

CO₂ AND H⁺ EXCRETION BY SWIMMING COHO SALMON, *ONCORHYNCHUS KISUTCH*

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SUMMARY

Coho salmon, *Oncorhynchus kisutch* (Walbaum), were swum at constant speed in a 'Brett-type' tunnel respirometer. Blood P_{O₂}, P_{CO₂} and pH as well as total CO₂ content and red blood cell pH were unchanged during swimming. The RE (respiratory exchange ratio) was slightly less than 0.7 when the fish was swimming in normal sea water, indicating that some CO₂ is retained by the fish. Lowering seawater bicarbonate concentration increased HCO₃[−] transfer, presumably because of passive bicarbonate loss. A reduction in seawater pH from 7.95 to 7.1 sharply reduced both CO₂ and hydrogen ion transfer, resulting in very low RE values of about 0.2. Hydrogen ion excretion was elevated during prolonged swimming following high speed swimming activity. It would appear that CO₂ and hydrogen ion transfer by fish need not be matched and changing internal and external conditions can have a marked and separate effect on hydrogen ion and CO₂ excretion and therefore on the RE value.

INTRODUCTION

Burst swimming activity in fish results in the production of large amounts of lactic acid which, at body pH of between 7.0 and 8.0, is almost completely dissociated and causes a marked fall in blood pH. The gill epithelia of teleost fish have been shown to be very permeable to hydrogen ions (McWilliams & Potts, 1978). Therefore, a change of internal or external pH will alter the net transport of protons across the gills. It is possible that an important component of acid-base regulation in fish following burst swimming is the diffusive loss of protons from fish across the gill epithelium. Similarly, a reduction of external pH by addition of acid to the water would reduce or even reverse hydrogen ion excretion and therefore impair the acid-base balance of the fish.

In this study we investigated the impact of various pH differences across the gills on H⁺ and CO₂ excretion by coho salmon swimming in sea water for prolonged

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periods. The pH difference between blood and water across the gill was altered by reducing seawater pH or by reducing blood pH by burst swimming. In addition, bicarbonate concentration difference between blood and water was reduced by lowering seawater bicarbonate levels.

METHODS

Experiments were carried out on seawater coho salmon, *Oncorhynchus kisutch*, in a non-reproductive stage, weighing between 0.5 and 1.0 kg, obtained from the Pacific Biological Station in Nanaimo, B. C., Canada. The fish were kept in fibreglass holding tanks of about 2 m diameter and supplied with flowing sea water (10–12°C) with a maximum water current of 0.3 m s⁻¹. The fish were fed daily with Moore-Clarke trout pellets and were treated for furunculosis and fibriosis before transportation and then afterwards with furazolidon to prevent skin infections.

Experiments were carried out in a large volume (120 l) tunnel respirometer at 13°C (Brett, 1964) flushed with local Bamfield sea water (salinity $32 \pm 1\text{‰}$). The respirometer was that used by Brett (1964), transported to Bamfield Marine Station and modified in the following ways. Pure oxygen was injected into the recirculating water of the closed respirometer to replace that used by the swimming fish and this permitted prolonged closure of the system without causing hypoxia. Oxygen was injected using a 50 ml calibrated glass syringe kept at room temperature and the injected volume measured at atmospheric pressure. The injected O₂ dissolved in the water and was completely mixed within 30 min. Oxygen was injected at intervals such that water O₂ levels never fell below 110 mmHg P_{O₂}. The O₂ content of the water was determined from the P_{O₂} measured with a Radiometer PHM71 blood gas analyser and thermostatted electrode and the Bunsen solubility coefficient for O₂ at the temperature and salinity of the sea water (Dejours, 1975). Oxygen uptake rate was determined from the change in oxygen content of the water, the total volume of water in the respirometer and the amount of oxygen injected into the system. Water sampled from the respirometer was replaced from a small head tank 30 cm above the respirometer.

Prolonged closure of the respirometer was required to create measurable changes in the CO₂ content of the water. In preliminary experiments, prolonged closure was shown to be associated with reductions in water pH. In subsequent experiments water pH was kept constant by injecting 0.25 N-NaOH (100% pure, CO₂-free) by means of a Harvard Linear displacement pump at rates between 0.5 and 2.5 ml min⁻¹. The pump was operated by a small electrical comparator connected to the output of a Radiometer pH meter (PHM65) and electrode, which were used to monitor water pH. If water pH fell below a predetermined setpoint the pump was activated and NaOH injected until the pH of the water was re-established at the setpoint level. The maximum variation in pH was never greater than 0.02 units.

Total carbon dioxide (T_{CO₂}) of sea water was determined using the method of Cameron (1971) with a Radiometer PHM71 acid-base analyser and associated CO₂ electrode. The bicarbonate concentration of sea water was measured by microtitration; 25 ml water samples were acidified to pH 3.5–3.8 with 0.1 N-HCl added *via* a Gilmont microburette. CO₂ was then expelled by bubbling with pure N₂ for about

4 min. The samples were returned to the initial pH by titrating with 0.02 N-NaOH via another Gilmont microburette. The difference between the amount of acid and base added represented the amount of bicarbonate in solution. The method was reproducible to 0.0005 mequiv l⁻¹ for concentrations of between 0.1 and 2.0 mM and gave 100 % recovery of pure NaHCO₃.

