

THE RELATIONSHIP BETWEEN OXYGEN UPTAKE AND ION LOSS IN FISH FROM DIVERSE HABITATS

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Summary

A recent examination of the relationship between O_2 uptake (\dot{M}_{O_2}) and diffusive sodium loss ($J_{\text{out}}^{\text{Na}}$) in a freshwater fish showed that Na^+ losses after exhaustive exercise exceeded those expected on the basis of \dot{M}_{O_2} , probably due to distortion of the paracellular tight junctions (the primary site of diffusive ion loss) and/or glomerular-type filtration caused by increased lamellar pressure. In the present study, an examination of this relationship in nine species of fish from diverse habitats supports this model. Under routine conditions, the rate of Na^+ loss per unit of O_2 consumed (termed the ion/gas ratio or IGR) was similar in all the species tested, averaging $61.6 \text{ pmol Na}^+ \text{ nmol}^{-1} \text{ O}_2$. After exhaustive exercise, the degree to which the IGR of each species increased relative to its routine levels (post-exercise IGR/routine IGR) was exponentially related to the relative rise in \dot{M}_{O_2} , i.e. greater rates of O_2 uptake led to even greater ion losses. Further analysis revealed that, although naturally active species had the lowest proportionate increase in \dot{M}_{O_2} , by virtue of their high routine rates, they had the highest post-exercise rates of O_2 uptake. In fact, there was an inverse correlation between post-exercise IGR and \dot{M}_{O_2} , i.e. species with low \dot{M}_{O_2} values lost more Na^+ per mole of O_2 taken up than did species with high ones. Thus, naturally active species, such as the common and golden shiner, were able to achieve higher rates of O_2 uptake while avoiding high rates of ion loss. Surprisingly, species such as banded sunfish, yellow perch and smallmouth bass did not limit ion loss associated with exercise despite their apparent ability to do so. They demonstrated a strong ability to limit ion losses caused by a brief osmotic shock and by exposure to soft water (both of which distort tight junctions).

Introduction

It has been argued that in gill-breathing organisms increased O_2 consumption will lead to proportionately elevated diffusive ion losses, since both are dependent upon functional surface area (Randall *et al.* 1972). This has been termed the osmorepiratory compromise (Nilsson, 1986). Closer consideration of the components of diffusive oxygen uptake and ion loss suggested, however, that the magnitude of the increased ion loss should be less

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than that of O_2 uptake (Gonzalez and McDonald, 1992). The main components of interest are gill functional surface area and diffusion gradient. Increases in functional surface area with exercise would influence both O_2 uptake and ion loss to the same extent but, although the O_2 gradient across the gill would increase as blood P_{O_2} dropped, there is no reason to expect the ion gradient to change. Upon examination of the relationship in a naturally active fish, the rainbow trout, results at $18^\circ C$ indicated that, after exhaustive exercise, the rise in diffusive sodium efflux was actually disproportionately greater than the elevation in \dot{M}_{O_2} (Gonzalez and McDonald, 1992). The ratio of Na^+ loss to O_2 uptake, termed the ion/gas ratio or IGR, increased by about 60 % relative to levels at rest. This disproportionate increase in ion loss was believed to be largely a consequence of rising intralamellar pressure stretching the paracellular tight junctions and decreasing their resistance to ion diffusion. The increased branchial pressure was probably due to the stressful nature of the exhaustive exercise protocol mediated by the release of catecholamines. Nonetheless, it clearly demonstrated that diffusive ion loss and oxygen uptake were not linked as tightly as was previously thought.

