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EFFECT OF ENVIRONMENTAL WATER SALINITY ON ACID–BASE REGULATION DURING ENVIRONMENTAL HYPERCAPNIA IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

Acid–base regulation in rainbow trout acclimated to about 3, 100 and 300 mmol l^{-1} Na^+ and Cl^-, at constant water [HCO_3^-], was assessed during 24 h of exposure to 1 % CO_2 and during recovery. The respiratory acidosis induced by a rise in plasma P_{CO_2} to about 1.15 kPa (8.5 mmHg, 3 mmol l^{-1}), 1.33 kPa (10 mmHg, 100 mmol l^{-1}) or 1.5 kPa (11.2 mmHg, 300 mmol l^{-1}) was partially compensated for by accumulation of plasma HCO_3^-. The degree of pH compensation depended on the salinity of the environmental water, being about 61, 82 and 88 % at 3, 100 and 300 mmol l^{-1} Na^+ and Cl^-, respectively. [HCO_3^-] in animals acclimated to 100 and 300 mmol l^{-1} rose to higher values than that in fish at 3 mmol l^{-1}.

Plasma [Cl^-] decreased during hypercapnia as compared to control concentrations in all groups of fish. Plasma [Na^+] rose during the first 8 h of hypercapnia in fish acclimated to all three salinities, but recovered towards control values during the remainder of hypercapnia. The rise in plasma [HCO_3^-] was significantly related to the fall in plasma [Cl^-], whereas the changes in plasma [Na^+] were unaffected by simultaneous changes in plasma [HCO_3^-]. Time courses of changes in plasma [Na^+] and total ammonia concentration, [T_{amm}], were similar but in opposite directions.

The transepithelial potential (TEP) of blood relative to water was negative, close to zero and positive, averaging −21, −5.8 and +6.2 mV for fish acclimated to 3, 100 and 300 mmol l^{-1} Na^+, respectively. After initiation of hypercapnia, which caused a quite heterogeneous response among groups, a clear trend towards depolarization was observed during the remainder of hypercapnia.

These results confirm the role of active HCO_3^-/Cl^- exchange processes for the compensation of extracellular pH during respiratory acidoses in fish.

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Key words: acid–base regulation, water salinity, hypercapnia, rainbow trout, *Oncorhynchus mykiss*, transepithelial potential.

Introduction

The acid–base regulation of fishes during exposure to hypercapnia is characterized by almost complete compensation of the respiratory acidosis by accumulation of additional HCO_3^- in the body fluids. During the initial stages of hypercapnia, the role of bicarbonate produced from CO_2 by nonbicarbonate buffering in the body fluids is relatively large, but it is gradually reduced as the degree of pH compensation increases, and approaches zero as a result of almost complete restoration of pH to control values. The net transfer of bicarbonate, furthermore, from the intracellular to the extracellular space, at the expense of reduced intracellular pH compensation, takes place only during the initial period of hypercapnia, before the intracellular pH is restored closer towards control values than the extracellular pH (see Heisler, 1986*b*). The major fraction of the final steady-state elevation in $[HCO_3^-]$, however, is brought about by acid–base-relevant transepithelial ion transfer across the gill epithelium (see Heisler 1980, 1984, 1986*b*).

The time course of pH compensation during hypercapnia has been described as being extremely variable among a number of fish species and conditions. The elasmobranch *Scyliorhinus stellaris* (Heisler *et al.* 1976) and the marine teleost fish *Conger conger* achieved almost complete compensation of plasma pH (to within 0.1 units of the control value) within about 8–10 h, whereas *Ictalurus punctatus* required 24 h (Cameron, 1980) and rainbow trout 22–75 h to achieve about the same degree of compensation (Eddy *et al.* 1977; Janssen and Randall, 1975; Perry *et al.* 1981). Apart from species differences, the ionic composition of the environmental water is obviously different between marine and freshwater species. The ionic composition of media used in the studies involving *Salmo gairdneri* in fresh water (Eddy *et al.* 1977; Janssen and Randall, 1975; Perry *et al.* 1981), furthermore, is variable and correlated with the time course of compensation, such that higher water electrolyte concentrations are associated with more complete and faster compensation of hypercapnia-induced respiratory acidoses (Heisler, 1982).

In experiments where various acidotic conditions were imposed on fish in combination with increases in water salinity from fresh water, by a moderate increment (Perry, 1986, hypercapnia) or to full-strength sea water (Tang and Boutilier, 1988, acid infusion; Tang *et al.* 1989, exhausting exercise), higher salinities, in all cases, caused an attenuated disturbance to extracellular pH (pHe) and/or a better recovery. The mechanisms involved, however, are unknown.

The basis for such a relationship between the concentration of ions in the environment and the net efflux of H^+ from the body rests in the evidence for the exchange of $H^+(NH_4^+)/Na^+$ and/or HCO_3^-/Cl^- across the gill epithelium (see Evans, 1986; Heisler, 1986*b*). To explain these differences in acid–base regulatory performance in fish at different salinities, studies must delineate possible factors such as the differences in permeability as well as ion transport processes of the epithelium in the gill, kidney and gut in freshwater- and seawater-adapted fish. The effect of the different buffering capacities of fresh water and sea water,

through the different HCO_3^- concentrations in these media, on the acid–base regulatory performance in fish must also be described. The experiments reported here address the latter aspect of this problem. The relationship between acid–base regulatory performance in rainbow trout acclimated to hypotonic, near-isotonic and hypertonic salinities at a fixed HCO_3^- concentration is described.

Materials and methods

Experimental animals

Specimens of rainbow trout [*Oncorhynchus mykiss* (Walbaum), mass between 594 and 1257 g, 932±44.1 g, mean±S.E., N=18] were obtained from a commercial hatchery and kept indoors on a 12 h/12 h light/dark photoperiod. They were acclimated at 10 °C in large glass aquaria of about 2 m^3 (approx. 150 l per fish) to one of three different water salinities for at least 1 month prior to experimentation. The aquaria were supplied with a continuous flow-through of fresh water of the same salinity. A sea-salt mixture was added to fresh dechlorinated Göttingen tap water ($[Ca^{2+}]$ 0.8–1.2 mmol l^{-1}, $[HCO_3^- + CO_3^{2-}]$ 0.6–0.8 mmol l^{-1}) to achieve the following mean ionic concentrations (mmol l^{-1}): $[Na^+]$=2.62, $[Cl^-]$=3.29; $[Na^+]$=97.72, $[Cl^-]$=113.82; or $[Na^+]$=320.72, $[Cl^-]$=333.82. The salinities will be referred to below as 3, 100 and 300 mmol l^{-1} Na^+. The fish were fed to satiation with commercial trout chow several times a day and food was withheld for about 48 h before an experiment.