In some experiments, the dorsal aortae of fish were cannulated (Smith, 1978) and the indwelling PE50 polyethylene cannula was used to sample blood while the fish was swimming. Blood P_{O₂}, P_{CO₂}, and pH were measured using a Radiometer PHM71 blood gas analyser and the appropriate electrodes thermostatted at the temperatures of the fish and calibrated with gases supplied by Wösthoff gas mixing pumps or pH standard solutions. Erythrocyte pH was measured using the quick freezing method of Zeidler & Kim (1977). Blood total CO₂ was measured using the method of Cameron (1971).

Swimming cannulated fish

The critical velocity of each cannulated fish (U_{crit}) was determined after it was anaesthetized lightly in MS222 in sea water (50 mg l⁻¹), placed in the open respirometer, continuously flushed with sea water, and allowed to recover overnight. The water velocity, and therefore the swimming speed of the fish, was increased using increments of 0.5 body lengths per second at 1-h intervals until the fish was exhausted (see Hoar & Randall, 1978, for details of calculation of critical velocity). Cannulated fish were allowed 2 days recovery from the operation before being placed in the respirometer. The U_{crit} of these fish was measured with the dorsal aortic cannula trailing in the respirometer.

Following an overnight rest, each cannulated fish was swum at 80 % U_{crit} and various blood parameters measured. Each fish was allowed 2 h rest and was then swum to exhaustion (burst swimming) at 120 % U_{crit}. Blood parameters were measured again 30 min after fatigue.

Swimming uncannulated fish

A further series of experiments were carried out on uncannulated fish. After determining the U_{crit}, each fish was subjected to four swimming periods of 6 h each, with an overnight rest between each swimming period. The respirometer was closed during each swimming period and changes in O₂, CO₂ and HCO₃⁻ in the sea water were determined. Oxygen was injected into the respirometer and seawater pH held constant by NaOH injection. The fish were subjected to different seawater conditions during each swimming period. Seawater pH was lowered by adding HCl to the respirometer. Seawater bicarbonate levels were reduced by first acidifying 450 l of sea water in a header tank with HCl to pH 3.5. The sea water was then aerated until the total CO₂ concentration was below 0.1 mM. The seawater pH was then elevated to 8.0 with NaOH and used to flush the respirometer with three times its own volume, giving a final total CO₂ level of 0.2 mM in the respirometer.

Fish were subjected to the following conditions:

(1) Fish were swum for 6 h at 80 % U_{crit} in sea water at pH 7.95. The pH of Bamfield sea water is around 8.1, so pH was lowered initially by injecting a small volume of 0.10 N-HCl.

(2) Fish were swum for 6 h at 80 % U_{crit} in low bicarbonate (0.2 mM) sea water. The fish was forced to swim in the closed respirometer and seawater pH was held at 7.95.

(3) Fish were swum for 6 h at 50 % U_{crit} in normal sea water acidified to pH 7.1 by adding HCl, and maintained at this pH by the pH stat device. The initial reduction in seawater pH caused a rise in seawater P_{CO_2} to 4.4 mmHg. Thus fish were subjected to hypercapnia as well as reduced seawater pH during this experimental period.

(4) Fish were swum at 50 % U_{crit} following a burst swim in normal sea water at pH 7.95. The protocol was to swim the fish at 50 % U_{crit} for 1 h after closing the respirometer, then burst swim the fish (at 120 % U_{crit}) to fatigue before returning the swimming speed to 50 % U_{crit} for 6 h.

RESULTS

Cannulated coho salmon swimming at 80 % U_{crit} for 1 h showed little or no change in either P_{O_2} , P_{CO_2} , total CO_2 , haematocrit or plasma and erythrocytic pH. There was a non-significant increase in haematocrit and a decrease in plasma pH and blood

Table 1. *The effect of swimming at 80 % critical velocity on various parameters in blood sampled from the dorsal aorta of a coho salmon*

	Time (min)			
	Initial	10	30	60
Haematocrit (%) $N = 7$	16.2 ± 3.7	19.1 ± 2.8	18.1 ± 3.3	16.9 ± 3.7
Blood P_{O_2} (mmHg) $N = 7$	105 ± 48	94 ± 39	87 ± 35	102 ± 43
Blood P_{CO_2} (mmHg) $N = 6$	2.5 ± 0.9	2.0 ± 0.7	1.8 ± 0.4	2.1 ± 0.5
Total CO_2 (mmol) $N = 7$	6.2 ± 1.3	4.6 ± 1.2	4.9 ± 1.4	6.0 ± 0.8
Plasma pH $N = 7$	7.77 ± 0.07	7.68 ± 0.06	7.80 ± 0.05	7.78 ± 0.07
Erythrocyte pH $N = 7$	7.42 ± 0.07	7.43 ± 0.07	7.49 ± 0.07	7.45 ± 0.08

