Acid–base balance, ionic status, and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*)

HELVE HÕBE, PETER R. H. WILKES, RICHARD L. WALKER, CHRIS M. WOOD,[1] AND BRIAN R. MCMAHON
Department of Biology, University of Calgary, Calgary, Alta., Canada T2N 1N4
Received October 6, 1982

HÕBE, H., P. R. H. WILKES, R. L. WALKER, C. M. WOOD, and B. R. MCMAHON. 1983. Acid–base balance, ionic status, and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*). Can. J. Zool. **61**: 2660–2668.

Renal function was investigated in the stenohaline *Catostomus commersoni* held at water pH 7.3 for 5 days. Urine displayed remarkably low levels of Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+} and was very acidic (mean pH 6.5). Renal electrolyte conservation was characterized by almost complete reabsorption of NaCl and to a lesser degree, K^+, Ca^{2+}, and Mg^{2+}. Net H^+ excretion was $+11.2\ \mu equiv \cdot kg^{-1} \cdot h^{-1}$, resulting primarily from a high titratable acid content. The physiological consequences of exposure to ambient pH 4.3 for 4 days included disturbances in plasma acid–base status with relatively minor changes in both plasma ion levels and renal output (*E*). A mixed metabolic and respiratory acidosis developed, which was not compensated for by increased renal H^+ excretion. Both plasma NaCl levels and E_{Na^+} decreased but E_{Cl^-} remained unchanged. E_{K^+} was correlated with plasma hyperkalemia. No changes in plasma levels or excretion of Ca^{2+} or Mg^{2+} occurred. These findings provide some physiological evidence supporting the contention that suckers are relatively acid tolerant. It is suggested that the copious amounts of mucus covering their gills may have served a protective role by retarding proton entry and limiting branchial ion loss.

HÕBE, H., P. R. H. WILKES, R. L. WALKER, C. M. WOOD et B. R. MCMAHON. 1983. Acid–base balance, ionic status, and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*). Can. J. Zool. **61**: 2660–2668.

La fonction rénale a pu être étudiée chez des poissons sténohalins *Catostomus commersoni* exposés à un pH de 7,3 pendant 5 jours. L'urine contient des concentrations particulièrement basses de Na^+, Cl^-, K^+, Ca^{2+} et Mg^{2+} et est très acide (pH moyen de 6,5). La conservation des électrolytes par le rein exige une réabsorption presque complète du NaCl et une récupération importante des ions K^+, Ca^{2+} et Mg^{2+}. L'excrétion nette d'ions H^+ est de $+11,2\ \mu equiv \cdot kg^{-1} \cdot h^{-1}$, le résultat d'un contenu élevé en acides titrables. Une exposition à un pH de 4,3 pendant 4 jours entraîne, comme conséquences physiologiques, des dérèglements dans l'équilibre acide-base du plasma, ainsi que des changements relativement mineurs dans les concentrations d'ions plasmatiques et le débit rénal (E). L'acidose métabolique et respiratoire s'établit sans être compensée par une augmentation de l'excrétion rénale d'H^+. Les concentrations plasmatiques de NaCl et le débit d'excrétion de Na^+ diminuent, mais le débit de Cl^- reste le même. Le débit de K^+ est en relation avec l'hyperkaliémie plasmatique. Les concentrations plasmatiques et l'excrétion de Ca^{2+} et de Mg^{2+} ne changent pas. Ces données physiologiques appuient la thèse selon laquelle les catostomes peuvent tolérer les eaux acides. Il semble que les couches importantes de mucus qui couvrent leurs branchies aient en rôle protecteur et retardent l'entrée des protons et la perte des ions.

[Traduit par le journal]

Introduction

The occurrence of acid rain and the resultant acidification of many freshwater lakes and rivers has been recognized globally as an anthropogenic environmental problem. Studies of the physiological responses of adult fish to low ambient pH have demonstrated that disturbances in acid–base balance, ionoregulation, and respiratory function all occur, but their relative importance appears to be modulated by several factors: the severity of acid stress (i.e., pH < 4 versus pH > 4) and type of acid (HCl vs. H_2SO_4) as well as ambient calcium and carbon dioxide levels (see reviews by Fromm 1980; Spry *et al.* 1981; Haines 1981). However, few studies have monitored or accounted for these factors; notable exceptions have mainly focussed on an active, pelagic, euryhaline fish species, the hatchery-bred rainbow trout (*Salmo gairdneri*) (Neville 1979; McDonald *et al.* 1980; McDonald and Wood 1981; Milligan and Wood 1982). Since ecological and toxicological studies have established interspecies differences in tolerance to acid stress (Harvey 1979), it was of interest to determine whether this phenomenon also had a physiological basis.

While it is well established that fish regulate internal acid–base homeostasis by modulation of the strong ion difference (Heisler 1980), renal participation in acid–base regulation, relative to the gill, has just recently become appreciated (Wood and Caldwell 1978), and thus few species have been examined. The general pattern which has emerged is that the kidney appears to be more important in freshwater species which produce a copious urine (*Salmo gairdneri*, Wood and Caldwell 1978; *Ictalurus punctatus*, Cameron and Kormanik 1982) relative to marine forms whose urine output is low (*Parophrys vetulus*, McDonald *et al.* 1982).

[1]Present address: Department of Biology, McMaster University, Hamilton, Ont., Canada L8S 4K1.

Thus, the present study concentrated on the effects of low ambient pH on acid–base balance, ionic status, and renal function in the North American white sucker (*Catostomus commersoni*). This species was of interest because of its benthic habitat and stenohalinity, features which contrast markedly with those of the commonly used trout. It is also endemic to acid-stressed freshwaters (Harvey 1979). Since little is known about the renal physiology of suckers (Hickman 1965), a secondary aim was to characterize normal patterns of renal ion and acid–base output.