Surgery and apparatus

The fish were anaesthetized by immersion in a buffered solution ($NaHCO_3$, to pH approx. 7.5) of MS 222 at concentrations of 1:10 000 in water of the respective pre-acclimation salinity. After full stage III anaesthesia (Iwama *et al.* 1989) had been induced, the animals were transferred to the operating table and anaesthesia as well as oxygen supply were maintained by irrigation of the gills with aerated and thermostatted water of reduced MS 222 concentration (1:20 000). The animals were fitted with chronic indwelling polyethylene catheters (PE 60) in the dorsal aorta by application of a modified Seldinger technique, similar to the procedure of Soivio *et al.* (1972). In brief, PE 60 was pulled and tapered over a conically sharpened stainless-steel mandril, and cut such that the conical mandril tip just protruded from the catheter. After direct puncture of the vessel with this assembly, the mandril was removed and the catheter was advanced into the vessel. A longer catheter was then connected to the PE 60 tubing, as described by Ultsch *et al.* (1981).

After surgery, the animal was transferred into the experimental chamber, which was part of a water recirculation system that has been described previously (Heisler *et al.* 1976; Heisler, 1978) (Fig. 1). The total volumes of the chambers were either 12.74 l or 13.89 l, depending on whether they contained two spacers or one spacer, respectively. Spacers were used to maximize the ratio of fish/water volume. The water in the recirculation system contained the electrolyte

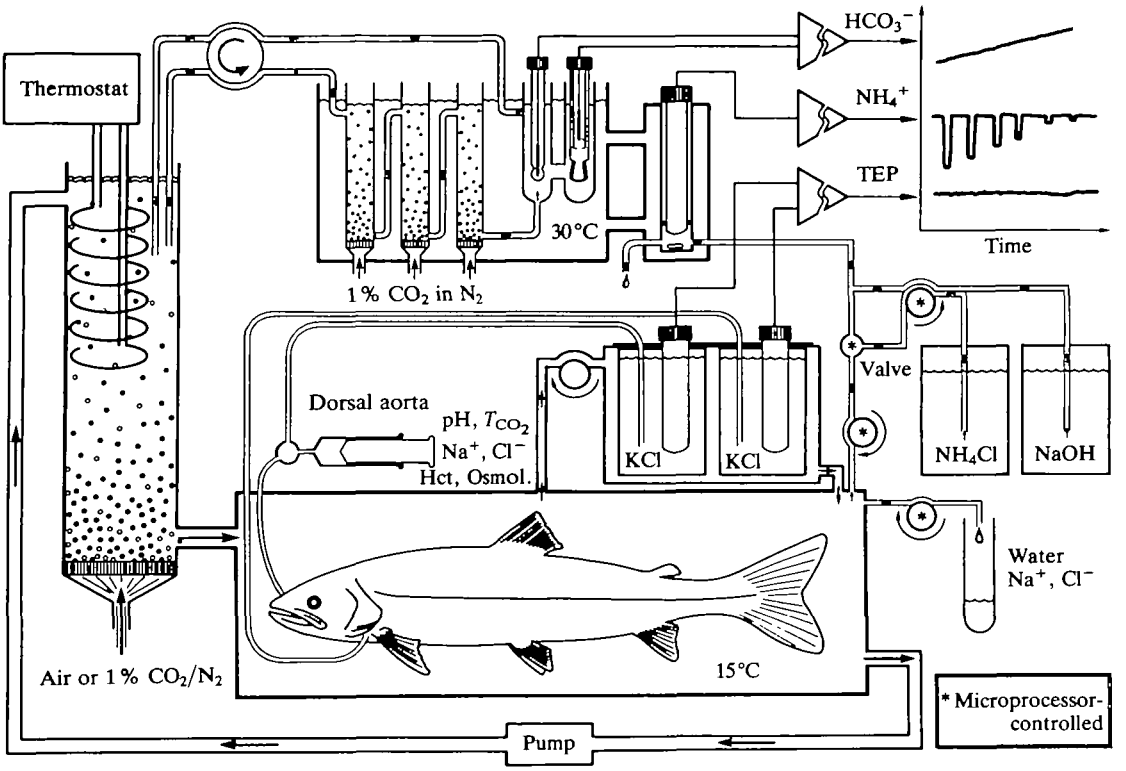

Fig. 1. Experimental apparatus showing the recirculating water system and apparatus used for automated determination of $[HCO_3^-]$ and $[NH_4^+]$ in the water. See text for details. TEP, transepithelial potential; T_{CO_2}, total CO_2 concentration; Osmol, osmolarity.

concentration the animal had been acclimated to, except for $[HCO_3^-]$, which had been pre-adjusted for all experimental series, by addition of HCl, to be the same as for the 3 mmol l^{-1} Na^+ salinity. The actual HCO_3^- concentrations were (mean±S.E.): 0.881±0.028 (3 mmol l^{-1}); 0.893±0.039 (100 mmol l^{-1}); 0.848±0.092 (300 mmol l^{-1}). The fish were allowed to recover after surgical procedures for at least 24 h in the recirculation system before experiments were initiated. During experimentation, the water in the recirculation system was flushed with thermostatted fresh water of the same ionic and respiratory gas composition every 24 h to prevent build-up of metabolic wastes.

Protocol

After an initial 24 h control period (control) the experimental animals were exposed to 1% CO_2 for 24 h (hypercapnia). The recovery from hypercapnia was monitored during a third 24 h period at normocapnia (recovery). Samples of environmental water and dorsal aortic blood were taken according to the following schedule: three control samples at −2 h, −1 h and −0.5 h; 15 min, 30 min, 1 h, 2 h,

4 h, 8 h, 20 h and 24 h after initiation of environmental hypercapnia; and 15 min, 30 min, 1 h, 5 h, 10 h, 20 h and 24 h after return to normocapnia.

At each sampling time 5 ml samples of water were stored at 4 °C for ion concentration analyses for a time not exceeding 72 h. The dorsal aortic catheter was connected to a three-way stopcock, which allowed blood sampling or connection with a KCl/agar bridge (3 mol l^{-1} KCl in 2 % agar) for transepithelial potential (TEP) measurements by means of a high-impedance voltmeter. After sampling of blood (1 ml), the extracted volume was replaced by heparinized (20 i.u. ml^{-1}) physiological saline, and contact with the KCl/agar bridge was established by switching to the other position of the three-way stopcock. Contact for monitoring TEP was maintained between blood samples, allowing continuous recording of TEP.