The initial values are for a fish resting in the respirometer just prior to the swim. Values $\bar{x} \pm s.e.$

Table 2. *The effect of burst swimming to exhaustion on parameters measured in blood sampled from dorsal aorta of coho salmon 30 min after fatigue*

	Normal	Post burst swim
Haematocrit (%)	16.2 ± 3.7	16.1 ± 3.2
Blood P_{O_2} (mmHg)	105 ± 48	83 ± 37
Blood P_{CO_2} (mmHg)	2.5 ± 0.9	8.0 ± 11.01
Total CO_2 (mmol)	6.2 ± 1.3	6.0 ± 1.7
Plasma pH	7.77 ± 0.07	7.68 ± 0.10
Erythrocyte pH	7.42 ± 0.07	7.48 ± 0.06

The normal values are those reported for resting fish in Table 1 and are repeated here for purposes of comparison.

total CO₂ after 10 min exercise (Table 1). Burst swimming also had little effect on measured blood variables (Table 2) but was associated with a non-significant increase in P_{CO₂} and decrease in plasma pH.

We found that each fish had to be swum for several hours in the closed respirometer before we could accurately measure changes in seawater CO₂ levels. Repetitive blood sampling over this prolonged period eventually impaired the swimming ability of the fish. As a result we decided to continue our experiments on uncannulated fish, varying only seawater pH and bicarbonate levels. In preliminary experiments, in

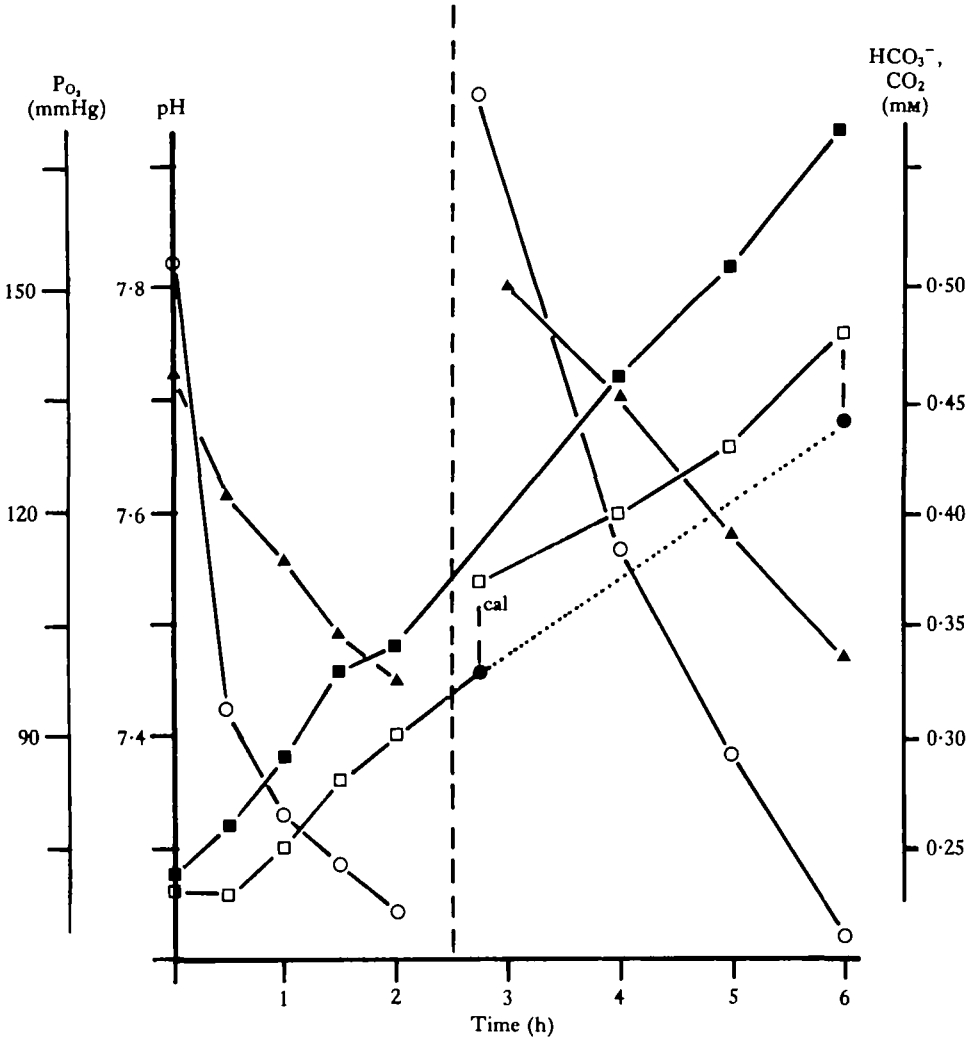


Fig. 1. Changes in pH (open circles), P_{O₂} (solid triangles) and total CO₂ (solid squares) and bicarbonate levels (open squares) in low bicarbonate sea water with a fish swimming at 80 % U_{crit} (critical swimming velocity) in the closed respirometer. After 2.5 h, oxygen and NaOH were added to the sea water to readjust pH and P_{O₂}. These results are from a preliminary experiment in which seawater pH was not regulated. The sudden rise in HCO₃⁻ at 2.5 h was due to the elevation in pH, the magnitude of this HCO₃⁻ increase was calculated (cal) and subtracted from the measured values, the result indicated by solid circles joined by a dotted line.

