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COMPENSATION OF PROGRESSIVE HYPERCAPNIA IN CHANNEL CATFISH AND BLUE CRABS

BY JAMES N. CAMERON AND GEORGE K. IWAMA*

Departments of Zoology and Marine Studies, The University of Texas Marine Science Institute, Port Aransas, TX 78373, USA

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SUMMARY

Channel catfish (*Ictalurus punctatus* Rafinesque) were progressively acclimated to CO_2 partial pressures of 7·5, 15, 30, 45 and 58 mmHg (1, 2, 4, 6 and 8 % CO_2 in air) and blue crabs (*Callinectes sapidus* Rathbun) to 15, 30 and 45 mmHg, with 24 h at each partial pressure. Measurements of both conventional acid–base parameters (pH, P_{CO_2}, total CO_2) and 'strong' ion concentrations (Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^-) were made at various times during each treatment. Intracellular $[Na^+]$, $[K^+]$ and $[Cl^-]$ were determined for red and white muscle in control and hypercapnic (8 %) catfish. Extracellular $[HCO_3^-]$ and strong ion difference (SID) both rose during hypercapnic compensation, with correlation coefficients (r) of 0·97 ($P<0·01$) for catfish and 0·41 (NS) for blue crabs. Since $[HCO_3^-]$ is calculated from two rapid measurements, and SID from four separate procedures, the former appears to be the measurement of choice. The results also dispel the notion of a 30 mequiv l^{-1} upper limit to bicarbonate compensation: $[HCO_3^-]$ values over 50 mequiv l^{-1} were achieved in both animals, and % pH regulation remained around 70 % at the highest P_{CO_2}. Due to superior intracellular buffering, the large change in extracellular SID did not lead to a measurable change in the concentrations of the major intracellular ions. The primary gill filaments of hypercapnic catfish showed a 30 % increase in numbers and a 75 % increase in area of apical crypts of chloride cells. The chloride cell 'patches' in crab gills increased in staining density after hypercapnia but did not enlarge.

INTRODUCTION

The response of aquatic animals to hypercapnia is familiar: pH is initially depressed, with a small bicarbonate increase due to non-bicarbonate buffering. After a time, the pH returns partially towards normal through the combined action of ion transport processes in the gills and renal net H^+ excretion, with a minimal contribution from increased ventilation (Cross *et al.* 1969; Cameron & Randall, 1972; Cameron, 1976, 1986; Heisler, 1986). Although this pattern has been shown repeatedly in many animals, two recent controversies prompt our re-examination:

*Present address: Department of Biology, Dalhousie University, Halifax, NS, Canada B3H 4J1.

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the advocacy of strong ion difference (SID) *vs* [HCO_3^-] approaches to acid–base analysis (Stewart, 1981), and the recent proposal (Heisler, 1986) that there is an upper limit to accumulation of [HCO_3^-] of about 30 mmol l^{-1} during compensation. There are only a handful of studies in which both acid–base and ionic variables have been measured simultaneously. Toews, Holeton & Heisler (1983), for example, measured [Na^+] and [Cl^-] but not other ions, so the SID cannot be calculated. There is also evidence that ionic responses to hypercapnia may differ in fresh- and saltwater fishes (reviewed by Heisler, 1986). Our first objective, then, was to measure both acid–base and ionic responses, allowing a comparison of SID and [HCO_3^-] approaches. The choice of the freshwater (FW) catfish and seawater (SW) blue crab allowed a further comparison since the FW catfish is an osmotic hyper-regulator with a fairly high rate of urine formation and the crab is an osmoconformer at SW salinity with a very low rate of urine formation.

The second hypothesis, that there is an upper limit to [HCO_3^-] compensation of about 30 mmol l^{-1} stems from observations that although pH compensation is always less than 100 %, neither alterations of external conditions nor direct infusions of bicarbonate increase the compensation (Claiborne & Heisler, 1984). This hypothesis seemed open to direct test by imposing a regime of progressive hypercapnia.

Finally, we attempted to assess the extent of changes in the intracellular ion concentrations attending the extracellular changes and examined the gills for gross morphological changes which might be associated with altered rates of ion transport.

MATERIALS AND METHODS

The experiments were performed on 700–1300 g channel catfish, *Ictalurus punctatus*, obtained from commercial fish farms, and on 120–250 g blue crabs, *Callinectes sapidus*, obtained from pots in Redfish Bay, Texas. The catfish were held in filtered recirculating tanks at 21–23 °C and fed with pelleted fish chow, while the crabs were held in flow-through seawater (salinity 20–25 ‰) systems at 19 to 20 °C and fed with cut fish. All the catfish experiments were performed at 23·0 ± 0·5 °C and the crab experiments at 20·0 ± 0·5 °C.

For each experiment, an animal was placed in a recirculating chamber system equipped with temperature control, filtration and an aeration reservoir. The dorsal aortas of the catfish were cannulated according to the method of Soivio, Nyholm & Westman (1975) under MS-222 anaesthesia, and the crabs were prepared for blood sampling by gluing a neoprene foam disc over a thinned spot in the dorsal carapace, directly over the lateral pericardial space. The crab chambers had a large stopper in the lid so that blood samples could be drawn by quickly inserting a syringe through the neoprene disc into the pericardial space. Animals were placed into the chambers on the day of cannulation or preparation, and allowed a full 24 h to recover and acclimate to their water equilibrated with room air (Control). Samples were then taken at two or three intervals of 1–3 h, after which the first CO_2 mixture (from Wösthoff gas mixing pumps) was introduced into the aeration reservoir. Each mixture was maintained for 24 h, after which a new mixture was begun. For the

crabs mixtures of 2, 4 and 6% CO_2 in air (P_{CO_2} = 15, 30 and 45 mmHg; 1 mmHg = 133·3 Pa) were employed, and for the catfish 1, 2, 4, 6 and 8% CO_2 in air (7·5, 15, 30, 45 and 58 mmHg).