Materials and methods

Experimental animals and test conditions

Catostomus commersoni Lacépède (200–800 g; both sexes, mature and immature) were collected from Lake Bonavista, Calgary, Alberta, using baited cylindrical wire-mesh traps. Fish were maintained in running dechlorinated tap water (12–15°C) for 2 weeks to 2 months prior to use in experiments. While in holding tanks, they were fed commercial dry pellets ad libitum but starved 7 days prior to and during experimentation to remove the influence of diet on renal acid excretion (Wood and Caldwell 1978).

In preparation for experiments, fish were surgically fitted with caudal arterial or venous catheters for repetitive blood sampling (Watters and Smith 1973; Wilkes *et al.* 1981) and (or) bladder catheters for continual urine collection (Wood and Caldwell 1978). During surgery, the gills were irrigated with an aerated, buffered, anaesthetic solution (MS 222, tricaine methanesulfonate; 1 : 10 000 dilution). Immediately after cannulation, fish were placed in individual, aerated, darkened Lucite chambers, each receiving a continuous flow (500–1000 $mL \cdot min^{-1}$) of either dechlorinated tap water (control group) or recirculated water (acid group), both maintained at 12.5 ± 1°C. At least 36–48 h of recovery was allowed before beginning experimentation (Wood and Caldwell 1978).

Ion levels (Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}) in each water system were measured daily over the experimental period (Table 1). In the recirculated system (45 L/kg fish), water was decarbonated prior to use, as outlined in McDonald *et al.* (1980). Water was acidified with sulfuric acid and maintained within pH range 4.0 to 4.5 by manual addition of 1.0 *N* H_2SO_4 or 1.0 *N* NaOH as required (Table 1); a Fisher Accumet 140A pH meter and combination electrode, calibrated with Fisher buffers (pH 4.0, pH 7.0) at the experimental temperature, were used.

Experimental protocol

Plasma acid–base and ionic status and renal output were monitored in suckers exposed to pH 7–7.5 (control group, mean weight = 371 ± 42 g; for blood data $n = 11$, for urine data $n = 5$) or pH 4–4.5 (acid group, mean weight = 316 ± 19 g; for blood data $n = 13$, for urine data $n = 9$). Following recovery from surgery, "day 0" values were measured in both experimental groups at water pH 7.3. Fish were subsequently either maintained at pH 7.3 (control group) or exposed to pH 4.3 (acid group) for 4 days (days 1 to 4 in figures).

Blood samples (600 μL) were withdrawn at 24-h intervals, usually at the end of two urine collection periods, and immediately analyzed for hematocrit, hemoglobin, plasma pH, total carbon dioxide content, and osmolality. Remaining plasma was stored (−10°C) for later ion analysis (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}). Ion measurements on aliquots of fresh and frozen plasma obtained from the same fish exhibited no significant differences ($p > 0.05$). The bladder was drained continually by a siphon into covered flasks. At 12-h intervals, urine was collected and immediately analyzed for volume, pH, and titratable acid minus bicarbonate content. Remaining urine was stored (−10°C) for later analysis of total ammonia and ionic content.

Analytical procedures

Hemoglobin (Hb) was determined using the cyanmethemoglobin technique (Blaxhall and Daisley 1973) and Hycel reagents. Mean corpuscular hemoglobin concentration (MCHC) was calculated as the ratio of Hb/hematocrit (Ht). Plasma pH and total carbon dioxide levels (C_{CO_2}) were measured using Radiometer microelectrodes and methodology outlined in McDonald *et al.* (1980). Plasma bicarbonate levels (HCO_3^-) and carbon dioxide tensions (P_{CO_2}) were then estimated using the Henderson–Hasselbalch equation with pH and temperature-corrected pK′ values (Severinghaus *et al.* 1956) and temperature-corrected αCO_2 values (Severinghaus 1965). ΔH_p^+, representing the change in concentration of H^+ ions (milliequivalents per litre) added to blood plasma by nonvolatile acids over time, was estimated from daily values (subscripts 1 and 2) of HCO_3^- and pH according to the formula, $\Delta H_p^+ = (HCO_{3_1} - HCO_{3_2}) - \beta(pH_1 - pH_2)$, where β represents the nonbicarbonate buffer capacity of true plasma (McDonald *et al.* 1980). The β value was calculated from the regression relationship, $\beta = -23.44\,(Ht) - 2.28$, derived by Wilkes *et al.* (1981) for true plasma and the mean Ht over the time interval in question.

Urine titratable acid minus bicarbonate content (TA − HCO_3^-) was determined as a single value in a double titration procedure (Hills 1973) using the control blood pH as the endpoint of the titration (i.e., absolute TA − HCO_3^-; see McDonald and Wood 1981). In fish with no blood catheter, urine samples were titrated to the average blood pH of cannulated fish on day 0. Urine ammonia levels were assessed colorimetrically using the phenol–hypochlorite method (Solorzano 1969) and considered virtually equivalent to $[NH_4^+]$ (Cameron and Wood 1978). Net renal acid excretion ($E_{\Sigma H^+}$) was calculated as urine $[NH_4^+]$ plus [TA − HCO_3^-] times urine flow rate (U_{FR}) (Hills 1973). Plasma osmolality (Osm) was determined with a Wescor Vapor Pressure Osmometer (Model 5100 B). Urine ($[Cl^-]_u$), plasma ($[Cl^-]_p$), and water ($[Cl^-]_w$) levels of chloride were measured by coulometric titration using a Digital Chloridometer (Buchler Model 4-2500); cation levels (Na^+, K^+, Ca^{2+}, Mg^{2+}) were measured, after appropriate dilution and swamping, by atomic absorption spectrophotometry (Jarrell Ash 850). Urine excretion rates (E_{Na^+}) were calculated using the equation, $E_{Na^+} = [Na^+]_u \times U_{FR}$. Renal ion clearances ($C_{Na^+}$) were estimated by the equation, $C_{Na^+} = ([Na^+]_u \times U_{FR})/[Na^+]_p$. Urine to plasma ratios (U/P) were also computed.

