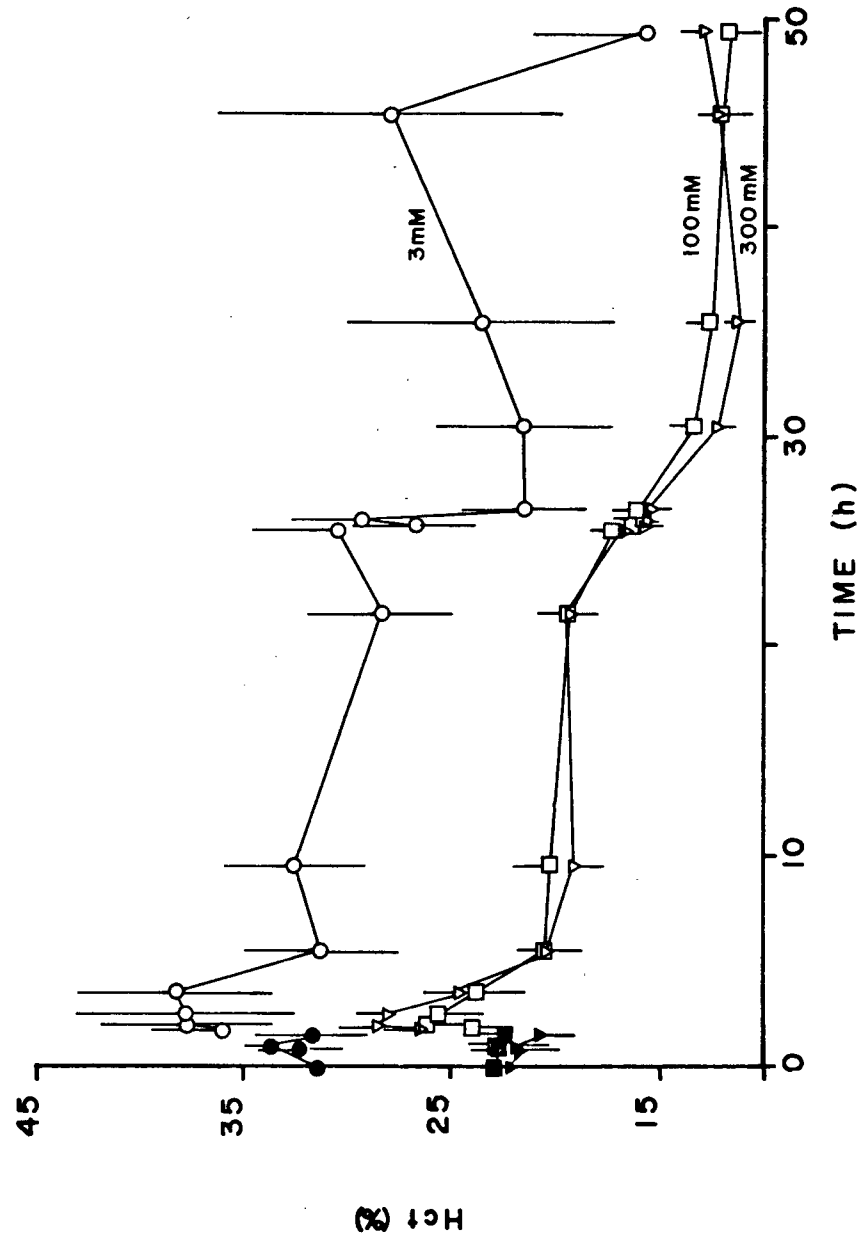

3mM
100mM
300mM
TIME (h)
0
10
30
50
15
25
35
45
Hct (%)

Figure 35. Means ± S.E. of net fluxes of Na^+(filled circles) and Cl^-(open circles) in carp during exposure to 5% environmental hypercapnia for 48h followed by 24h recovery. Fluxes are referenced to the control point (half filled circle). Control net fluxes for Na^+ and Cl^- are shown by the ..... and the ----- lines respectively and are extrapolations of net flux trends prior to the exposure period extended over the entire time course of the experiment. All data shown are for the water. Therefore positive values indicate efflux from the fish and negative values indicate uptake by the fish.

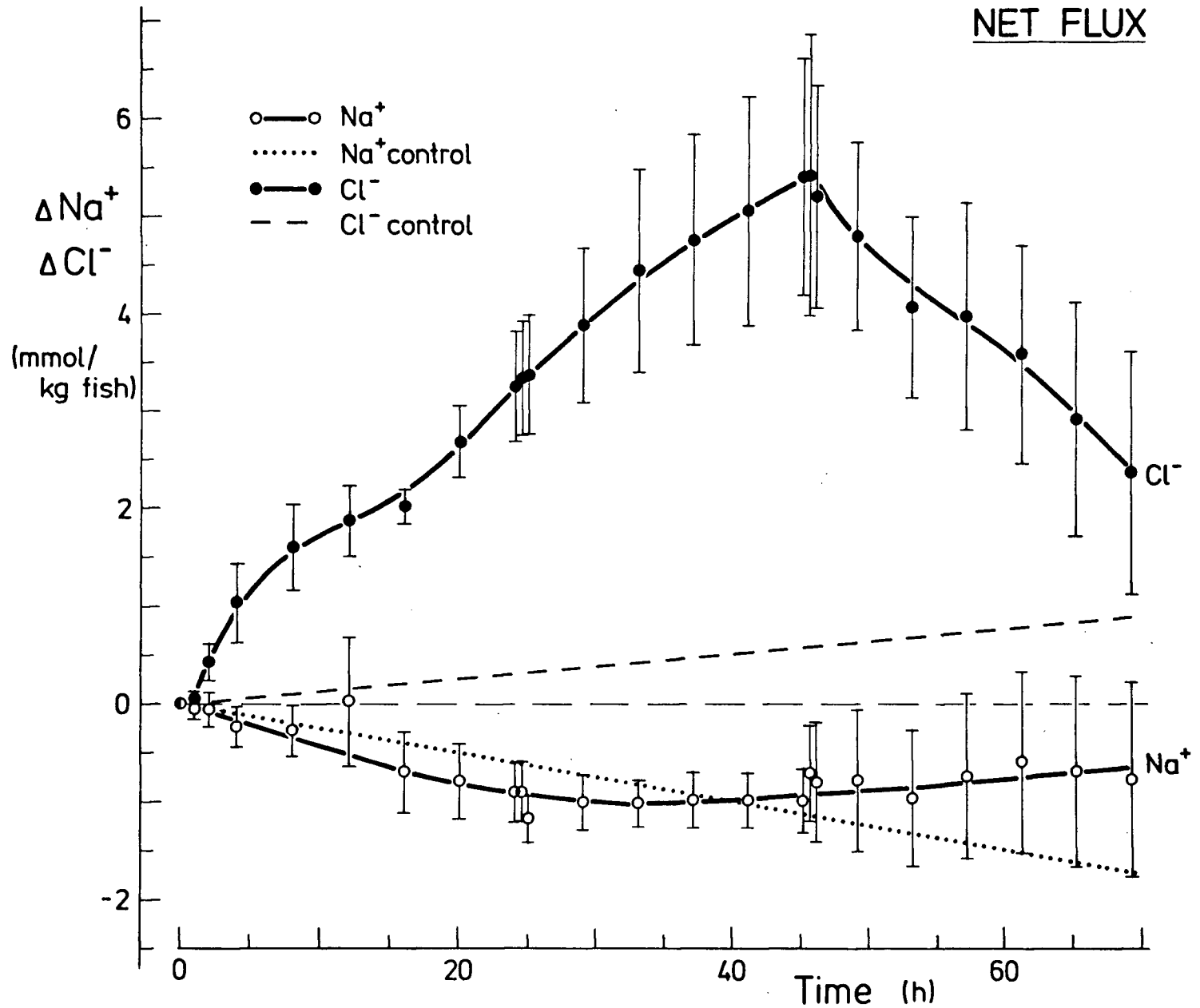
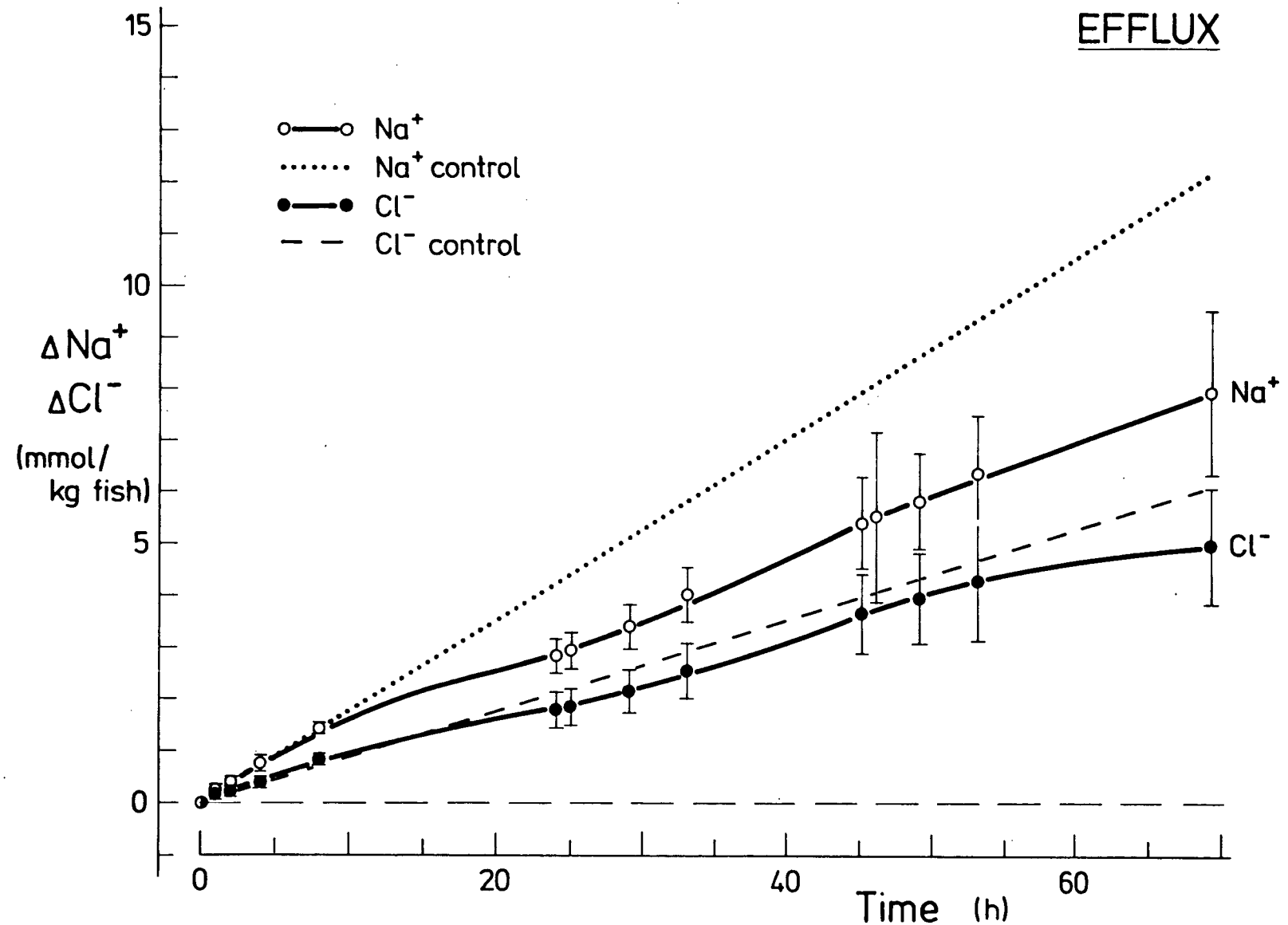

NET FLUX
Na+
Na+control
Cl-
Cl- control
ΔNa+
ΔCl-
(mmol/
kg fish)
6
4
2
0
-2
0
20
40
60
Time (h)
Cl-
Na+

Figure 36. Means ± S.E. of efflux rates for Na^+(filled circles) and Cl^-(open circles) in carp during exposure to 5% environmental hypercapnia for 48h followed by 24h recovery. Fluxes are referenced to the control point (half filled circle). Control effluxes for Na^+ and Cl^- are shown by the ..... and the ----- lines respectively and are extrapolations of efflux trends prior to the exposure period extended over the entire time course of the experiment.


EFFLUX
Na+
Na+ control
Cl−
Cl− control
15
10
5
0
ΔNa+
ΔCl−
(mmol/
kg fish)
0
20
40
60
Time (h)
Na+
Cl−

efflux of Cl^- from the blood, this would result in the observed decline n net flux from blood to water. Na^+ uptake from the water must have also been reduced to match the reduced efflux in order that an unchanged net flux from control trends could result.

DISCUSSION

Environmental hypercapnia caused a plasma acidosis in trout and conger and the initial fall in plasma pH was followed by a trend towards recovery with an associated accumulation of plasma HCO_3^-. These trends are characteristic of fish exposed to environmental hypercapnia in fresh water (carp, Claiborne and Heisler 1984; rainbow trout, Janssen and Randall 1975, Eddy et al. 1977, Lloyd and White 1967, Cameron and Randall 1972, Eddy 1976) and sea water (dogfish, Cross et al. 1969; spotted dogfish, Heisler et al. 1976,1980, Randall et al. 1976; coho salmon, Bubien and Meade 1979).

Water salinity had a positive effect in correcting the acidoses in trout acclimated to higher salinities as well as in marine conger eel conditioned to dilute waters. This supports the circumstantial evidence for the positive correlation between the ionic content of the water and the correction of acid-base disturbances from exposure to

environmental hypercapnia. Because the HCO_3^- concentration, and thus buffering, in the water was controlled so that they were nearly equivalent at the three salinities, the data was analysed with regard to the Na^+ and Cl^- concentrations in the water.

The accumulation of plasma HCO_3^- was correlated with reductions in plasma Cl^- in both trout and in conger. This relationship suggests that a Cl^-/HCO_3^- exchange mechanism plays a major role in the compensation of the acidosis in trout and conger caused by exposure to environmental hypercapnia. The link between Cl^- and HCO_3^- in transepithelial ion movements has been documented in numerous studies (Maetz and Garcia-Romeu 1964, De Renzis and Maetz 1973, De Renzis 1975, Kerstetter and Kirshner 1972, Kormanik and Evans 1979). Cameron (1976) and Claiborne and Heisler (1984) have shown that a Cl^-/HCO_3^- exchange process in the arctic grayling and carp, respectively, plays an important role in the compensation of plasma pH during environmental hypercapnia. While these studies also demonstrated $Na^+/H^+(NH_4^+)$ exchanges to play a significant role, net changes in ion concentrations in the trout experiment reported here did not show a significant role of this exhange process in the accumulation of plasma HCO_3^- and the consequent pH recovery. It is possible that this exchange was present but not detected because of a Na^+ leak from plasma to water which would have had to be roughly

equivalent to the active uptake of this ion in exchange for the efflux of H^+ to the water. While there were changes in plasma $[Na^+]$ in trout during the early hours after exposure to hypercapnia, these changes were not correlated to the changes in plasma HCO_3^-.

The reduction in plasma Cl^- concentrations associated with the HCO_3^- accumulation in the correction of plasma pH in carp exposed to environmental hypercapnia was effected by a reduction in the rates of Cl^- uptake from the water while maintaining normal efflux rates. This, of course, pertains to the condition in fresh water and the equivalent fluxes in fish at higher salinities are unknown.

The limitations to investigations of this nature at higher salinities are partially technical since the resolution of net fluxes through concentration changes is obscured by the high background concentrations of ions. While unrelated to any acid-base disturbance, Kormanik and Evans (1979) demonstrated increased Cl^- efflux from the marine toad fish, Opsanus beta, by increasing water HCO_3^-. The process of Cl^-/HCO_3^- exchange is therefore available to fish in salt water as a mechanism for HCO_3^- accumulation. It is not known, however, how this exchange process is modulated to effect the HCO_3^- accumulation. Chloride efflux could be stimulated or there could be a decrease in the passive influx of Cl^- which would result in the accumulation of plasma HCO_3^- through this exchange process.

There may be a general phenomenon of reducing active uptake of ions from the water because it seems that Na^+ uptake from the water in carp was also reduced upon exposure to environmental hypercapnia. Inhibition of active Na^+ uptake from the water has also been observed in trout exposed to acid waters (Wright and Wood 1985; Shaw 1960; McWilliams and Potts 1978; Packer and Dunson 1972; Ye, unpublished data). The irregularity here was that while normal Cl^- uptake rates are probably resumed during recovery from environmental hypercapnia, reduced Na^+ uptake from the water continued during recovery.

Modulation of the active Cl^- uptake step to accumulate the plasma HCO_3^- in freshwater carp provides *in vivo* evidence supporting previous *in vitro* studies showing the existence of an anion stimulated ATPase in the fish gill (Kerstetter and Kirschner 1974, DeRenzis and Bornancin 1977, Bornancin *et al*. 1980). Similar enzymes have been found in mammalian pancreas (Simon *et al*. 1972), intestinal and renal brush border membranes (Humphreys *et al*. 1979, Van Os *et al*. 1977, Kinne-Saffran and Kinne 1974, Liang and Sacktor 1976), and in the gills of *Necturus* (Wiebelhaus *et al*. 1971), eel (Morisawa and Utida 1976) and crab (Lee 1982). The microsomal localization of this enzyme in trout (Kerstetter and Kirschner 1974) and crab (Lee 1982) gills implicates its functional role to at least include anion exchanges related to iono- and acid-base regulation.