which uncannulated fish were swum for prolonged periods in the closed respirometer, there was a sharp drop in water pH (Fig. 1). In these experiments pH changes appeared to affect CO_2 excretion, so subsequent experiments were carried out at a constant pH (Fig. 2). The mean accumulated oxygen uptake, CO_2 excretion and net hydrogen ion transfer are illustrated in Fig. 3 and Table 3. Most rates did not change very much during each run (Fig. 3A–D), so mean rates were calculated for each 6-h period (Table 3). The data for conditions 2, 3 and 4 are presented as a fraction of the condition 1 value in Table 3 for ease of comparison. The oxygen uptake of coho salmon swimming at 80% U_{crit} in low bicarbonate sea water was reduced, compared with that of fish in normal sea water (condition 1). Oxygen uptake was further reduced in conditions 3 (low pH sea water) and 4 (following burst activity), as these fish were only capable of swimming at a reduced speed of 50% U_{crit} .

Coho salmon exposed to low pH sea water had a high rate of HCO_3^- excretion over the first 30 min of the swimming period and this was associated with a low rate of H^+ transfer (Fig. 3C). After burst activity the reverse was observed, namely a high H^+ transfer but depressed HCO_3^- excretion (Fig. 3D).

The amount of NaOH injected into the respirometer is equal to the H^+ excreted

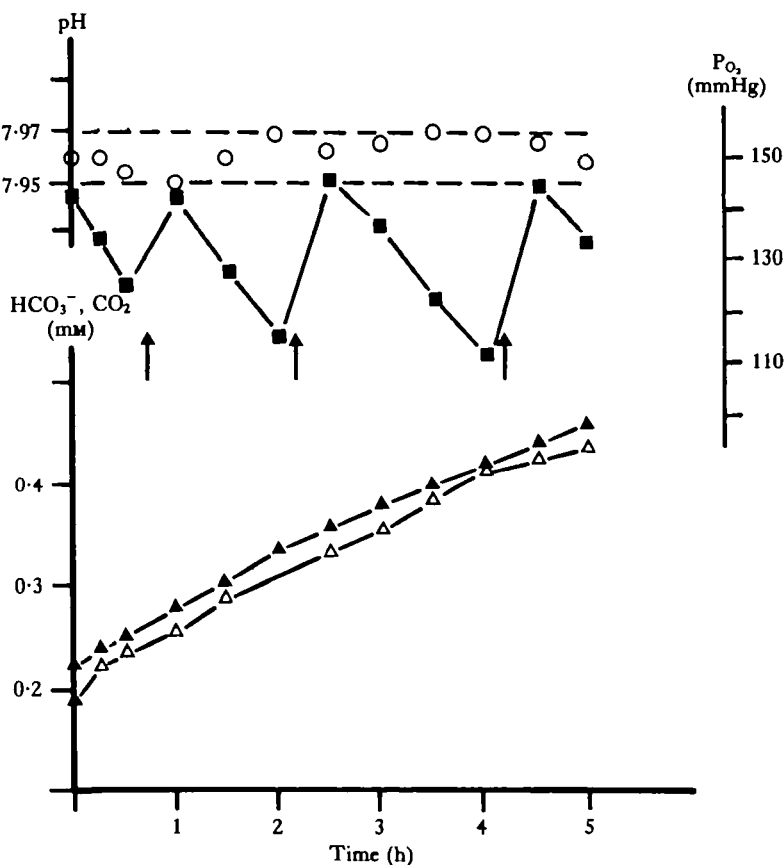


Fig. 2. Changes in pH (open circles), PO_2 (solid squares), total CO_2 (solid triangles) and bicarbonate (open triangles) in low bicarbonate sea water with fish swimming at 80% U_{crit} in the closed respirometer. The seawater pH was regulated by a pH stat device resulting in pH oscillations within the dashed lines. Oxygen was injected into the closed respirometer at the times indicated by arrows.

by the fish, if we regard anything that reduces seawater pH as contributing to H⁺ excretion by the fish (\dot{M}_{H^+}). CO₂ excreted into the water will reduce pH and cause an increase in seawater bicarbonate. CO₂ production by the fish was derived from the amount of CO₂ neutralized, i.e. the seawater HCO₃⁻ accumulation coupled to NaOH