Measurements

Immediately after drawing each blood sample, a 40–50 μl sample was aspirated into a capillary glass pH microelectrode (Radiometer) which had been standardized with phosphate buffers at the animal's temperature. In the catfish experiments, a second sample was taken for measurement of P_{CO_2} with a thermostatted electrode (Radiometer) and a third for haematocrit (Hct) measurement. The balance was centrifuged for about 15 s and the cells were discarded. A portion of the plasma was used immediately for total CO_2 analysis with a conductometric apparatus (Capni-Con). Approximately 20 μl of plasma was used for measurement of refractive index as an indicator of (protein) buffer value (Wood & Cameron, 1985; J. N. Cameron, unpublished data), and the balance was stored in a refrigerator for later analysis of specific ion concentrations. In the crab experiments a portion of the whole blood was used immediately for total CO_2 analysis, and the remainder allowed to coagulate. The clot was broken up with a glass rod, the sample centrifuged, and the supernatant saved for ion analysis.

The P_{CO_2} isolines for pH–HCO_3^- plots of crab blood were calculated using the Henderson–Hasselbalch equation with solubility values from Boutilier, Heming & Iwama (1984) and pK′ was estimated from Heisler's (1984) equation. For the catfish experiments, the pK′ values and P_{CO_2} isolines were calculated from measured values of P_{CO_2}, pH and total CO_2. Heisler's (1984) equation predicted pK′ and P_{CO_2} values for catfish which were somewhat lower than those calculated from our measured values. The reason for this discrepancy was not clear, since the calibrations for all measurement methods were replicated and cross-checked with at least one other method. The discrepancy, however, could be fully accounted for by a difference of only about 1% in each parameter, and made no difference to any of the conclusions of the study.

Sodium and potassium concentrations were measured on lithium-diluted samples with a flame photometer. Chloride concentrations were measured by amperometric titration (Buchler–Cotlove) and atomic absorption was used for measuring calcium and magnesium concentrations. Plasma protein concentrations were measured with a standard Coomassie blue dye method (Bio-Rad). Osmolarity was measured with a vapour pressure osmometer (Wescor 5100B). At the end of some of the catfish experiments, a 5 ml sample of blood was withdrawn, split into two portions, and put into a spinning equilibrator. Half was equilibrated with a low P_{CO_2}, the other half with a high P_{CO_2}, and both pH and total CO_2 were measured to estimate non-bicarbonate buffering. Previous data were also used to relate haematocrit (Hct), plasma protein and refractive index to buffer values (J. N. Cameron, unpublished data). The refractive index data were used to estimate buffering in the crabs, according to a previously established relationship (Wood & Cameron, 1985, and unpublished data).

Ion analyses of the water were performed for the catfish experiments: $[Na^+]$ and $[K^+]$ were measured by flame photometry, $[Cl^-]$ by amperometric titration, pH with an electrode, total CO_2 by conductometric methods, $[Ca^{2+}]$ and $[Mg^{2+}]$ by atomic absorption. The average values for the fresh water were: $[Na^+] = 5{\cdot}1$, $[K^+] = 0{\cdot}30$, $[Mg^{2+}] = 0{\cdot}37$, $[Ca^{2+}] = 1{\cdot}75$, $[Cl^-] = 4{\cdot}66$ and $[HCO_3^-] = 2{\cdot}75$, all in mequiv l^{-1}; P_{CO_2} was 0·9 mmHg and pH (control) was 8·04. In the blue crab experiments, only salinity was measured since the ion ratios of sea water are constant.

Intracellular ions

In seven of the catfish studied a bolus of ^3H- or ^{14}C-labelled mannitol was injected at least 4 h prior to the end of the experiment (i.e. at the highest ambient CO_2 concentration) as an extracellular marker. After the last blood samples had been taken, the fish were killed by a sharp blow to the skull, and six samples each of red and white muscle were removed. Each sample was weighed wet, dried at 65 °C to constant mass, and reweighed for estimation of total water content. Triplicate dried samples were then homogenized in doubly deionized water, the particulate material was removed by centrifugation, and the supernatant subjected to sodium, potassium and chloride analyses as described above. The matching triplicates were combusted in a sample oxidizer (Packard) so that the mannitol radioactivity could be used to calculate a correction for trapped extracellular fluid (and ions) in the tissues. The final intracellular ion concentrations were corrected to original tissue water minus any contribution of trapped extracellular fluid. As controls, six fish were treated similarly, except that after the initial recovery day, they were held for a second day with normal aeration, after which they were injected with mannitol, killed and analysed as above. In two of these experiments, the mannitol injection was made 24 h before the end and additional blood samples were taken in order to calculate mannitol clearance as an indicator of glomerular filtration rate (GFR) (Cameron, 1980).