The lack of consistent change in the osmolarity of trout during hypercapnia at 100 and 300 mM suggests that the ionic exhanges were taking place in balance as far as ionic charges were concerned. This is consistent with the findings of Heisler et al. (1976) and Toews et al. (1983) for the spotted dogfish and conger, respectively, that osmolarity remained constant in these fish during exposure to environmental hypercapnia. The reduction in osmolarity in trout exposed to hypercapnia in 3 mM suggests an imbalance in the ionic changes during the experiment.

The increase in hematocrit values and the depolarizing trend in the TEP during the initial sampling periods after the onset of hypercapnia in trout may have been due to the effects of catecholamines which may have been present during that time. This is likely since Perry (1986) has shown that catechlamines are released in trout in response to exposure to environmental hypercapnia. Nikinmma and Heustis (1984) and Baroin et al. (1984) reported red cell swelling which could explain the increase in hematocrit. Isaia et al.(1978), furthermore, observed increased water permeability in response to catecholamines in fish. An increase in water permeability of the gills suggests an increase in the general permeability to ions if the movement of water molecules are occurring through ion channels in membranes. Given these observations, the depolarizing trend observed here might be expected from the reduction of concentration gradients between blood and water.

Reports of TEP values in the literature are variable but the general trend of increasing values with the salinity of the acclimation medium is consistent with reported studies (Kerstetter et al. 1970; Potts and Eddy 1973; House and Maetz 1974; Eddy 1975; McWilliams and Potts 1978). These values also increase during hypercapnia and return towards control values during recovery from hypercapnia.

The entire data set in this Section demonstrates that the ionic strength of the water has a positive influence on the acid-base regulatory performance of fish during exposure to environmental hypercapnia over a wide range of salinities, including hypotonic, near isotonic and hypertonic media. Additionally, Cl^-/HCO_3^- plays a dominant role in this regulation through the accumulation of plasma HCO_3^-. This accumulation has been shown to be a modulation of the active Cl^- uptake process in freshwater (Cameron 1976; Wood et al. 1984). This is in contrast to the results of Perry et al. (1981) and Kerstetter and Mize (1976) who found no significant change in the influx rates of Na^+ and Cl^- in trout undergoing an acidosis. The reasons for this discrepency are not known.

SECTION 3.

FURTHER ANALYSIS OF THE TROUT-SALINITY-HYPERCAPNIA EXPERIMENT

INTRODUCTION

This Section presents further analysis of the ionic concentration and transepithelial potential (TEP) data from the Trout - Salinity - Hypercapnia experiment in Section 2. It was the general aim of these analyses to gain more understanding about the observed distribution of ions between the water and blood in trout during exposure to and recovery from environmental hypercapnia. Accordingly, three analyses were carried out. First, the electrochemical gradient between blood and water for Cl^- was analysed in a qualitative manner for the three salinity conditions of the experiment. The observed correlation between changes in plasma Cl^- and plasma HCO_3^- accumulation during exposure to hypercapnia implied that modulations of a Cl^-/HCO_3^- exchange process played an important role in extracellular pH regulation in trout in this type of acidosis. Data from the carp - hypercapnia experiment indicated that the active Cl^-/HCO_3^- exchange process was being inhibited during exposure to environmental hypercapnia to result in the observed HCO_3^- accumulation. The aim of this analysis was to determine whether these ion gradients were being maintained by passive or active processes.

Second, the apparent permeability of the gill epithelium

to Na^+ and Cl^- was determined by solving for these variables in the Goldman equation (Goldman 1943). The three data sets, one for each salinity, which included TEP and the concentrations of Na^+ and Cl^- for plasma and water enabled the solution.

Finally, the Nernst equation was applied to calculate the expected plasma concentrations based on a passive distribution of ions according to their electrochemical gradients. Comparisons of these expected concentrations to the measured plasma concentrations gave indications of the relative magnitude of active processes in maintaining the observed concentrations of each ion before, during and after exposure to environmental hypercapnia.

MATERIALS AND METHODS

The methods by which the values used in this Section were measured or calculated have been described in the Materials and Methods of Section 2. Several new parameters were derived based on some of those values: the permeability of gill to Na^+ (PNa^+) and to Cl^- (PCl^-) and the Nernst Ratio for Na^+, Cl^- and HCO_3^-. The Nernst Ratio is the ratio of the measured concentration of an ion divided by the expected concentration based on calculations using the Nernst equation. A value of 1.0, therefore, means that the ion is

distributed according to the existing electrochemical gradients.

The equations presented in Appendix II and Appendix III were used to calculate the Nersnt Ratio for Na^+, Cl^- and HCO_3^- ions as well as the relative permeabilities, $PNa^+/PHCO_3^-$, $PCl^-/PHCO_3^-$ and PNa^+/PCl^-.

RESULTS

I. ELECTROCHEMICAL GRADIENT ANALYSIS OF Cl^-.

Trout acclimated to 3, 100 and 300mM NaCl and then exposed to 1% hypercapnia showed an initial plasma acidosis and trends towards recovery in plasma pH over the 24h exposure period. pH recovery was greater in the 100 and 300mM acclimated fish than those acclimated in 3mM. The degree of recovery was proportional to the degree of plasma HCO_3^- accumulation.

There were changes in plasma ion concentrations associated with the changes in plasma HCO_3^- concentrations. Increases in plasma HCO_3^- concentrations were most strongly correlated to decreases in plasma Cl^- concentrations (Fig. 37 a.b.c.). At 3mM NaCl, the Cl^- gradient was from blood to water (Fig. 38 a.). On the basis of a possible Cl^-/HCO_3^- exchange process, the passive

Figure 37.a. Relationship of plasma HCO_3^- to plasma (Na^+ - Cl^-) for three salinities. Best fit regression lines represent data for trout during hypercapnia and recovery periods.
Figure 37.b. Relationship of delta HCO_3^- to delta Cl^- for plasma data from hypercapnia and recovery periods.
Figure 37.c. Relationship of delta HCO_3^- to delta Na^+ for plasma data from hypercapnia and recovery periods.
For Figures 37a.b.c. : _____ 3 mM; ------ 100 mM; ..... 300 mM. All units in mM.

	Correlation coefficient		Coefficients for best-fit line Y = a + b * X	
		a.		
	r	n	a	b
3mM	0.5281	64	0.9430	26.62
100mM	0.3676	57	0.7222	34.66
300mM	0.3503	85	1.4437	11.27
		b.		
	r	n	a	b
3mM	0.5084	58	-0.8470	-1.0584
100mM	0.3986	48	-0.5439	-0.0905
300mM	0.4139	74	-0.7463	-0.4684
		c.		
	r	n	a	b
3mM	0.0541	61	-0.2275	-1.0536
100mM	0.0255	59	-0.0424	0.1704
300mM	0.0361	74	0.0960	-0.3126

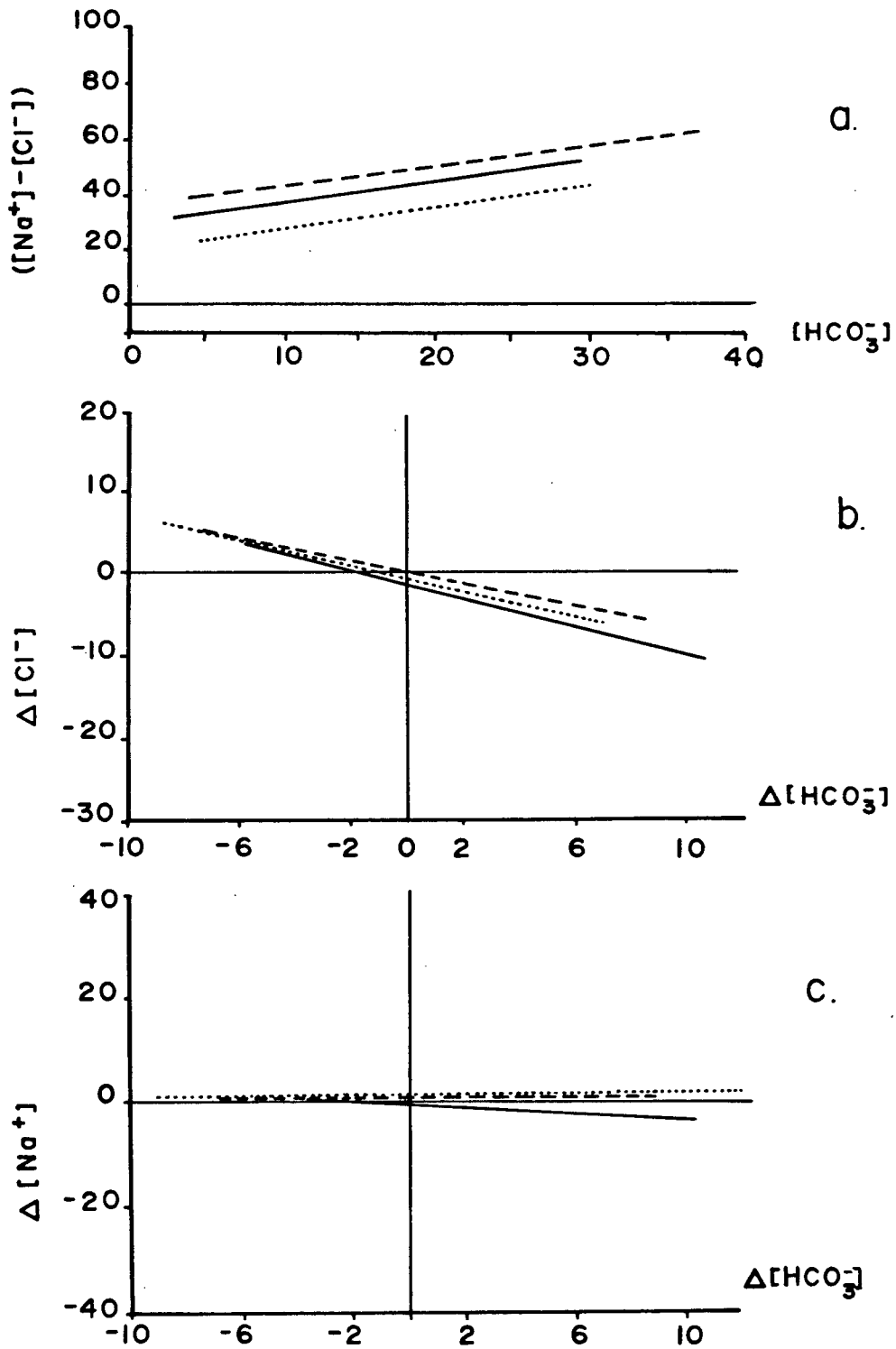
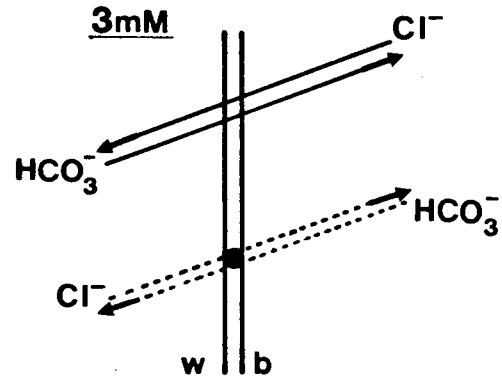
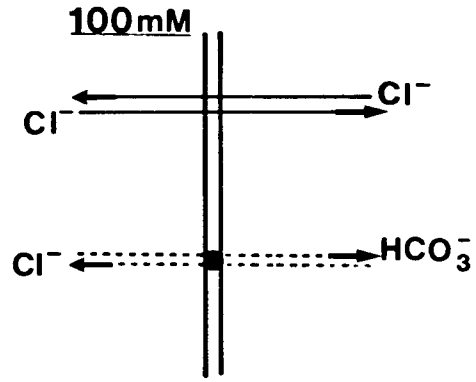

100
80
60
40
20
0
([Na+]-[Cl-])
a.
0
10
20
30
40
[HCO3-]
20
10
0
-10
-20
-30
Δ[Cl-]
b.
-10
-6
-2
0
2
6
10
Δ[HCO3-]
40
20
0
-20
-40
Δ[Na+]
c.
-10
-6
-2
2
6
10
Δ[HCO3-]

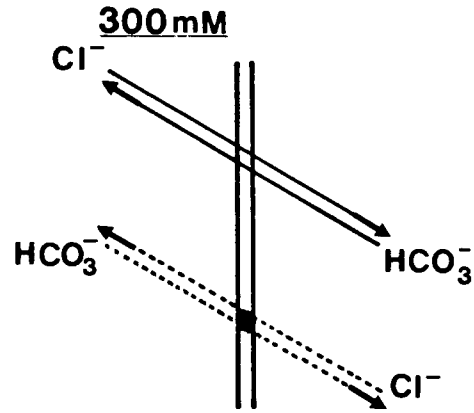
Figure 38. Hypothetical active and passive Cl^- movements across the trout gill at three salinities and the associated transepithelial potential (TEP) and plasma HCO_3^- accumulation characteristics. All data from trout exposed to environmental hypercapnia at three water salinities. The symbols w and b represent the water and blood compartments, respectively. Solid and broken lines with arrows represent the net directional movement of ions across the gill epithelium which is represented by the double vertical lines.



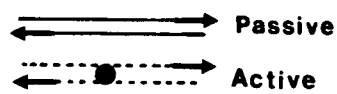
	T.E.P.	HCO_3^- ACCUM.
3mM	NEGATIVE	LOW



	T.E.P.	HCO_3^- ACCUM.
100mM	NEAR ZERO	HIGH



	T.E.P.	HCO_3^- ACCUM.
300mM	POSITIVE	HIGH



efflux of blood Cl^- could have caused the observed accumulation of plasma HCO_3^-. The negative TEP values also would have enhanced the passive efflux of Cl^-. At 100mM, the gradient for plasma Cl^-, in the same direction as in 3 mM, was very small (Fig. 38 b.). It is unlikely that the passive efflux of Cl^- along its small concentration gradient could have effected the observed large accumulation of plasma HCO_3^-. Similarly, the near zero potential across the gill epithelium at this salinity would have had a minor effect in the Cl^- and related HCO_3^- movements. At 300mM, the gradient for Cl^- was from water to blood (Fig. 38 c.). On the basis of passive movements and a Cl^-/HCO_3^- exchange mechanism, HCO_3^- would have been lost instead of gained. The inside-positive potential would have enhanced Cl^- uptake from the water on a passive basis as well.

This analysis of the possible movements of Cl^- shows that the degree of HCO_3^- accumulation in the fish acclimated to 100 and 300mM cannot be attributed to passive Cl^- movements. On the basis of passive Cl^- movements, the data set would predict the greatest reduction in plasma Cl^- and consequent HCO_3^- accumulation in 3mM, the least in 100mM and a loss of HCO_3^- in 300mM. Since the observed trend of HCO_3^- accumulation was in the opposite direction active processes must have accumulated the plasma HCO_3^- in fish exposed to hypercapnia at 100 and 300mM.

Although the fish in 3mM could have accumulated the plasma

HCO_3^- through a Cl^-/HCO_3^- exchange process linked to the passive efflux of Cl^-, experiment 2C., which involved studying the acid-base regulation in carp exposed to environmental hypercapnia, showed that active ion exchange processes in that fish in freshwater effected the accumulation of plasma HCO_3^-.

