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THE RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND ION LOSS IN A FRESHWATER FISH

BY RICHARD J. GONZALEZ* AND D. G. McDONALD

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

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Summary

An increase in the functional surface area (FSA) of the gills of freshwater fish facilitates increased oxygen consumption ($\dot{M}_{O_2}$) but also increases ion loss across the gills – a phenomenon that has been termed the osmorespiratory compromise. This study on the rainbow trout confirms that Na^+ loss (J_{out}^{Na}) accelerates with increasing $\dot{M}_{O_2}$ but also shows that the former always exceeds the latter, i.e. the ion/gas ratio (IGR, $J_{out}^{Na}/\dot{M}_{O_2}$) increases. Since an increase in FSA should affect J_{out}^{Na} and $\dot{M}_{O_2}$ equally, the increase in the IGR is attributed to an increase in ion permeability and is thought to arise through opening of paracellular diffusion channels *via* disruption of tight junctions – a conclusion supported by the effects of brief osmotic shock, low external Ca^{2+} concentration and catecholamine infusion on ion losses across the gills. By analogy with extensive studies on 'leaky' epithelia, these treatments disrupt tight junctions by cell shrinkage, by displacement of Ca^{2+} from tight junction surfaces or by increasing intralamellar pressure, respectively. While enforced exercise or handling stress substantially increased the IGR of the trout above routine levels, animals were also capable of reducing IGR to about one-tenth of routine levels. This regulation may, in part, be due to decreasing intralamellar pressure, achieved in part by down-regulation of adrenergic receptors, but there appears to be a significant additional level of control that is probably exerted directly at the tight junctions. Such a degree of control is only possible, however, if animals do not continue to exercise and if Ca^{2+} levels in the water are not reduced. With continued exercise, the IGR returns to routine levels. Consequently, ion losses remain substantially elevated, suggesting that they may ultimately limit maximum sustainable activity. Measurement of the IGR under routine conditions is proposed as a simple means of predicting ion losses during activity and of analyzing the nature of the osmorespiratory compromise.

Introduction

Randall *et al.* (1972) described the basic conflict between gas exchange and ion regulation in the gills of freshwater fish. A large, highly permeable gill membrane

* Present address: Department of Organismal Biology and Anatomy, The University of Chicago, 1025 East 57th Street, Chicago, IL 60637, USA.

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is required for efficient gas transfer, but a small, impermeable epithelium is needed to minimize diffusive ion losses. Any increase in surface area to promote oxygen uptake ($\dot{M}_{O_2}$) would accelerate Na^+ efflux (J_{out}^{Na}), while reductions of surface area to lower ion losses would reduce the ability of the gill to take up oxygen. This phenomenon has been termed the 'osmorespiratory compromise' (Nilsson, 1986).

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] appear to have at least partially solved the problem by utilizing only a fraction of their total gill surface area at rest, when oxygen demand is low, thereby minimizing the area available for diffusive ion loss. Only about 58 % of the secondary lamellae of rainbow trout are perfused at rest (Booth, 1978), and blood flow in the perfused lamellae is shunted through non-exchange pathways (Farrell *et al.* 1980). During exercise, however, oxygen demand increases, and functional surface area is elevated to meet the requirement (Booth, 1979). Consequently, elevated J_{out}^{Na} would be expected. Indeed, Randall *et al.* (1972) and Wood and Randall (1973*a*,*b*) found that active fish had higher rates of ^{22}Na efflux than resting fish.

Numerous studies have examined one or more aspects of this conflict, yet the relationship between J_{out}^{Na} and $\dot{M}_{O_2}$ has not been described or closely examined quantitatively. Our goals, therefore, were to quantify the relationship between oxygen uptake and ion loss in the rainbow trout, to describe what factors determine the nature of the relationship, and to examine some of the physiological implications of this relationship for activity in trout.

Materials and methods

Experimental animals

Rainbow trout (7–16 g for experiments 1–4, 130–180 g for experiment 5) were obtained from Rainbow Springs Fish Hatchery in Thamesford, Ontario, and maintained in for at least 2 weeks in 200 l, cylindrical tanks continuously supplied with dechlorinated, Lake Ontario tap water (1 mmol l^{-1} Ca^{2+}, 0.6 mmol l^{-1} Na^+, 0.3 mmol l^{-1} Mg^{2+}, 0.8 mmol l^{-1} Cl^-) at 18±1 °C. They were fed trout chow *ad libitum* until at least 2 days before the beginning of experiments. They were not fed during any experiments.

Experimental protocol

In this study, oxygen consumption ($\dot{M}_{O_2}$ in nmol g^{-1} min^{-1}) and diffusive Na^+ loss (J_{out}^{Na} in nmol g^{-1} min^{-1}) were investigated under four conditions: during routine activity, during recovery from enforced exercise, during chronic exercise and following intra-peritoneal saline or epinephrine injection. Both stop-flow and flow-through respirometry techniques were employed. A fifth series of experiments examined the relationship of Na^+ efflux and influx to external $[Na^+]$.

Respirometers were, in most instances, supplied with 'Na^+-free' hard water ($[Na^+]$<0.01 mequiv l^{-1}, Ca^{2+} 1 mmol l^{-1}, pH 7.5), and J_{out}^{Na} was evaluated by the appearance of Na^+ in the water. This approach could potentially underestimate

J_{out}^{Na} if external [Na^+] rises sufficiently such that significant amounts of Na^+ uptake (J_{in}^{Na}) occur. However, in practice [Na^+] rarely rose to more than 0.05 mequiv l^{-1} even at the end of trials where Na^+ losses were substantial. At these low levels, J_{in}^{Na}, estimated from uptake kinetic data for this species (Lauren and McDonald, 1987), could not have exceeded 10 % of J_{out}^{Na}. Thus, the underestimate of J_{out}^{Na} was essentially insignificant. 'Na^+-free' hard water was made by addition of $CaCl_2$ to deionized water with pH adjusted to 7.5 with 0.1 mol l^{-1} KOH. Water to flush the respirometers was supplied by gravity feed from a 150 l, recirculating water system. Water samples were analyzed for Na^+ with a Varian model 1275 atomic absorption spectrophotometer, and for P_{O_2} with a Radiometer E5046 P_{O_2} electrode connected to a Radiometer PHM 71 meter.