Scanning electron microscopy of catfish gills

Two fish were taken through the entire protocol outlined above, except that no samples were taken. After the final day at 8 % CO_2 in air, these hypercapnic fish were killed and several pieces of gill were removed for examination by scanning electron microscopy (SEM). The gill pieces were fixed for 1 h in 2·5 % glutaraldehyde in 0·15 mol l^{-1} sodium cacodylate (pH 7·4), followed by post-fixation for 20 min in osmium tetroxide (4 %). The samples were then washed three times with 0·15 mol l^{-1} NaCl and transferred to 20 % acetone in water. They were dehydrated by the vacuum/boiling acetone method to 100 % acetone overnight, changed to fresh 100 % acetone, and then dried in a critical point dryer. After gold coating, the samples were examined and photographed at up to 7000×. The hypercapnic fish gills were compared with gills treated in the same way from normal fish out of the holding tanks.

A similar examination of hypercapnic crab gills was performed by holding two crabs in 4 % CO_2 in air for 5 days, then removing some pieces of a posterior gill,

number 7, for fixation in 0·15 mol l^{-1} sodium cacodylate-buffered 2·5 % glutaraldehyde. After 1 h the gills were post-fixed for 20 min in 1 % osmium tetroxide, then rinsed and placed in buffer. The crab gills could be examined directly under a dissecting microscope since the chloride cells are confined to a discrete area (Copeland & Fitzjarrell, 1967) which stains more densely than the surrounding thin respiratory epithelium (Aldridge & Cameron, 1982).

Unless stated otherwise, all data are presented as means ± 1 S.E.

RESULTS

Acid–base responses

The combined responses of six catfish tested at 7·5, 15, 30 and 45 mmHg (1, 2, 4 and 6 % CO_2 in air) plus six more tested at 15, 30, 45 and 58 mmHg (2, 4, 6 and 8 % CO_2 in air) are shown in Fig. 1. The buffer line shown has a slope of −7·78 mequiv pH^{-1}, the average buffer value determined from eight fish with average Hct of 12·1 ± 1·9 % and plasma protein concentration of 30·7 ± 0·3 g l^{-1}. In a few preliminary experiments the hypercapnic treatments were continued for 48–72 h with no appreciable further rise in the plasma $[HCO_3^-]$. From the $[HCO_3^-]$

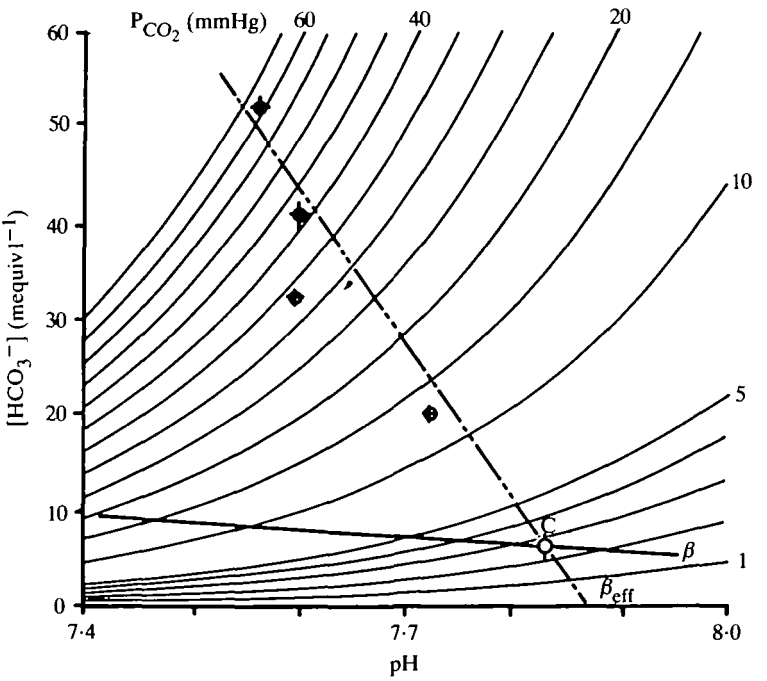

Fig. 1. pH–HCO_3^- diagram showing the responses of 12 catfish to progressive hypercapnia. The vertical and horizontal bars represent ±1 S.E. The *in vitro* plasma buffer line is designated by β (unbroken line), and the achieved or effective buffer value (using 24-h points for each treatment) by β_{eff} (broken line). Values for the P_{CO_2} isolines (in mmHg) are given. The C indicates control values.

and pH values achieved after 24 h at each treatment an effective or achieved buffer value (β_{eff}) of -148 mequiv pH^{-1} can be calculated (least squares, $r = -0{\cdot}95$).

The responses of 14 blue crabs to 15, 30 and 45 mmHg (2, 4 and 6 % CO_2 in air) are shown in Fig. 2, with the average buffer line slope of $-9{\cdot}8$ mequiv pH^{-1} for blood. In this series, samples were taken at 2, 4, 21 and 24 h after changing CO_2 concentrations in the water, showing the time course of the compensatory response. The effective or achieved buffer value, which was computed from the $[HCO_3^-]$ and pH reached after 24 h of each treatment, was -73 mequiv pH^{-1} (least squares, $r = -0{\cdot}98$). Maintenance of the CO_2 treatments for longer periods did not produce significant further compensation.

The % pH regulation, as originally defined by Siesjö (1971), for both catfish and blue crabs ranged from 61 to 72 % (Fig. 3).