The water [HCO_3^-] was continuously monitored by pumping environmental water at a rate of 20 ml min^{-1} into a 'Δ[HCO_3^-] system' (Heisler, 1978), where it was equilibrated in three successively connected bubble columns with 1 % CO_2, before pH, as an indicator of changes in [HCO_3^-], was continuously recorded by means of a special spherical glass electrode and a double electrolyte bridge sleeve diaphragm Ag/AgCl reference (for details, see Claiborne and Heisler, 1984; Heisler, 1989*b*). The complete electrode system was thermostatted at an elevated temperature (30±0.01 °C) to improve the stability and response time of the electrodes. The potential of the pH electrode was recorded *via* a high-impedance isolation amplifier (Knick model 87) and a conditioning amplifier on a chart recorder. Values corresponding to the above sample times were read from the chart recordings.

Measurements

Plasma pH was measured on whole blood using a glass capillary electrode (BMS III Mk 1, Radiometer, Copenhagen) calibrated with precision phosphate buffers and thermostatted to 10 °C. Haematocrit (Hct) was measured after centrifugation at 19 500 *g* in glass capillaries. Hct fell from control values of about 35 % at 3 mmol l^{-1} and 23 % at 100 and 300 mmol l^{-1} to values in the range of 15 % after 74 h of experimentation. Total CO_2 (T_{CO_2}) was determined in 20 μl samples of the supernatant plasma by differential conductivity measurement (Maffly, 1968), using a Capni-Con III apparatus (Cameron Instruments Inc., Port Aransas, Texas). The Capnicon was calibrated with standards produced from desiccated $NaHCO_3$.

A sample of plasma was acidified and frozen for total ammonia analysis by an enzymatic method (Boehringer, Mannheim, FRG.). The remaining plasma was analyzed for [Na^+] by atomic absorption spectroscopy and [Cl^-] was determined coulometrically (CMT10, Radiometer, Copenhagen). Plasma samples of 8 μl were analyzed for osmolarity with a water vapour micro-osmometer (Wescor). Plasma P_{CO_2} was calculated from plasma T_{CO_2} and pH by application of the Henderson–Hasselbalch equation, using the constants derived for rainbow trout from the polynomials reported by Heisler (1986*a*). Plasma [HCO_3^-] was calculated as

$T_{CO_2}-(P_{CO_2}\cdot\alpha_{CO_2})$. The general formula of Heisler (1986*a*) was used to determine α_{CO_2}.

The water samples were analyzed for $[Na^+]$ using the same techniques as for plasma. Water $[Cl^-]$ was determined by means of solid-state chloride-sensitive electrodes and double electrolyte bridge references connected to a microprocessor/ion analyzer. The electrode was calibrated with two NaCl standards bracketing the encountered range and referenced to particular standards close to the actual sample concentrations between measurements. Total ammonia concentration in the water was determined automatically using ammonia electrodes controlled by a microprocessor. Water was sampled by means of a roller pump from the fish system at the same times as plasma samples were collected and was alkalized to pH>12 by addition of about 2 % of 10 mol l^{-1} NaOH before being introduced into the chamber of the ammonia electrode (Ingold, Frankfurt, FRG) (Fig. 1). The signal from the electrode was amplified, filtered and recorded. Automatic calibration was performed with aqueous NH_4Cl standards before and after each measurement on environmental water.

The TEP between arterial plasma and the environment was recorded by means of Ag/AgCl electrode pairs, thermostatted to the animal's temperature. Sensing and referencing electrodes were connected to water and blood by polyethylene cannulae (size PE 50) filled with 3 mol l^{-1} KCl set in agar (see above). The agar junction of the reference electrode was placed close to the gills by threading it through a larger-diameter cannula sewn in place just inside the operculum, while the sensing electrode was connected to the dorsal aorta *via* the indwelling catheter, as described above. The potential across the electrodes was measured using a high-impedance voltmeter. The complete electrode chain was zeroed by placing both agar-bridge endings close together into the water. The offset voltage was always smaller than 1 mV.

Calculations and statistics

Net flux rates of ions were calculated as mmol kg^{-1} body mass from the changes in the amount per unit time of the specific ion in the environmental water compared to those during the control period; net H^+ transfer was calculated on the basis of the difference between changes in NH_4^+ and HCO_3^- in the water (for details see Heisler, 1978, 1986*a*, equation 44; 1989*b*). Analysis of variance and Scheffe's test (Edwards, 1967) were used to discern statistical significance among the means of any variable 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. Least-squares linear regression analysis was used to describe the relationship between certain data sets. The level of rejection in all cases was $P<0.05$.

Results

Acid–base status

The imposed rise in water P_{CO_2} by 1 kPa (7.5 mmHg) caused plasma P_{CO_2} to rise

to 4–6 times the control values (Fig. 2A), with somewhat higher values at the higher water salinities of 100 and 300 mmol l^{-1}. Although P_{CO_2} at 100 mmol l^{-1} was not significantly different from those at 3 and 300 mmol l^{-1}, the difference between P_{CO_2} at 3 mmol l^{-1} and that at 300 mmol l^{-1} was significant. After return to normocapnia, P_{CO_2} fell to about twice the control value almost immediately, but did not quite attain control values during the following 24 h of recovery. The fall in plasma pH induced by the rise in P_{CO_2} during hypercapnia was partially compensated by the end of the 24 h hypercapnic period (Fig. 2B). The degree of compensation depended on the salinity of the environmental water, being about 61, 82 and 88 % at 3, 100 and 300 mmol l^{-1} Na^+, respectively. The average pHe values after 22 and 24 h of hypercapnia were significantly different among the three salinity series.

The compensation of extracellular pH was effected by accumulation of HCO_3^- in the extracellular space (Fig. 2C). The [HCO_3^-] in animals acclimated to 100 and 300 mmol l^{-1} Na^+ rose to higher values than in fish at 3 mmol l^{-1} Na^+. The net flux of H^+ reversed from an influx under control conditions to an efflux during hypercapnia in all series (Fig. 3). Owing to the high variability, statistically significant differences among the three groups of fish could not be detected. At the end of the recovery period, net H^+ influx (or an equivalent HCO_3^- efflux) was resumed.

Total ammonia concentrations in the plasma declined in fish at all three salinities in the initial 2 h following the onset of hypercapnia (Fig. 4A). There was a general trend in all groups towards control values during exposure to hypercapnia. Although the data for the recovery period were highly variable, there was a general trend in the means of all groups to increase during each 24 h period.

Plasma ions

Plasma [Cl^-] decreased during hypercapnia compared to control concentrations in all groups of fish (Fig. 4B). After 8 h of exposure, the values were significantly different from control values in fish acclimated to 3 mmol l^{-1} and after 20 h the values were also significantly different for fish at 100 and 300 mmol l^{-1} Na^+. During the normocapnic recovery period, [Cl^-] recovered towards control values but, with the exception of the 3 mmol l^{-1} series, did not reach the pre-hypercapnia values during the course of the experiment. Plasma [Cl^-] was generally higher in fish acclimated to 300 mmol l^{-1} Na^+ than in animals at 3 and 100 mmol l^{-1} Na^+ throughout the experiment.