Experimental series

Routine $\dot{M}_{O_2}$ *and* J_{out}^{Na}

$\dot{M}_{O_2}$ and J_{out}^{Na} were assessed in separate experiments. $\dot{M}_{O_2}$ was measured with a stop-flow procedure. Fish (N=5) were transferred to individual 150 ml cylindrical respirometer chambers supplied with tap water (18°C) at 300 ml min^{-1} and allowed to acclimate to the chambers for 24 h. Care was taken to avoid disturbing the fish before or during the measurement period. $\dot{M}_{O_2}$ was determined by stopping flow to the chambers for a 10 min period. Since the ratio of water mass in the respirometers to fish mass was so small (<10:1) random movements and gill ventilation of the fish were sufficient to keep the water in the chamber well mixed during flow stoppage. Flow stoppage had no discernible effect on the fish (they showed no obvious signs of disturbance). This procedure was repeated for a second 24 h and the $\dot{M}_{O_2}$ values from the two runs were averaged.

J_{out}^{Na} was measured indirectly, by measuring J_{in}^{Na} and assuming that, over the long term, under routine conditions, fish would be in Na^+ balance and thus J_{in}^{Na} would equal J_{out}^{Na}. This approach was adopted because J_{in}^{Na} is easily measured by addition of isotope to water, while measurement of J_{out}^{Na} requires loading the fish with radioactive Na^+ *via* intraperitoneal injection or transferring fish to a high-activity loading bath for several hours. Both methods increase the amount of handling of the fish and could therefore be stressful. Eight fish were placed in a 10 l, black, Lucite box supplied at 300 ml min^{-1} with tap water from a 200 l recirculating system and left undisturbed for 24 h. At the end of that period, flow was stopped to the box containing the fish, and $^{24}NaCl$ was added (specific activity=1.9 kBq l^{-1}). After a 10 min mixing period, a 10 ml water sample was withdrawn for analysis of Na^+ (^{23}Na and ^{24}Na). Another water sample was taken 12 h later, and the fish were removed. The fish were rinsed in tap water for 1 min to remove external ^{24}Na, killed by a blow to the head, weighed and placed in vials for measurement of ^{24}Na activity using a Packard 5000 series gamma counter. At the end of the exposure period, internal ^{24}Na specific activity was estimated at 11.0 % or less of that of the external medium. Consequently, ^{24}Na back flux (i.e. blood to water) was judged to be insignificant.

Recovery from exercise

In these experiments, fish were vigorously exercised by manual chasing in 10 l of aerated water in a cylindrical chamber. After 5 min of exercise, the fish were transferred to individual respirometers where they were held for up to 6 h with simultaneous measurements of $\dot{M}_{O_2}$ and J_{out}^{Na} at regular intervals, by the stop-flow technique. Each measurement period was 10 min in duration. Between measurements, the respirometers were flushed with aerated water at a flow rate of 300 ml min^{-1}.

With this approach the following experiments were conducted.

Exercise control. Fish were exercised in normal tap water, and then transferred immediately to respirometers containing 'Na^+-free' water (1 mmol l^{-1} $CaCl_2$ at pH 7.5). The respirometers were flushed for 1 min, and the initial stop flow period was begun. Between measurements the respirometers were flushed with 'Na^+-free' water.

Recovery in NaCl-free water. This was similar to the exercise control, except that respirometers were supplied with 'NaCl-free' water. 'NaCl-free' water was prepared by adding 1 mmol l^{-1} $CaCO_3$ to deionized water. $CaCO_3$ was solubilized by bubbling with CO_2 for 24 h followed by vigorous aeration for 24 h to drive off excess dissolved CO_2. This approach permitted measurements of both Na^+ and Cl^- efflux. Water [Cl^-] was measured using the mercuric thiocyanate method (Zall *et al.* 1956).

Recovery in 'low-Ca^{2+}' water. Similar to the exercise control, except that, after exercise in tap water, fish were transferred to low-Ca^{2+} water (0.03 mmol l^{-1} $CaCl_2$, Na^+<0.01 mmol l^{-1}) adjusted to pH 7.5.

Exercise in 'high-NaCl' water. Similar to the exercise control, except that fish were exercised in tap water containing 200 mmol l^{-1} NaCl. Upon removal, the fish were rinsed for 1 min in tap water to remove salts adhering to the body, and then placed in the respirometers containing 'Na^+-free' water.

Chronic exercise

Fish were transferred to individual 3.2 l Beamish-type swimming respirometers (Beamish *et al.* 1989). The chambers were supplied with 1.5 l min^{-1} of Na^+-free water from a 600 l recirculated reservoir and fish were forced to swim at about 45 cm s^{-1} or 85 % of U_{crit} for this species (Graham and Wood, 1981). For $\dot{M}_{O_2}$ and J_{out}^{Na} determinations, flow to the respirometers were stopped for 30 min and 10 ml water samples were collected by syringe at end of this period. The procedure was repeated at regular intervals until 6.5 h had elapsed.

Intraperitoneal injection

In this series, three different experiments were performed.