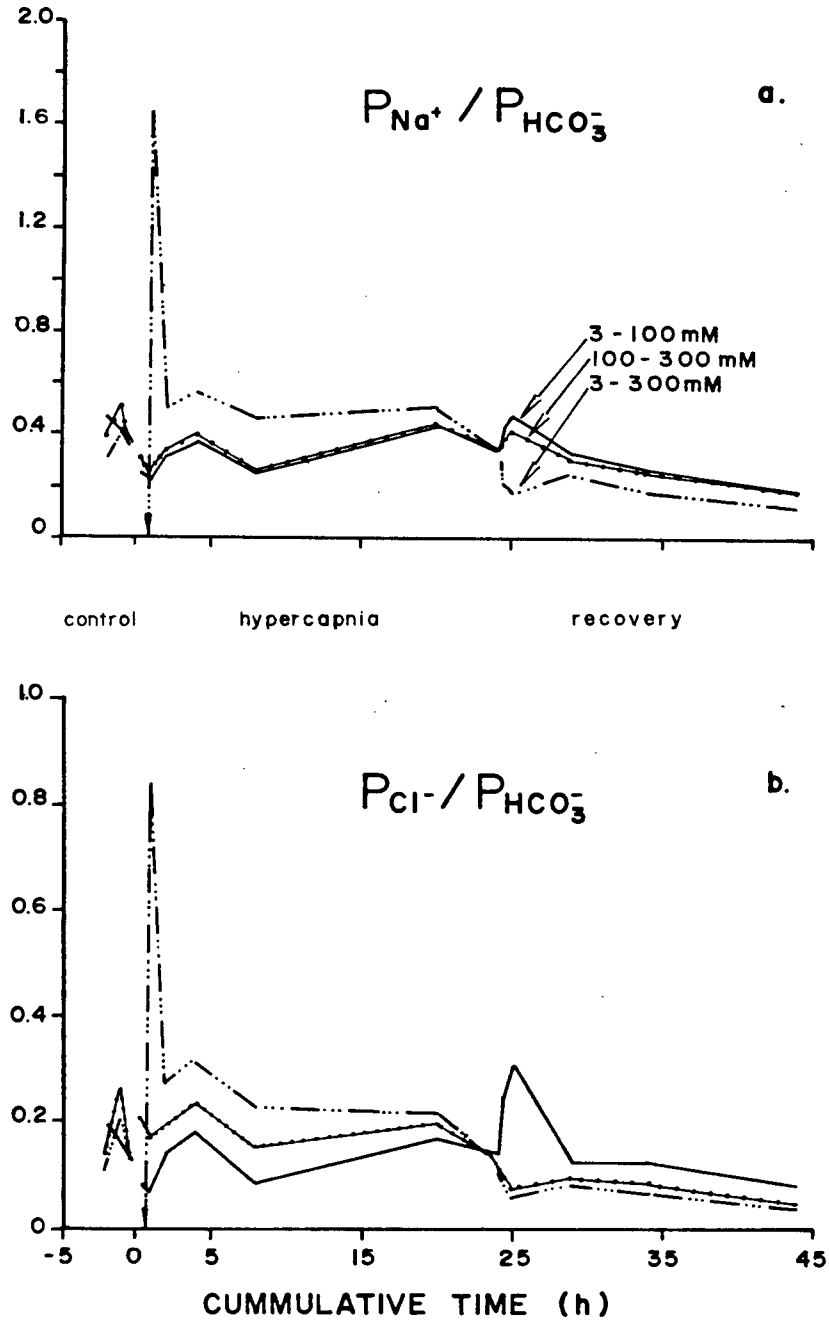
II. PERMEABILITY OF THE GILL TO Na^+ (PNa^+) AND Cl^- (PCl^-).

PNa^+ was about 0.4 of $PHCO_3^-$ whereas PCl^- was about 0.2 of $PHCO_3^-$ in trout before experimental procedures were initiated (Fig. 39 a.b.). Exposure to environmental hypercapnia caused slight transient changes in relative permeabilities which were greatest just after exposure and decreased to control values at the end of the 24h exposure. To a smaller degree, similar transients were seen after recovery from exposure to hypercapnia was started.

Both $PNa^+/PHCO_3^-$ and $PCl^-/PHCO_3^-$ for the 3-300mM data set showed large changes within the first 2h after the beginning of hypercapnia (Fig. 39 a.b.). Both values changed from control values to large negative values and then changed to about 4 times control values at the 1h sampling period.

$PNa^+/PHCO_3^-$ calculated for the data sets: 3-100mM and 100-300mM showed similar values as well as trends

Figure 39.a. and b. Permeabilites of Na^+ ($PNa^+/PHCO_3^-$) and Cl^- ($PCl^-/PHCO_3^-$) relative to HCO_3^- for the gill of trout during and after exposure to environmental hypercapnia. These two parameters were derived by simultaneous solution of the Goldman equation for three unique pairs of data from the three water salinities investigated. The three sets of solutions from this analysis are represented by the three lines on each graph.


2.0
1.6
1.2
0.8
0.4
0
$P_{Na^+} / P_{HCO_3^-}$
a.
3 - 100 mM
100 - 300 mM
3 - 300 mM
control
hypercapnia
recovery
1.0
0.8
0.6
0.4
0.2
0
$P_{Cl^-} / P_{HCO_3^-}$
b.
-5
0
5
15
25
35
45
CUMMULATIVE TIME (h)

throughout the experiment (Fig. 39 a.). These differed from values derived from the 3-300mM data set. They were lower during exposure to hypercapnia and higher during recovery compared to 3-300mM values.

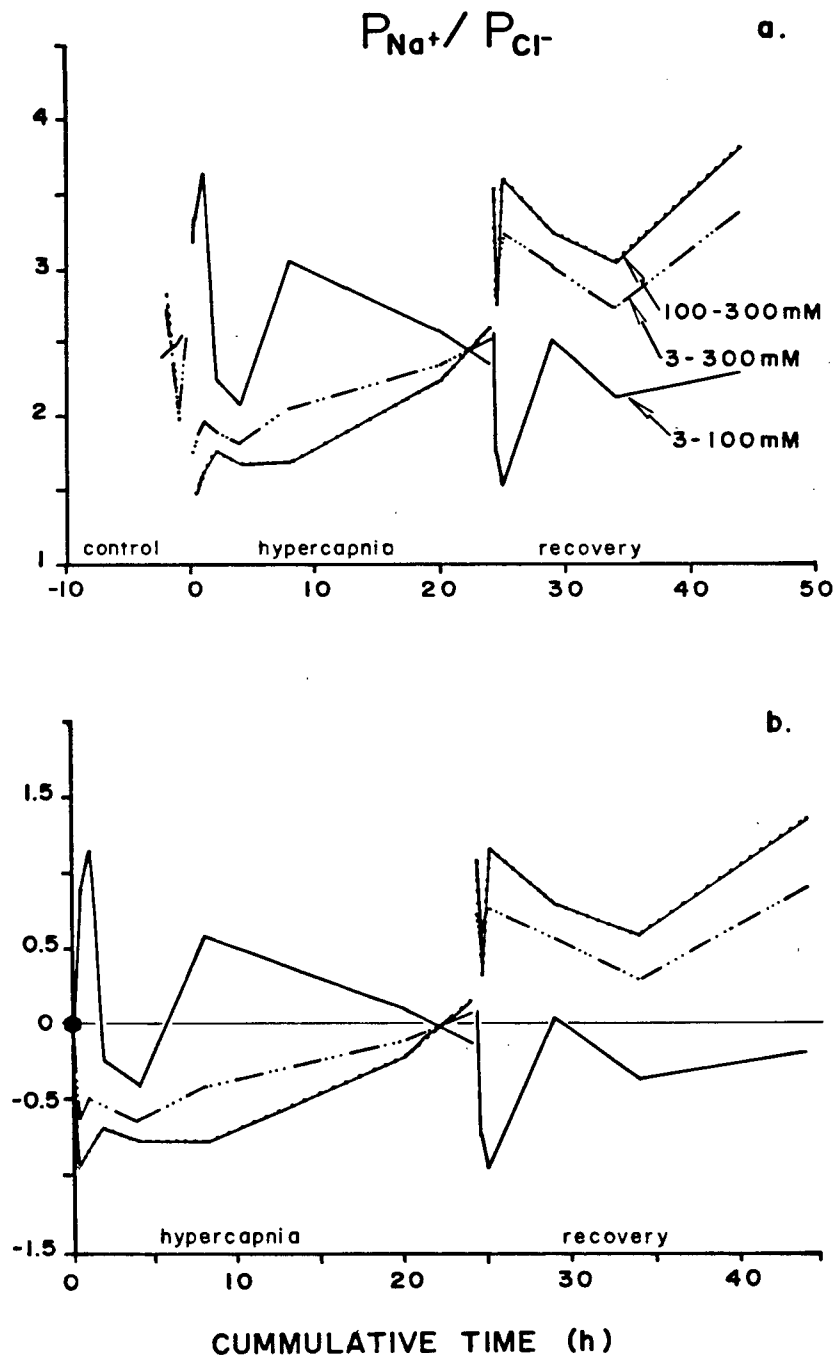
During exposure to hypercapnia, $PCl^-/PHCO_3^-$ values were lowest when the 3-100mM data set was used (Fig. 39 b.). They were highest when the 3-300mM data set was used and intermediate for the 100-300mM data set. The differences were approximately equal during hypercapnia which gradually decreased over the 24 h exposure period. Deviations from the range of control values during hypercapnia were in the $PCl^-/PHCO_3^-$ values for the 3-100mM data set. During recovery, $PCl^-/PHCO_3^-$ calculated for the 100-300 and 3-300mM data sets were similar in value and trend and were lower than those values calculated for the 3-300mM data set. There was an increase in the $PCl^-/PHCO_3^-$ values in the first hour of recovery for the 3-100mM data set.

The PNa^+/PCl^- values averaged about 2.5 for the control period (Fig. 40 a.b.). This ratio was changed during and after exposure to environmental hypercapnia. Changes which occurred during hypercapnia were abolished by the end of the 24h exposure.

The ratios of PNa^+/PCl^- for the data sets: 100-300mM and 3-300mM were similar in their response during the hypercapnia period. Those values decreased relative to average control values after onset of hypercapnia and

Figure 40.a. Permeability of Na^+ relative to Cl^- for the trout gill during and after exposure to environmental hypercapnia. Calculated by division of $PNa^+/PHCO_3^-$ by $PCl^-/PHCO_3^-$; see figure legends 39a. and 39b. above.

Figure 40.b. Data of 40.a. referenced to the average control values. Exptl.(PNa^+/PCl^-)-Ave. Control(PNa^+/PCl^-).


P_{Na^+}/P_{Cl^-}
a.
4
3
2
1
100-300mM
3-300mM
3-100mM
control
hypercapnia
recovery
-10
0
10
20
30
40
50
b.
1.5
0.5
0
-0.5
-1.5
hypercapnia
recovery
0
10
20
30
40
CUMMULATIVE TIME (h)

increased gradually to control levels by the 24h sampling time. In contrast, PNa^+/PCl^- for the 3-100mM data set increased relative to control levels immediately after onset of hypercapnia and generally declined back to control values by the 24h sampling time except for a period between 2-4h when there was a transient decline to near control levels. The end of hypercapnia caused the PNa^+/PCl^- ratios for the 100-300mM and 3-300mM data sets to increase and remain higher than control levels for the remainder of the experiment. This was caused by lower $PCl^-/PHCO_3^-$ values relative to $PNa^+/PHCO_3^-$ values for this period. The lower PNa^+/PCl^- values in the initial sampling periods in recovery from hypercapnia for the 3-100 mM data set was due to increased $PCl^-/PHCO_3^-$ values relative to $PNa^+/PHCO_3^-$ values which stayed near control levels. Other than this change, PNa^+/PCl^- ratios remained near control values during the recovery period for the 3-100 mM data set. The PNa^+/PCl^- ratio for the 3-300 mM data set decreased sharply 1 h after the end of hypercapnia after which it increased to near control levels for the remainder of the experiment.

The difference in PNa^+/PCl^- values between the 3-100 mM data set and the other data sets, 3-300 and 100-300 mM, suggests that the apparent permeability to these ions changed over the salinity range investigated and occurred in the range of 3-100 mM. The similarity in trends in data whenever the

300 mM data set was included in the calculations indicates that some aspect of this high salinity had a strong influence on the parameters in these calculations.

III. NERNST RATIO ANALYSIS

The Nernst ratio for plasma Na^+ during the control period averaged about 34, 1.3 and 0.7 for fish in 3mM, 100mM and 300mM respectively (Fig. 41 a.b.c.). There was a general increase in this ratio during exposure to hypercapnia in all three salinities. Values during the recovery period were generally variable and showed no consistent difference from the hypercapnia period except for the 300mM group of fish where there was a clear trend towards control values.

The average Nernst ratios for plasma Cl^- were about 20, 0.85 and 0.58 for 3mM, 100mM and 300mM respectively (Fig. 42 a.b.c.). There was little change in this ratio at 3mM during hypercapnia and recovery periods. The ratios at 100mM during hypercapnia were generally higher than control values but variability masked the differences. There was a general trend towards the control value during recovery. There was a significant initial decline in Nernst ratio at 300mM after which there was a recovery back to the control value by the end of the 24h. The end of hypercapnia caused an increase which subsequently declined to the control value.

The average Nernst ratios for plasma HCO_3^- were about

Figure 41.a.b.c. Means ± S.E. of ratios of measured to expected plasma Na^+ concentrations for trout during and after exposure to environmental hypercania at three water salinities. 'Expected' values calculated from the Nernst equation. Filled circles are the average control points.

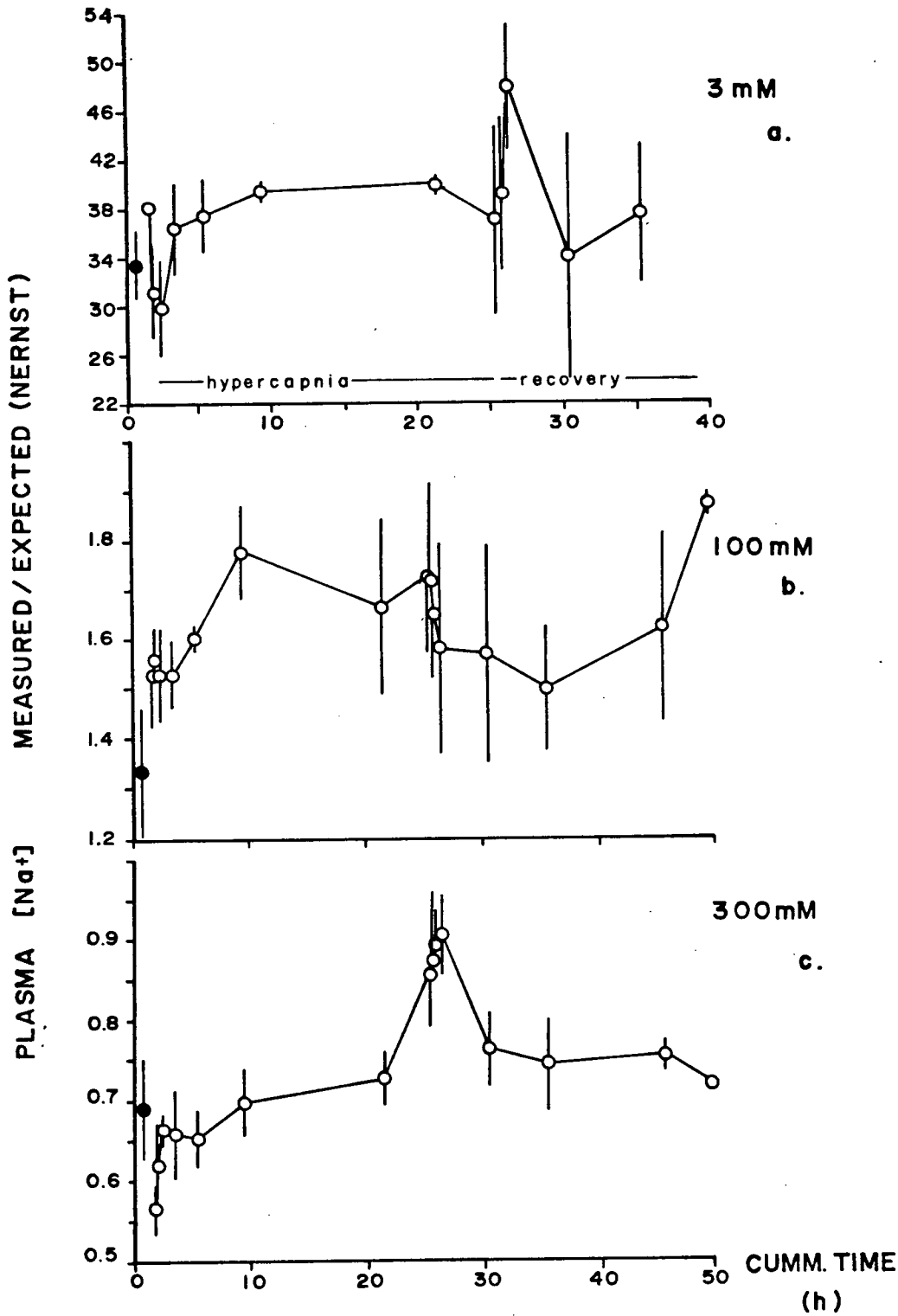

PLASMA [Na+] MEASURED / EXPECTED (NERNST)
3 mM
a.
hypercapnia
recovery
100 mM
b.
300 mM
c.
CUMM. TIME (h)

Figure 42.a.b.c. Means ± S.E. of ratios of measured to expected plasma Cl^- concentrations for trout during and after exposure to environmental hypercania at three water salinities. 'Expected' values calculated from the Nernst equation. Filled circles are the average control points.