Plasma [Na^+] generally rose during the first 8 h of exposure to hypercapnia in fish acclimated to all three salinities (Fig. 4C), but recovered towards control values during the remainder of the hypercapnic period. The recovery period was marked by a large variability in plasma [Na^+], with a general trend of further recovery towards pre-hypercapnia values at 100 and 300 mmol l^{-1} Na^+. At 3 mmol l^{-1} Na^+, plasma [Na^+] rose markedly during the initial period of recovery before drifting downwards to attain control values at the end of the experiment.

The plasma osmolarity in fish acclimated to 3 mmol l^{-1} Na^+ was reduced

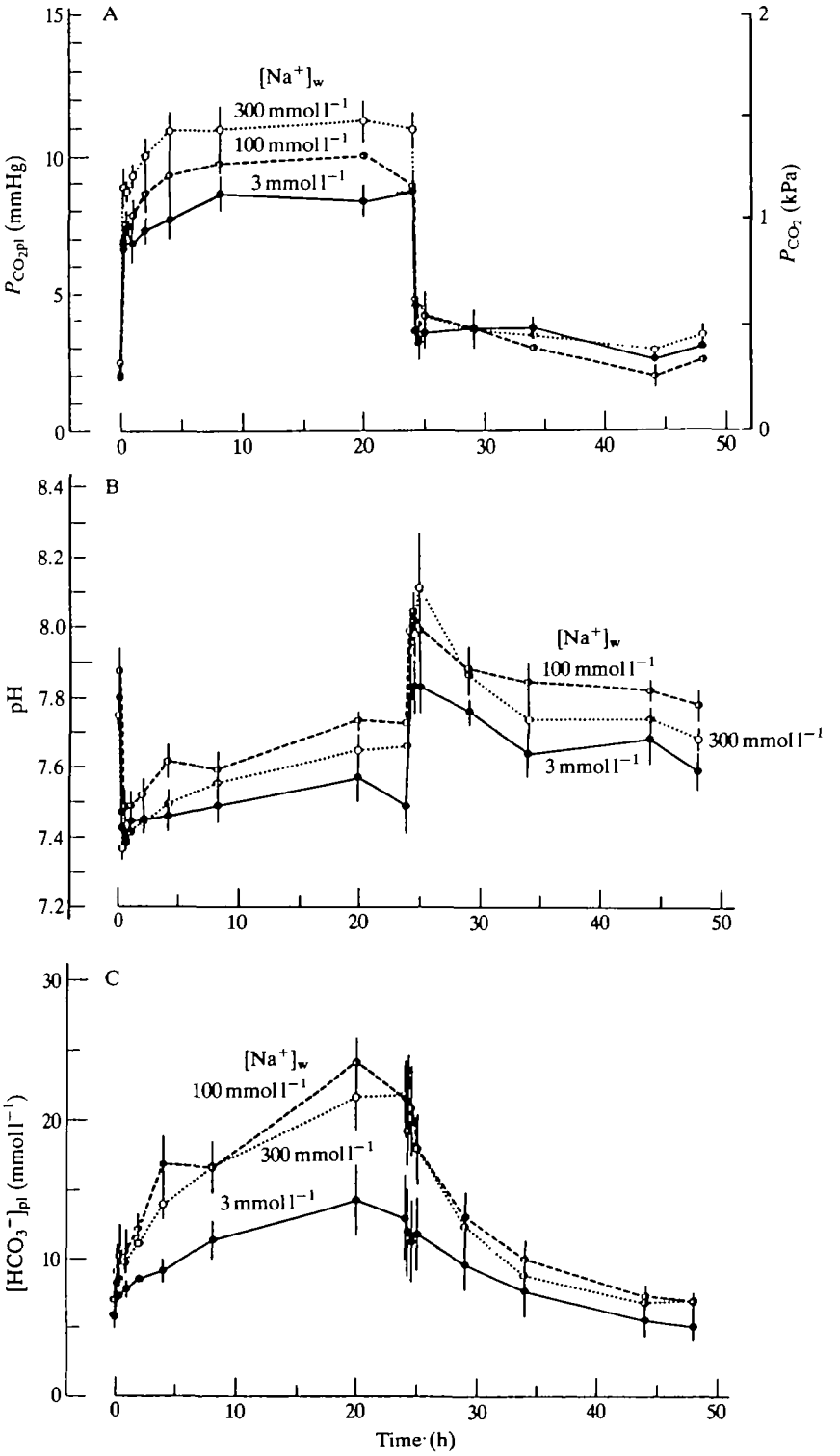
A
$[Na^+]_w$
300 mmol l^{-1}
100 mmol l^{-1}
3 mmol l^{-1}
P_{CO_2pl} (mmHg)
P_{CO_2} (kPa)
B
pH
$[Na^+]_w$
100 mmol l^{-1}
300 mmol l^{-1}
3 mmol l^{-1}
C
$[HCO_3^-]_{pl}$ (mmol l^{-1})
$[Na^+]_w$
100 mmol l^{-1}
300 mmol l^{-1}
3 mmol l^{-1}
Time (h)

Fig. 2

Fig. 2. Plasma P_{CO_2} (A), pH (B) and $[HCO_3^-]$ (B) in rainbow trout acclimated to 3, 100 and 300 mmol l^{-1} NaCl and exposed to environmental hypercapnia (mean±S.E., N=6 for each salinity).

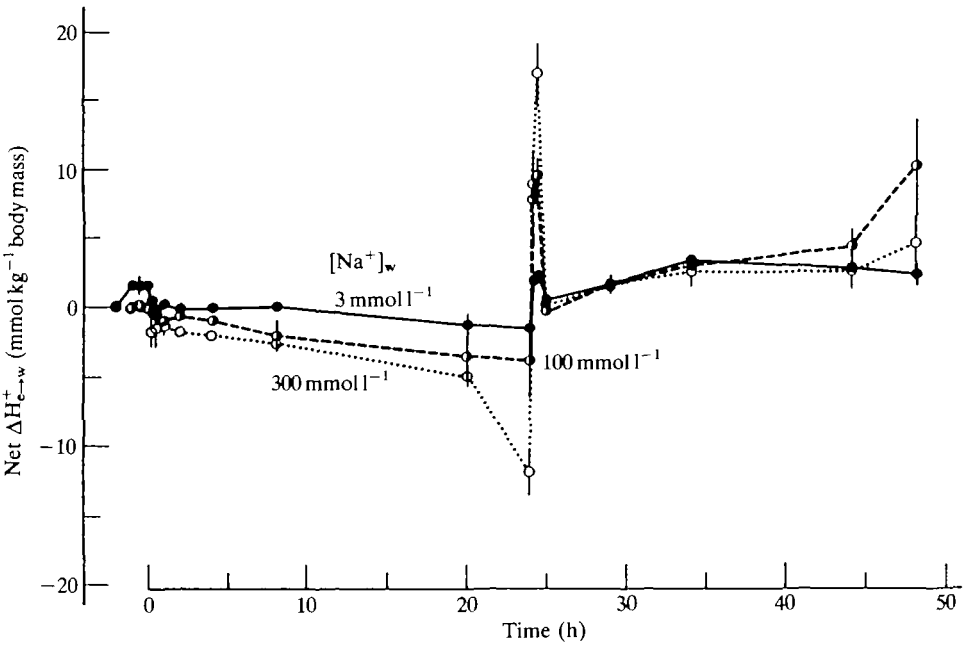

Fig. 3. Net flux of H^+ in rainbow trout acclimated to 3, 100 and 300 mmol l^{-1} NaCl and exposed to 1 % environmental hypercapnia (mean±S.E., N=3 for 3 mmol l^{-1}, N=5 for 100 mmol l^{-1}, N=6 for 300 mmol l^{-1} Na^+).