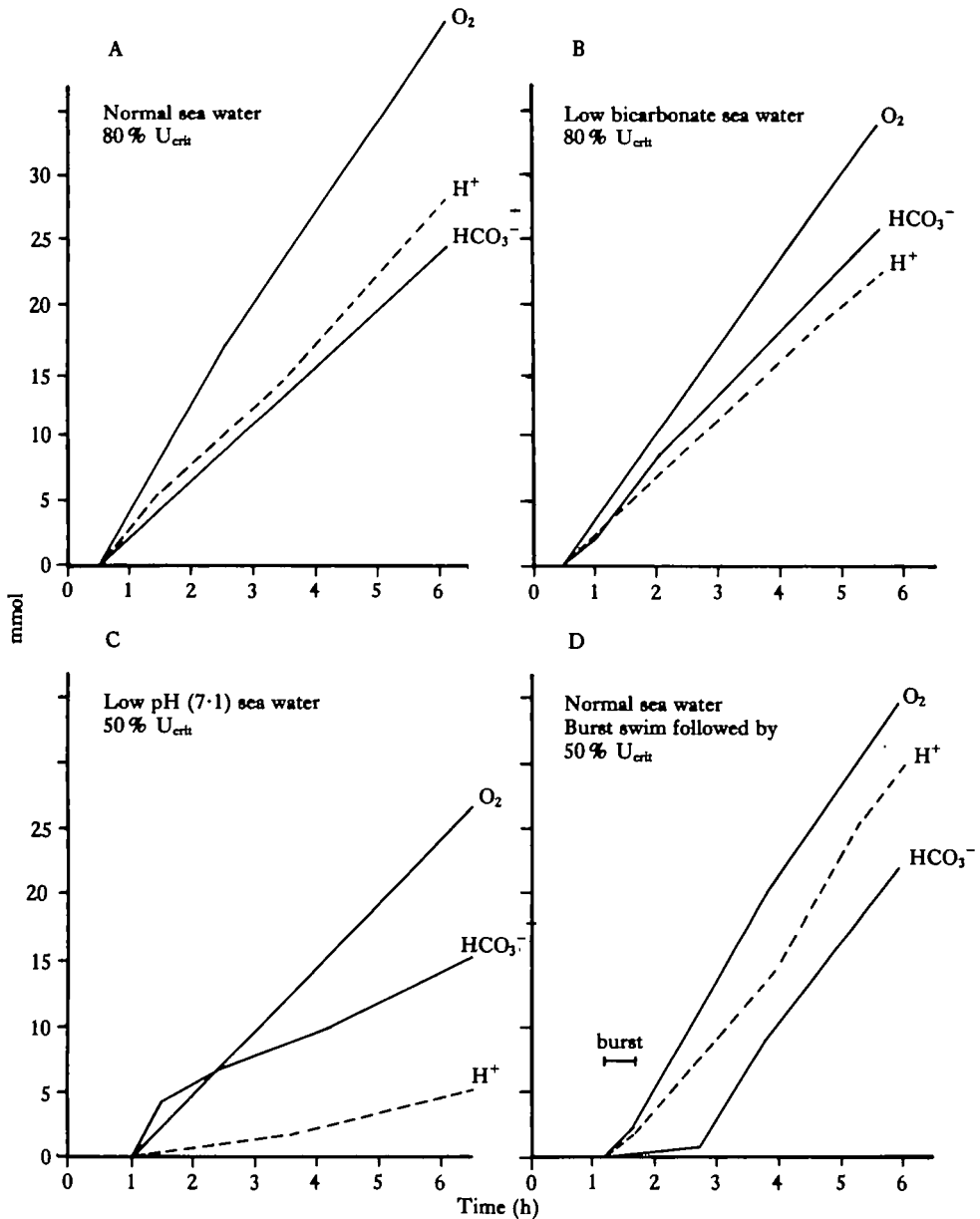


Fig. 3. Accumulated oxygen uptake and hydrogen ion and bicarbonate excretion into sea water containing the same swimming coho salmon (600 g wt) at 13 °C in a closed respirometer under four different conditions. (A) Sea water pH 7.95, normal sea water, 80% U_{crit}. (B) Sea water pH 7.95, low bicarbonate sea water, 80% U_{crit}. (C) Sea water pH 7.10, normal sea water, 50% U_{crit}. (D) Sea water pH 7.95, normal sea water, 50% U_{crit} after a burst swim at 120% U_{crit} to exhaustion.

Table 3. *Oxygen uptake and CO₂ and hydrogen ion production by swimming coho salmon (weight = 600 g) measured under four conditions over a 6-h period of swimming at 13 °C*

Condition	Oxygen uptake	CO ₂ excretion	Hydrogen ion production	HCO ₃ ⁻ ion production
	mmol h ⁻¹		mequiv h ⁻¹	
1 (N = 6)	7.20 ± 1.32	4.45 ± 0.75	4.86 ± 0.82	4.39 ± 0.74
Values presented as a fraction of condition 1				
2 (N = 6)	0.88 ± 0.14 (< 0.05)	0.95 ± 0.29 (N.S.)	0.85 ± 0.33 (< 0.05)	1.08 ± 0.32 (N.S.)
3 (N = 5)	0.75 ± 0.26 (< 0.005)	0.25 ± 0.13 (< 0.005)	0.18 ± 0.12 (< 0.005)	0.61 ± 0.16 (< 0.005)
4 (N = 4)	0.74 ± 0.23 (< 0.03)	0.76 ± 0.28 (N.S.)	0.84 ± 0.31 (N.S.)	0.76 ± 0.26 (N.S.)