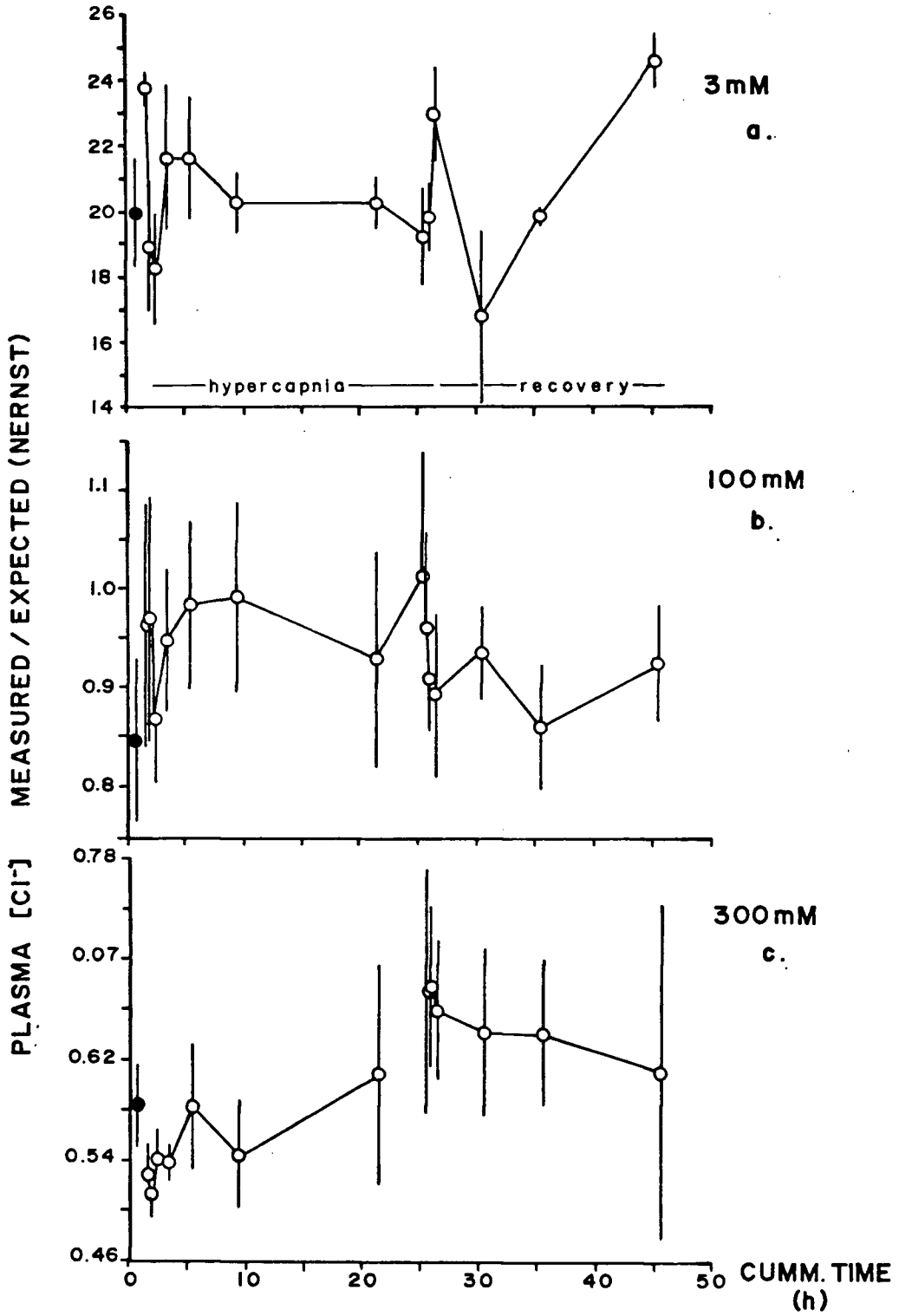
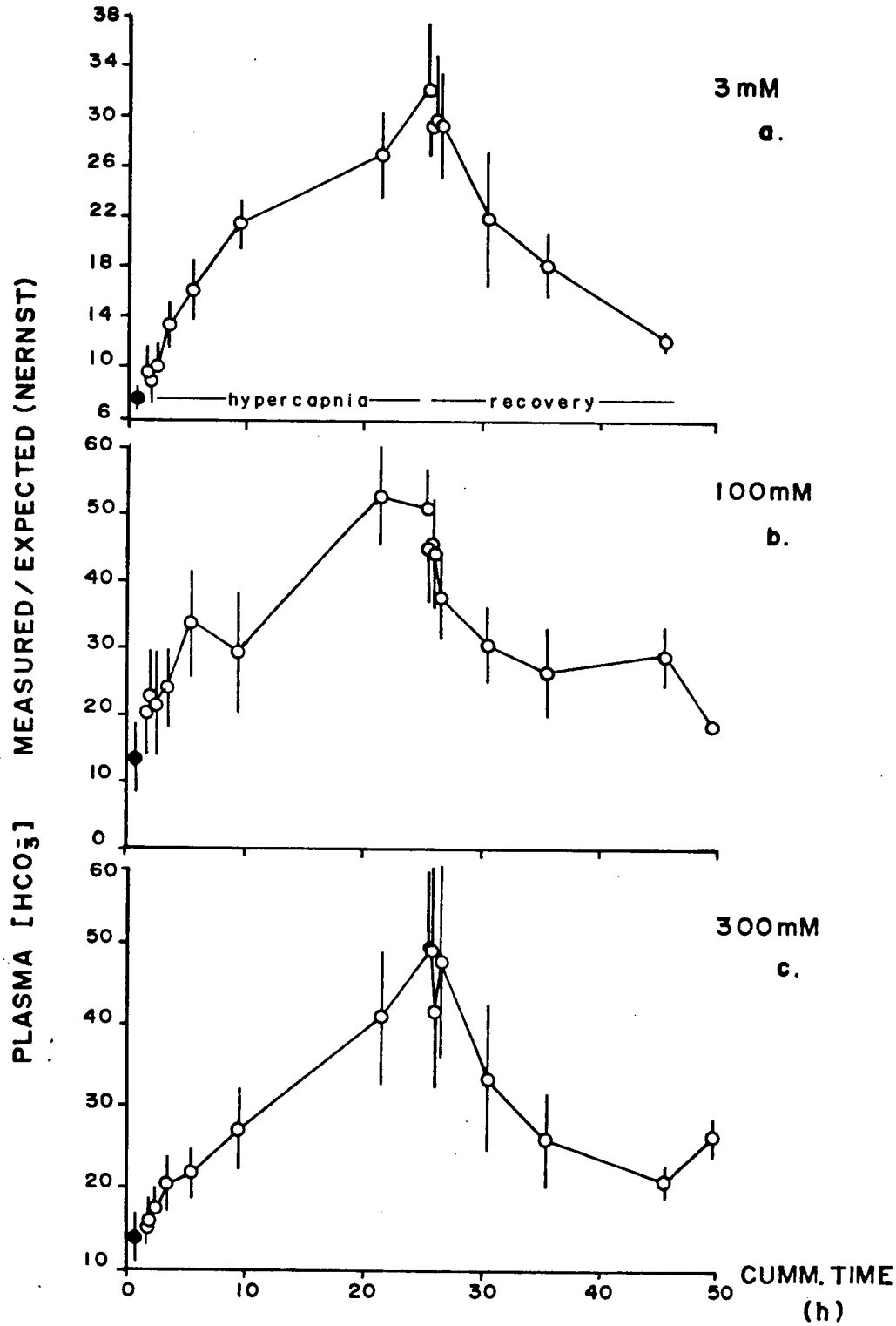

PLASMA [Cl⁻] MEASURED / EXPECTED (NERNST)
3mM
a.
hypercapnia
recovery
100mM
b.
300mM
c.
CUMM. TIME (h)
26
24
22
20
18
16
14
1.1
1.0
0.9
0.8
0.78
0.07
0.62
0.54
0.46
0
10
20
30
40
50

Figure 43.a.b.c. Means ± S.E. of ratios of measured to expected plasma HCO^-_3 concentrations for trout during and after exposure to environmental hypercania at three water salinities. 'Expected' values calculated from the Nernst equation. Filled circles are the average control points.


PLASMA [HCO3] MEASURED / EXPECTED (NERNST)
3mM
a.
hypercapnia
recovery
100mM
b.
300mM
c.
CUMM. TIME
(h)

8, 13, and 13 for 3mM, 100mM and 300mM respectively (Fig. 43 a.b.c.). Unlike the ratios for Na^+ and Cl^-, trends in the Nernst ratios for HCO_3^- showed consistent trends during both hypercapnia and recovery periods. The ratios increased 4-5 times during the hypercapnia period and returned to near-control levels by the end of the recovery period.

DISCUSSION

The analyses in this Section provide a basis for a series of qualitative statements about the nature of the distribution of Na^+ and Cl^- between blood and water in trout acclimated to three water salinities and exposed to environmental hypercapnia.

Active rather than passive processes involving a Cl^- for HCO_3^- exchange effected the accumulation of plasma HCO_3^- in trout exposed to 1% environmental hypercapnia at all three salinities investigated. This agrees with the results of the carp hypercapnia study of Section 2 which showed that not only was this exchange process important in regulation of plasma pH during a hypercapnic acidosis in carp but also that it was the inhibition of this process that was responsible for plasma HCO_3^- accumulation in fresh water. The analyses in this Section suggest that the Cl^-/HCO_3^- exchange process was stimulated in trout acclimated to the two higher salinities in order to accumulate the plasma HCO_3^-;

that is, an active step was involved in the efflux of Cl^- from blood to water and also in the linked uptake of HCO_3^- from water to blood. Cameron (1976) showed that an active Cl^-/HCO_3^- exchange process in the gill of Thymallus arcticus played an important role in the regulation of extracellular acid-base balance when subjected to environmental hypercapnia or to a step change in temperature, both of which disturbed plasma pH. The discussion in Section 2 presented the published information for the existence of an anion stimulated ATPase in the fish gill which would be the regulated enzyme in this case.

The deviations of Nernst ratios for HCO_3^- also support the conclusion that active processes were involved in accumulation of plasma HCO_3^- during hypercapnia in trout acclimated at all salinities. Nernst ratios for HCO_3^- during control periods as well as for experimental periods were similar for fish acclimated to 100 and 300 mM NaCl, both of which were greater than ratios for fish acclimated to 3 mM at all sampling times. These results are also consistent with the degree of HCO_3^- accumulation in fish at the three salinities reported in Section 2. Plasma HCO_3^- accumulation was similar in fish acclimated at 100 and 300 mM NaCl, both of which were greater than the accumulation at 3 mM.

The proposed active nature of the Cl^-/HCO_3^- exchange mechanism in fish acclimated to the three salinities is

further supported by another aspect of the Nernst ratio data . The Nernst ratios for Cl^- were highest for the fish acclimated in 3 mM and near unity for those acclimated at 100 and 300 mM. The Nernst ratios for HCO_3^-, on the other hand, were lowest for the fish acclimated to 3 mM and highest for those acclimated at the two high salinities. Because the Nernst ratio is defined as the ratio of the measured plasma concentration of an ion over that expected according to a passive distribution of the ion between blood and water, the proposed link between Cl^- and HCO_3^- by an active exchange process would predict the observed results for each ion which complemented each other.

The Nernst ratios for Na^+, Cl^- and HCO_3^- all suggest that there were two groups of fish with regard to ion distribution between blood and water. One group was fish acclimated to 3 mM NaCl which showed large positive ratios and the other consisted of the fish acclimated to 100 and 300 mM, both of which showed ratios near one. Both Na^+ and Cl^- Nernst ratios showed that plasma concentrations of these ions were actively maintained higher than that expected on the basis of passive distribution according to existing electrochemical gradients in fish acclimated to 3 mM. For fish acclimated at 100 and 300 mM, Nernst ratios of Na^+ and Cl^- were close to one relative to the fish at 3 mM. Fish in 300 mM NaCl showed Nernst ratios for Na^+ and Cl^- which were less than one. The reciprocal of the Nernst ratios

during the control periods show that plasma Na^+ and Cl^- concentrations were maintained about 1.43 and 1.72 times lower than concentrations expected on the basis of a passive distribution of ions.

While it is tempting to ascribe the stimulation of Cl^- and the associated HCO_3^- uptake to chloride cells, at least for the fish acclimated in 300 mM, there are several reasons that argue against involvement of cellular recruitment or even synthesis of new anion-stimulated ATPase for ion pumping as shown by Lee (1982) in the blue crab, Callinectes sapidus, acclimated to dilute waters, in the Cl^-/HCO_3^- exchange observed in this experiment. First, fish acclimated to 100 mM NaCl, a medium which is not hypertonic to body fluids, showed a similar rate of HCO_3^- accumulation as fish in 300 mM. Since chloride cells have been shown to develop in fish in hypertonic waters and function to regulate body ion concentrations they would not be expected to develop in the fish acclimated in 100 mM. An exception would be the possibility of their development in response to a stimulus from an acid-base disturbance such as in this experiment. There is, however, no evidence for such a response.

Another argument against involvement of cellular and molecular recruitment in active Cl^-/HCO_3^- exchange in fish acclimated in 100 and 300 mM is the difference in the time course of these processes. The time course of net changes in plasma ions, in the order of hours, strongly

suggests that there was stimulation of existing transport mechanisms rather than possible development of cellular and molecular structures, which require time spans in the order of days. It is possible that stimulation of the transport mechanisms present in the gill of fish in 100 mM NaCl was sufficient to accumulate plasma HCO_3^- observed in fish at both high salinities. The graded accumulation of plasma HCO_3^- in the conger, reported in Section 2, according to the water salinity supports this possibility which precludes the requirement for chloride cell development to effect the degree of plasma Cl^- and HCO_3^- concentration changes. The conger were not acclimated to the new salinities levels for more than three hours, a period too short for such cellular recruitment to take place.

Both Na^+ and Cl^- are less permeable than HCO_3^- across the gill. The relative permeabilites of $PNa^+/PHCO_3^-$ and $PCl^-/PHCO_3^-$ differed in fish in the different salinities during exposure to hypercapnia and during recovery as shown by the differences when different data sets were used to derive these values. Both permeabilities were slightly greater than the average control values when the 3-300mM data set was used. The reasons for large fluctuations in these permeabilities during the initial sampling times cannot be explained. The PNa^+/PCl^- data for the 3-300 mM data set show that large fluctuations in both $PNa^+/PHCO_3^-$ and $PCl^-/PHCO_3^-$ occurred in concert;

i.e. PNa^+/PCl^- values changed very little. $PCl^-/PHCO_3^-$ was reduced when the 3-100mM data set was used. While it is not possible to say how individual permeabilities were changed to give the observed results, these data were useful in explaining some aspects of the subsequently calculated ratio PNa^+/PCl^-.

The trout gill is about 2.5 times more permeable to Na^+ than to Cl^- under steady state conditions. Relative permeability estimates of near unity have been reported by Isaia and Masoni (1976) for the eel and by McWilliams and Potts (1978) for brown trout. These results are supported by previous studies showing a greater permeability of the fish gill to Na^+ than to Cl^-. While Isaia and Masoni (1976) showed the permeabilities of these ions to be approximately equal in the eel, Anguilla anguilla, in fresh water and distilled water changes in Ca^{++} and magnesium (Mg^{++}) had a larger effect on PNa^+ than PCl^-. This suggests a greater Na^+ permeability since divalent ions are knwon to mainly affect passive ion permeability characteristics of the gill. Eddy (1975) also found brown trout gills to be more permeable to Na^+ than to Cl^-. The Nernst ratios for Na^+ were consistently greater than those for Cl^- by about 1.5-2.0 for the salinity range in this study. The agreement of this estimate with the estimate of PNa^+/PCl^- of about 2.5 implies that the passive leak of Na^+ was being compensated by active processes under steady state conditions.

The relative permeability of the gill, PNa^+/PCl^-, changed with salinity when exposed to hypercapnia and during recovery. An assumption in these calculations is the constant permeability of HCO_3^- to both Na^+ and Cl^-. It is clear from calculations of relative permeabilities in this Section that these terms represent apparent permeabilities, and therefore do not indicate the mechanisms by which the observed distribution of ions took place. An increase in the $PNa^+/PHCO_3^-$ value, for example, for fish in freshwater may lead to reductions in plasma Na^+ concentrations toward the water values. However, it does not specify how this is taking place. The passive leak of Na^+ may be increasing with normal Na^+ uptake rates, the passive leak of Na^+ from blood to water may be constant while the active uptake step is reduced or both events might be occurring simultaneously. The PNa^+/PCl^- values also tend to be very sensitive to changes in either $PNa^+/PHCO_3^-$ or $PCl^-/PHCO_3^-$ values. This can be seen in the two points in the 3-100 mM data set which fall below the 0 control level early in the hypercapnia period. Those values are low because the increase in $PCl^-/PHCO_3^-$ is slightly greater than the increase in $PNa^+/PHCO_3^-$. Conclusions were therefore only based on consistent trends in this parameter.

The 3-100 mM data set showed generally higher PNa^+/PCl^- values compared to control values. This was due to a reduced $PCl^-/PHCO_3^-$ values relative to

corresponding $PNa^+/PHCO_3^-$ values. This reduced apparent Cl^- permeability can be explained for the 3 mM salinity data set by the Cl^- flux observed in the carp hypercapnia experiment, assuming that similar fluxes would have occurred in trout acclimated at 3 mM and exposed to environmental hypercapnia. On that basis, the apparent permeability of Cl^- may have been due to the reduced uptake of Cl^- without any change in the passive leak from blood to water.

The two data sets, 100-300 mM and 3-300 mM both showed lower PNa^+/PCl^- values during hypercapnia. It was the greater reduction in $PNa^+/PHCO_3^-$ values compared to the $PCl^-/PHCO_3^-$ that produced this result in the 100-300 mM data set. In contrast, it was increased $PCl^-/PHCO_3^-$ over the $PNa^+/PHCO_3^-$ which resulted in low PNa^+/PCl^- values during hypercapnia in the 3-300 mM salinity. In spite of these differences, it is tempting to speculate that some aspect of the 300 mM salinity, such as the active efflux of Cl^- from blood to water, influenced this parameter. This would result in the decreased apparent $PCl^-/PHCO_3^-$ value over $PNa^+/PHCO_3^-$ such as was the case for the 300 mM data set.