compared to the level for that salinity during the control period (mean 282 mosmol l^{-1}) during exposure to hypercapnia. The osmolarity for that group tended to rise towards control levels during the later period of recovery, but never regained control values. There were no significant changes in osmolarity in the groups of fish acclimated to 100 (mean 307 mosmol l^{-1}) and 300 mmol l^{-1} (mean 330 mosmol l^{-1}) Na^+.

Acid–base and ion correlations

There were weak but significant correlations between plasma ($[Na^+]-[Cl^-]$) and plasma $[HCO_3^-]$ at all three salinities (Fig. 5A). The reason for using ($[Na^+]-[Cl^-]$) as a parameter was that the transepithelial fluxes of both ions in appropriate directions could have resulted in the accumulation of plasma HCO_3^- *via* Na^+/H^+ as well as Cl^-/HCO_3^- exchanges. Since Na^+ and Cl^- transepithelial fluxes would occur in opposite directions in order for HCO_3^- to be accumulated in plasma, ($[Na^+]-[Cl^-]$) would be expected to mirror the net changes in $[HCO_3^-]$

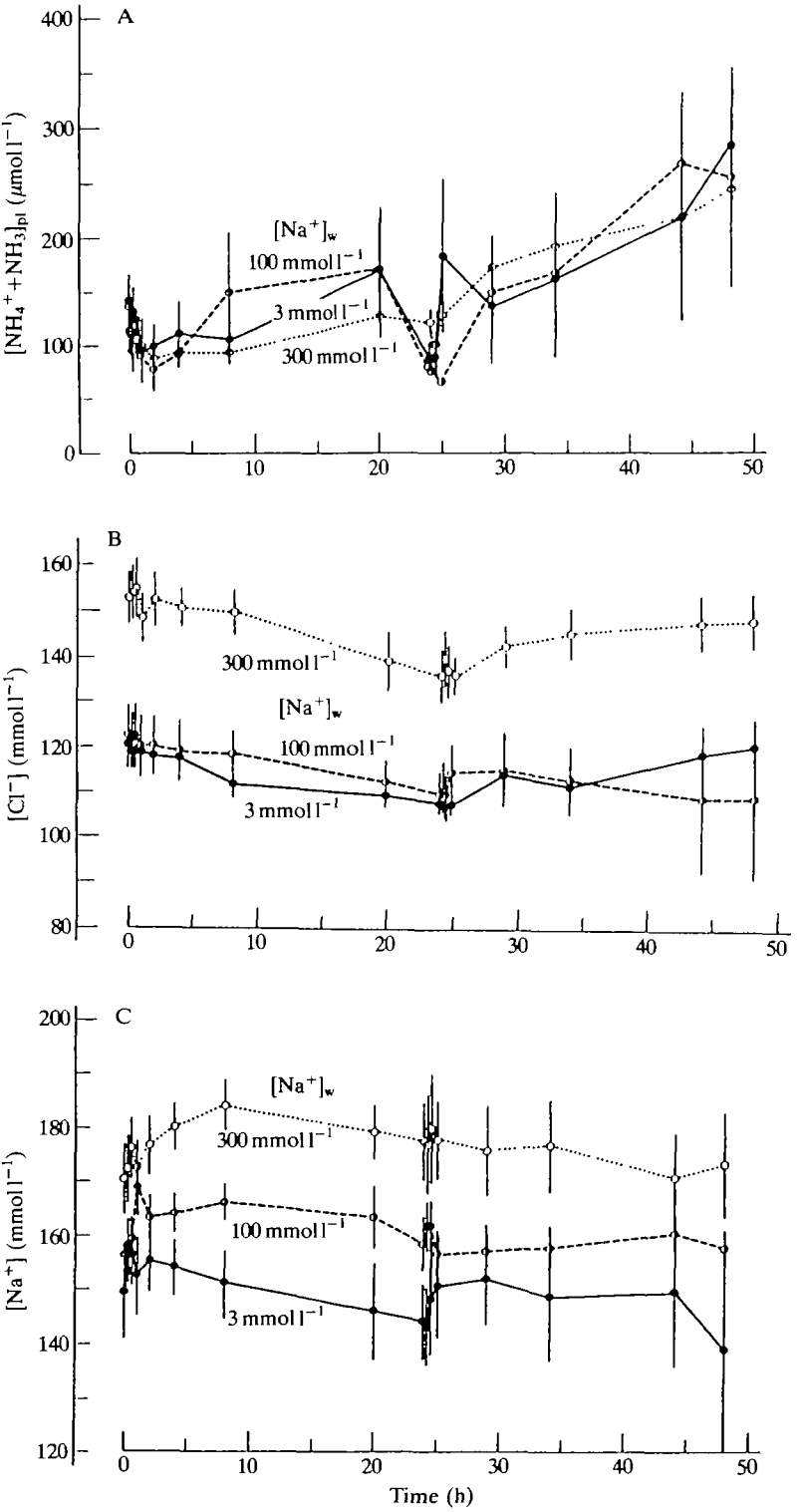
A
$[NH_4^+ + NH_3]_{pl}$ (μmol l^{-1})
400
300
200
100
0
$[Na^+]_w$
100 mmol l^{-1}
3 mmol l^{-1}
300 mmol l^{-1}
0
10
20
30
40
50
B
$[Cl^-]$ (mmol l^{-1})
160
140
120
100
80
300 mmol l^{-1}
$[Na^+]_w$
100 mmol l^{-1}
3 mmol l^{-1}
0
10
20
30
40
50
C
$[Na^+]$ (mmol l^{-1})
200
180
160
140
120
$[Na^+]_w$
300 mmol l^{-1}
100 mmol l^{-1}
3 mmol l^{-1}
0
10
20
30
40
50
Time (h)

Fig. 4

Fig. 4. Plasma [total ammonia] (A), $[Cl^-]$ (B) and $[Na^+]$ (C) in rainbow trout acclimated to 3, 100 and 300 mmol l^{-1} NaCl exposed to 1 % environmental hypercapnia (mean±S.E., N=6 for each salinity).

and indicate the net effect of these mechanisms. Further analysis, however, suggested that this correlation was indicative of net Cl^-/HCO_3^- exchange rather than of Na^+/H^+ exchange. The rise in plasma $[HCO_3^-]$ was significantly related to the fall in plasma $[Cl^-]$ at all three salinities (Fig. 5B), whereas the changes in plasma $[Na^+]$ were statistically unaffected by the simultaneous changes in plasma $[HCO_3^-]$ in fish acclimated to all three salinities (Fig. 5C). Furthermore, while the slopes of the best-fitting lines deviated from the line of identity for the relationship between changes in plasma $[HCO_3^-]$ and $[Na^+]$, the relationship between changes in plasma $[HCO_3^-]$ and $[Cl^-]$ was approximately 1:1.