(1) Swimming at 80 % U_{crit} in normal sea water.(2) Swimming at 80 % U_{crit} in low bicarbonate sea water.(3) Swimming at 50 % U_{crit} in sea water of pH = 7.1.(4) Swimming at 50 % U_{crit} in normal sea water following a burst swim.

For more details, see text.

Values for conditions 2, 3, and 4 are presented as fractions of the condition 1 value. Statistical differences as compared to the condition 1 value are based on Wilcoxon's Q-test. N.S. = non-significant, *P* values in brackets. Values presented as mean ± s.d.

Table 4. *Comparison of CO₂ production (\dot{M}_{CO_2}), hydrogen ion production (\dot{M}_{H^+}) and oxygen uptake (\dot{M}_{O_2}) in swimming coho salmon under a variety of conditions (see Table 3)*

Condition	$\dot{M}_{CO_2}/\dot{M}_{O_2}$	$\dot{M}_{H^+}/\dot{M}_{O_2}$	$\dot{M}_{H^+}/\dot{M}_{HCO_3^-}$	$\dot{M}_{H^+}/\dot{M}_{CO_2}$
1 (N = 6)	0.62 ± 0.07	0.68 ± 0.10	1.11 ± 0.07*	1.09 ± 0.07
2 (N = 6)	0.65 ± 0.05 (N.S.)	0.63 ± 0.65 (N.S.)	0.81 ± 0.08* (< 0.005)	0.96 ± 0.07 (N.S.)
3 (N = 5)	0.21 ± 0.13 (< 0.005)	0.17 ± 0.12 (< 0.01)	0.31 ± 0.20* (< 0.005)	0.71 ± 0.19 (< 0.005)
4 (N = 4)	0.64 ± 0.07 (N.S.)	0.79 ± 0.10 (N.S.)	1.19 ± 0.16* (N.S.)	1.22 ± 0.19 (N.S.)

Statistical differences as compared to condition 1 are based on Wilcoxon's Q-test. Data presented as mean ± s.d. N.S. = non-significant, *P* values in brackets.

* All $\dot{M}_{H^+}/\dot{M}_{HCO_3^-}$ values are significantly different from unity at $P < 0.01$.

addition, plus the increase in molecular CO₂ calculated using the Henderson-Hasselbalch equation and appropriate values of pK' and CO₂ solubility in sea water (Dejours, 1975; Strickland & Parsons, 1968).

Table 4 shows the calculated respiratory exchange ratios (RE) based on our estimates of CO₂ excretion (\dot{M}_{CO_2}) and oxygen uptake (\dot{M}_{O_2}). Lipids are the major fuel for prolonged swimming in coho salmon (Krueger *et al.* 1968) and as a result one would predict a respiratory quotient of 0.7. Thus, if there were no changes in body

CO₂ and O₂ content one would expect a respiratory exchange ratio of 0.7 in these experiments on swimming coho salmon. The $\dot{M}_{\text{CO}_2}/\dot{M}_{\text{O}_2}$ ratio, however, was low, less than 0.7 in all cases. The ratio of \dot{M}_{H^+} to \dot{M}_{O_2} , however, was only less than 0.7 when seawater pH was reduced to 7.1 (Table 4).

If CO₂ is the only substance excreted by the fish into the sea water that affects pH, then one would expect \dot{M}_{H^+} to equal \dot{M}_{CO_2} . The $\dot{M}_{\text{H}^+}/\dot{M}_{\text{CO}_2}$ ratio, however, is less than unity in low pH and low bicarbonate sea water and greater than unity in normal sea water and following a burst swim (Table 4).

DISCUSSION

The fish gill epithelium is very permeable to CO₂, permeable to hydrogen ions (McWilliams & Potts, 1978), but probably not very permeable to bicarbonate ions (Perry, Davie, Daxboeck & Randall, 1982). Swimming coho salmon excrete CO₂ which reacts with water and reduces pH and elevates the HCO₃⁻ content of the sea water in the respirometer. Most of the CO₂ must enter the water as molecular CO₂ but there may be a separate and occasionally large flux of protons. Thus seawater pH is reduced by the addition of both CO₂ and protons to the water. Fish excrete ammonia and ammonium ions into sea water; at the pH of sea water, excreted ammonia will combine with protons to produce ammonium ions. Thus our values of hydrogen ions excreted, as measured by the amount of NaOH injected to maintain seawater pH at a given level, are probably underestimated because we have not allowed for ammonia excretion. Ammonia production is probably low, however, because the swimming coho salmon were fasting while in the respirometer. If ammonia excretion was similar to that of fasting sockeye salmon (Brett & Zala, 1975), we estimate that ammonia excretion results in an underestimate of hydrogen ion excretion of about 6–8%. Ammonia excretion will have no significant effect on our estimate of CO₂ excretion.