Extracellular ionic changes

In the catfish the plasma $[Ca^{2+}]$ for control fish was $2{\cdot}81 \pm 0{\cdot}25$ mequiv l^{-1} and the plasma $[Mg^{2+}]$ was $1{\cdot}03 \pm 0{\cdot}07$ mequiv l^{-1}; $[Ca^{2+}]$ did not change significantly at any hypercapnic level and $[Mg^{2+}]$ was unchanged except for a small increase of $0{\cdot}16$ mequiv l^{-1} at the highest P_{CO_2}. The concentrations of Na^+, K^+ and Cl^- as well as the computed SID are shown in Fig. 4. $[Na^+]$ rose by only 4 mequiv l^{-1} ($P = 0{\cdot}03$), while the major component of the change in SID was the reduction in

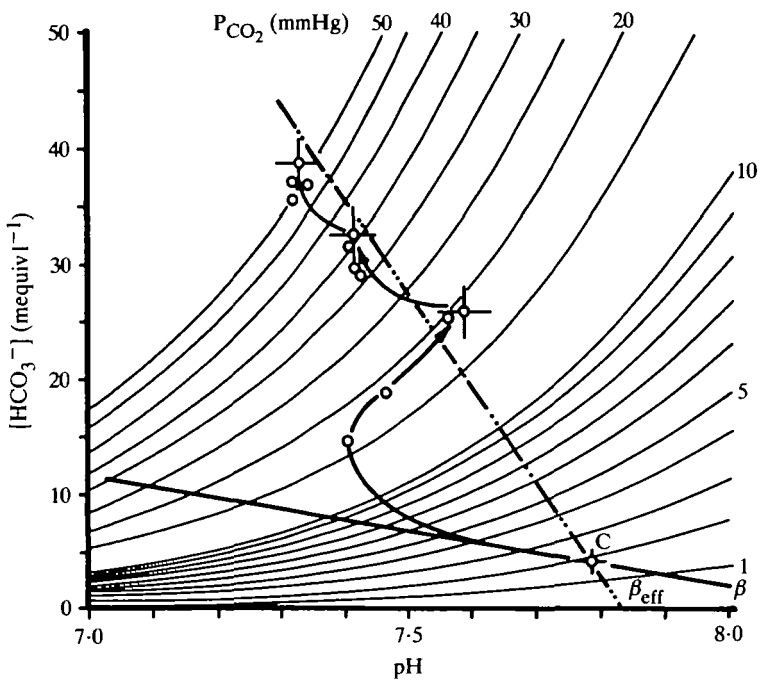

Fig. 2. pH–HCO_3^- diagram showing the responses of 14 blue crabs to progressive hypercapnia. The vertical and horizontal bars represent ±1 S.E. The *in vitro* blood buffer line is designated by β, and the achieved or effective buffer value by β_{eff}. Values for the P_{CO_2} isolines (in mmHg) are given.

$[Cl^-]$ ($P < 0{\cdot}001$). The correlation (r^2) between the measured change in $[HCO_3^-]$ and the computed change in SID was 0·94 (Fig. 6). At the highest CO_2 treatment the water pH dropped to 6·43 at constant $[HCO_3^-]$.

Ionic changes were more difficult to detect in the osmoconforming SW blue crabs (Fig. 5), partly due to day-to-day fluctuations in salinity, which changed the concentrations of all ions. No single ion accounted for the observed change in SID.

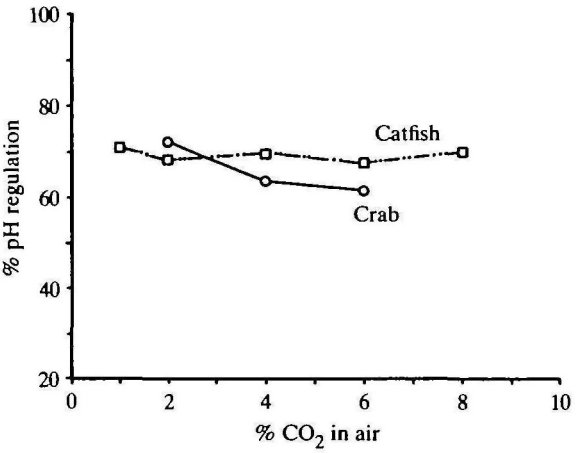

Fig. 3. The percentage pH regulation, calculated from the formula of Siesjö (1971), for catfish and blue crabs as a function of the ambient $[CO_2]$.