The disproportionate rise in ion loss in rainbow trout raised questions as to the degree of linkage between diffusive ion loss and oxygen uptake in fish in general. Fish inhabit a wide variety of environments and display enormous variation in lifestyles. Different habitats place different degrees of emphasis on energetics (and thus on oxygen uptake) and ion regulation. For instance, the rainbow trout are naturally active fish migrating between the sea and freshwater streams or living their whole life in fast-flowing streams. They can routinely experience exhaustive exercise when migrating upstream or while pursuing prey. In contrast, other species inhabit environments that are far less demanding energetically, but place a premium on control of diffusive ion loss. Certain sunfish, for example, live in swamps and bogs, where the water current is extremely low but the water is acidic. The primary mechanism for survival in such environments is the tight control of branchial ion loss, which is stimulated by protons. The main aim of the present research was to compare the IGR among a variety of species in order to determine whether the relationship between ion loss and oxygen uptake, as seen in the rainbow trout, holds for other species of fish from greatly differing habitats. A second goal was to investigate the permeability characteristics of the gills of these species (by exposing them to osmotic shock and soft water) in order to assess the ability of the species to resist tight junctional distortions that would lead to elevated ion losses.

We examined nine species of fish (five families) that occupy a broad spectrum of habitats, including two cyprinids, the common shiner (*Notropis cornutus*) and the golden shiner (*Notemigonus chryssoleucas*); two salmonids, the lake trout (*Salvelinus namaycush*) and the rainbow trout (*Oncorhynchus mykiss*); the yellow perch (*Perca flavescens*, Percidae); the killifish (*Fundulus heteroclitus*), a cyprinodontid, and three centrarchids, the smallmouth bass (*Micropterus dolomieu*), the black banded sunfish (*Enneacanthus chaetodon*) and the banded sunfish (*Enneacanthus obesus*). The common shiner inhabits fast-flowing streams, whereas the golden shiner is more typically found in lakes, although it does occur in streams (Lee *et al.* 1980). Both are schooling species and active swimmers. Lake trout live in cold northern lakes, while rainbow trout inhabit cold fast-moving headwater streams (Scott and Crossman, 1973). Smallmouth bass and

yellow perch can be found in streams, but are more common in lakes (Scott and Crossman, 1973). Banded and black banded sunfish dwell almost exclusively in shallow densely vegetated swamps with very little current (Sweeney, 1972). The killifish is a euryhaline native to estuarine marshes all along the Atlantic coast of the United States (Lee *et al.* 1980). The lake trout and rainbow trout are considered to be stenothermal cold-water species preferring temperatures of 10 and 14 °C, respectively (Scott and Crossman, 1973; Reynolds and Casterlin, 1979). The remaining species are more eurythermal and inhabit waters that vary widely in temperature (Magnuson *et al.* 1979). As a compromise, all species were tested at 18 °C and the salmonids were re-tested at 10 °C.

Materials and methods

Animals

Common shiners (11.5 ± 0.6 g, $N=20$) and golden shiners (11.5 ± 0.8 g, $N=15$) were purchased from a local bait shop in Hamilton, Ontario. Rainbow trout (11.3 ± 0.3 g, $N=5$) were obtained from Rainbow Springs Fish Hatchery in Thamesford, Ontario. Lake trout (13.8 ± 0.6 g, $N=19$) were acquired from Harwood Fish Culture Station, Ministry of Natural Resources, Harwood, Ontario. Yellow perch (13.0 ± 0.3 g, $N=25$) were purchased from Coolwater Farms, Pickering, Ontario. Smallmouth bass (15.4 ± 0.4 g, $N=30$) were purchased from Aqua Research Ltd, North Hatley, Quebec, and banded (4.8 ± 0.2 g, $N=25$) and black banded (6.7 ± 0.3 g, $N=5$) sunfish were netted from ponds in Burlington Co., New Jersey. Killifish (5.1 ± 0.3 g, $N=5$) were collected from estuarine marshes in the vicinity of Antigonish, Nova Scotia. The fish were held in 101 black Lucite boxes connected to a 1501 recirculating flow-through system, containing dechlorinated Hamilton tap water ($1 \text{ mmol l}^{-1} \text{ Ca}^{2+}$, $0.6 \text{ mmol l}^{-1} \text{ Na}^{+}$, $0.3 \text{ mmol l}^{-1} \text{ Mg}^{2+}$, $0.8 \text{ mmol l}^{-1} \text{ Cl}^{-}$) at 18 °C. The fish were fed *ad libitum* until at least 24 h before the start of experiments. All fish were held at 18 °C. In addition, a group of rainbow trout and lake trout was acclimated to 10 °C for 4 weeks. Additional data from a recent report (Gonzalez and McDonald, 1992) on rainbow trout at 18 °C were utilized for comparison.