Epinephrine injection. Fish were injected with 1.9 μmol of epinephrine bitartrate in 50 μl of 0.6 % NaCl, and immediately transferred to individual 150 ml

cylindrical respirometers. $\dot{M}_{O_2}$ and J_{out}^{Na} were measured on a flow-though basis with flow adjusted to 30 ml min^{-1}.

Sham injection. Same as epinephrine injection, except that the fish were injected with 50 μl of the saline vehicle only.

Control. Similar to epinephrine injection except that the fish were transferred directly to the respirometers without injection and with a minimum of handling.

Concentration dependence of sodium influx and efflux

The purpose of this experiment was to establish the influence of external [Na^+] on Na^+ influx and Na^+ efflux, particularly the latter. J_{in}^{Na} and J_{out}^{Na} were measured simultaneously at increasing external [NaCl] up to 1.07 mmol l^{-1}. J_{in}^{Na} was measured by the disappearance of ^{24}Na from the medium and J_{out}^{Na} by the appearance of ^{22}Na in the medium. Rainbow trout (130–180 g) were fitted with catheters in the dorsal aorta, transferred to 3 l flux boxes supplied at 0.5 l min^{-1} with tap water and allowed to recover from surgery and handling for 48 h. Larger fish were used for this experiment because smaller ones could not be fitted with catheters. They were then infused by the catheter with ^{22}Na (0.6 kBq g^{-1}), which was allowed to equilibrate overnight. At the start of the experiment, the flux boxes were flushed with NaCl-free tap water for 20 min and then $^{24}NaCl$ (1.7 kBq μmol^{-1}) was added to give a [Na^+] of 0.14 mmol l^{-1}. Water samples for analysis of Na^+ (^{22}Na, ^{23}Na and ^{24}Na) were drawn at time 0 and at 15 min intervals thereafter, and [Na^+] was increased to 0.55 mmol l^{-1} at +2 h, and to 1.07 mmol l^{-1} at +4 h by further addition of $^{24}NaCl$. Blood samples for analysis of Na^+ (^{22}Na, ^{23}Na and ^{24}Na) were drawn at the end of each hour. Backflux correction was possible, since tracer measurements were made on both plasma and water, but was found to be unnecessary.

Statistics

Means±1 S.E.M. are reported throughout. Among-group comparisons were analyzed by repeated-measures analysis of variance (ANOVA) ($P<0.05$). If significant, pairwise comparisons were made using Fisher's PLSD (protected least significant difference).

Results

Routine conditions

Routine $\dot{M}_{O_2}$ of rainbow trout juveniles acclimated to tap water at 18°C was 72.0±2.2 nmol g^{-1} min^{-1} (Table 1). Routine J_{in}^{Na}, averaged over a 12 h period, was 8.8±0.8 nmol g^{-1} min^{-1}. Particular care was taken to ensure that fish were not disturbed during this time, so it is likely that the trout were in Na^+ balance. Consequently, the average J_{out}^{Na} would also be 8.8 nmol g^{-1} min^{-1}. Thus, under routine conditions, trout lost 122.2 pmol Na^+ for each nmol of O_2 absorbed (the ion/gas ratio or IGR; see Figs 1A, 2A).

Table 1. *Sodium efflux (J^{Na}_{out}), oxygen consumption ($\dot{M}_{O_2}$) and the ion/gas ratio ($J^{Na}_{out}/\dot{M}_{O_2}$) of juvenile rainbow trout under routine conditions, over the first 0.5 h of exercise at 85 % of U_{crit} (chronic exercise) and immediately following 5 min of enforced exercise, with various treatments indicated (post-exercise)*

Treatment	N	J^{Na}_{out} (nmol g^{-1} min^{-1})	$\dot{M}_{O_2}$ (nmol g^{-1} min^{-1})	Ion/gas ratio (pmol Na^+ $nmol^{-1}$ O_2)
Routine	8, 5	8.8±0.8	72.0±2.2	122.2†
Chronic exercise	6	45.6±7.6	244.0±22.2	189.1±27.5
Post-exercise				
Control	18	39.7±2.3	200.7±5.4	200.0±12.3
1 mmol l^{-1} $CaCO_3$	5	25.1±1.1*	180.5±2.9	138.9±5.9
Low-Ca^{2+}	5	56.7±7.0*	203.8±12.5	286.6±44.4*
High-NaCl	5	74.9±12.3*	182.0±4.3	412.4±70.1*

Values are means±S.E.

Asterisks indicate significant difference from control values ($P<0.05$).

Body mass=11.5±0.3 g.

† Calculated from the routine J^{Na}_{out} ($N=8$) and $\dot{M}_{O_2}$ values ($N=5$) measured on separate groups of fish.

Recovery from exercise

In fish that were exercised and then transferred to 1 mmol l^{-1} $CaCl_2$ water (control) for recovery, $\dot{M}_{O_2}$ (1–11 min post-exercise) was elevated by slightly less than threefold relative to routine levels, while J^{Na}_{out} was elevated slightly more than fourfold. Consequently, the ion/gas ratio, IGR, rose to 200 pmol $nmol^{-1}$, an increase of 64 % relative to routine levels (Table 1). Virtually identical increases in $\dot{M}_{O_2}$, J^{Na}_{out} and IGR were observed over the first 0.5 h of chronic exercise at 85 % U_{crit} (Table 1).

In fish that were acutely exercised but then transferred to 1 mmol l^{-1} $CaCO_3$, the IGR was only slightly greater than the routine value (Table 1). The elevation in $\dot{M}_{O_2}$ was similar to that during control exercise, but J^{Na}_{out} was stimulated less. Allowing fish to recover in 1 mmol l^{-1} $CaCO_3$ permitted simultaneous measurement of the diffusive efflux of Cl^-. J^{Cl}_{out} was virtually identical to J^{Na}_{out} (26.4±1.0 *vs* 25.1±1.1 nmol g^{-1} min^{-1}, respectively) in these animals.