Transepithelial potentials

The transepithelial potentials (TEP) of blood relative to water were negative, close to zero and positive, averaging −21, −5.8 and +6.2 mV for fish acclimated to 3, 100 and 300 mmol l^{-1} Na^+, respectively (Fig. 6). Initiation of hypercapnia caused mixed results: while TEP remained initially unaffected in fish acclimated to 3 mmol l^{-1} Na^+, TEP in animals at 100 mmol l^{-1} Na^+ depolarized immediately by about 2.5 mV and in fish at 300 mmol l^{-1} it hyperpolarized by about 2.8 mV. In contrast to these immediate responses, TEP in fish acclimated to 3 mmol l^{-1} Na^+ depolarized to about −16 mV 2 h after the onset of hypercapnia. This initial effect was generally followed by a trend towards depolarization during the remainder of the period of hypercapnia. The initial effects of hypercapnia were essentially reversed after the return to normocapnia. During the recovery period, TEP tended to recover towards control values, although the actual values were not re-attained during the experimental observation period. The effect of hypercapnia was much less than that of acclimation to different salinities.

Discussion

The fixed level of environmental hypercapnia (1 % CO_2) applied in this series of experiments resulted in considerably higher P_{CO_2} values in the body fluids at elevated environmental salinities. The mechanism for this highly significant phenomenon is unknown. Interestingly, the absolute level at 300 mmol l^{-1} (about 1.46 kPa; 11 mmHg) is in the same range as in the elasmobranch *Scyliorhinus stellaris* (Heisler *et al.* 1976, 1980) and the marine teleost *Conger conger* (Toews *et al.* 1983) exposed to the same level of environmental hypercapnia. In contrast, trout at 3 mmol l^{-1} water salinity exhibit a difference between control P_{CO_2} and values during hypercapnia similar to that of carp at the same environmental P_{CO_2} (Claiborne and Heisler, 1984, 1986). At 3 mmol l^{-1} the inspired −arterial P_{CO_2} difference during hypercapnia is reduced to less than half the control value, suggesting either a considerable degree of hyperventilation or an improved

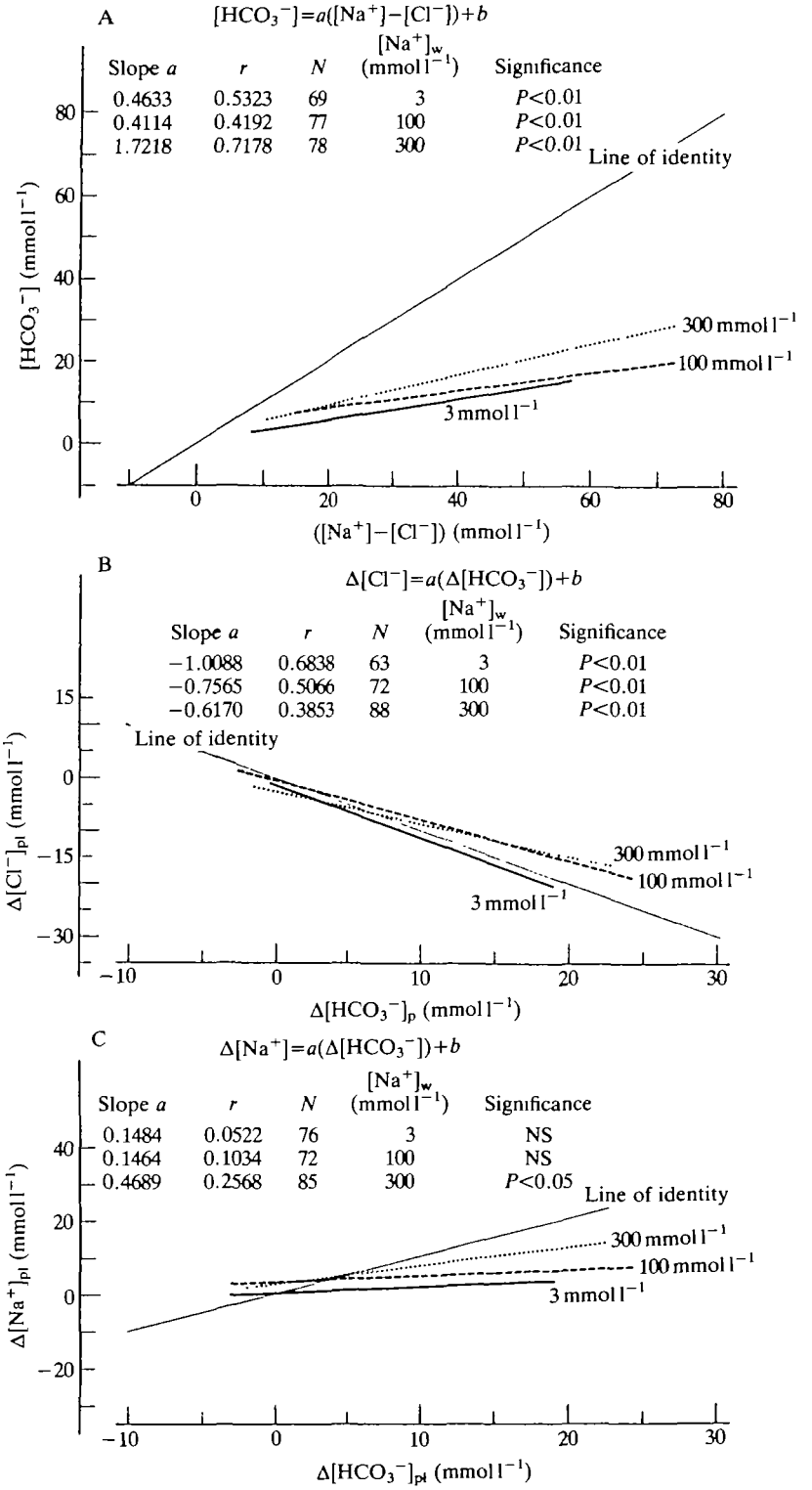
A
$[HCO_3^-]=a([Na^+]-[Cl^-])+b$
Slope a | r | N | $[Na^+]_w$ (mmol l^{-1}) | Significance
0.4633 | 0.5323 | 69 | 3 | P<0.01
0.4114 | 0.4192 | 77 | 100 | P<0.01
1.7218 | 0.7178 | 78 | 300 | P<0.01
Line of identity
300 mmol l^{-1}
100 mmol l^{-1}
3 mmol l^{-1}
$[HCO_3^-]$ (mmol l^{-1})
$([Na^+]-[Cl^-])$ (mmol l^{-1})
B
$\Delta[Cl^-]=a(\Delta[HCO_3^-])+b$
Slope a | r | N | $[Na^+]_w$ (mmol l^{-1}) | Significance
−1.0088 | 0.6838 | 63 | 3 | P<0.01
−0.7565 | 0.5066 | 72 | 100 | P<0.01
−0.6170 | 0.3853 | 88 | 300 | P<0.01
Line of identity
300 mmol l^{-1}
100 mmol l^{-1}
3 mmol l^{-1}
$\Delta[Cl^-]_{pl}$ (mmol l^{-1})
$\Delta[HCO_3^-]_p$ (mmol l^{-1})
C
$\Delta[Na^+]=a(\Delta[HCO_3^-])+b$
Slope a | r | N | $[Na^+]_w$ (mmol l^{-1}) | Significance
0.1484 | 0.0522 | 76 | 3 | NS
0.1464 | 0.1034 | 72 | 100 | NS
0.4689 | 0.2568 | 85 | 300 | P<0.05
Line of identity
300 mmol l^{-1}
100 mmol l^{-1}
3 mmol l^{-1}
$\Delta[Na^+]_{pl}$ (mmol l^{-1})
$\Delta[HCO_3^-]_{pl}$ (mmol l^{-1})