Experimental protocol

Stop-flow respirometry was used to measure simultaneously diffusive Na^{+} loss ($J_{\text{out}}^{\text{Na}}$) and O_2 uptake (\dot{M}_{O_2}) except for the routine estimates that were done separately (see below). Individual fish were placed in 150 ml cylindrical respirometers supplied with 'Na⁺-free' hard water ($<0.01 \text{ mmol l}^{-1} \text{ Na}^{+}$, $1 \text{ mmol l}^{-1} \text{ Ca}^{2+}$, pH 7.5) flowing at a rate of 300 ml min^{-1} . Water for the respirometers was supplied by gravity feed from a 1501 recirculating system. At the beginning of a measurement period, a 10 ml water sample was removed from the respirometer and flow was stopped. After 10 min, another water sample was taken, and flow was restored. The respirometry chambers were small enough that fish ventilation was sufficient to mix the contents of the chamber during the stop-flow period. Water samples were analyzed for P_{O_2} with a Radiometer E5046 P_{O_2} electrode connected to a Radiometer PHM 71M, and for Na^{+} with a Varian model 1275 atomic absorption spectrophotometer. \dot{M}_{O_2} and $J_{\text{out}}^{\text{Na}}$ were calculated from changes in the water P_{O_2} and Na^{+} concentrations, respectively.

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

Routine \dot{M}_{O_2} and J_{out}^{Na} were measured separately in order to minimize manipulation of the fish and, thereby, give a better indication of resting values. In order to measure routine \dot{M}_{O_2} , fish were placed in individual 150 ml respirometers supplied with tap water at 300 ml min^{-1} and allowed to recover for at least 6 h before the 10 min measurement period. In a previous study, \dot{M}_{O_2} was shown to return to resting levels by 6 h (Gonzalez and McDonald, 1992). Routine IGR was estimated by dividing average J_{out}^{Na} by average \dot{M}_{O_2} . Consequently, there was only one value for each species.

Routine J_{out}^{Na} was estimated indirectly by measuring Na^+ influx (J_{in}^{Na}) isotopically over 12 h at rest. It was assumed that, over the long term, the fish would be in Na^+ balance and therefore $J_{in}^{Na} = J_{out}^{Na}$. Several studies have shown this to be a reasonable assumption (McDonald *et al.* 1980, 1983; McDonald and Wood, 1981). This approach was preferred because it is easily accomplished by addition of isotope to the water without disturbing the fish, whereas direct measurement of J_{out}^{Na} requires loading the fish with isotope, either by injection or by placement in a high-activity bath. Both increase the stress experienced by the fish.

Fish were placed in aerated, 10 l Lucite boxes connected to a 200 l recirculating system containing tap water (18°C) and allowed to acclimate overnight. At the beginning of the flux measurement, flow to the boxes was stopped and $^{24}\text{NaCl}$ (1.9 kBq l^{-1}) was added. After a 10 min mixing period, a 10 ml water sample was removed from each box. The fish were left undisturbed for 12 h, at which time another water sample was drawn. The fish were removed, rinsed in tap water for 1 min, killed, weighed and placed in assay vials. The water samples and fish were assayed for gamma radiation using a Packard 5000 series gamma counter. Water samples were analyzed for Na^+ with the atomic absorption spectrophotometer. J_{in}^{Na} was calculated from the number of counts accumulated by the fish and the average specific activity of the test water.