Statistical data analyses

The data presented in this study represent pooled values for two replicate control series and three replicate acid-exposure

series; data for fish with blocked catheters or ruptured bladders (determined at the end of experiments) have not been included. Values throughout have been expressed as means ± SEM, unless otherwise stated. Differences between mean day 0 values of control and acid groups at water pH 7.3 were tested using a Student's two-tailed *t*-test (unpaired design); significance determined from this analysis has only been reported in the text. Possible relationships between some variables were evaluated by computing simple Pearson's correlation coefficients.

Control group data were analyzed according to the multiple comparison procedure of Dunnett (1955) which dictated the use of the residual mean square, derived from a single-factor repeated measures ANOVA, as an estimate of the error variance, for computation of a Dunnett's *t*-statistic (critical values in Winer 1971). Acid group data were analyzed in an identical manner in cases where sampling effects in the control group were not significant ($p > 0.05$). In figures, one asterisk (*) represents significance ($p < 0.05$) demonstrated with this within-group comparison of means. A two-factor repeated measures ANOVA (Winer 1971) was employed on both control and acid group data (days 1 to 4 only) for any variables where significant sampling effects were demonstrated. Time-dependent acid responses were then tested using an unpaired Student's two-tailed *t*-test. In figures, two asterisks (**) represent significance ($p < 0.05$) shown with this between-group comparison of means. Data computations were performed using BMDP (Dixon and Brown 1979) and SPSS (Hull and Nie 1979) computer program packages.

Results

Ion levels and pH of the control and acid water systems are listed in Table 1. The marked difference in $[Na^+]_w$ between the two systems was attributed to the gradual addition of 1.0 *N* NaOH for water pH control. The higher levels of other ions in the acid relative to the control water further suggested that some net body ion loss probably ensued during acid exposure.

Cannulation and repetitive blood sampling had some effect on blood data; minor but statistically significant changes occurred in Ht, Hb (data not included), pH_a, pH_v, $[HCO_3^-]_v$ (Fig. 1), Osm, $[Cl^-]_p$, and $[Ca^{2+}]_p$ (Fig. 2). There were no significant differences between any of the repeated samples taken over 5 days for any of the urinary variables (Figs. 3, 4) with the exception of $[H^+]_u$ (Fig. 3B).

The regression equation relating urine flow rate and inulin clearance, $C_{IN} = (U_{FR} - 0.010)/0.64$ ($r = 0.92$), derived by MacKay and Beatty (1968), was used to estimate C_{IN}; a mean value of 4.2 ± 0.29 was calculated from data for fish with both blood and urinary catheters (day 0 values, $n = 8$). U/P ratios and renal ion clearances were estimated (Table 2) and the magnitude of the latter compared with C_{IN} (Pitts 1974), to gain some information on renal tubular processes (i.e., reabsorption or secretion; see Discussion). To test for relationships between urinary variables, the control group data (10 samples per fish, $n = 5$, 50 complete data sets) were subjected to simple correlation analysis and results are shown in Table 3 (see Discussion).

TABLE 1. Ion concentrations (milliequivalents per litre) and pH of the control and acid water systems. Water samples were collected daily over the experimental period. Values are means ± SEM, ($n = 20$)

Variable	Control system	Acid system
pH	7.30±0.04	4.30±0.04
$[Na^+]$	0.051±0.001	0.640±0.040*
$[K^+]$	0.013±0.001	0.041±0.010
$[Cl^-]$	0.040±0.008	0.215±0.010
$[Ca^{2+}]$	1.29±0.05	1.78±0.04
$[Mg^{2+}]$	0.86±0.10	0.92±0.012

*1.0 *N* NaOH added for pH control

Exposure to ambient pH 4.3 resulted in a slight transitory elevation of both Ht (70%) and Hb (44%), from initial control values of 17.0 ± 1.5% and 4.23 ± 0.04 g%, respectively, and thus, MCHC (0.249 ± 0.001 $g \cdot mL^{-1}$) remained unchanged; over days 2 to 4, Ht and Hb gradually declined, probably as a consequence of repetitive blood sampling, as seen in the control group, rather than acid stress (data not included).

Plasma acid–base responses were progressive, and hence, by day 4, significant reductions had occurred in pH_a (0.24 units), pH_v (0.26 units), $[HCO_3^-]_a$ (25%), and $[HCO_3^-]_v$ (28%) (Figs. 1B, 1C). Plasma Pa_{CO_2} (35%) and Pv_{CO_2} (21%) increased concomitantly (Fig. 1A). ΔH_p^+ was relatively constant over the 4 days with a total accumulation of 4.3 $mequiv. \cdot L^{-1}$.

Changes in plasma ion levels were minor (Fig. 2) in comparison with acid–base responses (Fig. 1). $[Na^+]_p$ declined on day 2 with a net change of 10.3 $mequiv. \cdot L^{-1}$ by day 4 (Fig. 2B). This pattern reflected Osm which decreased by 6% over the 4 days (Fig. 2A). The depression in $[Cl^-]_p$ was not significant as similar changes occurred in the control group (Fig. 2C). $[K^+]_p$ increased on day 2, stabilizing thereafter, with an overall rise of 0.36 $mequiv. \cdot L^{-1}$ (Fig. 2D). No significant decrease in either $[Ca^{2+}]_p$ or $[Mg^{2+}]_p$ occurred (Figs. 2E, 2F), suggesting that the drop in Osm was mainly due to loss of other ions (i.e., Na^+, Cl^-, HCO_3^-) rather than to water gain.