Exposure to low-Ca^{2+} medium (0.03 mmol l^{-1} $CaCl_2$) following acute exercise led to a significantly greater increase in IGR, 43 % greater than the effects of exercise alone; while exposure to 200 mmol l^{-1} NaCl (1 mmol l^{-1} Ca^{2+}) during exercise gave rise to an even larger increase in IGR, 106 % greater than that caused by the effects of exercise alone. These significant elevations of the IGR were solely the result of stimulated J^{Na}_{out} compared to values for control exercise, since $\dot{M}_{O_2}$ values were not different.

With continued recovery, the IGR of control fish (in 'Na^+-free', 1 mmol l^{-1} $CaCl_2$ water) fell from initially elevated levels to one-tenth of *routine* values by 6 h (Fig. 1A). This decline was due to a marked drop in J^{Na}_{out} below routine levels

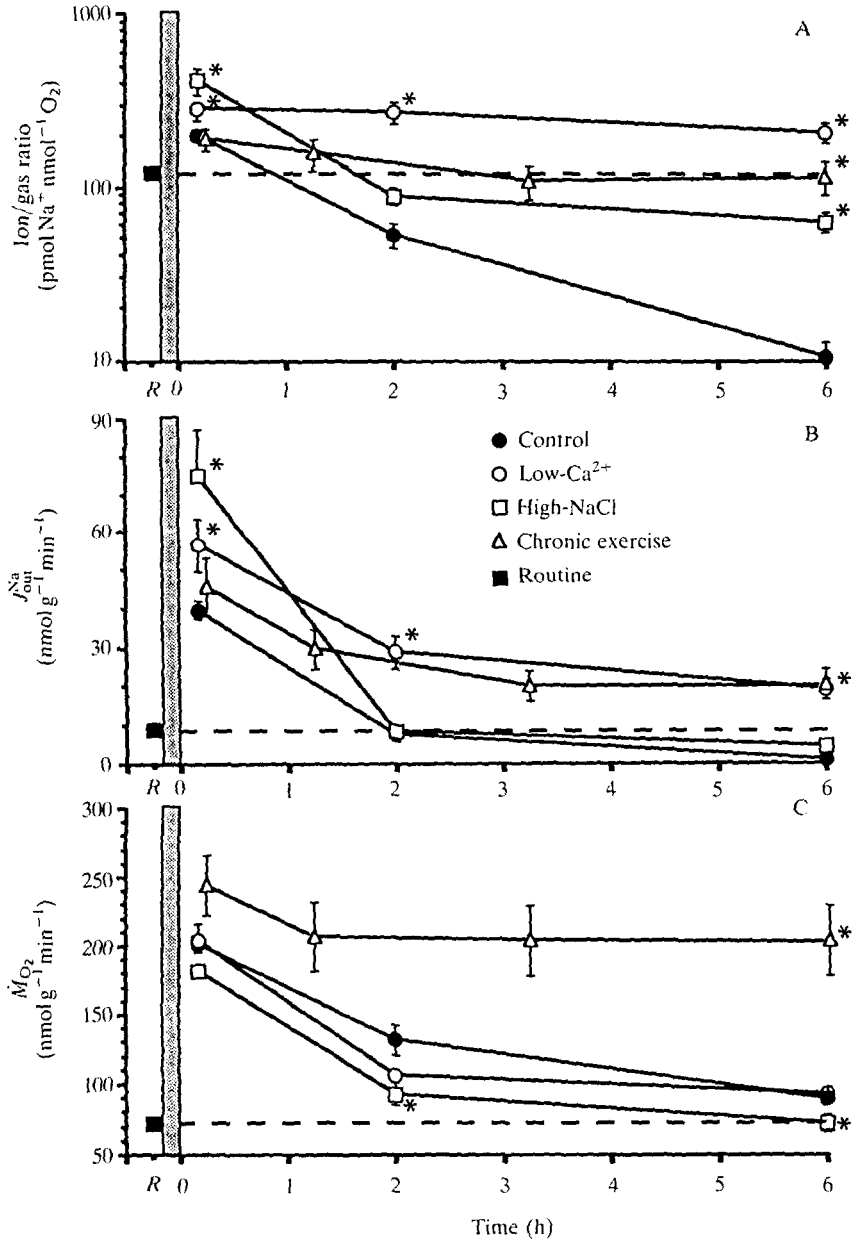

Fig. 1. (A) Ion/gas ratio, (B) Na^+ efflux (J_{out}^{Na}) and (C) oxygen consumption ($\dot{M}_{O_2}$) in juvenile rainbow trout (body mass=11.3±0.3 g) at 18°C. Shaded bar indicates the 5 min period of enforced exercise for controls (N=18), low-Ca^{2+} (N=5) and high-NaCl (N=5). The chronic exercise group (N=6) were exercised continuously from time 0 at 85% of U_{crit}. Controls recovered in 1 mmol l^{-1} $CaCl_2$ (pH 7.5), low-Ca^{2+} fish recovered in 0.03 mmol l^{-1} $CaCl_2$, and high-NaCl fish were exercised in 200 mmol l^{-1} NaCl and recovered in 1 mmol l^{-1} $CaCl_2$. Estimates of routine levels from Table 1 are given for comparison (dashed line). Asterisks indicate means significantly different (P<0.05) from controls. R, routine. Values are mean±S.E.M.