SECTION 4.

CATECHOLAMINE RELEASE IN ACID INFUSED TROUT

INTRODUCTION

An increase in circulating catecholamines characterizes the acute stress response in fish (Nakano and Tomlinson 1967) and can have varied effects on blood flow patterns (Richards and Fromm 1969; Bergman, Olson and Fromm 1974; Wood 1974, 1975; Payan and Girard 1977; Booth 1978), ionic fluxes (Girard and Payan 1977; Perry 1981), and general permeability of the fish gill (Isaia et al. 1978). Acid-base disturbances are no exception to this phenomenon and plasma catecholamines in fish have been observed to increase in response to severe metabolic (Primmett et al. 1986; Ye, in prep.) and respiratory (Perry 1986) acidoses.

Several effects of catecholamines on ion transfer processes across the erythrocyte membrane and gill epithelium of fishes are known. In freshwater, beta receptor stimulation inhibits Cl^-/HCO_3^- exchange (Perry et al. 1984) and stimulates $Na^+/H^+(NH_4^+)$ exchange (Payan et al. 1975; Girard and Payan 1977; Payan 1978). The result of the modulation of these ion exchange processes is to restore the acid-base balance of the intracellular and extracellular fluids. The intracellular pH of the trout red cell is increased in vitro with the addition of catecholamines (Cossins and Richardson 1985). This serves to offset the Bohr

and Root shifts that are exhibited and thus protects the oxygen carrying capacity of the blood. This effect has been shown *in vivo* for trout after a bout of anaerobic exercise which causes a plasma acidosis (Primmett *et al*. 1986). Holeton *et al*. (1983) also showed that a metabolic acidosis of similar magnitude to that of Primmett *et al*. (1986) induced net ion exchanges across the gill epithelium of trout that was associated with correction of the acid-base disturbance in the blood. It is not clear, however, whether the catecholamine release in response to metabolic acidoses in the above experiments was due to the acid-base disturbance or to some other aspect of the exercise.

The two *in vivo* experiments reported in this Section tested the hypothesis that catecholamines are released in trout during a bout of anaerobic exercise in response to increase in the acid load of the blood and that the functional significance of this release is to maintain erythrocytic pH and thus the oxygen carrying capacity of the blood in face of the plasma acidosis.

MATERIALS AND METHODS

This Section describes two series of experiments with identical treatment but with different measurements and slightly different sampling times. The first experiment was

designed to investigate the possibility of catecholamine release with acid infusion and its role in red cell pH regulation and the second investigated effects this treatment had on ionoregulation.

EXPERIMENT 4A. CATECHOLAMINE RELEASE AND RED CELL pH REGULATION :

ANIMALS :

Rainbow trout, Salmo gairdneri, weighing from 221.5 to 460 g were obtained from a commercial hatchery and held outdoors in fiberglass tanks supplied with flowing dechlorinated Vancouver tap water (8-10°C; pH 6.9-7.1; $CaCO_3$ 4 ppm). Fish were fed ad libitum from self feeders containing dry trout pellets. In the laboratory, fish were kept in blackened perspex aquaria at 10°C and starved for 48 h prior to surgical operations and experimentation.

SURGERY :

The dorsal aorta of all experimental animals were chronically cannulated with PE50 cannulas by the procedures of Smith and Bell (1964)

PROTOCOL :

Fouteen animals were infused through the dorsal aortic cannula with 5 $ml*Kg^{-1}$ body weight of a 0.05 N HCl solution made up in 120 mM physiological saline. Five fish treated in an identical fashion were infused with the saline alone and served as controls. Infusion times averaged about 5 minutes. Arterial blood samples of 500 ul were taken before the infusion and at time periods of 5, 30, 60 and 120 min post-infusion. Portions of each blood sample were analyzed for pHe, total oxygen content and hemoglobin concentration; the remainder of the blood sample was centrifuged anaerobically. The resulting plasma was taken up into a chilled syringe and transferred to an Eppendorf vial for storage at -40°C. Stored plasma was subsequently analyzed for catecholamines. The red cell fraction after centrifugation was used for intracellular pH measurement.

ROOT EFFECT DETERMINATION :

Blood samples were drawn from quiescent fish fitted with dorsal aortic cannulas as described above. Blood samples were immediately pooled, transferred to an intermittently rotating tonometer, and equilibrated against humidified gas mixtures containing either 0.2 or 1.0 % CO_2 in air (mixed with Wosthoff pumps). After 30-40 min of tonometry at 10°C,

blood was taken up into a positive displacement gas-tight syringe (Hamilton) and measurements were made of blood and red cell pH, hemoglobin concentration and oxygen content. Adrenaline and noradrenaline concentrations were measured in the plasma of a 1 ml sample of the blood pool taken during the initial stages of the equilibration procedure.

ANALYTICAL PROCEDURES :

The procedures for whole blood and red cell pH determinations as well as for Pco_2 measurements are described in General Materials and Methods. Hemoglobin concentrations were determined spectrophotometrically (Sigma bulletin no. 525). Blood oxygen contents were measured with the Lex-O_2-Cont apparatus (Lexington Instruments, Mass., USA). Plasma adrenaline and noradrenaline concentrations were measured using high pressure liquid chromatography by a method described by Woodward (1982).

STATISTICAL PROCEDURES :

The Student's t test (paired and unpaired as appropriate) was used to discern statistical significance between means with a 5 % level of rejection. Various data sets were also described by linear regression analysis.

EXPERIMENT 4B. ION REGULATION AFTER ACID INFUSON :

The Materials and Methods for this experiment were similar to that for the experiment 4A. Only the differences are noted below.

ANIMALS AND PREPARATION :

The Rainbow trout, Salmo gairdneri, weighed between 280 to 378 g. All fish in this experiment were fitted with urinary catheters through which urine was carried out of the recirculating water system to waste. The urinary catheters were made of PE 60 which were led up the ureter just past the sphincter muscle. Tissue glue was applied to the outside of the catheter wall to secure it in place. As an additional security, cotton sutures were anchored to the two flaps which cover the urinary papillae and wrapped around the cather and firmly tied in place. The end of the catheter was positioned at a slight negative head relative to its origin.

The experimental chamber consisted of a black perspex box for a single fish connected to a pump and a reservoir. The system constituted a recirculating closed system for non-volatile ions and chemicals. An aeration stone mixed water in the reservoir and equilibrated the system with air. The entire water volume was approximately 10 times the weight of the fish. It was hoped that this system would be sensitive

enough to reflect the net transfers of ions between fish and water. The system water was renewed several times a day but not during the short experimental protocol described below.

PROTOCOL :

Eight animals were infused with HCl and 5 were sham injected with the saline. The dorsal aortic cannula was connected to the KCl/agar bridge for a transepithelial potential (TEP) reading. Once stable reading was obtained (< 1 min), the connection was broken and a 300 ul whole blood sample was collected for the measurements below. Samples were collected before infusion, which served as another control value, and at time periods of 5, 15, 40, 60 and 120 min after the infusion of either acid or saline was complete.

MEASUREMENTS :

Plasma ion concentrations of Na^+, Cl^-, K^+, Ca^{++} and Mg^{++} were determined by spectrophotometry. Plasma NH_4^+ concentration was measured by a modification of the Solarzano method.

Ion concentrations above were measured for each water sample by spectrophotometry. Water HCO_3^- concentrations were determined by equilibrating each sample with 1 % CO_2 (Wosthoff pump) and determinig the Tco_2 content of that

sample. Water HCO_3^- concentration was calculated using these values and the solubility constant from Boutilier et al. (1985).

TEP values were determined according to the methods described in General Materials and Methods and Nernst ratios as described in Section 3. were calculated for plasma ions.

STATISTICS :

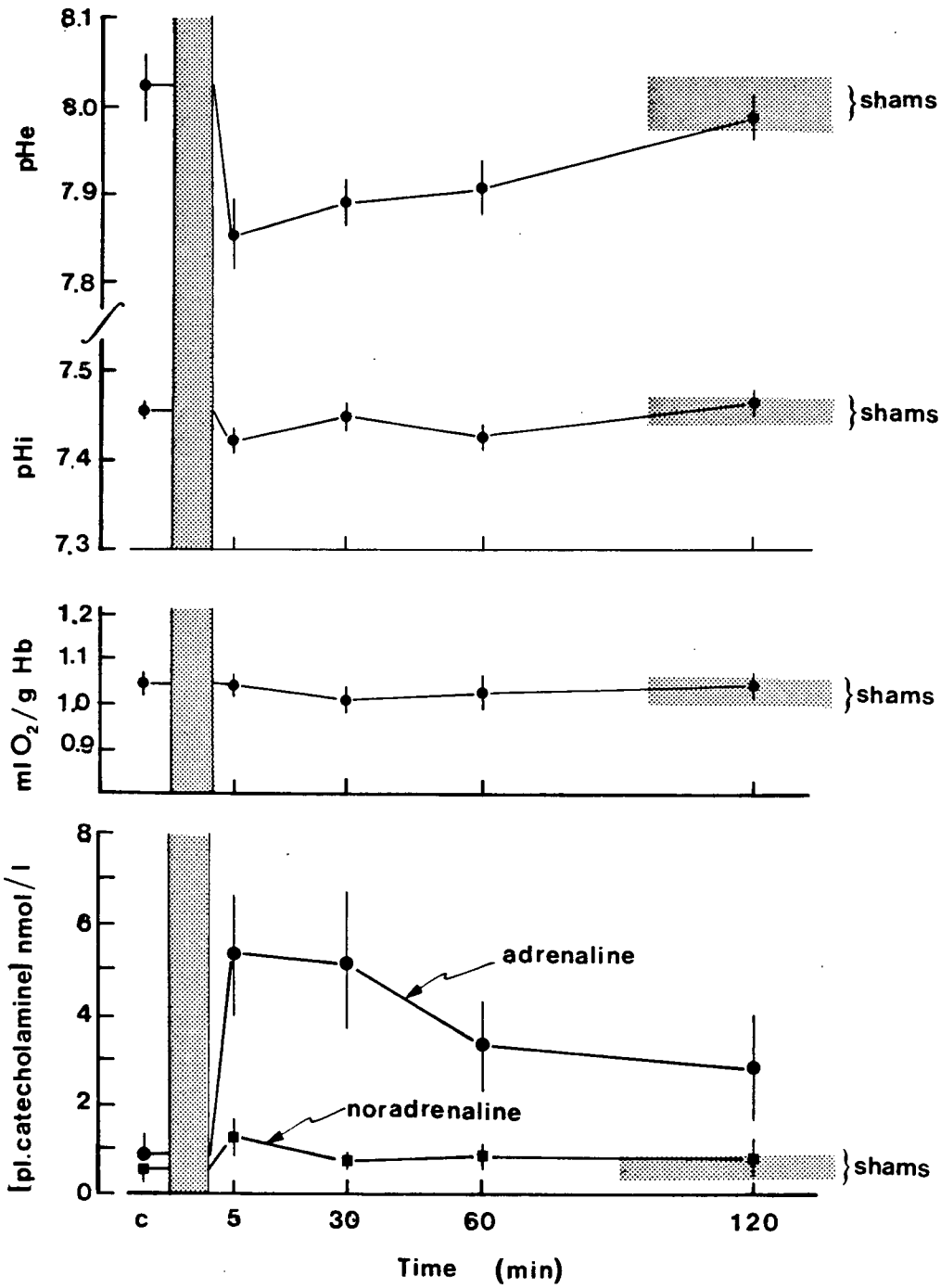
Paired and unpaired Student's t tests were used to discern statistical significance between means using 5 % as the rejection level.

RESULTS

EXPERIMENT 4A. CATECHOLAMINE RELEASE & RED CELL pH REGULATION

The maximum change in plasma pH occurred in the first sampling period which was 5min after infusion of acid ended. Plasma pH fell 0.167 units below that of pre-infusion control levels at that time (Fig. 44 a). At the same time as plasma adrenaline concentrations increased by 6-fold whereas plasma noradrenaline increased only slightly (Fig. 44 d). Red cell pH declined slightly at the 5min mark (Fig. 44 b). In 4 out of the 14 fish, red cell pH increased relative to pre-infusion

Figure 44. Means ± S.E. of a. Arterial plasma pH (pHe), b. red cell pH (pHi), c. mls O_2 bound per gram of haemoglobin (mls O_2*gHg^{-1}) and d. plasma catecholamine concentrations following intra-arterial infusion of a 5 ml*Kg^{-1} body weight 0.05N HCl solution of 120mM saline in 14 rainbow trout. Shaded vertical bar represents the infusion time period. Shaded horizontal bars (labelled shams) represent ± 1 standar error of the combined mean data of five control experiments designed to examine the influence of the saline vehicle alone (i.e. 5 ml*Kg^{-1} of 120mM saline injections). The shading is representative of ± 1 S.E.M. for both adrenaline and noradrenaline levels in the bottom panel. C = pre-infusion control. (N = 14).


8.1
8.0
7.9
7.8
pHe
shams
7.5
7.4
7.3
pHi
shams
1.2
1.1
1.0
0.9
ml O2/g Hb
shams
8
6
4
2
0
[pl.catecholamine] nmol/l
adrenaline
noradrenaline
shams
c
5
30
60
120
Time (min)

levels in the face of a fall in plasma pH. Relative to the control animals there was no change in O_2 content per gram of hemoglobin throughout the course of the experiments (Fig. 44 c). In the 2h following the 5min mark of maximum change in these parameters, plasma pH and catecholamine levels gradually returned toward control values (Fig. 44 a.d). Saline infused control fish showed no significant post-infusion changes in any of the measured variables relative to their pre-infusion values (Fig. 44 a.b.c.d. and Table 4).

Changes in circulating catecholamine concentrations are proportional to changes in pHe in acid infused fish of the present experiment. The greater the decrease in pHe, the greater the increase in catecholamine concentration (Fig. 45).

Rainbow trout blood exhibit a Root shift. Reduction of plasma pH <u>in vitro</u> by equilibration with high CO_2 tensions caused a reduction of red cell pH in the absence of increased catecholamine levels. The amount of oxygen bound to hemoglobin decreased as well. It is assumed that acidification by the addition of acid and in the absence of elevated catecholamine levels would have had the same effect (Fig. 46).

EXPERIMENT 4B. PLASMA AND WATER IONS AND TEP CHANGES AFTER HCl INFUSION

As in the previous experiment, plasma pH also declined

Table 4. Means ± S.E. of arterial plasma pH(pHe), red cell pH(pHi), mls O_2 bound per gram of hemoglobin($mlsO_2*gHb^{-1}$), adrenaline([A]) and noradrenaline([NA]) concentrations before and following intra-arterial infusion of a 5 $ml*Kg^{-1}$ 120mM saline solution in five animals. None of the means values are significantly different from those of the pre-infusion controls.

PARAMETER	PRE-INFUSION	POST-INFUSION			
	CONTROL	+ 5min	+ 30min	+ 60min	+ 120min
pHe	7.948 ± 0.034	7.963 ± 0.033	7.954 ± 0.028	7.947 ± 0.026	7.947 ± 0.030
pHi	7.413 ± 0.027	7.418 ± 0.032	7.400 ± 0.018	7.382 ± 0.019	7.415 ± 0.029
$mlsO_2*gHb^{-1}$	1.04 ± 0.03	1.04 ± 0.03	1.03 ± 0.04	1.03 ± 0.05	1.04 ± 0.04
[A] $nmol*L^{-1}$	0.31 ± 0.11	0.45 ± 0.12	0.34 ± 0.11	0.76 ± 0.36	0.74 ± 0.17
[NA] $nmol*L^{-1}$	0.27 ± 0.04	0.23 ± 0.05	0.22 ± 0.03	0.17 ± 0.03	0.23 ± 0.03

Figure 45. Relationship between the changes in plasma pH and the corresponding changes in plasma adrenaline concentrations between pre-infusion control samples and the + 5min post-infusion samples for each of 14 animals contributing to the mean data in Fig. 2.1. Linear regression analysis of the data points were used to generate the best fit line, Delta [Adrenaline] = 42.46 - 2.28*Delta pHe, r^2 = 0.92.