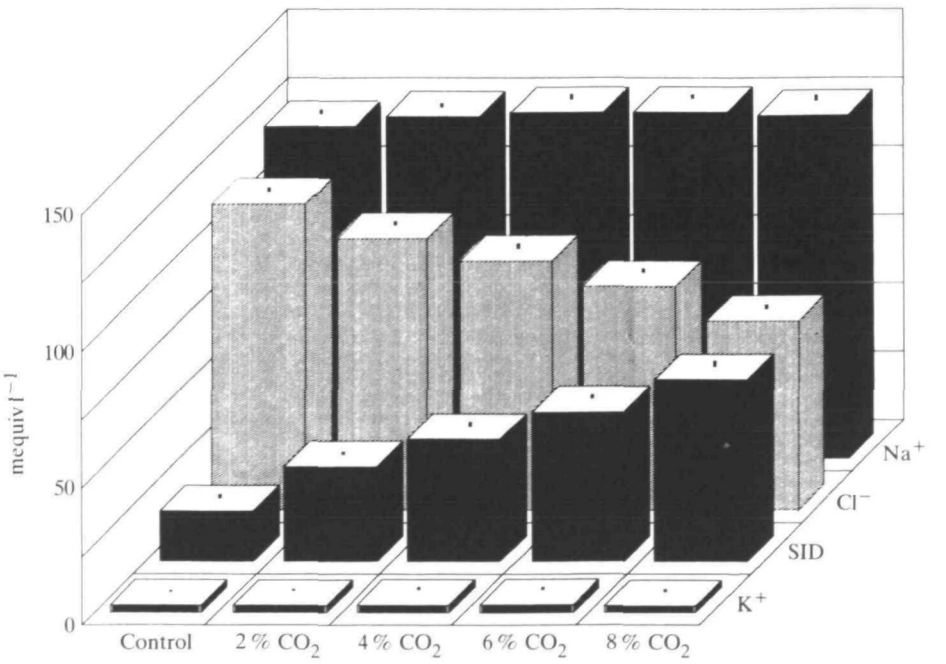

Fig. 4. Changes in concentrations of K^+, Na^+ and Cl^- and the calculated SID from hypercapnic catfish. The small bars represent 1 S.E.

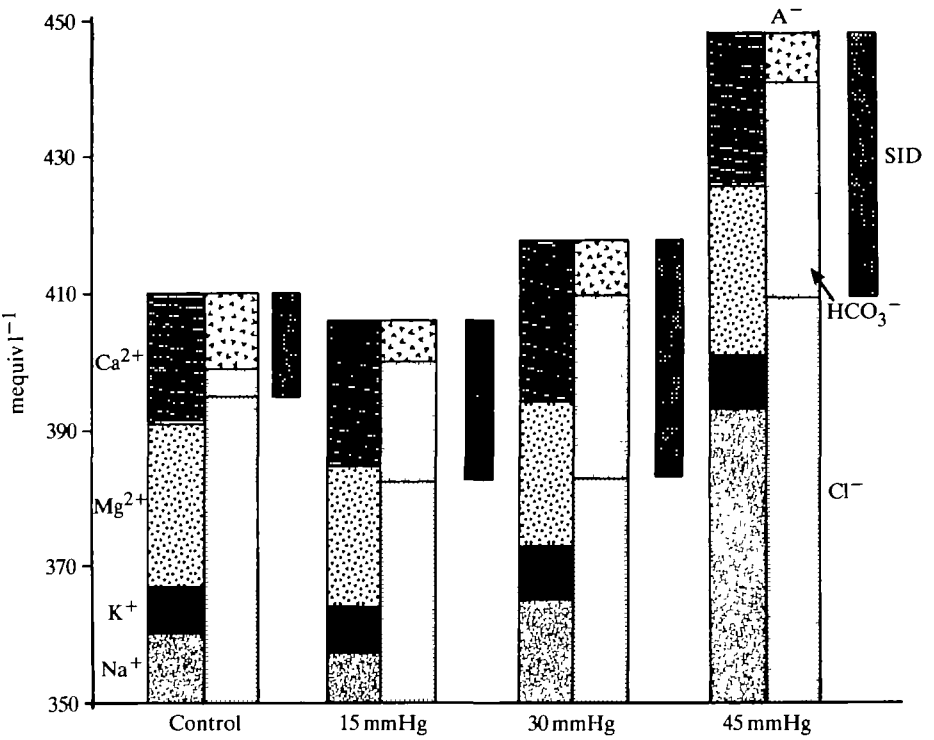

Fig. 5. Changes in ionic status of hypercapnic blue crabs at different ambient $[CO_2]$. The computed SID is shown alongside the plot for each ambient CO_2 level. A^-, unidentified anions.

Calcium concentration increased significantly, but the changes in $[K^+]$ and $[Mg^{2+}]$ were small or insignificant. The combined effect of all of the changes, however, was to produce significant progressive increases in the SID (t-test), but with a low overall correlation coefficient ($r = 0{\cdot}41$, NS) with the measured $[HCO_3^-]$ values (Fig. 6).

Intracellular ions

The major intracellular strong ion, K^+, did not change significantly with hypercapnia in either red or white muscle (Table 1). There was, however, considerable variation in the data, so small changes could have gone undetected. In white muscle, both $[Na^+]$ and $[Cl^-]$ rose slightly ($P < 0{\cdot}05$) but in red muscle, $[Cl^-]$ rose and $[Na^+]$ fell. Since free intracellular $[Mg^{2+}]$ and $[Ca^{2+}]$ are very small, the SID was taken to be $[Na^+] + [K^+] - [Cl^-]$; there was no significant change in SID in either tissue type.

Urinary HCO_3^- retention

The glomerular filtration rates (GFR) calculated from mannitol clearance in two catfish at 8% CO_2 were 11·8 and 18·1 ml kg^{-1} h^{-1}, roughly three times the resting

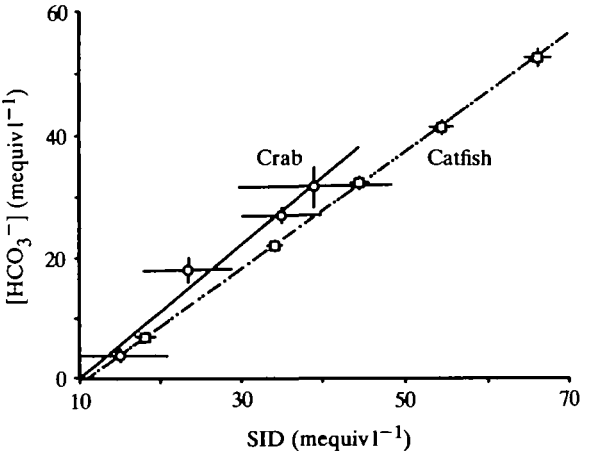

Fig. 6. The correlation between calculated SID and $[HCO_3^-]$ values for catfish (squares) and blue crabs (circles) at various levels of hypercapnia. The least squares regression line calculated from all individual catfish data was $[HCO_3^-] = -6{\cdot}09 + 0{\cdot}874SID$ ($r^2 = 0{\cdot}94$, $P < 0{\cdot}01$) and for all individual blue crab data was $[HCO_3^-] = -12{\cdot}26 + 0{\cdot}23SID$ ($r^2 = 0{\cdot}17$, NS). Bars represent ±1 S.E.

rate for catfish at this temperature (Cameron, 1980). At the mean $[HCO_3^-]$ of 52·8 mequiv l^{-1} (Fig. 1), the filtered HCO_3^- load would be 790 μequiv kg^{-1} h^{-1}.