Recovery from exercise

Controls. All the species were vigorously exercised for 5 min by chasing in 10 l cylindrical chambers filled with tap water (18°C). They were then transferred to respirometers containing ' Na^+ -free' water ($1 \text{ mmol l}^{-1} \text{ CaCl}_2$, pH 7.5). Simultaneous measurements of \dot{M}_{O_2} and J_{out}^{Na} were made immediately after exercise and at regular intervals for 6 h.

Soft water exposure. Common shiners, golden shiners, smallmouth bass, yellow perch and banded sunfish were exercised in tap water (18°C) in the same way as the controls, and were then placed in respirometers supplied with ' Na^+ -free', low- Ca^{2+} ($0.03 \text{ mmol l}^{-1} \text{ CaCl}_2$, pH 7.5) water for periodic simultaneous measurements of J_{out}^{Na} and \dot{M}_{O_2} .

Brief osmotic shock. Common shiners, golden shiners, smallmouth bass, yellow perch and banded sunfish were exercised in tap water supplemented with $200 \text{ mmol l}^{-1} \text{ NaCl}$ (18°C) for 5 min. They were rinsed for 1 min to remove salts adhering to their body and

then placed in the respirometers containing 'Na⁺-free' water for simultaneous measurements of J_{out}^{Na} and \dot{M}_{O_2} .

Salmonids at 10 °C. The two salmonid species were acclimated to 10 °C tap water for 4 weeks. J_{out}^{Na} and \dot{M}_{O_2} were measured as in controls, but in a system maintained at 10 °C.

Statistical analysis

All data are reported as means \pm one standard error. Means were compared using analysis of variance (ANOVA, overall $P \leq 0.05$) with multiple comparisons (Scheffe test) if ANOVA proved significant.

Results

Routine measurements

The temperature at which the two salmonid species were tested greatly affected how they responded to exercise and, since we only had routine measurements at 18 °C, they are omitted from this section. There were significant differences in routine \dot{M}_{O_2} among the warm-water species tested (Table 1). The two shiner species had the highest values, smallmouth bass and yellow perch values were intermediate, and the banded sunfish had the lowest values (routine measurements were not made for the killifish or black banded sunfish). During the 12 h while J_{in}^{Na} was being measured, care was taken to ensure that the fish were not disturbed, so it is likely that they were in Na⁺ balance. Consequently, it can be assumed that $J_{in}^{Na} = J_{out}^{Na}$. On this basis, the golden shiners exhibited the highest J_{out}^{Na} and the banded sunfish the lowest, while the common shiner, smallmouth bass and yellow perch showed intermediate values (Table 1). Despite these interspecific differences in routine \dot{M}_{O_2} and J_{out}^{Na} , the ion/gas ratio (IGR) only ranged from 40.5 pmol Na⁺ nmol⁻¹ O₂ for the common shiner to 90.1 pmol Na⁺ nmol⁻¹ O₂ for the golden shiner (Table 1). For comparison, IGRs for the same five species after 6 h of recovery in 'Na⁺-free' water from enforced exercise are also presented in Table 1. IGRs of four of the five species had either

Table 1. Routine Na⁺ efflux (J_{out}^{Na}), O₂ consumption (\dot{M}_{O_2}), ion/gas ratio ($J_{out}^{Na}/\dot{M}_{O_2}$; IGR) and IGR after 6 h of recovery from exercise at 18 °C

Species	N	Routine J_{out}^{Na} (nmol g ⁻¹ min ⁻¹)	N	Routine \dot{M}_{O_2} (nmol g ⁻¹ min ⁻¹)	IGR (pmol Na ⁺ nmol ⁻¹ O ₂)		
					Routine	N	Recovery
Common shiner	11	4.8 \pm 0.5*	5	118.6 \pm 13.5*	40.5	5	32.2 \pm 5.6
Golden shiner	10	12.7 \pm 1.3†	5	141.0 \pm 16.6*	90.1	5	33.8 \pm 7.4
Smallmouth bass	8	3.5 \pm 0.3‡	10	74.7 \pm 8.6†	46.9	10	11.4 \pm 2.3
Yellow perch	5	4.2 \pm 0.4‡	5	72.7 \pm 5.7†	57.8	5	59.1 \pm 11.6
Banded sunfish	11	3.0 \pm 0.3‡	10	41.3 \pm 3.4‡	72.6	10	116.2 \pm 16.6

Routine measurements of J_{out}^{Na} and \dot{M}_{O_2} were made on separate groups of fish. Recovery IGR measurements were made on the same fish.