Renal output varied markedly with time (Figs. 3, 4). U_{FR} decreased by 10% on day 2, returning to control rates by day 4 (Fig. 3A). The transitory elevation in $E_{TA-HCO_3^-}$ (Fig. 3C) and sustained rise in $E_{NH_4^+}$ (Fig. 3D) were not significant and hence, $E_{\Sigma H^+}$ was unchanged (Fig. 3E). $[H^+]_u$ (measured as pH) exhibited a gradual increase which was significant on day 4 (Fig. 3B). E_{Na^+} decreased by 45% on day 2 (Fig. 4A) in concert with U_{FR} (Fig. 3A). E_{K^+} displayed a peak increase of 120% over days 2 and 3 but returned to con-

TABLE 2. Urine concentrations, U/P ratios, and renal clearance rates of ions (x) in *Catostomus commersoni* maintained in water pH 7.3 at 12.5 ± 1°C. Values are means ± SEM with n in parentheses for day 0 measurements of control and acid groups combined (two 12-h urine collection periods averaged; single blood sample)

	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	H^+
$[x]_u$, mequiv.·L^{-1}	2.15±0.29 (14)	2.09±0.32 (14)	1.67±0.23 (14)	1.47±0.23 (14)	1.21±0.10 (14)	29.33±4.54* (14)
U/P	0.018±0.002 (8)	0.022±0.005 (8)	0.920±0.117 (8)	0.381±0.089 (8)	0.726±0.059 (8)	19.60±3.86 (8)
C_x, μequiv.·kg^{-1}·h^{-1}	0.048±0.007 (8)	0.056±0.011 (8)	2.423±0.358 (8)	1.016±0.217 (8)	1.947±0.162 (8)	— (8)

*$[H^+]_u = 1 \times 10^{-8}$ M.

TABLE 3. Correlations between several urine variables in *Catostomus commersoni*. The analysis was performed on the control group data (10 samples per fish, $n = 5$; 50 complete data sets). Only those correlation coefficients significant at the 5% level are shown. (*, $p < 0.01$). With the exception of urine flow, concentrations of each of the variables (e.g., $[Na^+]_u$) were used in the analysis

	Flow	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	H^+	NH_4^+	TA − HCO_3^-
Na^+	−0.591*								
Cl^-	—	0.435*							
K^+	−0.544*	—	—						
Ca^{2+}	−0.396*	—	—	—					
Mg^{2+}	−0.281	—	—	0.553*	—				
H^+	−0.477*	0.613*	—	—	—	—			
NH_4^+	−0.367*	—	—	0.280	—	—	—		
TA − HCO_3^-	−0.546*	0.329	—	0.505*	—	0.366	0.504*	0.283	
H^+	−0.587*	0.317	—	0.515*	0.304	0.324	0.509*	0.687*	0.892*

trol values by day 4 (Fig. 4C). E_{Cl^-} (Fig. 4B), $E_{Ca^{2+}}$ (Fig. 4D), and $E_{Mg^{2+}}$ (Fig. 4E) were all not significantly affected by acid exposure.

These physiological responses were further accompanied by a progressive accumulation of mucus on sucker gills (visual observation). All fish survived ambient pH 4.3 for 4 days.

Discussion

Normal blood and renal physiology

Plasma acid–base and ion levels in cannulated suckers are in agreement with those reported by Wilkes *et al.* (1981); Hb, Osm, and $[Mg^{2+}]_p$ have not been previously measured but lie within the range found in other freshwater teleosts (Hickman and Trump 1969). The marked difference in $[Ca^{2+}]_p$ between the control and acid groups on day 0 (Fig. 2E) was attributed to the greater proportion of mature females in the control group; mature females had significantly higher $[Ca^{2+}]_p$ than males ($p < 0.05$), as found by Beamish *et al.* (1975).

The nephron of a freshwater teleost kidney is typified by a glomerulus, ciliated neck segment, first and second proximal tubular segments, a distal segment, and a collecting tubule and duct, but lacks a loop of Henle (Hickman and Trump 1969). The bladder has been shown to function as part of this complex, at least in euryhaline fish species (e.g., Renfro 1975). Since the catheterization technique employed in the present study rendered the urinary sphincters incompetent so that fluid drained from the bladder as soon as it was produced, any modification in urine volume or composition by transport mechanisms in the bladder wall (e.g., Renfro 1975; Beyenbach and Kirschner 1975) would therefore not be seen. Thus, the following discussion pertains to the features of urethral urine in stenohaline white suckers.

Urine flow was quite variable with time (Fig. 3A) and probably reflected intermittent glomerular filtration (Hickman 1965; MacKay and Beatty 1968). The inverse relationship between urine flow and electrolyte levels (Table 3) implied that in suckers, renal ion absorption and water movement were probably dissociated processes. Urine $[Na^+]$ and $[Cl^-]$ were comparatively low (cf. euryhaline species, Kobayashi and Wood 1980; Cameron 1980) and even lower than those reported by Hickman (1965) for this species (larger specimens). Movements of Na^+ and Cl^- were probably coupled to some extent, as shown by the significant correlation

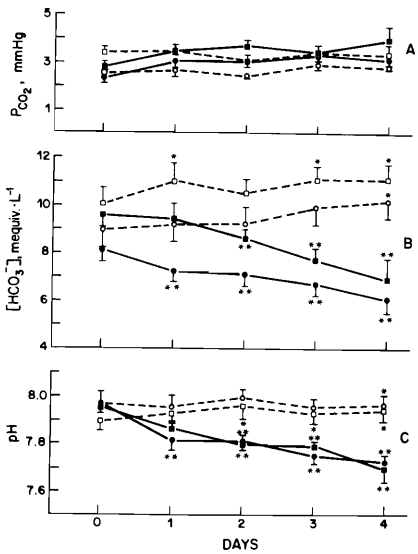

FIG. 1. Blood acid–base status (means ± SEM) in white suckers held at pH 7.3 (day 0) followed by 4 days of exposure to pH 4.3 (—) or maintained at pH 7.3 throughout (---), for arterial and venous samples (acid group arterial samples: ●—●, $n = 8$; control group arterial samples: ○---○, $n = 6$) (acid group venous samples: ■—■, $n = 5$; control group venous samples: □---□, $n = 5$). Asterisks represent significance ($p < 0.05$) obtained either by a "within group" (*) or "between group" (**) comparison of means (see text for details). For the sake of clarity, significant changes in P_{CO_2} were not illustrated but occurred in arterial (days 2, 3, 4) and venous samples (day 4) in the acid group only (*).