(Fig. 1B) while $\dot{M}_{O_2}$ returned simply to routine levels (Fig. 1C). The effects of brief exposure to 200 mmol l^{-1} NaCl waned quickly, but the IGR was still significantly higher than controls at 6 h (Fig. 1A). Furthermore, recovery in low-Ca^{2+} water was associated with a persistent elevation of the IGR (Fig. 1A). After 2 h, the IGR had not dropped from initial levels, and by 6 h it had declined only slightly. This prolonged elevation in the IGR was due to continued elevation of J_{out}^{Na}, relative to controls (Fig. 1B); $\dot{M}_{O_2}$ in the low-Ca^{2+} group was not different from controls at any time (Fig. 1C).

Chronic exercise

During 6.5 h of exercise at 85 % U_{crit}, $\dot{M}_{O_2}$ declined only slightly from initial values at the start of exercise (Fig. 1C) while J_{out}^{Na} declined to about half of the initial level (Fig. 1B). Consequently, the IGR also stabilized by 3 h at about half of the initial value (Fig. 1A), and by that time was almost identical to the routine IGR (113 *vs* 122 pmol $nmol^{-1}$).

Effects of intraperitoneal injection

Elevations of the IGR, J_{out}^{Na} and $\dot{M}_{O_2}$, similar to those for exercised fish (controls in Table 1), were achieved simply by transferring fish to a flow-through respirometer with a minimum of handling (Table 2, controls). The additional stress associated with handling and injection of saline alone (sham) led to a doubling of the IGR, while epinephrine injection resulted in an almost sevenfold increase in the IGR, 2.5-fold greater than that due to the injection alone. In injected fish, the massive increase in the IGR was due to stimulation of J_{out}^{Na}; $\dot{M}_{O_2}$ was not significantly different from controls.

Although the effect of injection of epinephrine on the IGR was substantial, the disturbance subsided rapidly (Fig. 2A), and the measured values were not different from controls after 60 min. This drop in the IGR was the result of significant reductions in J_{out}^{Na} (Fig. 2B), since the $\dot{M}_{O_2}$ of all three groups did not change significantly over the 120 min test period (Fig. 2C).

Table 2. *Sodium efflux (J_{out}^{Na}), oxygen uptake ($\dot{M}_{O_2}$) and ion/gas ratio ($J_{out}^{Na}/\dot{M}_{O_2}$) for juvenile rainbow trout 10 min after sham (saline) injection and epinephrine injection*

Treatment	*N*	J_{out}^{Na} (nmol g^{-1} min^{-1})	$\dot{M}_{O_2}$ (nmol g^{-1} min^{-1})	Ion/gas ratio (pmol Na^+ $nmol^{-1}$ O_2)
Control	5	40.8±11.7	199.5±20.2	197.8±39.6
Sham	12	84.3±12.5*	171.8±9.3	525.3±94.5*
Epinephrine	5	188.3±29.5*	151.0±19.8	1334.3±275.2*

Controls were transferred to the respirometers with minimum handling.
Values are means±S.E.
Asterisks indicate significant difference from control values ($P<0.05$).
Body mass=12.8±0.6 g.