Fig. 5

Fig. 5. Relationships between plasma ($[Na^+]-[Cl^-]$) and plasma $[HCO_3^-]$ (A), between changes in plasma $[Cl^-]$ and corresponding changes in plasma $[HCO_3^-]$ (B) and between changes in plasma $[Na^+]$ and corresponding changes in plasma $[HCO_3^-]$ (C) during exposure to, and recovery from, 1% hypercapnia in rainbow trout acclimated to 3, 100 and 300 mmol l^{-1} NaCl. Lines were established on the basis of experimental data by linear regression analysis.

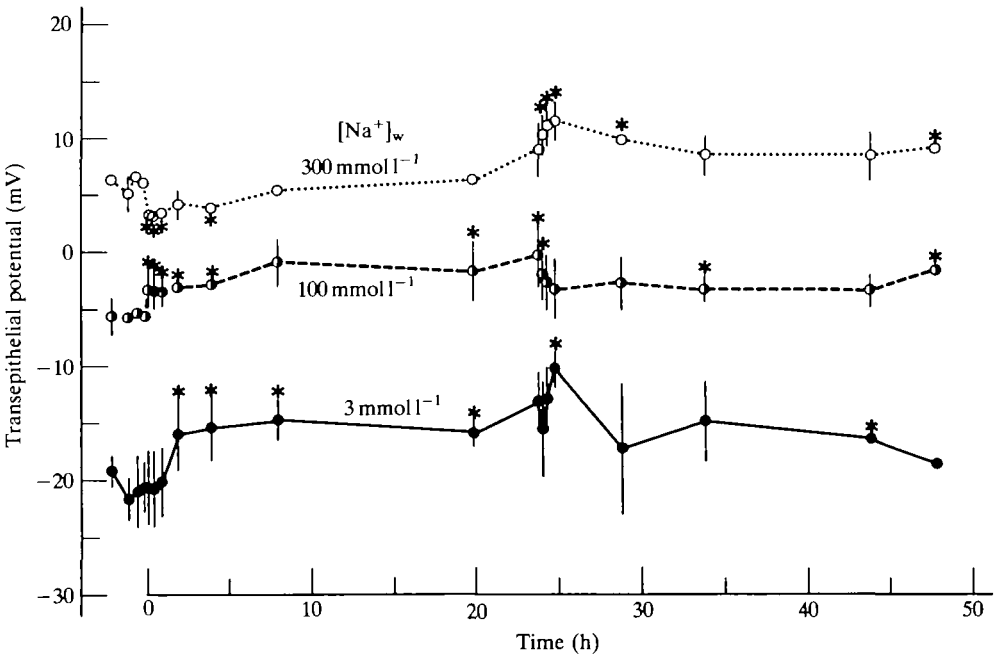

Fig. 6. Transepithelial potential values during the control period and during exposure to and recovery from 1% environmental hypercapnia in rainbow trout acclimated to 3, 100 and 300 mmol l^{-1} NaCl (mean±S.E., $N=6$ for each salinity, * significantly different from controls).

efficiency of CO_2 gas exchange (Heisler, 1989*a*) caused by general changes in gill morphology; conversely, the enhanced inspired–arterial differences at 300 mmol l^{-1} salinity could be due to a deterioration of gas exchange conditions caused by factors such as mucus formation on the secondary lamellae upon transfer to higher salinities (as suggested by Bath and Eddy, 1979).

The initial fall in plasma pH upon exposure to hypercapnia, the trend towards recovery and the associated accumulation of HCO_3^- in the extracellular space are characteristic of freshwater fish (carp: Claiborne and Heisler, 1984; Heisler, 1986*b*; rainbow trout: Janssen and Randall, 1975; Eddy *et al.* 1977; Lloyd and White, 1967; Cameron and Randall, 1972; Eddy, 1976) and marine species (dogfish: Cross *et al.* 1969; spotted dogfish: Heisler *et al.* 1976, 1980; Randall *et al.* 1976; coho salmon: Bubien and Meade, 1979) exposed to environmental hypercap-

nia. An elevation of the environmental Na^+ and Cl^-concentrations had a clearly supportive effect for the compensation of the respiratory acidosis. Both the rate of recovery of plasma pH and the absolute degree of compensation were enhanced at higher water salinities. This finding confirms previous evidence for a positive correlation between the ionic content of the water and the kinetics and degree of pH recovery during environmental hypercapnia. Interestingly, the increase in the rate of elevation of plasma $[HCO_3^-]$ was associated exclusively with the step from 3 to 100 mmol l^{-1} water salinity, whereas the additional increase to 300 mmol l^{-1} resulted in a tendency to a slightly slower accumulation (Fig. 2). In spite of generally lower bicarbonate concentrations, the degree of compensation was still larger at 300 mmol l^{-1} salinity, owing to a higher P_{CO_2} and the correspondingly larger change in pH expected at constant water $[HCO_3^-]$.