Values are means \pm S.E.M. Values within columns with different symbols were found to be significantly different ($P \leq 0.05$).

Table 2. Na^+ efflux ($J_{\text{out}}^{\text{Na}}$), O_2 consumption (\dot{M}_{O_2}), and ion/gas ratio ($J_{\text{out}}^{\text{Na}}/\dot{M}_{\text{O}_2}$; IGR) immediately following 5 min of enforced exercise at 18 °C

Species	<i>N</i>	$J_{\text{out}}^{\text{Na}}$ (nmol g ⁻¹ min ⁻¹)	\dot{M}_{O_2} (nmol g ⁻¹ min ⁻¹)	IGR (pmol Na ⁺ nmol ⁻¹ O ₂)
Common shiner	5	9.3±0.8*	191.8±15.0*	50.3±6.8*
Golden shiner	5	18.1±1.2*†	192.6±10.4*	94.9±8.0*†
Smallmouth bass	10	21.3±2.3†	152.0±4.6†	141.0±14.8†
Yellow perch	5	21.9±1.5†‡	133.4±4.6†	164.2±10.2†
Killifish	5	22.4±4.5†‡	132.2±5.0†	165.2±27.2†
Black banded sunfish	5	22.4±1.6†	113.8±5.8‡	198.9±21.3†
Banded sunfish	10	28.9±3.7‡	90.1±3.6‡	317.6±35.7‡

Values are means ± S.E.M. Values within columns with different symbols were found to be significantly different ($P \leq 0.05$).

returned to or fallen below routine levels. Recovery of IGR by the smallmouth bass was particularly striking: it declined to about one-quarter of the routine value. Only the IGR of banded sunfish failed to return to routine levels.

Post-exercise measurements

Immediately after exercise in tap water (18 °C) there were significant differences in \dot{M}_{O_2} , $J_{\text{out}}^{\text{Na}}$ and IGR among the species tested (Table 2). Common and golden shiners had the highest \dot{M}_{O_2} values, followed by the smallmouth bass, yellow perch and killifish whose values were about 25 % lower than those of the shiners. The black banded and banded sunfish had the lowest values; the \dot{M}_{O_2} of the banded sunfish was less than half that of the two shiner species. Conversely, common shiners had the lowest $J_{\text{out}}^{\text{Na}}$, and golden shiner, smallmouth bass, yellow perch, killifish and black banded sunfish all had values about twice as high. Banded sunfish had the highest value, losing Na⁺ at three times the rate of the common shiner. Consequently, there were significant differences in IGR among the species (Table 2). Common and golden shiners had the lowest post-exercise IGRs. The yellow perch, smallmouth bass, killifish and black banded sunfish had significantly higher IGRs than the shiners, and the banded sunfish exhibited the highest IGR (six times greater than that of the common shiner).

The temperature in which rainbow trout and lake trout were tested significantly affected \dot{M}_{O_2} and $J_{\text{out}}^{\text{Na}}$, but it had a much greater influence on $J_{\text{out}}^{\text{Na}}$ than on \dot{M}_{O_2} (Table 3). For both species, exercise in water at 18 °C increased \dot{M}_{O_2} by 20–25 % relative to the values at 10 °C, but raised $J_{\text{out}}^{\text{Na}}$ by about 150 %. As a result, the IGRs of the two salmonids at 18 °C were about double the values at 10 °C.