The measured respiratory exchange ratio ($\text{RE} = \dot{M}_{\text{CO}_2}/\dot{M}_{\text{O}_2}$) for conditions 1, 2, 3 and 4 was less than the expected 0.7 for fat metabolism. Oxygen uptake is a reflection of oxygen utilization by the tissues because oxygen stores are small in fish. Thus the low RE value, of less than 0.7, is caused either by a reduction in CO₂ production by the tissues or by an increase in the CO₂ stores in the fish. It is possible that some CO₂ produced by fat metabolism could be utilized by other metabolic pathways, but this seems unlikely because pathways that consume CO₂ are anabolic and probably are not operative during exercise. Thus, the most probable explanation for the low RE values is that some CO₂ is retained in the body of the fish during swimming. The extent of CO₂ retention has been calculated assuming a respiratory quotient for fat metabolism of 0.7 and comparing this with the measured RE value for swimming coho salmon (Table 5). In all cases there was CO₂ retention in the body.

If we assume that ammonia excretion has a negligible effect, then we can calculate the acid-base imbalance of the fish from the difference between acid and bicarbonate transfer into sea water. The accumulated imbalance over 6 h is presented in Table 5. As can be seen in Fig. 3, the imbalance was built up in a linear fashion for conditions 1 and 2, while for 3 and 4 the imbalance was mainly due to initial changes.

The initial concentration differences across the blood-water barrier were used to calculate the driving forces for passive diffusion of H⁺ and HCO₃⁻ ions (Table 6).

Table 5. *Acid-base balance in swimming coho salmon under four conditions (see Table 3) comparing measured values with those for CO₂ production assuming a respiratory quotient of 0.7*

Condition	1	2	3	4
	mmol per 6 h			
(a) expected \dot{M}_{CO_2} ($\dot{M}_{O_2} \times 0.7$)	30.24	26.00	23.39	22.55
(b) measured \dot{M}_{CO_2}	26.70	24.96	6.96	21.06
(c) retained CO ₂	3.45	1.04	16.43	1.49
	mequiv per 6 h			
(d) measured \dot{M}_{H^+}	29.16	23.82	5.46	25.62
(e) measured $\dot{M}_{HCO_3^-}$	26.34	28.44	14.08	20.04
(f) imbalance (d - e)	+2.82	-4.62	-8.62	+5.58

The values presented are the total mmol or mequiv for a 600 g coho salmon swimming for 6 h.

Table 6. *H⁺ and HCO₃⁻ concentration gradients and calculated Nernst potentials (E) and driving force (F) for both ions over the gills in swimming coho salmon*

H ⁺	Condition	Water pH _o	Blood ¹ pH _i	E (mV) ²	F (mV) ³
	1	7.95	7.80	-8.5	+18.5
	2	7.95	7.80	-8.5	+18.5
	3	7.10	7.80	+39.5	-29.5
	4	7.95	7.68	-15.8	+25.8
HCO ₃ ⁻	Condition	[HCO ₃ ⁻] _o	[HCO ₃ ⁻] _i ¹	E (mV)	F (mV)
		mequiv l ⁻¹	mequiv l ⁻¹		
	1	2.0	6.0	+27	-17
	2	0.2	6.0	+83	-73
	3	1.7	6.0	+31	-21
	4	2.0	5.8	+26	-16

¹ Values from Tables 1 and 2.

² $E = \frac{RT}{nF} \ln \frac{C_i}{C_o}$, where C_i is blood and C_o is water concentration.

³ F = TMP - E, where TMP (transepithelial potential) is +10 mV. See Perry, Davie, Daxboeck & Randall (1982).

The potential is expressed as the blood value compared with the water at zero potential.

As can be seen in Table 6 the passive H⁺ ion flux is reversed in condition 3 (pH 7.1), directed inwardly. This is consistent with the highly suppressed acid production of the fish (Fig. 3C). In some cases the seawater pH did not change at all for over 1 h, although the [HCO₃⁻] increased markedly. Maybe here the H⁺ influx equalled the CO₂ excretion which would result in [HCO₃⁻] increase without pH change. The initial H⁺ driving force in condition 4 is enlarged, indicating a higher H⁺ loss by passive diffusion. The excess acid transfer must be due to the anaerobic lactate production during burst swimming. In condition 2, the low [HCO₃⁻] in the sea water results in a high driving force for HCO₃⁻ ions and will therefore cause a higher HCO₃⁻ loss by passive diffusion. These initial conditions corroborate with the observed HCO₃⁻ loss (Fig. 3B and Table 5).

The very low RE of 0.21 in condition 3, with even more reduced hydrogen ion excretion ($\dot{M}_{H^+}/\dot{M}_{O_2} = 0.17$), was maintained for the duration of the experiment, that is for 6-h in sea water pH 7.1 (Table 4). If one assumes a tissue RQ of 0.7, then the hydrogen ion load on the buffering capacity of the body is large. A 600 g coho will retain 16 mequiv of acid over the 6-h period, or 27 mequiv kg⁻¹. The buffering capacity of steelhead trout muscle was found to be 60 mequiv pH unit⁻¹ (G. Somero, unpublished communication). Heisler (1980) calculated the whole body buffer capacity for dogfish to be 38 mequiv pH unit⁻¹. Assuming similar buffering capacities in coho, then body pH should drop 0.7–1.0 pH unit during the course of the experiment. Salmonids can tolerate such changes (Hillaby & Randall, 1979) but a fall in body pH probably impairs many body functions. We observed that, in sea water of pH 7.1 or following a burst swim, the maximum prolonged swimming speed of coho salmon was reduced and could only be maintained for 6 h at 50 % U_{crit} of that in normal sea water. The reduction in body pH may limit aerobic activity either by reducing the blood oxygen capacity *via* the Root effect or may impair metabolism directly by the inhibitory effect of low pH on a variety of enzyme activities. Either or both actions may have impaired the ability of the fish to maintain high levels of prolonged swimming in conditions 3 and 4.