Changes in the gills

Upon visual examination the gills of hypercapnic catfish appeared to have more apical crypts of chloride cells per unit area, and the crypts appeared to be enlarged (Fig. 7). A series of photographs taken from random locations along the filament surfaces yielded estimates of $7{\cdot}61 \pm 0{\cdot}45 \times 10^6$ crypts cm^{-2} for control fish and $9{\cdot}88 \pm 0{\cdot}32 \times 10^6$ crypts cm^{-2} for hypercapnic fish, a 30% increase. The area per

Table 1. *Intracellular concentrations of ions from red and white muscle of catfish*

	Control (N = 7,21)	8% CO_2 (N = 6,18)
White muscle		
Na^+	5·45 ± 0·55	7·43 ± 0·31*
K^+	145·8 ± 3·4	141·8 ± 2·4
Cl^-	6·82 ± 0·60	11·04 ± 0·66*
SID	144·1 ± 3·0	136·2 ± 3·2
Red muscle		
Na^+	17·68 ± 2·54	8·18 ± 1·72*
K^+	126·7 ± 4·8	142·8 ± 7·1(*)
Cl^-	9·18 ± 1·38	20·22 ± 2·71*
SID	135·2 ± 4·9	142·4 ± 9·0

* Significant at 5%; (*) $P < 0{\cdot}1$.
All data presented as mequiv l^{-1} ± 1 S.E.

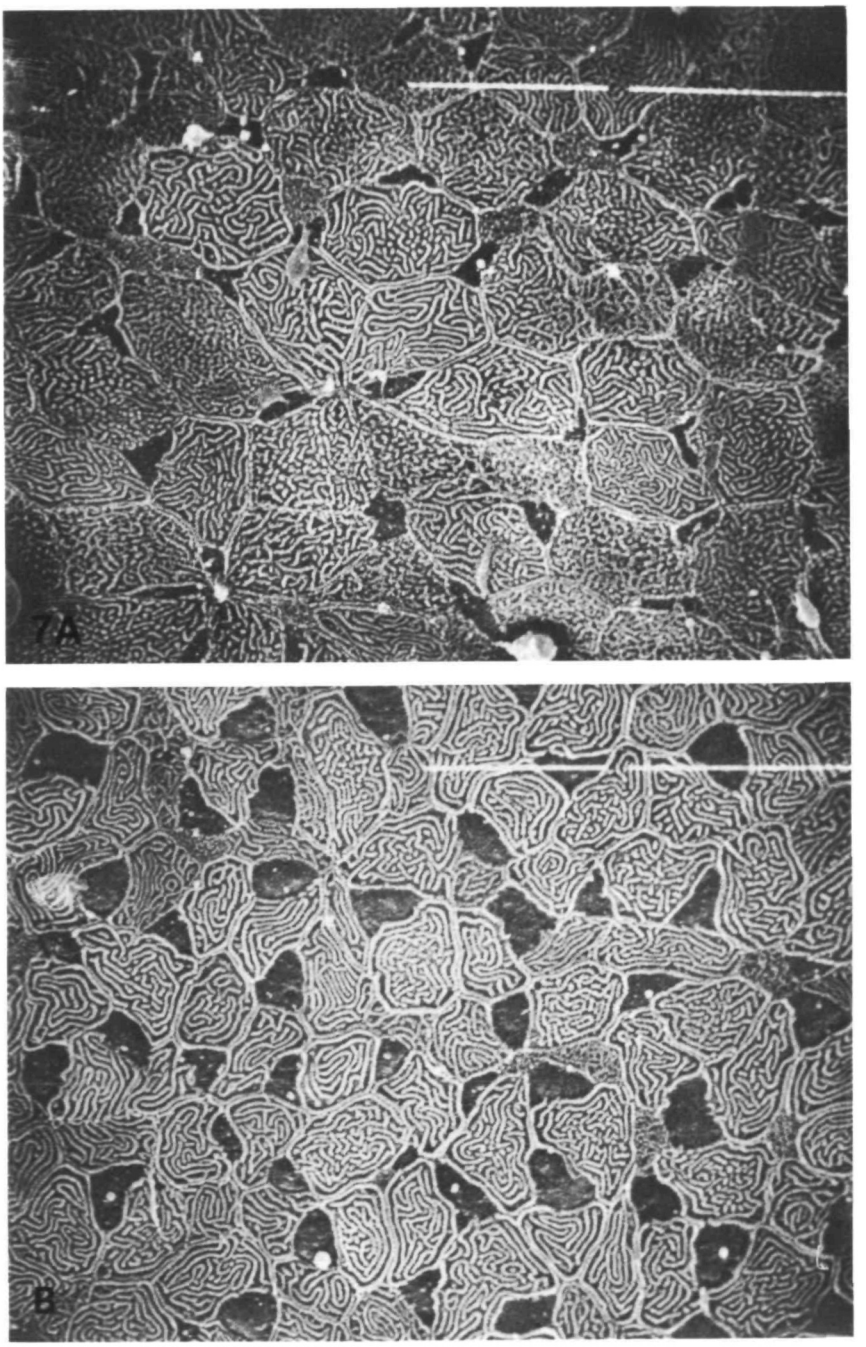

Fig. 7. Scanning electron micrographs of catfish gill filaments under control conditions (A) and after a 4-day stepwise progression to a P_{CO_2} of 56 mmHg (B). The left-hand bar in the photograph is 5 μm.