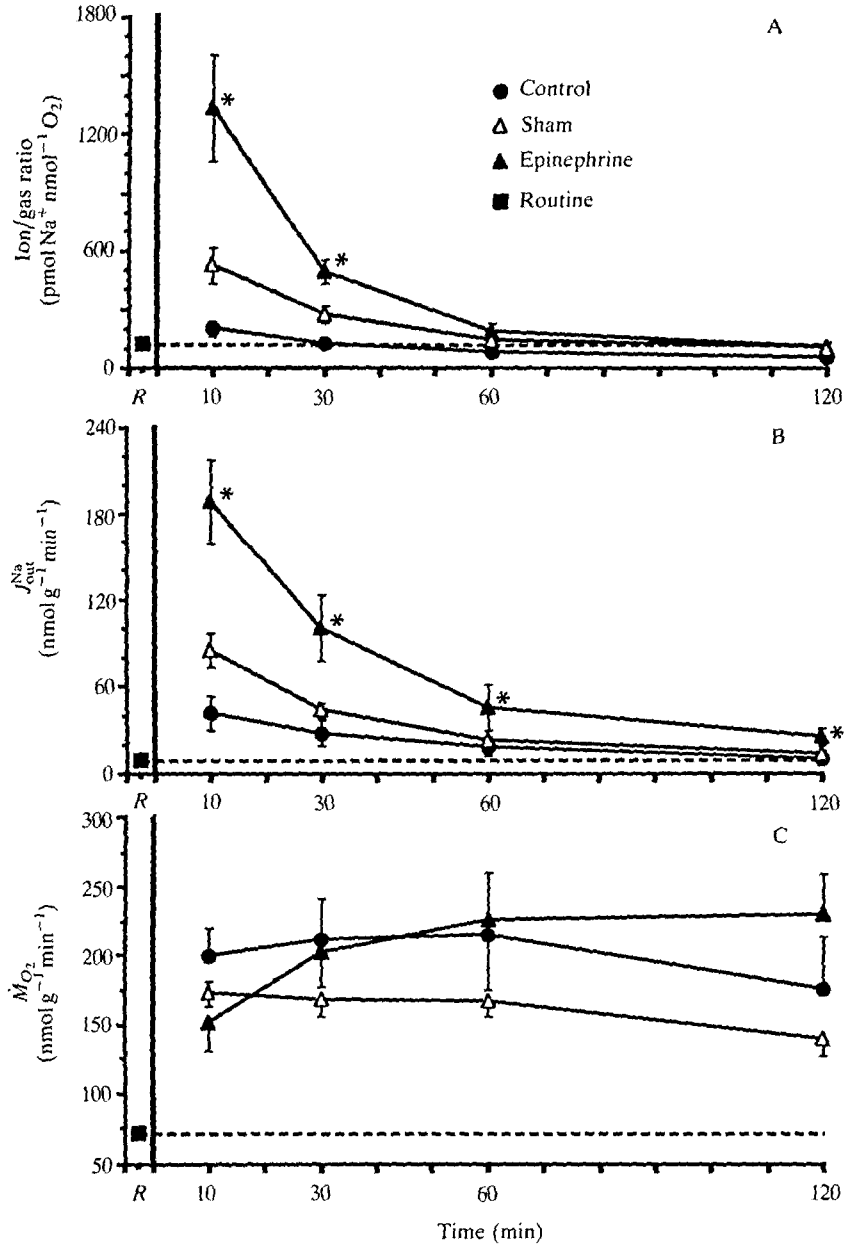

Fig. 2. (A) Ion/gas ratio (IGR), (B) Na^+ efflux (J_{out}^{Na}) and (C) oxygen consumption ($\dot{M}_{O_2}$) in juvenile rainbow trout (body mass=12.8±0.6 g) at 18°C during 120 min of recovery from saline injection (sham, N=12) or epinephrine injection (1.9 μmol epinephrine bitartrate per fish, N=5), or transfer to the respirometer with minimum handling (controls, N=5). The solid line indicates the beginning of the post-injection period. Routine values for IGR, J_{out}^{Na} and $\dot{M}_{O_2}$ from Table 1 are given for comparison (dashed line). Asterisks indicate means significantly different (P<0.05) from controls. R, routine. Values are mean±S.E.M.

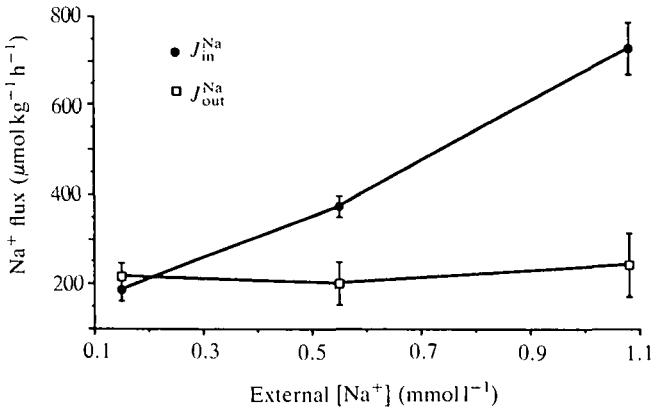

Fig. 3. The effect of external [Na^+] on Na^+ influx (J_{in}^{Na}) and efflux (J_{out}^{Na}) in rainbow trout (150±20 g, N=7) at 18°C. Fish were held at each [Na^+] for 2 h. Values are mean±S.E.M.

Concentration dependence of sodium influx and efflux

One assumption in this study is that Na^+ is lost across the gills by simple diffusion. This would only be the case if there were no Na^+/Na^+ exchange occurring, i.e. carrier-mediated, apparent uptake/loss which requires no energy expenditure. Such exchange, if it exists, should be sensitive to external [Na^+]. As illustrated in Fig. 3, it is apparent that efflux is unaffected by external [Na^+]. Influx rose fourfold with a sevenfold increase in external concentration, but efflux remained constant (Fig. 3).

Discussion

While the focus of this study is on the relationship between O_2 uptake and Na^+ loss at the gills, we have actually measured fluxes across the whole animal. This does not constitute a problem for measurements of $\dot{M}_{O_2}$, since O_2 fluxes across the skin can be considered as insignificant in rainbow trout, but the kidneys do contribute to ion loss. At rest, the contribution is no more than about 10 % of the total losses (McDonald and Wood, 1981). During exercise, increases in cardiac output and blood pressure lead to increased urine flow rate and urinary Na^+ excretion (Wood and Randall, 1973*a*,*b*; Hofmann and Butler, 1979). No direct measurements, of which we are aware, have been made of urinary Na^+ excretion during exercise. However, by using urine flow rates during exercise from Hofmann and Butler (1979) and urinary [Na^+] values from McDonald and Wood (1981), we estimate that urinary Na^+ excretion (on an hourly basis) during exercise is unlikely to exceed 1.0 nmol g^{-1} min^{-1}. Even if the animals were to void their bladders completely in the respirometer as a result of handling, we estimate that the Na^+ loss would be unlikely to exceed 10 nmol g^{-1}, based on values for maximum bladder volume of Curtis and Wood (1991). In comparison, each fish lost at least

370 nmol g^{-1} over the first 10 min in the respirometer. These calculations, therefore, justify the conclusion that the ion losses were occurring predominantly across the gills.

Although we have focused on Na^+ losses, our finding that Cl^- losses were similarly accelerated suggests that the effects upon the gills are not specific to the movements of Na^+ but apply more generally to the movement of all permeable electrolytes across the gills.

The osmorespiratory compromise at the gills

This study shows a considerable interrelationship between $\dot{M}_{O_2}$ and J_{out}^{Na} in the freshwater-adapted rainbow trout; Na^+ loss across the gills increased substantially whenever oxygen consumption increased. Furthermore, the increase in J_{out}^{Na} was usually greater than the increase in $\dot{M}_{O_2}$: anywhere from at least 1.6 times to over 10 times. To understand why Na^+ efflux increased more than O_2 influx it is necessary first to examine the factors that govern their respective diffusion.

Oxygen diffusion across the gills is a function of diffusion distance, O_2 permeation coefficient (i.e. unit O_2 permeability of the epithelium), functional surface area, FSA, which may be as little as 60 % of the total anatomical surface area in resting trout (Booth, 1978), and mean water-to-blood O_2 diffusion gradient. The increase in $\dot{M}_{O_2}$ largely results from increases in the FSA and the O_2 gradient, although there may also be a reduction in diffusion distance through lamellar thinning and, if circulating catecholamines increase, an increase in O_2 permeability (Randall and Daxboeck, 1984). The FSA is thought to increase (Nilsson, 1986) *via* lamellar recruitment (the opening of closed lamellae and the increased perfusion of open lamellae) and intralamellar shunting of blood from basal to more central channels, while the O_2 gradient increases because of a combination of increased water P_{O_2} at the exchange surface (resulting from increased ventilation and reduced physiological dead space) and decreased mean venous P_{O_2} (due to increased tissue O_2 consumption).