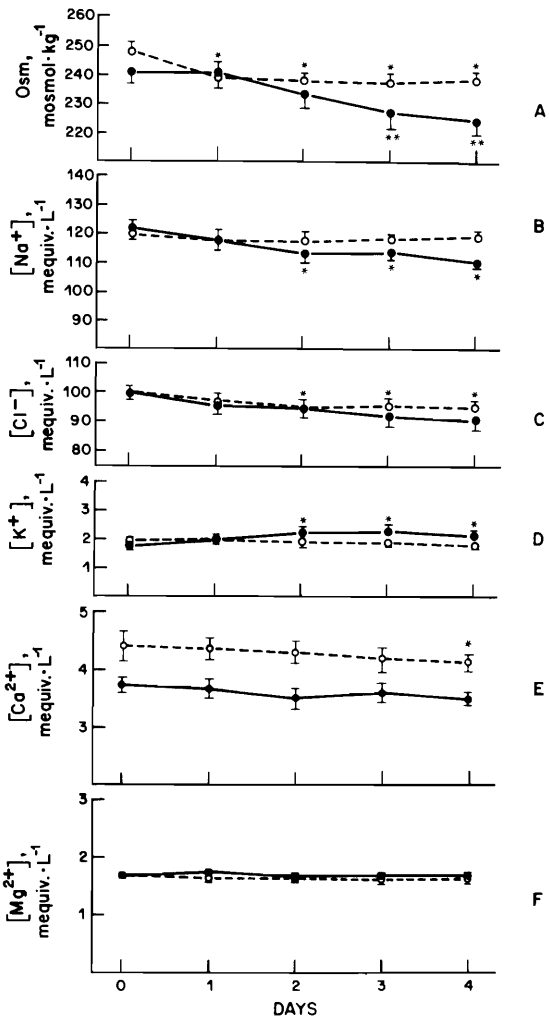

FIG. 2. Plasma osmolality and monovalent and divalent ion concentrations (means ± SEM) in which suckers held at pH 7.3 (day 0) followed by 4 days of exposure to pH 4.3 (●—●, $n = 13$) or maintained at pH 7.3 throughout (○---○, $n = 11$). Asterisks denote significance as described in Fig. 1.

between respective concentrations (Table 3) and equimolar excretion rates (Figs. 4A, 4B). Since U/P ratios were well below 1, and C_{NA^+} and C_{Cl^-} much less than C_{IN} (Table 2), almost complete reabsorption of both ions probably occurred from the filtrate (cf. Hickman 1965). Patterns of renal K^+ output were at variance with those noted by Hickman (1965); $[K^+]_u$, the U/P ratio, and C_{K^+} were all lower (Table 2), findings which favored net tubular reabsorption of K^+ ($C_{K^+} < C_{IN}$), although tubular secretion cannot be entirely discounted. The inverse relationship between $[K^+]$ and $[Na^+]$ found by Hickman which implicated countertransport of these ions via an Na^+/K^+ exchanger, was also not observed (Table 3). No such correlation has been reported in either *Salmo gairdneri* (Wood and Randall 1973) or *Ictalurus punctatus* (Cameron 1980). While the reasons for these discrepancies are uncertain, they may be related to the shorter sampling regime (i.e., 24 h) and (or) small number of fish employed in Hickman's study.

Urinary divalent cation levels (Table 2) were quantitatively similar to those reported in *Salmo gairdneri* (Wood and Randall 1973) but twofold less than in *Salvelinus fontinalis* (Hammond 1969). The coupled movement of Ca^{2+} and Mg^{2+} implicated in studies of euryhaline species (Miles 1971; Wood and Randall 1973), was not observed (Table 3), suggesting that in suckers, independent renal processes probably regulate movement of these ions. Since U/P ratios were less than one and renal clearances less than C_{IN} (Table 2), some tubular reabsorption of both Ca^{2+} and Mg^{2+} probably occurred. Calcium is strongly reabsorbed against its concentration gradient in the euryhaline *Channa argus* (Oguri 1968) but apparently follows tubular volume reabsorption in *Anguilla rostrata*, either by simple diffusion or across an electrochemical gradient (Fenwick 1981).

The sucker kidney further demonstrated a definite

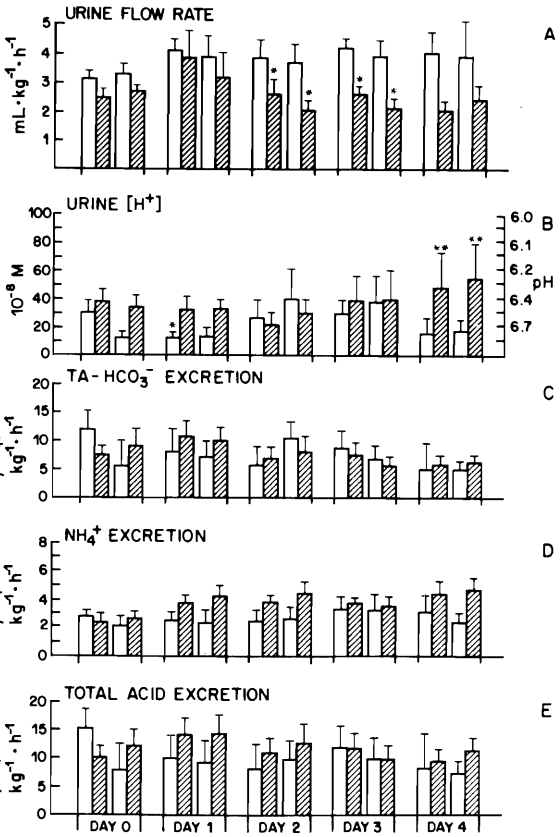

FIG. 3. Changes in urine flow rate and variables denoting urine acidity in white suckers held at pH 7.3 (day 0) followed by 4 days of exposure to pH 4.3 (▨, $n = 9$) or maintained at pH 7.3 throughout (□, $n = 5$). Each bar represents a 12-h collection period (means + SEM). Asterisks denote significance as described in Fig. 1.