crypt increased by 35%, leading to an overall increase in crypt area of 75% ($P < 0{\cdot}01$).

There was no significant enlargement of the chloride cell patches of crab gills after 5 days of hypercapnia, but the patches were more sharply defined and stained more densely than in the control crabs. It was not possible to quantify this change, but the difference was quite noticeable and consistent, when a large number of filaments were examined from control and hypercapnic groups.

DISCUSSION

The present study shows clearly that for two quite different aquatic animals, the hyper-regulating freshwater channel catfish and the osmoconforming seawater blue crab, there is no fixed upper limit of 30 mequiv l^{-1} for HCO_3^- compensation of hypercapnic acidosis (Figs 1, 2). The ability of these two animals to regulate pH is as good at an ambient CO_2 concentration of 8% as it is at 1 or 2% (Fig. 3). In re-evaluating the data reviewed by Heisler (1986) it appears that the similarity in achieved [HCO_3^-] in various aquatic animals may simply be the coincidental result of the similar range of CO_2 concentrations used in the various studies, usually 1–4%. Instead of an absolute limitation, it appears that at each ambient CO_2 level the animal reaches a new steady-state set-point at which pH is regulated in much the same way as in control (normocapnic) conditions. This is supported by the work of Claiborne & Heisler (1984) showing that extra bicarbonate infused into the circulation was promptly excreted, and that changes in external [Na^+] or pH did not affect the new set-point. Resting, normocapnic fish also excrete infused bicarbonate (Cameron & Kormanik, 1982).

Part of the explanation for the pattern observed is suggested by the diagrams in Figs 1 and 2: for pH to be 100% compensated at the highest CO_2 concentrations we employed, the [HCO_3^-] would have to have risen to 148 mequiv l^{-1} in the catfish and to 119 mequiv l^{-1} in the blue crab, with attendant changes in appropriate strong ions. The resulting gradients would impose a substantial energetic cost and would probably lead to impairment of cellular functions. However, the HCO_3^- concentrations reached at the highest P_{CO_2} values would have been sufficient for 100% pH regulation at the lower tensions. The steady-state set-point, then, probably represents a compromise between the requirements for pH and ion regulation.

In that regard it may seem surprising that the 48 mequiv l^{-1} change in extracellular SID in the catfish was not accompanied by any detectable change in the intracellular SID (Table 1). The admittedly simplified determinations of intracellular ions were carried out as much as a test of the practicality of such measurements as in the hope of seeing any significant change. For the intracellular SID to be calculated, the following measurements had to be made: wet mass, dry mass, plasma mannitol radioactivity, tissue mannitol radioactivity, tissue homogenate volume (for dilution correction), tissue [Na^+], plasma [Na^+], tissue [K^+], plasma [K^+], tissue [Cl^-] and plasma [Cl^-]. The plasma radioactivity and ion data are used to correct for trapped

extracellular ions in the tissue and, finally, the tissue ion concentrations are calculated correcting for dilutions and original water content. The analytical power of the method is further reduced since different animals must be used for control and hypercapnic estimates, and different tissue samples must be used for radioactivity measurement and ion analyses, introducing two extra degrees of freedom. Intracellular free $[Mg^{2+}]$ and $[Ca^{2+}]$ were assumed to be insignificant, and concentrations were assumed to be the same as activities. Our confidence in the final estimates is therefore rather low. About the best that can be said for the intracellular SID data is that they show that there was no large change. In the only other published study of muscle ion concentrations in hypercapnia, Eddy, Smart & Bath (1979) actually found a decrease in $([Na^+] + [K^+] - [Cl^-])$, but since no extracellular correction was made these values cannot be taken to represent the intracellular ionic environment.

Since the non-bicarbonate buffer value of the intracellular compartment is much greater than that of blood, the change in SID need not be great, depending upon the actual pH change and buffer value. For example, under control conditions in catfish the intracellular pH (pH_i) is on average 0·51 units below blood pH (pH_e) (Cameron, 1980), which would result in an intracellular $[HCO_3^-]$ of 1·93 mequiv l^{-1} and pH_i of 7·32 in this study. At 8 % ambient CO_2, the pH_e fell to 7·56 (Fig. 1). If pH_i had changed by 80 % of the decrease in pH_e, the pH_i would be 7·10 and the intracellular $[HCO_3^-]$ would be 18·3 mequiv l^{-1}. Using a buffer value for white muscle of 40 (Cameron, 1980; Castellini & Somero, 1981), 8·8 mequiv l^{-1} of the increase would be supplied by non-bicarbonate buffering, and only 7·6 mequiv l^{-1} need be supplied by changes in SID or total weak acid (cf. Stewart, 1981). For 100 % pH regulation, i.e. no change in pH_i, a 28 mequiv l^{-1} change in SID would be required intracellularly. These calculations only show probable upper and lower limits of the intracellular ionic changes brought about by pH regulation, but emphasize again that the intracellular steady-state set-point is probably a compromise between the need for pH regulation and for maintenance of ionic balance.