Simple Na^+ diffusion across the membrane is a function of the same factors: Na^+ permeation coefficient, diffusion distance, FSA, and mean blood-to-water Na^+ gradient, but also, of course, the electrical gradient, the transepithelial potential (TEP). Clearly, the increase in FSA for O_2 diffusion should also increase Na^+ diffusion but there is no reason to suppose that there would be any corresponding increase in the Na^+ gradient. On the basis of these factors alone, one would therefore expect smaller increases in Na^+ efflux than in O_2 uptake. The fact that the reverse was always true means that Na^+ diffusion was enhanced either by an increase in TEP (i.e. inside more positive) or by an increase in Na^+ permeability or a combination of both. While changes in TEP cannot be ruled out, it is likely they would make a relatively small contribution. For example, according to calculations by Potts and McWilliams (1989), a positive increase of 30 mV in TEP would increase Na^+ efflux by only 1.7 times, whereas Na^+ efflux increased by at least fourfold in the present study. In any case, an increase in TEP of greater than 5 mV, in the absence of any change in the chemistry of the external

environment, is very unlikely (Eddy, 1975; McWilliams and Potts, 1978). Indeed, lowering the external [Ca^{2+}] of the environment from 1.0 to 0.1 mmol l^{-1} causes the TEP to become more negative (by about 10 mV) which would impede Na^+ diffusion (Eddy, 1975; McWilliams and Potts, 1978). Thus, it can be concluded that the two main contributors to the increase in J_{out}^{Na} are an increase in FSA and an increase in Na^+ permeability of the gills, the latter being greater than any increase in O_2 permeability that might be occurring. Furthermore, since the ion/gas ratio (IGR) expresses ion loss per unit O_2 diffusion, it corrects for any influence of FSA on diffusion and can thus be used as an approximate, and conservative, measure of the change in Na^+ permeability of the gills.

The challenge, then, is to explain the increase in Na^+ permeability. At present the location and nature of the ion diffusion channels across the gills are far from certain. In theory, ions may be lost in several ways: by simple diffusion across or between cells (transcellular and paracellular routes) or by more complex routes through the cells by carrier-mediated exchange diffusion or by a leaky pump (Potts and McWilliams, 1989). The latter two pathways would, however, be influenced by changes in external ion concentration. Consequently, our observation of no effect of external [Na^+] on Na^+ efflux would tend to rule these out (Fig. 3). As for the simple diffusion pathways, the structural characteristics of the paracellular pathway in the gills of the freshwater-adapted trout (Sardet *et al.* 1979), in particular those features of the tight junctions (depth and number of strands) that limit the permeability of this route, suggest that the fish gill is characteristic of a typically 'tight' epithelium, i.e. one where the contribution of the paracellular pathway to electrolyte permeability is less than 50 % (Schneeberger and Lynch, 1984). However, the increases in Na^+ permeability observed in the present study in response to handling, exposure to osmotic shock (200 mmol l^{-1} NaCl) or low-Ca^{2+} medium, are more readily explained if they occur largely *via* opening of the paracellular pathway as we have recently argued (McDonald and Prior, 1988; McDonald *et al.* 1989, 1991) rather than through increased transcellular diffusion.

Previous studies of factors affecting paracellular permeability have largely been confined to 'leaky' epithelia, such as mammalian gall bladder and renal proximal tubule (reviewed by Kottra and Frömter, 1983). Nonetheless, very similar treatments to those employed here have been shown in these studies to increase the ion permeability of the paracellular pathway: hypertonicity in the external medium (which causes cell shrinkage and consequent distortion of tight junctions), low external [Ca^{2+}] (which removes Ca^{2+} bound to negative fixed charges on adjacent membranes within the tight junctions) and slight increases in hydrostatic pressure on the vascular side of the epithelium (which also distorts and widens tight junctions). The increase in J_{out}^{Na} following brief exposure to 200 mmol l^{-1} NaCl (Fig. 1B) and during exposure to low a external Ca^{2+} concentration (Fig. 1B) are comparable to the first two of these treatments. Based on increases in the IGR (Fig. 1A) it would appear that permeability was at least doubled by these treatments, relative to that caused by handling/exercise alone.

The analogous response in the gills to a hydrostatic pressure increase would be

an increase in intralamellar pressure. Such an increase is likely to occur whenever there is an increase in cardiac output (and, therefore, in systemic blood pressure) and would be exacerbated by any increase in levels of circulating catecholamines. Amongst the myriad effects of the latter, dilation of afferent lamellar arterioles (Farrell, 1980) and constriction of lamellar efferents (Petterson, 1983) would be particularly important in determining intralamellar pressure.