The compensation was mainly brought about by uptake of bicarbonate-equivalent ions from (or extrusion of H^+-equivalent ions to) the environment. The mechanisms involved cannot easily be deduced from the present data.

The present experiments set out to evaluate the influence of elevated water electrolyte concentrations on the efficiency of transepithelial ion transfer processes for acid–base regulation. Since $[HCO_3^-]$ in the water was kept independent of salinity, the increased capability for net uptake of HCO_3^- from the water cannot be attributed to this parameter, or to differences in environmental pH, but must be related to the differences in water $[Na^+]$ and $[Cl^-]$ or other ions.

There was a close correlation between the changes in plasma $[HCO_3^-]$ and $[Cl^-]$, indicating that, in net terms, water HCO_3^- was exchanged with Cl^- in the body fluid. The relationship, furthermore, approximated a 1:1 ratio, as described by Jensen and Weber (1985). This relationship suggests that a net Cl^-/HCO_3^- exchange mechanism plays a significant role in the accumulation of plasma HCO_3^- for acid–base regulation during 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 Kirschner, 1972; Kormanik and Evans, 1979). Cameron (1976) and Claiborne and Heisler (1984) have demonstrated the role of the Cl^-/HCO_3^- exchange process for acid–base regulation during hypercapnia in the arctic grayling and the carp, respectively. While unrelated to any acid–base disturbance, an increased Cl^- efflux was demonstrated in the marine toadfish, *Opsanus beta*, exposed to elevated increasing water $[HCO_3^-]$ (Kormanik and Evans, 1979). This suggests that this mechanism is also available to fish in water of elevated osmolarity.

The correlation between plasma $[HCO_3^-]$ and $[Cl^-]$, however, does not necessarily describe the only mechanism involved. Bicarbonate may also have been produced from CO_2 associated with the active extrusion of H^+ and/or NH_4^+ in exchange for Na^+. The exchange of H^+ for Na^+, however, is not osmotically neutral (Heisler, 1989*a*). If this mechanism is used, the osmotically active $Na^+ + HCO_3^-$ is accumulated in the body fluids (Δosmolarity$=\Delta HCO_3^- + \Delta Na^+ >$net $\Delta H^+_{w\rightarrow e}$), in contrast to the osmotically balanced conditions during

Cl^-/HCO_3^- exchange (Δosmolarity$=\Delta HCO_3^- - \Delta Cl^- = 0$). In the present study, significant changes in osmolarity were not observed during hypercapnia, a result consistent with previous findings in the larger spotted dogfish (Heisler *et al.* 1976) and in the conger eel (Toews *et al.* 1983) during exposure to environmental hypercapnia. While this supports the possibility that Cl^-/HCO_3^- exchange predominates the compensatory mechanism, it still does not provide positive evidence against the possible involvement of active $H^+(NH_4^+)/Na^+$ exchange mechanisms. The potential changes in osmolarity may simply have been masked by effective osmoregulation. Cameron (1976) and Claiborne and Heisler (1984) demonstrated that $Na^+/H^+(NH_4^+)$ exchanges play a significant role in the compensation of plasma pH during exposure to environmental hypercapnia. While the changes in plasma $[Na^+]$ were not correlated with the accumulation of plasma HCO_3^- in this study, the time course of the rise in plasma $[Na^+]$ coincided more closely with the initial decline in $[T_{amm}]$, which would have been virtually all in the NH_4^+ form, in the initial hours of hypercapnia.

Control TEP values in trout acclimated to the three salinities applied in this study are in agreement with TEP values reported in the literature (see Potts, 1984). Those values also increased during hypercapnia and returned towards control values during recovery from hypercapnia. This consistent trend of TEP to increase during hypercapnia may reflect the accumulation of HCO_3^- in the plasma, although a definitive conclusion cannot be drawn. The trend of TEP to depolarize in trout during the first one-third of the hypercapnic period may be attributable to an increase in general branchial permeability. This notion is in accordance with data on the elevation of circulating catecholamine levels in response to environmental hypercapnia (Perry, 1986), which is associated with an increased water permeability (Isaia *et al.* 1978) and possibly with a corresponding rise in ionic permeability.

The TEP values, $[Cl^-]$ gradients and the degree of HCO_3^--accumulation were used to conduct a qualitative analysis of the electrochemical gradients between body fluids and water. At 3 mmol l^{-1}, the Cl^- gradient is from blood to water. On the basis of a possible Cl^-/HCO_3^- exchange process, the passive efflux of blood Cl^- could effect the observed accumulation of plasma HCO_3^-. The blood-side negative TEP would enhance the passive efflux of Cl^-, while hindering the influx of HCO_3^-. At 100 mmol l^{-1}, the gradient for plasma Cl^-, although in the same direction as at 3 mmol l^{-1}, is very small. It is unlikely that the passive efflux of Cl^- along its relatively small electrochemical gradient could effect a large influx of HCO_3^- from the water, causing the observed large accumulation of plasma HCO_3^-. Although Cl^- would be close to equilibrium, the large outward chemical gradient for HCO_3^- suggests active uptake from the water in order to achieve the observed accumulation of plasma HCO_3^-. At 300 mmol l^{-1}, the gradient for Cl^- is from the water to the blood. On the basis of strictly passive movements and a Cl^-/HCO_3^- exchange mechanism, HCO_3^- would have been lost instead of gained. The inside positive potential would also have enhanced Cl^- uptake from the water on a passive basis. Since the observed trend in HCO_3^- accumulation is

opposite to what would be predicted by strictly passive movements of these ions at all three salinities, active processes must have been involved in the elevation of plasma [HCO_3^-].

Active Cl^-/HCO_3^- exchange processes on the fish gill probably play a significant role in the compensation of pHe following exposure to environmental hypercapnia. Regardless of the HCO_3^- levels in the water, the availability of the Cl^- counterion in the water significantly affects the mechanisms that result in the accumulation of blood HCO_3^- to accomplish the observed compensation. The larger [HCO_3^-] in water with a larger seawater content may actually provide an enhanced basis for rapid compensation and protection against acidoses compared to that observed during the present experiment. Unfortunately, the data on the net H^+ fluxes were highly variable, making it difficult to explain the different levels of net HCO_3^- accumulation strictly on the basis of net ionic exchanges between blood and water. This variability may be attributable to inter- and intra-individual cycling of ionic fluxes, owing to incomplete adaptation of the gill epithelium to the changed salinity levels. Further research into the possible mechanisms for the enhanced acid–base regulatory performance in waters of higher salinity may clarify this point. Future studies on intracellular gill epithelial parameters may provide a closer insight into the mechanisms involved in the regulation of bulk extracellular pH during adaptation to different salinities.

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