If ammonia excretion were to account for the apparent reduction in acid excretion in conditions 2 and 3, then it can be calculated, assuming an RQ of 0.7, that the fish would have to excrete 4.6 and 8.6 mmol NH₃ per 600 g fish over the 6-h experimental period, respectively. The body stores of ammonia are small, less than 0.25 mmol, so this NH₃ would have to be produced metabolically. There are several possible sources: (i) hydrolysis of amides (e.g. glutamine, asparagine and lysine), (ii) purine-nucleotide cycle activity, (iii) degradation of nucleic acids, and finally (iv) protein catabolism followed by amino-acid oxidation. Amino-acid oxidation is probably most important from a quantitative point of view, the NH₃/CO₂ ratio being 0.28. The RQ for protein oxidation, however, is 0.97 (Thillart & Kesbeke, 1978) and ammonia excretion will neutralize only some 30 % of the acidity resulting from CO₂ excretion. In fact, the $\dot{M}_{H^+}/\dot{M}_{CO_2}$ ratio expected from lipids and proteins will be about the same, that is 0.7. Thus, even if the swimming coho salmon produced some ammonia, it is very unlikely that ammonia excretion can account for the very low rates of apparent acid excretion observed in condition 3, where $\dot{M}_{H^+}/\dot{M}_{O_2}$ values were reduced to 0.17.

It is possible that swimming fish subjected to acid waters are able to activate metabolic pathways that consume acids to ameliorate the effects of acid accumulation within the body. Gluconeogenesis, for example, will alkalize tissues, as will every pathway that converts acids into fats and sugar. Although these mechanisms are important during rest, it is questionable whether they are activated in swimming fish. It seems more probable that the regulatory enzymes controlling the rates of these proton consuming pathways will be inhibited by a reduction in the energy charge of the cell as the adenylate pool is decreased when the fish swims.

What is apparent from these studies is that CO₂ and hydrogen ion production by the fish need not be matched and that changing internal and external conditions can have a marked and separate effect on hydrogen ion and carbon dioxide excretion. Also, CO₂ is not necessarily excreted in a constant ratio with O₂ uptake according to oxidative metabolism, but appears to be influenced by the acid-base condition of the fish.

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REFERENCES

- BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd Can.* **21**, 1183–1226.
- BRETT, J. R. & ZALA, C. A. (1975). Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. *J. Fish. Res. Bd Can.* **32**, 2479–2486.
- CAMERON, J. N. (1971). A rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**, 632–634.
- DEJOURS, P. (1975). *Principles of comparative Respiratory Physiology*, 253 p. Amsterdam, Oxford and New York: North Holland/American Elsevier.
- HOAR, W. S. & RANDALL, D. J. (1978). Editors, *Fish Physiology: Locomotion*, Vol. 7. New York: Academic Press.
- HEISLER, N. (1980). Regulation of the acid-base status in fish. In *Environmental Physiology of Fishes*, (ed. M. A. Ali), pp. 123–162. New York: Plenum Press.
- HILLABY, B. A. & RANDALL, D. J. (1979). Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd Can.* **36**, 621–629.
- KRUEGER, H. M., SEDDLER, J. B., CHAPMAN, G. A., TINSLEY, I. J. & LOWRY, R. R. (1968). Bioenergetics, exercise and fatty acids of fish. *Am. Zool.* **8**, 119–129.
- MCWILLIAMS, P. G. & POTTS, W. T. M. (1978). The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta*. *J. comp. Physiol.* **126**, 277–286.
- PERRY, S. F., DAVIE, P. S., DAXBOECK, C. & RANDALL, D. J. (1982). A comparison of CO₂ excretion in a spontaneously ventilating, blood perfused trout preparation and saline perfused gill preparations. Contribution of the branchial epithelium and red blood cell. *J. exp. Biol.* **101**, 47–60.
- SMITH, D. G. (1978). Neural regulation of blood pressure in rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* **56**, 1678–1696.
- STRICKLAND, J. D. H. & PARSONS, T. R. (1968). *A practical Handbook of seawater Analysis*. Ottawa: Fish. Res. Bd Can. 311p.
- THILLART, G. V. D. & KESBEKE, F. (1978). Anaerobic production of carbon dioxide and ammonia by goldfish *Carassius auratus*. *Comp. Biochem. Physiol.* **59A**, 393–400.
- ZEIDLER, R. & KIM, H. D. (1977). Preferential hemolysis of post natal calf red cells induced by internal alkalinization. *J. gen. Physiol.* **70**, 385–401.