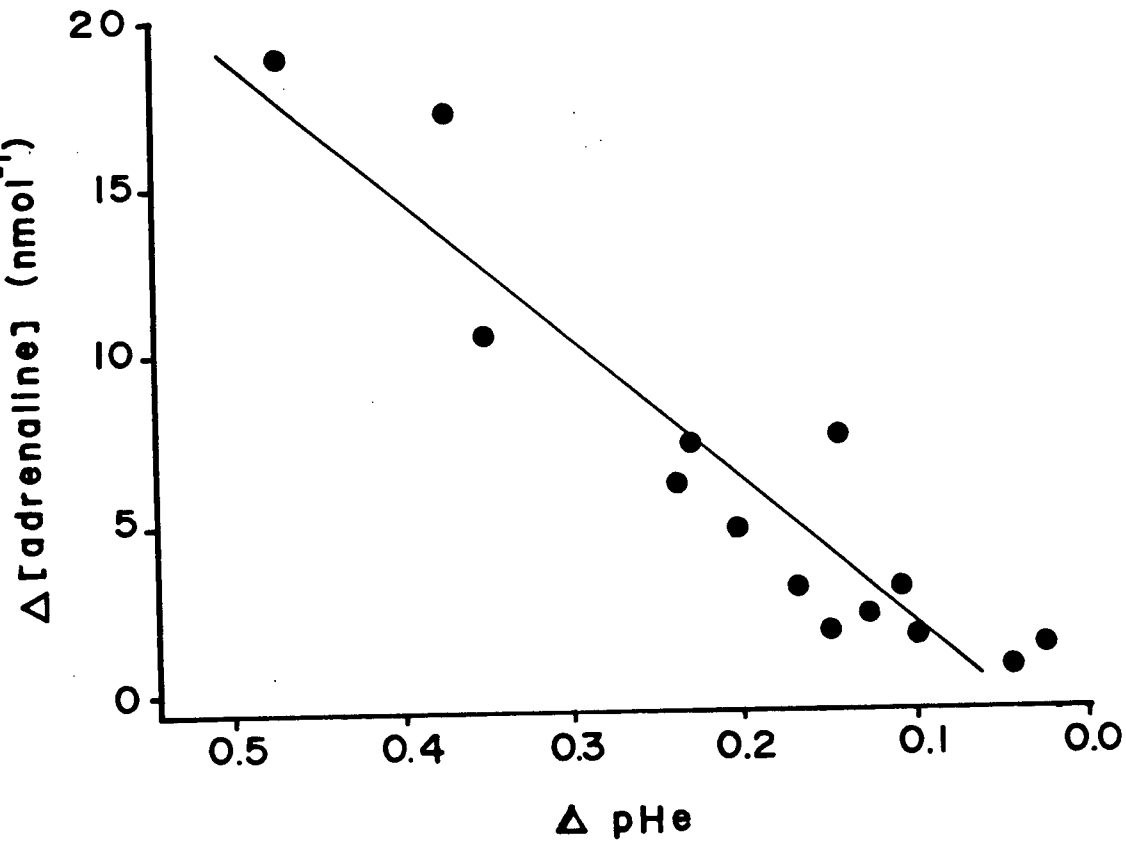
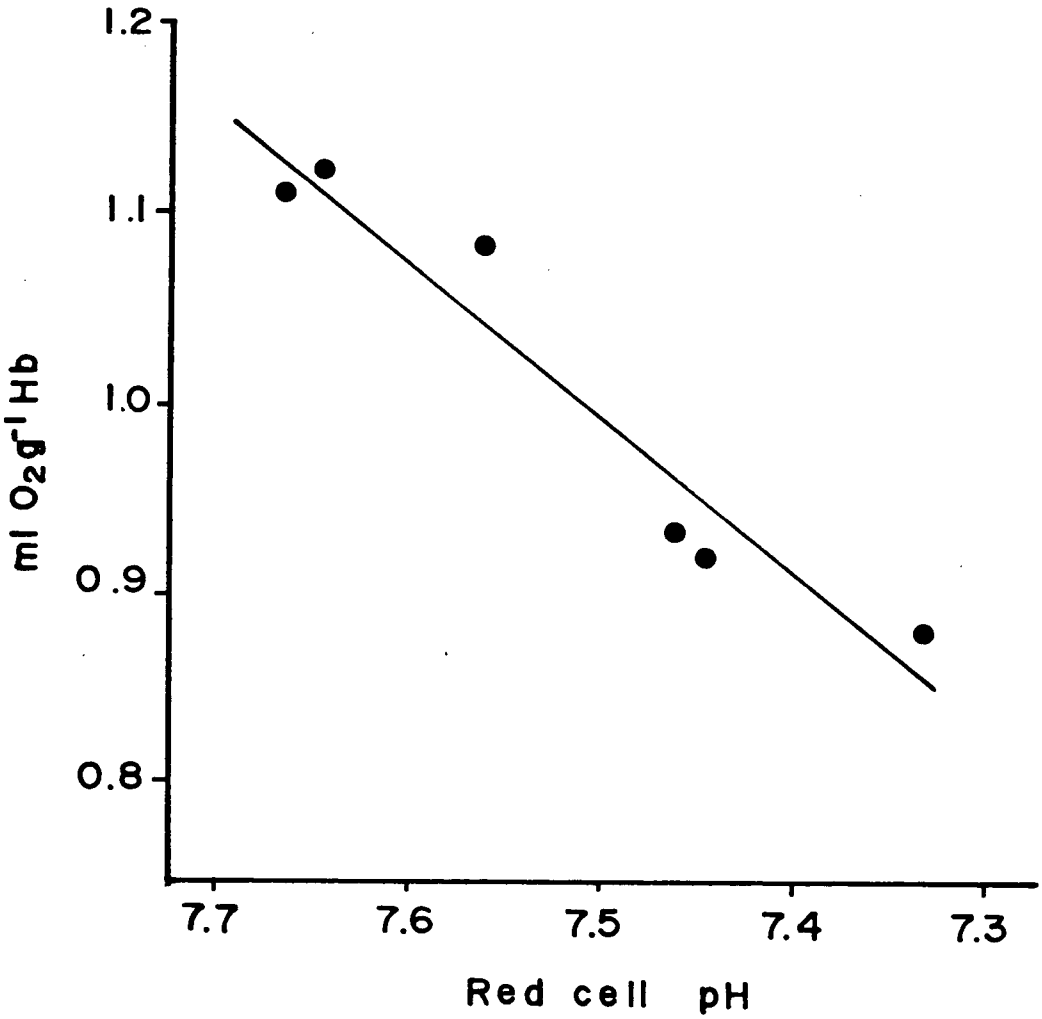

20
15
10
5
0
Δ [adrenaline] (nmol^{-1})
0.5
0.4
0.3
0.2
0.1
0.0
Δ pHe

Figure 46. Relationship between O_2 capacity per gram of haemoglobin and red blood cell pH in rainbow trout blood equilibrated *in vitro* (10°C) against gas mixtures having an oxygen partial pressure of 152 mmHg and a CO_2 partial pressure of 2.5 mmHg. Data are individual measurements on a single blood pool from three animals. Haemoglobin concentration of the blood pool = 8.4 $gHb*100ml^{-1}$. The line of best fit was generated by linear regression analysis where $mls\ O_2*g^{-1}[Hb] = -5.005 + 0.800*pHi$ ($r = 0.94$).


1.2
1.1
1.0
0.9
0.8
ml $O_2 g^{-1} Hb$
7.7
7.6
7.5
7.4
7.3
Red cell pH

about 0.17 units relative to the pre-infusion control value (Fig. 47 a). Plasma pH values for saline infused sham control fish did not change in the early sampling periods during which maximum changes in the acid infused fish occurred. There was a slight and transient increase in plasma pH of the sham controls at 40 and 60min post-infusion. By 120min, at the end of the experiment, both acid and saline infused fish showed plasma pH values which were not different from their respective pre-infusion control levels. Plasma pH of the acid infused fish was not significantly different from values of the saline infused fish.

Plasma HCO_3^- concentrations in saline infused fish were variable and showed increasing trends during the 5, 15 and 40min sampling periods (Fig. 47 b). Mean values, however, declined close to control levels by the 120min mark. Plasma HCO_3^- concentrations in HCl infused fish declined in the first 5min following infusion and gradually increased toward pre-infusion levels over the course of the experiment. While the 120min HCO_3^- concentration for acid-infused fish was not significantly different from either the pre-infusion level or the level for fish infused with saline, the mean value was 0.56mM lower than the pre-infusion control value.

There was no difference in the Hct values between saline and acid infused fish throughout the course of the experiment. Values in both groups declined with sampling throughout the experiment (Fig. 48). There were no

Figure 47. Means ± S.E. of a. Plasma pH and b. plasma HCO_3^- concentrations in rainbow trout infused with HCl and saline in the dorsal aorta via a chronic indwelling catheter (at the 0 time mark which represents the end of a 5min infusion routine) over the time course of the experiments. [HCO_3^-] in mM.

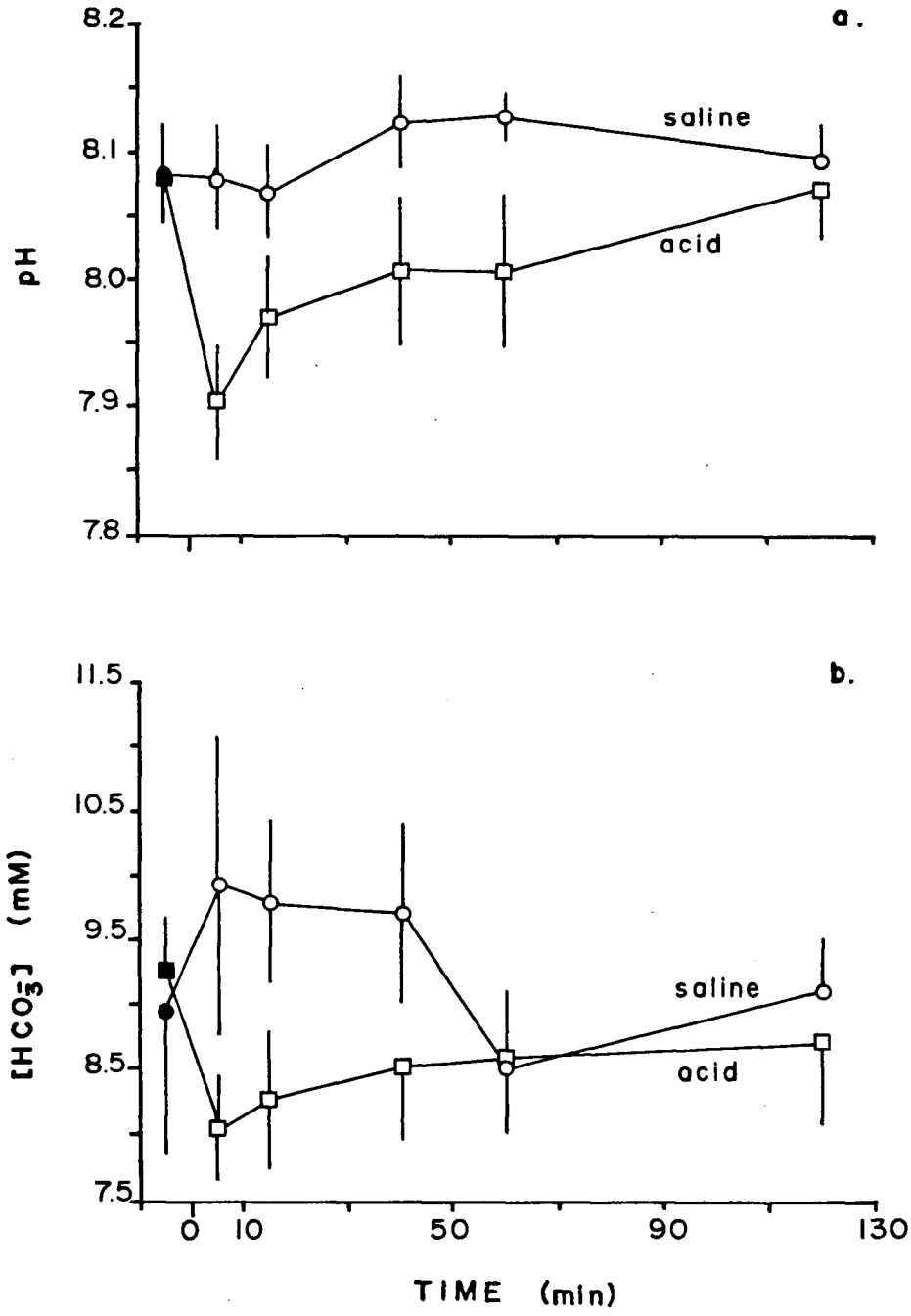
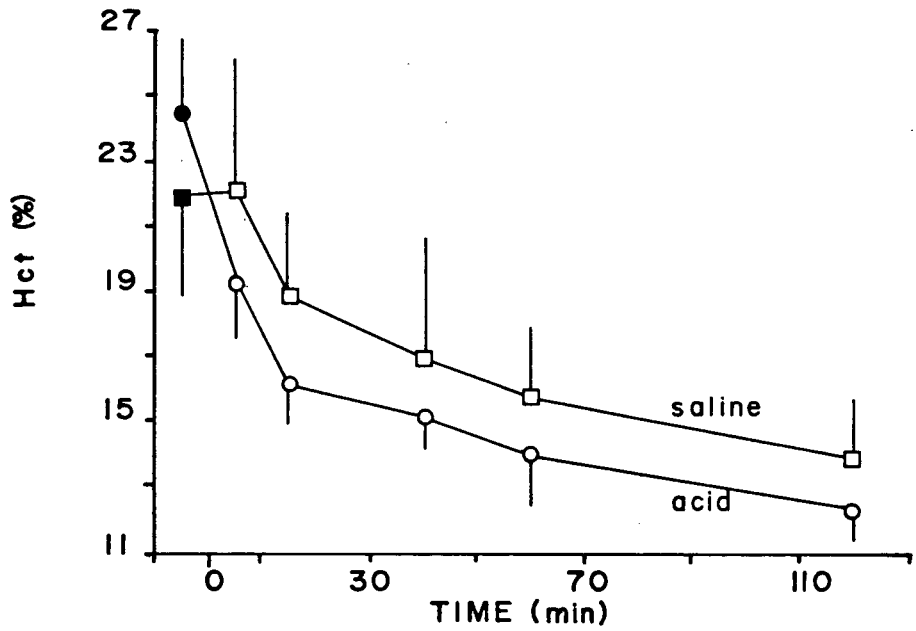

a.
8.2
8.1
8.0
7.9
7.8
pH
saline
acid
b.
11.5
10.5
9.5
8.5
7.5
[HCO3-] (mM)
saline
acid
0
10
50
90
130
TIME (min)

Figure 48. Means ± S.E. of a. Hematocrit (%) of rainbow trout infused with HCl and saline in the dorsal aorta via a chronic indwelling catheter (at the 0 time mark which represents the end of a 5min infusion routine) over the time course of the experiments.


27
23
19
15
11
Hct (%)
0
30
70
110
TIME (min)
saline
acid

significant trends in measured plasma ion concentrations for the duration of the course of the experiment for either saline or acid infused fish (Table 5). No consistent trends were observed in any of the measured water ion concentrations in either saline or acid infused fish (Table 6).

Transepthelial potential values were highly variable for both saline and acid infused fish throughout the experiment. Relative to its own pre-infusion control value, there was a transient increase in TEP in the first 15min following acid infusion. TEP for acid infused fish fluctuated about values for the saline infused fish for the reminder of the experiment. These latter values were not significantly different from the pre-infusion mean (Fig. 49).

Table 7 shows Nernst ratios calculated for Na^+, Cl^-, K^+, HCO_3^-, NH_4^+, Ca^{++}, and Mg^{++} for each sampling period throughout the experiment. The results were generally variable and no significant trends were observed for Cl^-, HCO_3^-, K^+ and Mg^{++} ratios. Na^+ ratios increased above pre-infusion control values throughout the experiment (Fig. 50 a). There was a rapid decline in NH_4^+ ratio in the first 15min following acid infusion after which the values remained stable to the end of the experiment (Fig. 50 b). Ca^{++} Nernst ratios declined to levels below control values at the 60 and 120min sampling periods (Fig. 51). Nernst ratios for all ions are not equal to unity.

Table 5. Means ± S.E. of plasma ion concentrations for rainbow trout infused with a-HCl(N = 8) and s-saline(N = 5). All concentrations in mM.

ION	CONTROL	+ 5min	+ 15min	+ 40min	+ 60min	+ 120min
a-Na^+	143.52 ± 3.94	144.68 ± 5.56	149.97 ± 5.81	145.90 ± 5.27	147.12 ± 5.64	149.03 ± 4.16
s-Na^+	119.76 ± 9.62	120.10 ± 8.02	121.07 ± 8.83	116.41 ± 9.42	120.32 ± 7.88	118.57 ± 7.64
a-Cl^-	114.15 ± 6.30	114.86 ± 6.27	111.16 ± 5.23	116.17 ± 6.59	115.78 ± 6.21	111.16 ± 5.27
s-Cl^-	107.94 ± 12.25	110.41 ± 12.78	110.20 ± 12.30	106.28 ± 12.69	114.03 ± 12.01	113.12 ± 12.34
a-K^+	3.69 ± 0.35	3.48 ± 0.22	3.92 ± 0.53	3.38 ± 0.15	3.46 ± 0.15	3.92 ± 0.53
s-K^+	4.93 ± 0.38	4.14 ± 0.37	4.44 ± 0.54	4.97 ± 1.27	4.16 ± 0.40	4.15 ± 0.39
a-Ca^{++}	1.24 ± 0.17	1.08 ± 0.10	0.91 ± 0.10	1.03 ± 0.11	1.05 ± 0.14	0.91 ± 0.10
s-Ca^{++}	1.57 ± 0.41	1.35 ± 0.26	1.21 ± 0.24	1.13 ± 0.29	1.18 ± 0.26	1.16 ± 0.25
a-Mg^{++}	0.57 ± 0.08	0.56 ± 0.06	0.52 ± 0.09	0.59 ± 0.09	0.58 ± 0.07	0.52 ± 0.09
s-Mg^{++}	0.60 ± 0.09	0.55 ± 0.05	0.54 ± 0.05	0.54 ± 0.06	0.55 ± 0.04	0.59 0.04
a-NH_4^+	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01

Table 6. Means ± S.E. of water ion concentrations for rainbow trout infused with a-HCl(N = 8) and s-saline(N = 5). All concentrations in mM.