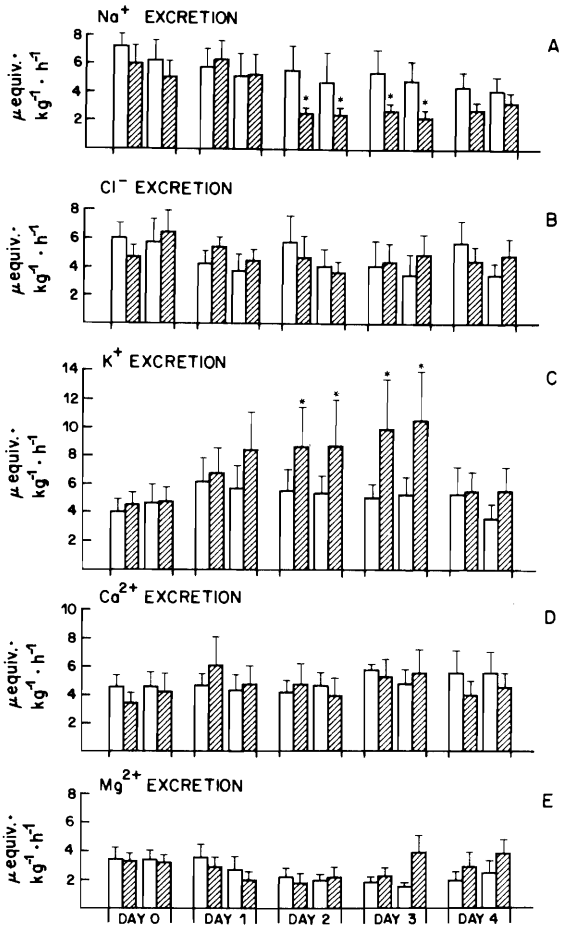

FIG. 4. Renal ion excretion (means + SEM) in white suckers held at pH 7.3 (day 0) followed by 4 days of exposure to pH 4.3 (▨, $n = 9$) or maintained at pH 7.3 throughout (□, $n = 5$). Each bar represents a 12-h collection period. Asterisks denote significance as described in Fig. 1.

capacity to acidify urine (mean pH 6.5, Table 2), at variable rates (Fig. 3). Renal excretion of H^+ apparently depends upon the availability of urinary buffers such as titratable acids (TA − HCO_3^-; mainly dibasic phosphates) and ammonia ($NH_3 + H^+ \rightarrow NH_4$; Heisler 1980). Since TA − HCO_3^- largely accounted for the observed pattern of net acid rather than base excretion (i.e., 77%; Fig. 3), its role in defending the normal acid–base balance of suckers probably superseded that of NH_4^+. Moreover, the level of urine pH appeared to have contributed more substantially to the generation of TA − HCO_3^- than to NH_4^+, as shown by the strong positive correlation between urine $[H^+]$ and [TA − HCO_3^-] (Table 3). Few interrelationships between renal acid and electrolyte levels were apparent; in fact, the positive correlation between either $[Na^+]$ or $[K^+]$ and various components of urine acidity (Table 3) was exactly opposite to that expected if Na^+/H^+ or K^+/H^+ exchange processes regulated urine pH (mammalian kidney, Pitts 1974). Relationships of this nature have also not been reported in euryhaline species such as *Salmo gairdneri* (Kobayashi and Wood 1980) and *Ictalurus punctatus* (Cameron 1980). In contrast with suckers, however, these species normally excrete a more alkaline urine (pH > 7.0) with NH_4^+ as the predominant component of $E_{\Sigma H^+}$.

The mechanisms of urinary acidification and ion transport in teleosts clearly deserve further investigation.

Effects of acid exposure

The physiological consequences of exposure to ambient pH 4.3 included disturbances in both plasma acid–base status (Fig. 1) and to a lesser degree plasma ion levels (Fig. 2), findings which qualitatively agree with those found in other fish species maintained under comparable ambient pH and $[Ca^{2+}]$ (*Salmo gairdneri*, McDonald and Wood 1981; *Cyprinus carpio*, Ultsch *et*

al. 1981); however, the nature of the plasma pH depression was different. The observed respiratory acidosis (i.e., CO_2 excess; Fig. 1A) was atypical and implicated some limitation in CO_2 diffusion and (or) catalyzed HCO_3^- dehydration (Cameron 1979) at the gill. Endogenous (i.e., lactic acid; Krishna Murthy *et al.* 1981) and exogenous factors (i.e. H^+ influx or base efflux, McDonald and Wood 1981; Ultsch *et al.* 1981) as well as changes in the relative intensities of the counterion exchanges across the gill (i.e., Na^+/H^+ or NH_4^+ and Cl^-/HCO_3^- or OH^-, Maetz 1973), may all have contributed to the observed metabolic acidosis (i.e., bicarbonate depletion; Fig. 1B). Since an increase in gill H^+ permeability has been documented at low ambient pH (McWilliams and Potts 1978), enhanced branchial H^+ influx would be the most plausible explanation for the observed acidosis. However, without measurements of ion flux rates and indices of anaerobiosis, other causal factors (as above) cannot be entirely discounted.