The utility of SID vs $[HCO_3^-]$

The conventional approach to acid–base analysis is based upon measurements of pH, P_{CO_2} and total CO_2 combined with calculation of $[HCO_3^-]$ using the Henderson–Hasselbalch equation. On theoretical grounds, Stewart (1978, 1981) has objected to this analysis because two of the three measured parameters (pH and total CO_2) are dependent variables, and the pK′ employed in the Henderson–Hasselbalch equation is not a true constant, but a complex function of many variables. Arguing from first principles, he has described an alternative mathematical and graphical analysis based upon P_{CO_2}, total weak acid (mainly protein) and the strong ion difference. The theoretical arguments for and against this approach are too involved for discussion here, but at least the present study serves as a comparison of the results obtained using both approaches. Our data provide values for the P_{CO_2} and the SID, but only indirectly for total weak acid. That is, the buffer value as conventionally measured provides an indicator of the total weak (buffer) acid present.

Based upon our results, we can see no advantage of the SID approach over the conventional HCO_3^- analysis. On the contrary, a complete evaluation of acid–base status can be completed with the HCO_3^- approach in 3 min, whereas the data for SID analysis were usually not available for several days and involved several additional steps which could produce errors. In animals such as the blue crab with high background ion concentrations the utility of the SID approach is low, since even small analytical errors obscure changes related to acid–base status.

HCO_3^- retention

The ability of the catfish kidney and of the gills of both catfish and blue crabs to retain HCO_3^- against a steep outward-directed gradient is impressive. The three-fold diuresis observed in the catfish coupled with the high plasma [HCO_3^-] leads to the observation that about 800 μequiv kg^{-1} h^{-1} are filtered. Since the urine of hypercapnic catfish maintains a net H^+ excretion at 2% hypercapnia (Cameron, 1980) and apparently remains acidic in rainbow trout at 5% (Eddy *et al.* 1979), this large filtered load must be fully resorbed by renal acidification. Retention in the kidney is less of a problem for marine fish and for the seawater blue crab, since the rates of urine formation in marine animals are much lower than in freshwater forms.

The maintenance of steep HCO_3^- gradients across the gills, which have a very large surface area, implies a very low passive HCO_3^- permeability, a very high rate of inward HCO_3^- transport, or both. Under control conditions the HCO_3^- gradient was only 2 or 3 to 1, whereas at the highest ambient CO_2 the gradients increased to about 20 to 1.

Changes in the gills

Whether the morphological changes observed in the gills are directly related to the ionic changes involved in acid–base regulation is not clear. The primary function of the chloride cells is generally thought to be Cl^- secretion, not acid–base regulation (Karnaky, 1986; Girard & Payan, 1980). The changes observed in apical crypt numbers and areas in the catfish and the suspected increase in chloride cell density in the crabs might indicate an increase in acid–base-related active transport in the gills. Part of this transport may be Cl^-/HCO_3^- exchange (Maetz & Garcia-Romeu, 1964; DeRenzis & Maetz, 1973; Cameron, 1976, 1978), moving Cl^- outwards which would counter the passive loss of HCO_3^- and produce the lower [Cl^-] values observed (Figs 4, 5). The increased activity may also be due to the maintenance of an increased inward-directed H^+ gradient. Passive H^+ influx is presumably countered by increased Na^+/H^+ exchange (DeRenzis & Maetz, 1973; Cameron, 1976, 1978). The changes observed in the hypercapnic animals are also similar to those described by Steinmetz (1986) for turtle bladder when acidification is stimulated experimentally. He describes changes in the apical regions of transporting cells which correspond closely to the changes in apical crypts observed in SEM examination of catfish gills (Fig. 7). An increased density of chloride cell patches in the crab gill might also correspond to changes in cellular organelles associated with increased transport activity.

The idea of a fixed 30 mequiv l^{-1} upper limit for blood [HCO_3^-] during hypercapnic compensation is shown by this study to be incorrect. This idea was suggested by Heisler (1986) on the basis of the similarity of maximum values achieved in a number of studies that he reviewed. Further support was provided by Claiborne & Heisler (1984), who showed that after an apparent maximum compensation level had been reached in carp, infused bicarbonate was quite quickly excreted. Rather than showing that 30 mequiv l^{-1} is an absolute upper limit, these data and those of the present study suggest to us that at each level of ambient hypercapnia a new steady-state set-point for pH regulation is reached. This set-point then appears to be defended just as the normal (control) set-point would be. New questions are posed, then: why is pH not 100% regulated; or what determines the new set-point? Concentrations of HCO_3^- are reached at high CO_2 tensions which would be sufficient for 100% regulation of lower tensions (Figs 1, 2). We can only speculate that the new set-point represents a compromise between the opposing ion transports required for pH and for ion regulation. This hypothesis is supported by the studies cited above and by the influence of salinity upon the ability of animals to compensate hypercapnia (Iwama, 1986).

Our studies further show that bicarbonate retention by gills and kidney can continue in ambient CO_2 tensions of up to at least 56 mmHg, and that pH regulation as indicated by Siesjö's (1971) formula is not impaired at these levels. We have not examined the kidney for evidence of increased active transport activity, but morphological changes in the gills of both catfish and crabs might indicate an increase in pH-related transport.

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