ION	CONTROL	+ 5min	+ 15min	+ 40min	+ 60min	+ 120min
a-HCO_3^-	0.61 ± 0.11	0.52 ± 0.11	0.57 ± 0.11	0.57 ± 0.10	0.51 ± 0.12	0.57 ± 0.11
s-HCO_3^-	0.27 ± 0.12	0.22 ± 0.05	0.43 ± 0.23	0.34 ± 0.14	0.34 ± 0.12	0.63 ± 0.32
a-Na^+	0.89 ± 0.06	0.84 ± 0.04	0.77 ± 0.04	0.79 ± 0.03	0.83 ± 0.04	0.77 ± 0.04
s-Na^+	0.90 ± 0.07	0.85 ± 0.08	0.84 ± 0.07	0.80 ± 0.07	0.85 ± 0.08	0.84 ± 0.07
a-Cl^-	0.92 ± 0.04	0.91 ± 0.04	0.90 ± 0.05	0.89 ± 0.04	0.09 ± 0.04	0.90 ± 0.05
s-Cl^-	0.88 ± 0.03	0.85 ± 0.02	0.85 ± 0.04	0.85 ± 0.05	0.85 ± 0.05	0.85 ± 0.04
a-K^+	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
s-K^+	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
a-Ca^{++}	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
s-Ca^{++}	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
a-Mg^{++}	0.01 ± variability undetectable	0.01	0.01	0.01	0.01	0.01
s-Mg^{++}	0.01 ± variability undetectable	0.01			0.01	0.01 0.01
a-NH_4^+	0.03 ± 0.13	0.32 ± 0.14	0.36 ± 0.15	0.31 ± 0.13	0.35 ± 0.15	0.36 ± 0.15

Table 7. Means ± S.E. of plasma ratios of : Measured [ion] / Expected [ion] by the Nernst equation for trout infused with HCl.

ION	CTRL.	+5min	+15min	+40min	+60min	+120min
Na^+	82.682 ± 10.861	84.475 ± 8.113	95.660 ± 9.187	90.071 ± 6.662	100.221 ± 11.696	108.297 ± 11.712
Cl^-	226.910 ± 28.901	261.204 ± 21.257	254.701 ± 25.862	271.775 ± 34.589	266.697 ± 39.165	250.564 ± 31.741
K^+	60.181 ± 24.384	66.312 ± 21.032	71.870 ± 18.538	73.194 ± 21.961	79.919 ± 22.469	94.506 ± 54.794
HCO_3^-	24.934 ± 4.548	29.144 ± 4.970	33.043 ± 5.312	30.043 ± 6.694	22.956 ± 4.379	28.542 ± 7.212
NH_4^+	0.108 ± 0.020	0.080 ± 0.020	0.060 ± 0.010	0.060 ± 0.010	0.064 ± 0.010	0.061 ± 0.009
Ca^{++}	10.075 ± 2.398	12.262 ± 2.304	12.518 ± 2.111	9.095 ± 0.963	6.905 ± 1.354	7.640 ± 1.043
Mg^{++}	15.948 ± 1.266	14.318 ± 2.094	17.086 ± 4.272	17.890 ± 5.664	11.219 ± 2.915	14.829 ± 4.266

Figure 49. Means ± S.E. of trans-epithelial potentials (TEP) in rainbow trout infused with 5ml*Kg^{-1} body weight of a 0.05 N HCl solution made up in 120mM physiological saline and the saline alone.

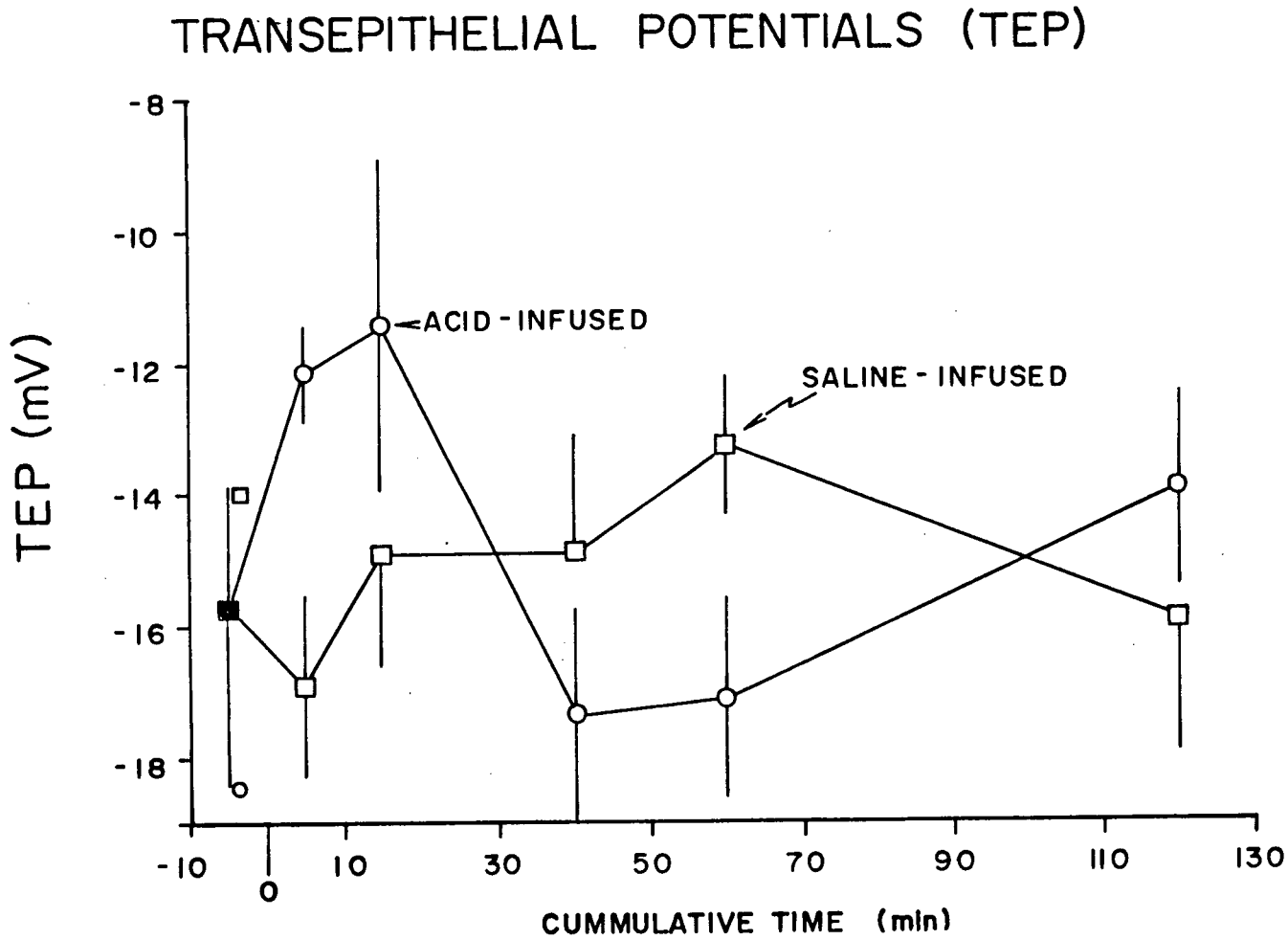

TRANSEPITHELIAL POTENTIALS (TEP)
TEP (mV)
-8
-10
-12
-14
-16
-18
ACID - INFUSED
SALINE - INFUSED
-10
0
10
30
50
70
90
110
130
CUMMULATIVE TIME (min)

Figure 50. Means ± S.E. of a. nernst ratio for plasma Na^+ in trout infused with 5ml*Kg^{-1} body weight 0.05 N HCl. Filled circle is the pre-infusion control point. b. As a. except that it pertains to NH_4^+.

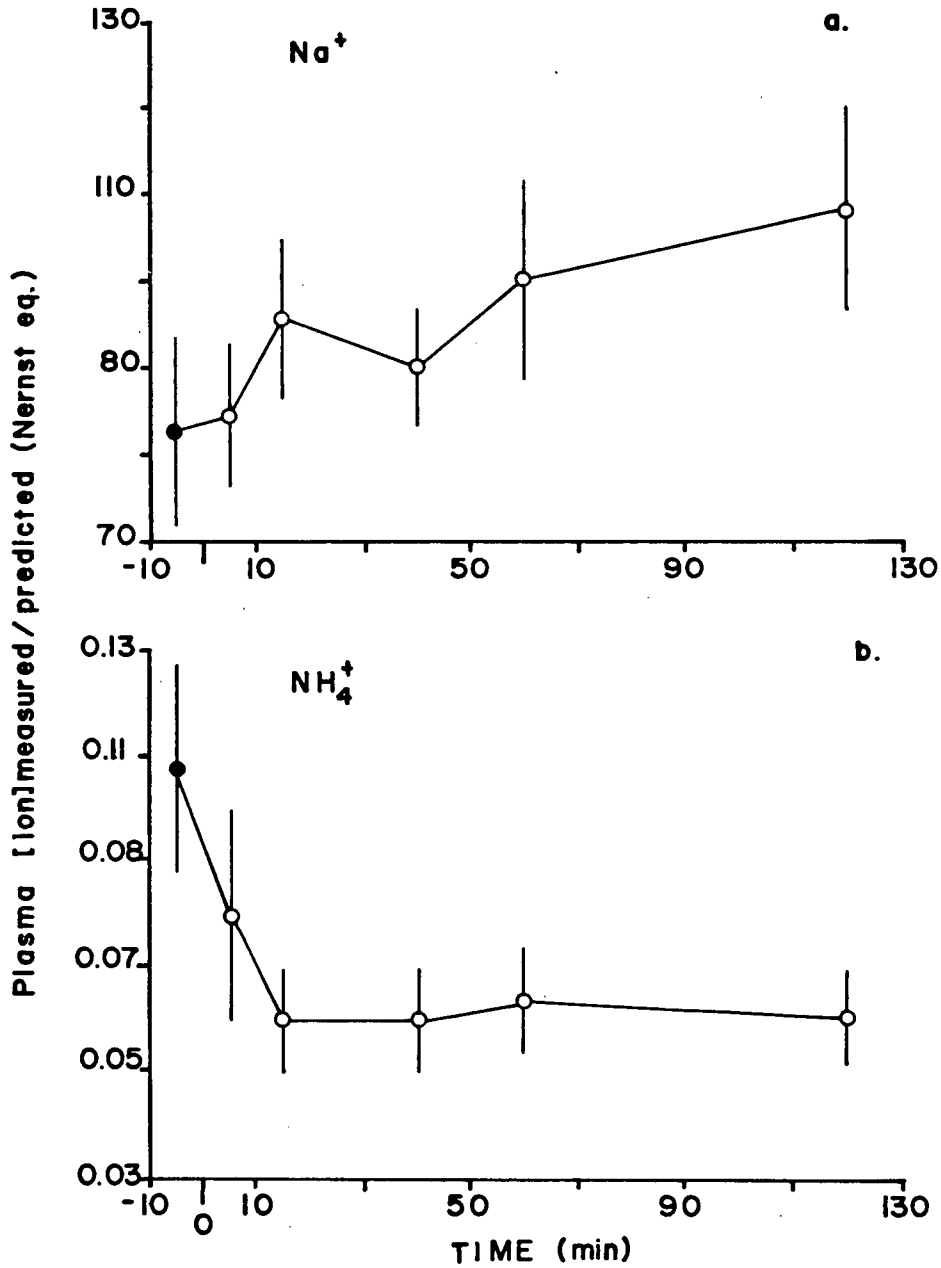
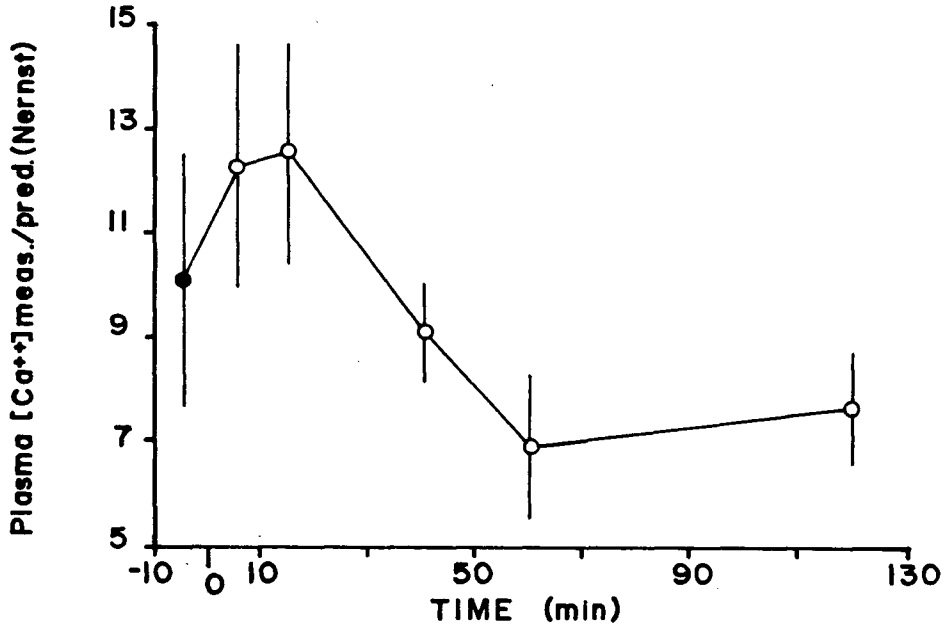

a.
Na^+
130
110
80
70
-10
10
50
90
130
b.
NH_4^+
0.13
0.11
0.08
0.07
0.05
0.03
-10
0
10
50
90
130
TIME (min)
Plasma [Ion]measured/predicted (Nernst eq.)

Figure 51. Means ± S.E. of the nernst ratio for plasma Ca^{++} in trout infused with 5ml*Kg^{-1} body weight 0.05 N HCl. Filled circle is the pre-infusion control point.


Plasma [Ca++]meas./pred.(Nernst)
15
13
11
9
7
5
-10
0
10
50
90
130
TIME (min)

DISCUSSION

These acid infusion experiments demonstrate that the increased acid load in trout during a bout of anaerobic exercise elicits a significant release of the catecholamines, adrenaline and noradrenaline. This evidence suggests that the release of catecholamines in fish in response to acidoses (eg. Primmett et al. 1986) is, at least partially, due to the excess H^+ load in the circulatory system. This increase in catecholamine concentration in extracellular fluid of fishes reduces the change in erythrocytic pH and this in turn maintains the O_2-Hb affinity and saturation of the blood in face of the plasma acidosis. This in vivo evidence supports the recent in vitro study by Cossins and Richardson (1985) which showed that this maintenance of O_2-Hb affinity was due to attenuation of the Bohr and Root shifts shown by the blood of trout. The proportional relationship between circulating catecholamines and the change in plasma pH from control values further highlights the apparent functional significance of this phenomenon in the maintenance of blood O_2-Hb affinity and carrying capacity. The need for this protection is greatest when the degree of acidosis, or delta pHe, is also maximum.

As expected, the nature of this increase in catecholamine concentration is a transient phenomenon. This is consistent

with the acute stress response in fish (Nakano and Tomlinson 1967) and also with available data where transient increases in catecholamine concentrations have been observed in trout during a burst swim (Primmett et al. 1986). The results of experiment 1A in Section 1.,in which animals in steady state conditions of acidosis 24h after an alteration in ventilatory volume and in which catecholamine levels were consistent with resting values, also suggests that increase in catecholamines in response to an acid-base disturbance is a transient phenomenon.

While Holeton et al. (1983) showed a number of net changes in plasma and water ion concentrations in response to metabolic acidosis induced in excercised trout, no significant changes in ion concentrations were observed in the present experiments. The acidosis induced in the two experiments in this Section represent about one half the change in pH demonstrated by other studies (Holeton et al. 1983 and Primmett et al. 1986) where metabolic acidoses have been experimentally induced; although the pattern of acid-base disturbance was similar to those studies. Changes in pH in the order of 0.5 and 0.3 units from the control values were recorded for the Holeton study above where trout were prodded to exhaustion and where trout were swum to exhaustion (Primmett et al. 1986), respectively. The insult in our study may have been too mild to induce net ion changes in a magnitude that could be detected in the water, given the

fish/water volume ratio. It is also possible that the acid-base disturbance that was induced effected changes in transepithelial ion fluxes which were offset by other compensatory ion exchanges caused by some aspect of the treatment, such as the increase in circulating catecholamines.