The role of the sucker kidney in compensating for the plasma acidosis was negligible since the rate of net H^+ excretion was virtually unchanged by acid exposure (Fig. 3E). By contrast, at least 50% of the incoming proton load was excreted by the kidney in acid-exposed *Salmo gairdneri* (McDonald and Wood 1981). The extent of renal participation in acid–base homeostasis in other fish species, however, appears to be dictated not only by the prevailing environmental conditions but also by the nature (metabolic or respiratory) of the acid–base disorder. For example, Cameron and Kormanik (1982) found that in *Ictalurus punctatus*, the kidney clearly adjusted for the blood pH disturbance resulting from infusions of both NH_4Cl and $NH_4HCO_3^-$, yet its response to either mineral acid infusion (HCl) or environmental hypercapnia was relatively minor (Cameron 1980). Thus, it is conceivable that the contribution of the sucker kidney may become significant, perhaps under conditions of more severe external acid stress.

Some compensatory adjustments in renal output were notable (Figs. 3A, 4A, 4C). Firstly, a reduction in the total permeability of the body surface to water was suggested from the drop in U_{FR} which in turn, probably accommodated the depression in plasma Osm (Fig. 2A). Secondly, increased renal Na^+ reabsorption was implicated from the decrease in E_{Na^+}, perhaps in response to the plasma hyponatremia (Fig. 3B). Finally, the rise in EE_{K^+} may have resulted from increased renal K^+ secretion, probably to minimize the plasma hyperkalemia (Fig. 3D). Thus, the sucker kidney appeared to be important in restoring, to some extent, circulating ion levels in plasma during acid exposure.

The present findings further indicate that suckers are physiologically acid-tolerant since net H^+ accumulation (i.e., ΔH_p^+) and NaCl loss from blood plasma over 4 days of acid exposure were both less than one half those in *Salmo gairdneri* (McDonald and Wood 1981). In the absence of definite information, one can only speculate on the possible strategies involved in this greater tolerance. These could include intracellular buffering and intercompartmental redistribution of body electrolytes, or simply a resistance of the branchial epithelium to the effects of acid. In the latter regard, the copious buildup of mucus detected on the gills of suckers may be important. Enhanced mucous secretion has been noted at pH 4.0 in *Salvelinus fontinalis* (Dively *et al.* 1977) and in *Fundulus grandis* (MacFarlane 1981) but not in rainbow trout (McDonald and Wood 1981). Since mucus, to some degree, can restrict both diffusive ion movement (Shephard 1982) and respiratory gas exchange (Ultsch and Gros 1979), it must have provided less resistance to gas transfer than to ion flow, for it to have served as a protective barrier. Studies of the chemical nature and physiological properties of mucus secreted by acid-exposed fish clearly warrant future experimentation.

Acknowledgements

We wish to thank L. Linton and B. Smith for advice on data analysis and computer programming. The staff of Lake Bonavista Recreation Centre helped greatly with the collection of fish. Financial support was provided by an operating grant (A5762) to B. R. McMahon from the Natural Sciences and Engineering Research Council of Canada and a Research Allowance to H. Hōbe from the Alberta Heritage Fund for Medical Research. C. M. Wood's travel was supported by an NSERC strategic operating grant in environmental toxicology. H. Hōbe was supported by an NSERC postgraduate scholarship.

BEAMISH, R. J., W. L. LOCKHART, J. C. VANLOON, and H. H. HARVEY. 1975. Long-term acidification of a lake and resulting effects on fish. Ambio, **4**: 998–102.

BEYENBACH, K. W., and L. B. KIRSCHNER. 1975. Kidney and urinary bladder functions of the rainbow trout in Mg and Na excretion. Am. J. Physiol. **229**: 389–393.

BLAXHALL, P. C., and K. W. DAISLEY. 1973. Routine hematological methods for use with fish blood. J. Fish Biol. **5**: 771–781.

CAMERON, J. N. 1979. Excretion of CO_2 in water-breathing animals—a short review. Mar. Biol. Lett. **1**: 3–13.

——— 1980. Body fluid pools, kidney function, and acid–base regulation in the freshwater catfish, *Ictalurus punctatus*. J. Exp. Biol. **86**: 171–183.

CAMERON, J. N., and G. A. KORMANIK. 1982. The acid–base responses of gills and kidneys to infused acid and base loads in the channel catfish, *Ictalurus punctatus*. J. Exp. Biol. **99**: 143–160.

CAMERON, J. N., and C. M. WOOD. 1978. Renal function and acid–base regulation in two Amazonian erythrinid fishes: *Hoplias malabaricus*, a water breather, and *Hoplerythrinus unitaeniatus*, a facultative air breather. Can. J. Zool. **56**: 917–930.

DIVELY, J. L., J. E. MUDGE, W. F. NEFF, and A. ANTHONY. 1977. Blood P_{O_2}, P_{CO_2}, and pH changes in brook trout (*Salvelinus fontinalis*) exposed to sublethal levels of acidity. Comp. Biochem. Physiol. A, **57**: 347–351.

DIXON, W. J., and M. B. BROWN. 1979. Biomedical computer programs, P-series. University of California Press, Berkeley.

DUNNETT, C. W. 1955. The multiple comparisons procedure for comparing several treatments with a control. J. Am. Stat. Assoc. **50**: 1096–1121.

FENWICK, J. C. 1981. The renal handling of calcium and renal $Ca^{2+}(Mg^{2+})$-activated adenosinetriphosphatase activity in freshwater- and seawater-acclimated North American eels (*Anguilla rostrata*, LeSueur). Can. J. Zool. **59**: 478–485.

FROMM, P. O. 1980. A review of some physiological and toxicological responses of freshwater fish to acid stress. Environ. Biol. Fishes, **5**: 79–93.

HAINES, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. **110**: 669–707.

HAMMOND, B. R. 1969. Renal function and the effects of arginine vasotocin in lake trout, *Salvelinus namaycush* (Walbaum). Ph.D. thesis, Cornell University, Ithaca, NY.

HARVEY, H. H. 1979. The acid deposition problem and emerging research needs in the toxicology of fish. Proceedings of the 5th Annual Aquatic Toxicity Workshop, Hamilton, Ont., November 7–9, 1978. Tech. Rep. Fish. Mar. Serv. (Can.) No. 862. pp. 115–128.