While a number of studies have now shown that catecholamine levels are not elevated during steady-state aerobic exercise (Ristori and Laurent, 1985; Butler *et al.* 1986; Axelsson and Nilsson, 1986; Hughes *et al.* 1988), catecholamine mobilization is rapid (within 30 s) and substantial (up to 1000-fold above basal levels) whenever fish are acutely handled or otherwise stressed (Mazeaud and Mazeaud, 1981). In the present study, fish were briefly handled while being transferred to respirometers (controls; Fig. 2), forced to swim for 5 min (controls, Fig. 1), handled and injected with saline (shams, Fig. 2) or injected with epinephrine (Fig. 2). Assuming that the first three treatments of this series were progressively more stressful, then this series represents progressively increasing blood catecholamine levels with corresponding increases in IGR relative to resting levels: 1.6 times, 1.6 times, 4.3 times and 10.9 times, respectively (Tables 1 and 2). The epinephrine dose was calculated to provide a blood concentration of 300 nmol l^{-1} (based on a 62 % distribution space; Mazeaud and Mazeaud, 1981), well below the possible levels resulting from severe stress (Mazeaud and Mazeaud, 1981). These findings suggest that the ion permeability of the gills varies in proportion to the catecholamine concentration in the blood, presumably through progressive increases in intralamellar pressure. Perhaps more notable is the magnitude of the effect of catecholamines. For example, the rate of Na^+ loss immediately after epinephrine infusion would approach, if sustained, a rate of 21 % of total body Na^+ per hour! While the beneficial role of catecholamines in preparing the organism to deal with stressful circumstances is well known, it is difficult to imagine the advantages obtained from ion losses of this magnitude.

Regulation of the ion permeability of the gills

Following all treatments, except for low external $[Ca^{2+}]$, there was a rapid reduction in J_{out}^{Na} relative to declining $\dot{M}_{O_2}$, as reflected in a decrease in the IGR (Figs 1 and 2). This suggests a regulated decrease in ionic permeability. If a major contributor to the increase in Na^+ permeability were circulating catecholamines, then it follows that the reduction in Na^+ permeability is at least partly attributable to either declining catecholamine levels in the blood or reduction in catecholamine receptor density (down-regulation). The latter is more likely in view of the fact that the biological half-life of epinephrine is substantially longer than the half-life of its effect; about 60 min (Mazeaud and Mazeaud, 1981) compared to about 15 min for the decline in IGR following epinephrine infusion (Fig. 2). Indeed, in an earlier study of the effects of catecholamines on ionic efflux, McDonald and Rogano (1986) showed that, during a 2 h period of epinephrine infusion, Na^+ and Cl^- efflux, initially elevated about sixfold above resting levels, declined to resting

levels by about 15 min after the start of infusion and remained at or below resting levels for the rest of the infusion period.

It is nonetheless striking that over the longer term the IGR fell to less than 10 % of routine, i.e. pre-disturbance, levels (Fig. 1A, controls). If we assume that under routine conditions catecholamine levels were not elevated, this reduction cannot solely be the result of down-regulation of receptors but must reflect another level of ion permeability control in the gills. In 'leaky' epithelia there is now substantial evidence (reviewed by Schneeberger and Lynch, 1984; Madara, 1988) that such regulation is exerted by intracellular second messengers, cyclic AMP and Ca^{2+}, interacting with cytoskeletal elements that regulate the permeability and perm-selectivity of tight junctions. The possibility that such control exists in the freshwater fish gill has previously been proposed (McDonald *et al.* 1983; McDonald and Rogano, 1986) but the present study provides the most direct evidence to date. While it cannot be stated with any certainty how such control is exerted, the relatively slow time course for reduction in the IGR (Fig. 1A) suggests a hormonal involvement. Rather more clear is the requirement for external Ca^{2+}. When Ca^{2+} levels in the water were very low, the IGR remained high for at least 6 h, even though $\dot{M}_{O_2}$ had fallen to near routine levels (Fig. 1A). This serves to illustrate the critical role played by Ca^{2+} in regulating epithelial permeability in freshwater fish, presumably through its effect on tight junction integrity. It is also interesting to note that the reduction in IGR was much less following brief osmotic shock (Fig. 1A). If one assumes that the increased leak immediately following the osmotic shock was due to opening of tight junctions through cell shrinkage, it follows that the restoration of cell volume is a relatively slow process in the freshwater trout gill. This has important implications to ion balance for fish moving into brackish waters on a seaward migration. Finally, it is apparent that the regulation of ion permeability was not as effective during chronic exercise (Fig. 1A). Although there was a decline in IGR from initially high levels at the start of exercise, it stabilized subsequently at about 90 % of routine IGR, in contrast to the 10 % of routine IGR seen in resting fish. Perhaps the increased cardiac output during exercise and the resulting increase in intralamellar pressure counteract the effects of any regulation of tight junction dimensions.

Significance of ion losses during exercise

These results have important implications for the maintenance of ion balance in active fish. Assuming, conservatively, that the IGR remains at routine levels when trout become more active, then when the animal is respiring at $\dot{M}_{O_2max}$, around 330 nmol g^{-1} min^{-1} at 15 °C for a 10 g rainbow trout (R. W. Wilson and C. M. Wood, unpublished observations), J_{out}^{Na} would approach 40 nmol g^{-1} min^{-1}. Even with Na^+ uptake at V_{max} (9 nmol g^{-1} min^{-1}), the net Na^+ loss would be 30 nmol g^{-1} min^{-1} (1.8 μmol g^{-1} h^{-1}) or about 3 % of the total body Na^+ per hour. Losses of this magnitude would soon cause ionoregulatory difficulties and could be expected to inhibit the continuation of swimming activity. This calculation serves to illustrate, in concrete terms, the nature of the osmorespiratory compromise

and, indeed, suggests that the upper limit to aerobic activity in fish may well be set by the need to defend ion balance, rather than, strictly speaking, by the diffusion and perfusion capacitance of the gas exchange system. Of particular practical significance to future investigations of the osmorespiratory compromise is our finding that the ion/gas ratio increased acutely with exercise but returned to approximately routine levels with continued exercise. This suggests that the IGR, determined easily under conditions of routine activity, would be a useful analytical tool for predicting ion losses during increased activity and for evaluating the influence on the osmorespiratory compromise of such factors as body size, adaptation to various salinities and interspecific differences (e.g. benthic, sluggish species *vs* active, pelagic species).

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