The increases in the Nernst ratios for Na^+ during the experiment indicate that there was a stimulation of the active uptake of Na^+ with acid infusion. On this basis, the fact that there were no significant net changes in $[Na^+]$ in the blood suggests that the permeability of the gill epithelium increased to match increased activity of the ion pump. This proposed process could theoretically reduce the excess H^+ load of the plasma through a $Na^+/H^+(NH_4^+)$ exchange across the gill epithelium. There is evidence for a Na^+/NH_4^+ exchange process in fish (Maetz and Garcia-Romeu 1964, Evans 1977, Payan 1978) and Maetz (1973) suggested that NH_4^+ and H^+ may compete for similar sites on a transport vehicle in exchange for Na^+. Such a stimulation has been reported for trout in response to adrenaline through the stimulation of beta adrenergic receptors (Payan et al. 1975; Girard and Payan 1977; Payan 1978). The reduced Nernst ratio for NH_4^+ means that it was being actively pumped out of the animal against the existing electrochemical gradients and it supports the possibility that this active exchange process was being stimulated through the action of catecholamines in response to

the acid infusion.

Two conditions must accompany the above hypothesis of an active $Na^+/H^+(NH_4^+)$ exchange playing a major role in the reduction of H^+ from the blood of trout after acid infusion; that is the increase in gill permeability to Na^+ and the concomitant increase in the efflux of an anion or an influx of another cation to maintain electrical neutrality. Isaia et al. (1978) demonstrated that there was an increase in the gill permeability to water in response to catecholamines. This suggests that there was a general increase in permeability to ions as well. The analyses in Section 3. suggested that Na^+ was more permeable than Cl^- across the trout gill epithelium. The proposed stimulation of the cation exchange could have taken place, assuming a general increase in permeability and this differential between PNa^+ and PCl^-. The permeability of the gill to Cl^- would also have increased, but the active uptake of this ion to compensate the increased leak may have had to be minimal given the mild degree of acidosis in general. Given the lack of evidence that Cl^- was involved for charge balance in this proposed hypothesis, it is possible that Ca^{++} might have been taken from the water against its electrochemical gradient to balance the negative cation balance in the blood (Fig. 51).

GENERAL DISCUSSION

GENERAL DISCUSSION

Fish regulate blood pH in response to an acid-base disturbance (see Heisler 1985 for review). That is, pH is returned toward resting levels over time either following a short-term perturbation such as a burst swim or during a prolonged treatment such as the exposure to environmental hypercapnia. A stimulation of ventilation as well as ion exchange processes across the gill epithelium characterise the response of fishes to acidotic conditions. Acid-base disturbances also elicit a generalized stress response and several acidotic conditions have been shown to raise levels of catecholamines above resting levels in fish (Primmett et al. 1986; Perry 1986). The results of the studies in this thesis confirm these general statements regarding the response of fish to acid-base disturbances and describe some specific processes which result in the restoration of the acid-base status of the blood.

The data from the experiments reported in this thesis suggest that there is a greater potential for regulating the acid-base status of the blood in fishes through ion exchange processes between blood and water rather than through regulating the Pco_2 tension of the blood by adjustments of ventilation volume.

While ventilation increases in response to acid

conditions, the limited range for adjusting the Pco_2 tension in the blood reduces its potential in correcting acid-base disturbances. The first experiment in Section 1. showed that in steady state conditions, Pco_2 can only be increased by about 2 mmHg by reducing ventilation below normal levels. Pco_2 tensions probably rise in any acidosis. This will occur during exposure to environmental hypercapnia due to the diffusion of CO_2 from water to blood. An excess load of H^+ ions of a metabolic origin will also titrate blood HCO_3^- to molecular CO_2 and thereby increase Pco_2 tensions. The functional role of the stimulation of ventilation in acidotic conditions is limited to those circumstances where the Pco_2 is endogenous, such as in the case of HCO_3^- titration by H^+ from metabolism. The Pco_2 tension might be reduced in that case toward normal values by increased gill water flow. Alkalotic conditions might also be corrected by reducing ventilation and thereby limiting CO_2 excretion.

Given this limited range for adjusting CO_2 excretion by ventilation, the possibility becomes attractive that the functional significance of the stimulation of ventilation in response to acid conditions is the enhancement of oxygen uptake to offset Bohr and Root shifts . While the sensitivity of ventilation in fish to oxygen is well documented, this simple rule has many exceptions. Elasmobranchs which increase ventilation under acid conditions have blood which does not

exhibit either Bohr or Root shifts. Cyprinids have been reported to show little ventilatory response to exposure to hypercapnia while their blood shows Bohr and Root shifts. It has also been shown that the reduction in blood oxygen carrying capacity in acidotic conditions can be offset by catecholamines (Boutilier et al. 1986, Cossins and Richardson 1986) which are released in fish under such conditions (Boutilier et al. 1986, Primmett et al. 1986, Perry 1986, in prep.). The mechanism by which this effect is offset is the alkalinization of the erythrocyte cytosol. The acid infusion study in Section 4. demonstrated this effect in vivo by showing no significant reduction in blood oxygen carrying capacity in spite of a plasma acidosis. The stimulation of ventilation in acidotic conditions is, therefore, not clearly attributed to reductions in the blood oxygen carrying capacity as might be demonstrated in vitro. While in vivo studies have shown an attenuation of this effect with hyperoxic conditions (Smith and Jones 1982), some residual sensitivity to the acidotic condition seems to exist.

The ventilation study of the dogfish in Section 1 showed that the stimulation of ventilation was most sensitive to blood pH. Given the lack of Bohr and Root shifts and the equivocal nature of the direct relationship between ventilatory stimulation under acid conditions and the Bohr and Root shifts shown by teleost blood, it is possible that a sensitivity of ventilation to the actual acid-base status of

the blood in teleosts exists. Some inconsistencies in the published data must, however, be reconciled. One obvious question is why raising the oxygen content of water would attenuate the increased ventilation in trout exposed to hypercapnic conditions (Smith and Jones 1982) when it has been shown that catecholamines offset the Bohr and Root shifts *in vivo* (Boutilier *et al*. 1985; Primmett *et al*. 1986) and *in vitro* (Cossins and Richardson 1986) and that catecholamines are released in that species exposed to environmental hypercapnia (Perry 1986).

The potential for correcting acid-base disturbances in general and the acidotic conditions reported in the experiments in this thesis is clearly greater through adjustments of ion exchange processes between blood and water than through changes in gill water flow. With a few exceptions (Perry *et al*. 1981b., Kerstetter and Mize 1976) net changes in ion transfer rates (Cameron 1976, carp experiment in this thesis) and blood ion concentrations (Claiborne and Heisler 1984, Holeton *et al*. 1983, conger and trout experiments in this thesis) change in fish in various situations of acidoses. Transepithelial ion transfers seem to predominate over a renal function in this regard (see review by Heisler 1985). The accumulation of blood HCO_3^- characterizes the recovery of blood pH after the initial fall in acidotic conditions.

In the trout, conger and carp, active Cl^-/HCO_3^-

exchange processes between the blood and water were observed to reduce blood Cl^- and increase blood HCO_3^- concentrations in response to environmental hypercapnia. This is consistent with the results of other studies where fish have been exposed to environmental hypercapnia (Lloyd and White 1967; Cross et al. 1969; Cameron and Randall 1975; Janssen and Randall 1975; Heisler et al. 1976; Borjeson 1976; Randall et al. 1976; Bubien and Meade 1979; Heisler et al. 1980; Claiborne and Heisler 1984). These ionic exchanges resulted in plasma pH values returning towards control values.

It is clear from the experiments in this thesis that active rather than passive processes were involved in the accumulation of plasma HCO_3^- through exchange processes between blood and water with Cl^-. The unidirectional ion flux study with the carp showed that it was an inhibition of the active Cl^- uptake from the water which effected the net changes in that ion and the associated accumulation of plasma HCO_3^- in response to hypercapnia. It is likely that similar processes were present in conger and trout under fresh water or near fresh water conditions. This inhibition can occur rapidly over a few hours and since this mechanism of Cl^- uptake is reportedly present on the respiratory cells of the gill epithelium (Girard and Payan 1977), it is likely that this could have occurred even in the conger which were tempered for only three hours in fresh water. It was concluded from the experiments in Section 2 on trout that

active processes must have effected accumulation of plasma HCO_3^- in fish acclimated to 100 mM and 300 mM NaCl. It seemed most likely that this involved the stimulation of Cl^- efflux for HCO_3^- uptake through transport mechanisms already present on the gill rather than involvement of chloride cell recruitment or synthesis of new transport protein specifically for this process. This hypothesis might also be extended to include the conger eel which also showed a graded response in recovery from hypercapnic acidosis according to environmental salinity. Further support for the active nature of the Cl^-/HCO_3^- exchange at all three salinities investigated in the trout study was seen in the Nernst ratio analysis for these ions. The Nernst ratios for Cl^- were higher at 3 mM than those for fish in 100 mM and 300 mM whereas Nernst ratios for HCO_3^- was lower in fish at 3 mM than those for 100 mM and 300 mM.

While net changes in plasma ion concentrations were not observed in trout infused with acid, as reported in Section 4, Nernst ratio analysis suggested that there might have been an active $Na^+/H^+(NH_4^+)$ exchange mechanism working to reduce the H^+ load of the plasma, and thereby correcting blood pH. It is hypothesized that this active uptake of Na^+ from water to blood was matched with an equivalent increase in a passive leak for this ion, since a net accumulation of Na^+ was not observed in the plasma.

There was general agreement between permeability estimates

of the gill and degree of active net flux for Na^+ and Cl^-. Under steady state conditions in trout prior to exposure to environmental hypercapnia, there was good agreement between the estimate for PNa^+/PCl^- of 2.5 to the Nernst ratio for Na^+ which was 1.5 to 2.0 times greater than those ratios for Cl^- at all three salinity levels. The apparent permeability of Cl^- was lower than that for Na^+ over the 3-100 mM salinity range in trout during exposure to hypercapnia. This also agreed with the observation in carp that in fresh water, Cl^- influx was reduced in response to exposure to environmental hypercapnia. Both the decrease in the influx rates given normal efflux rates, would result in the observed net reduction in plasma Cl^- concentration.

The *in vivo* release of catecholamines in trout in response to acid conditions has been reported by Perry (1986) for exposure to hypercapnia, by Boutilier *et al*. (1986) and by Primmett *et al*. (1986) for burst exercise. The increased hematocrit and depolarizing trend in the trout exposed to hypercapnia at all three salinities also pointed to the release of catecholamines. The infusion of acid in the trout, reported in Section 4, also showed an increase in catecholamines in proportion to the change in plasma pH. These results should be expected since a significant change in the acid-base status of the blood perturbs many physiological functions which include cardiovascular and ion transport systems. An inhibition of Cl^-/HCO_3^- exchange (Perry *et*

al. 1984) and an increase in $Na^+/H^+(NH_4^+)$ exchange through beta receptor stimulation (Payan et al. 1975; Girard and Payan 1977; Payan 1978) has been reported for the fish gill. This may explain inhibition of Cl^-/HCO_3^- exchange seen in the carp in fresh water exposed to hypercapnia, although an actual increase in catecholamines in that species under similar conditions has not been reported. While stimulation of Na^+/H^+ exchange was suggested for acid-infused trout in Section 4 where catecholamine levels were shown to be high, such a stimulation in the trout study of Section 2 was not seen. There is a possibility that it was present and masked as in the acid-infused trout. Nernst ratios for Na^+ in trout exposed to environmental hypercapnia significantly deviated from unity at all sampling times and increased during hypercapnia, especially at the 100 mM and 300 mM levels. There was no clear agreement in the PNa^+/PCl^- data with this possible stimulation of net Na^+ flux between blood and water. The Na^+ - Cl^- would have been expected to increase at these salinities, specifically due to an increase in the PNa^+ component over PCl^- in order to mask any net accumulation of Na^+ in the blood as a result of increased Na^+ uptake.

Another known effect of catecholamines is the inhibition of Na^+ and Cl^- fluxes from chloride cells by an alpha and beta receptor stimulation (Pic et al. 1975; Girard 1976; Payan and Girard 1978; Shuttleworth 1978; Degnan et al. 1977). This

supports one conclusion of Section 2 that the active efflux of Cl^- from blood to water that effected the HCO_3^- accumulation in trout at 100 mM and 300 mM salinities was not due to the recruitment of chloride cells. The likely presence of elevated catecholamines would have inhibited Cl^- efflux rather than stimulated it at the high salinities. This therefore, suggests that the set of mechanisms that was actually responsible for the increased Cl^- efflux and consequent HCO_3^- uptake would have had to overcome this inhibition of Cl^- efflux from the chloride cells and effect the net reduction in plasma choride concentration. This also suggests either that there are locations for the anion-stimulated ATPase on sites other than the reported one on the chloride cell or that other mechanisms for active Cl^- efflux besides chloride cells exist.

It should also be noted that the results of experiments on the effects of catecholamines on ion transport across biological barriers are not consistent. For example, the inhibition of Cl^- transport by catecholamines has been reported for the rabbit colon by Halm et al. (1983) but as that author points out, that result seems anomalous since beta adrenergic agonists are believed to act through cyclic AMP (cAMP) and exogenous cAMP is a stimulant of colonic Cl^- transport in the rat (Foster et al. 1983). While it is possible that actions of a secondary messenger such as Ca^{++} is stimulating the Cl^- transport in the presence of

exogenous cAMP and thus masking the beta adrenergic effect, the results are not definitive.

While the data set on the movements of H^+, HCO_3^- and NH_4^+ across the fish gill epithelium shows that these movements are modulated during recovery from acid-base disturbances and their link to the movements of Na^+ and Cl^- are well documented, the factors determining these exchange processes are not well understood. Studies on acid-base regulation in fishes have been largely descriptive and inroads are starting to be made, such as in the effect of catecholamines on these exchange processes, into the mechanisms by which these movements occur. The linkage of these two groups of ions places obvious demands on the regulatory processes in that both osmoregulation and acid-base regulation are affected by these ion transfers. Acid-base balance cannot occur beyond the point where ionic and osmotic regulation of the animal is compromised beyond its adaptive range. The opposite relationship is also true. While expansion of the data base regarding the role of ventilation and ion transfer processes in acid-base regulation is needed, further research into the possible controls over this regulation would also be most desirable.

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APPENDICES

APPENDIX I.

Table A.1. Ionic concentrations for the sea salt used to make up the various salinities in experiment 2A. in Section 2. These salts were purchased from Wiegandt GmbH & Co., Sterkenhofweg 13, D-4150 Krefeld 1, F.R.G. All concentrations in mM.

ION	CONCENTRATION	ION	CONCENTRATION
Na^+	468.0	Cl^-	545.7
K^+	10.0	Br^-	0.8
Mg^{++}	53.3	I^-	0.003
Ca^{++}	10.4	SO_4^-	28.1
Sr	0.2	HCO_3^-	2.4
F	0.07	H_3BO_3	25

APPENDIX II.

NERNST RATIO CALCULATIONS

$$TEP = \frac{RT}{ZF} * \ln \frac{[cation]w \text{ or } [anion]pl}{[cation]pl \text{ or } [anion]w}$$

TEP in volts; Concentrations in moles; ln = natural log

To calculate what the plasma cation concentration, for example, would be given the TEP and [cation]w and assuming the conditions for the Nernst equation,

$$e^{TEP/(RT/ZF)} = [cation]w / [cation]pl$$

$$[cation]pl^* = [cation]w / e^{TEP/(RT/ZF)}$$

NERNST RATIO = [cation]pl measured / [cation]pl expected*

APPENDIX III.
PERMEABILITY CALCULATIONS

$$TEP = \frac{RT}{ZF} * \ln \frac{PNa^+[Na^+]w + PCl^-[Cl^-]pl + PHCO_3^-[HCO_3^-]pl}{PNa^+[Na^+]pl + PCl^-[Cl^-]w + PHCO_3^-[HCO_3^-]w}$$

$$C = e^{\wedge}(TEP/RT/ZF)$$

$$C\,(PNa^+[Na^+]pl + PCl^-[Cl^-]w + PHCO_3^-[HCO_3^-]w) = (PNa^+[Na^+]w + PCl^-[Cl^-]pl + PHCO_3^-[HCO_3^-]pl)$$

divide by $PHCO_3^-$

$$C(PNa^+/PHCO_3^-[Na^+]pl + PCl^-/PHCO_3^-[Cl^-]w + [HCO_3^-]w) = (PNa^+/PHCO_3^-[Na^+]w + PCl^-/PHCO_3^-[Cl^-]pl + [HCO_3^-]pl)$$

set to 0

$$PNa^+/PHCO_3^-(C[Na^+]pl - [Na^+]w) + PCl^-/PHCO_3^-(C[Cl^-]w - [Cl^-]pl) + (C[HCO_3^-]w - [HCO_3^-]pl) = 0$$

$$PNa^+/PHCO_3^-(C[Na^+]pl - [Na^+]w) + PCl^-/PHCO_3^-(C[Cl^-]w - [Cl^-]pl) = -(C[HCO_3^-]w - [HCO_3^-]pl)$$

SOLVED FOR $PNa^+/PHCO_3^-$ & $PCl^-/PHCO_3^-$ FOR 2 SALINITIES

3 EXPERIMENTAL SALINITIES ALLOWED 3 UNIQUE SALINITY PAIRS WHICH ALLOWED 3 ESTIMATES OF EACH OF THESE PARAMETERS

$$PNa^+/PCl^- = PNa^+/PHCO_3^- \;/\; PCl^-/PHCO_3^-$$

THESE ESTIMATES WERE MADE FOR EACH SAMPLING TIME THROUGH THE EXPERIMENT

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