HEISLER, N. 1980. Regulation of the acid-base status in fishes. *In* Environmental physiology of fishes. *Edited by* M. A. Ali. Plenum Publishing Corp., New York. pp. 77–85.

HICKMAN, C. P., JR. 1965. Studies on renal function in freshwater teleost fish. Trans. R. Soc. Can. **3**(4): 213–236.

HICKMAN, C. P., JR., and B. F. TRUMP. 1969. The kidney. Fish Physiol. 1969, **1**: 91–239.

HILLS, A. G. 1973. Acid–base balance—chemistry, physiology, pathophysiology. Williams and Wilkens Co., Baltimore.

HULL, C. H., and N. H. NIE. 1979. SPSS update 7–9. New procedures and facilities for releases 7–9. McGraw-Hill Book Co., New York.

KOBAYASHI, K. A., and C. M. WOOD. 1980. The response of the kidney of the freshwater rainbow trout to true metabolic acidosis. J. Exp. Biol. **84**: 227–244.

KRISHNA MURTHY, V. K., P. REDDANNA, and S. GOVINDAPPA. 1981. Hepatic carbohydrate metabolism in *Tilapia mossambica* (Peters) acclimated to low environmental pH. Can. J. Zool. **59**: 400–404.

MAETZ, J. 1973. Na^+/NH_4^+, Na^+/H^+ exchanges and NH_3 movement across the gill of *Carassius auratus*. J. Exp. Biol. **58**: 255–275.

MACFARLANE, R. B. 1981. Alterations in adenine nucleotide metabolism in the gulf killifish (*Fundulus grandis*) induced by low pH water. Comp. Biochem. Physiol. B, **68**: 193–202.

MACKAY, W. W., and D. D. BEATTY. 1968. The effect of temperature on renal function in the white sucker fish, *Catostomus commersoni*. Comp. Biochem. Physiol. **26**: 235–245.

MCDONALD, D. G., H. HÖBE, and C. M. WOOD. 1980. The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri* to low environmental pH. J. Exp. Biol. **88**: 109–131.

MCDONALD, D. G., R. L. WALKER, P. R. H. WILKES, and C. M. WOOD. 1982. H^+ excretion in the marine teleost *Parophyrys vetulus*. J. Exp. Biol. **98**: 403–414.

MCDONALD, D. G., and C. M. WOOD. 1981. Branchial and renal acid and ion fluxes in acid exposed rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **93**: 101–118.

MCWILLIAMS, P. G., and W. T. W. POTTS. 1978. The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta*. J. Comp. Physiol. **126**: 277–286.

MILES, H. M. 1971. Renal function in migrating adult coho salmon. Comp. Biochem. Physiol. A, **38**: 787–826.

MILLIGAN, C. L., and C. M. WOOD. 1982. Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol. **99**: 397–415.

NEVILLE, C. M. 1979. Sublethal effects of environmental acidification on rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **36**: 84–87.

OGURI, M. 1968. Urinary constituents of snake-head fish, with special reference to urine sugar. Bull. Jpn. Soc. Sci. Fish. **34**: 6–10.

PITTS, R. F. 1974. Physiology of the kidney and body fluids. 3rd ed. Yearbook Medical Publishers, Inc., Chicago.

RENFRO, J. L. 1975. Water and ion transport by the urinary bladder of the teleost, *Pseudopleuronectes americanus*. Am. J. Physiol. **228**: 420–431.

SEVERINGHAUS, J. W. 1965. Blood gas concentrations. Handb. Physiol. Sect. 3: Respir. 1964–1965, **2**: 1475–1487.

SEVERINGHAUS, J. W., M. STUPFEL, and A. F. BRADLEY. 1956. Variations of serum carbonic acid pk′ with pH and temperature. J. Appl. Physiol. **9**: 197–200.

SHEPHARD, K. L. 1982. The influence of mucus on the diffusion of ions across the esophagus of fish. Physiol. Zool. **55**(1): 23–34.

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr. **14**: 799–801.

SPRY, D. L., C. M. WOOD, and P. V. HODSON. 1981. The effects of environmental acid on freshwater fish with particular reference to the softwater lakes in Ontario and the modifying effects of heavy metals in the environment. A literature review. Can. Tech. Rep. Fish. Aquat. Sci. No. 999. pp. 1–444.

ULTSCH, G. R., and G. GROS. 1979. Mucus as a diffusion barrier to oxygen: possible role in O_2 uptake at low pH in carp (*Cyprinus carpio*) gills. Comp. Biochem. Physiol. A, **62**: 685–689.

ULTSCH, G. R., M. E. OTT, and N. HEISLER. 1981.

Acid–base and electrolyte status in carp (*Cyprinus carpio*) exposed to low environmental pH. J. Exp. Biol. **93**: 65–80.

WATTERS, K. W., and L. S. SMITH. 1973. Respiratory dynamics of the starry flounder *Platichthys stellatus* in response to low oxygen and high temperature. Mar. Biol. **19**: 133–148.

WILKES, P. R. H., R. L. WALKER, D. G. MCDONALD, and C. M. Wood. 1981. Respiratory, ventilatory, acid–base, and ionoregulatory physiology of the white sucker (*Catostomus commersoni*): the influence of hyperoxia. J. Exp. Biol. **91**: 239–254.

WINER, B. J. 1971. Statistical principles in experimental design. 2nd ed. McGraw Hill Book Co., New York.

WOOD, C. M., and F. H. CALDWELL. 1978. Renal regulation of acid–base balance in a freshwater fish. J. Exp. Zool. **205**: 301–307.

WOOD, C. M., and D. J. RANDALL. 1973. The influence of swimming activity on sodium balance in the rainbow trout (*Salmo gairdneri*). J. Comp. Physiol. **82**: 207–233.