For the five species for which we had both post-exercise and routine data, the increase in post-exercise $J_{\text{out}}^{\text{Na}}$, relative to routine levels, was related to the relative increase in \dot{M}_{O_2} (Fig. 1A). $J_{\text{out}}^{\text{Na}}$ appears to increase exponentially with the increase in \dot{M}_{O_2} . Similarly, the increase in post-exercise IGR appeared to be dependent upon the relative increase in \dot{M}_{O_2} in an exponential manner (Fig. 1B). In contrast, the actual post-exercise IGR was inversely related to \dot{M}_{O_2} for the seven warm-water species at 18 °C and the two salmonids at 10 °C, i.e. species with higher \dot{M}_{O_2} values had lower IGRs (Fig. 2). It is interesting to

Table 3. Na^+ efflux (J_{out}^{Na}), O_2 uptake (\dot{M}_{O_2}), and ion/gas ratio ($J_{out}^{Na}/\dot{M}_{O_2}$; IGR) after enforced exercise at 18 °C and 10 °C for lake trout and rainbow trout

Temperature (°C)	N	J_{out}^{Na} (nmol g ⁻¹ min ⁻¹)	\dot{M}_{O_2} (nmol g ⁻¹ min ⁻¹)	IGR (pmol Na ⁺ nmol ⁻¹ O ₂)
Lake trout				
18	10	67.3±6.8	145.0±5.9	477.6±60.3
10	5	27.3±2.3*	114.0±4.2*	239.1±14.1*
Rainbow trout				
18†	18	39.7±2.3	200.7±5.4	200.0±12.3
10	5	16.3±1.9*	167.9±4.7*	97.5±11.9*

Values are means ± S.E.M. Asterisks indicate a significant difference from 18 °C values ($P \leq 0.05$).

†Data from Gonzalez and McDonald (1992).

Table 4. The effects of brief osmotic shock (5 min in 200 mmol l⁻¹ NaCl) during enforced exercise and soft water (0.03 mmol l⁻¹ CaCl₂) after exercise on J_{out}^{Na}

Species	J_{out}^{Na} (nmol g ⁻¹ min ⁻¹)		
	Control	Brief osmotic shock	Soft water
Common shiner	9.3±0.8	49.1±5.7*	34.5±5.2*
Golden shiner	18.1±1.2	87.7±7.4*	24.8±2.4
Smallmouth bass	21.3±2.3	46.5±5.9*	28.7±2.9
Yellow perch	21.9±1.5	52.7±3.7*	17.7±1.4
Banded sunfish	28.9±3.7	42.0±7.2	18.6±2.0*

Control fish were exercised and recovered in tap water (0.6 mmol l⁻¹ Na⁺, 1 mmol l⁻¹ Ca²⁺).

Values are means ± S.E.M. $N=5$ for the common shiners, golden shiners and yellow perch. $N=10$ for smallmouth bass and banded sunfish.

Asterisks indicate a significant difference from control values ($P \leq 0.05$).

note that the values for the salmonids tested at 18 °C lay far above the regression line, indicating that they had much higher J_{out}^{Na} values than predicted from their \dot{M}_{O_2} values (data not included in Fig. 2).

Soft water

Recovery in soft water (0.03 mmol l⁻¹ Ca²⁺) from enforced exercise had no effect on \dot{M}_{O_2} , relative to controls, in the species tested (data not shown). However, the J_{out}^{Na} of two species was immediately sensitive to soft water exposure (Table 4), although in opposite ways: J_{out}^{Na} of the common shiner more than tripled, relative to control values, while that of the banded sunfish actually dropped by one-third. During continued exposure to soft water, the responses of the species could be divided into two categories (an example of each is presented in Fig. 3). In the first category, exemplified by the common shiner, J_{out}^{Na} did not recover during the 6 h exposure period (Fig. 3A). The golden shiner was included in this category. While the rise in J_{out}^{Na} of the golden shiner was not significant initially, it failed to decline as rapidly as controls; consequently, it was significantly higher after

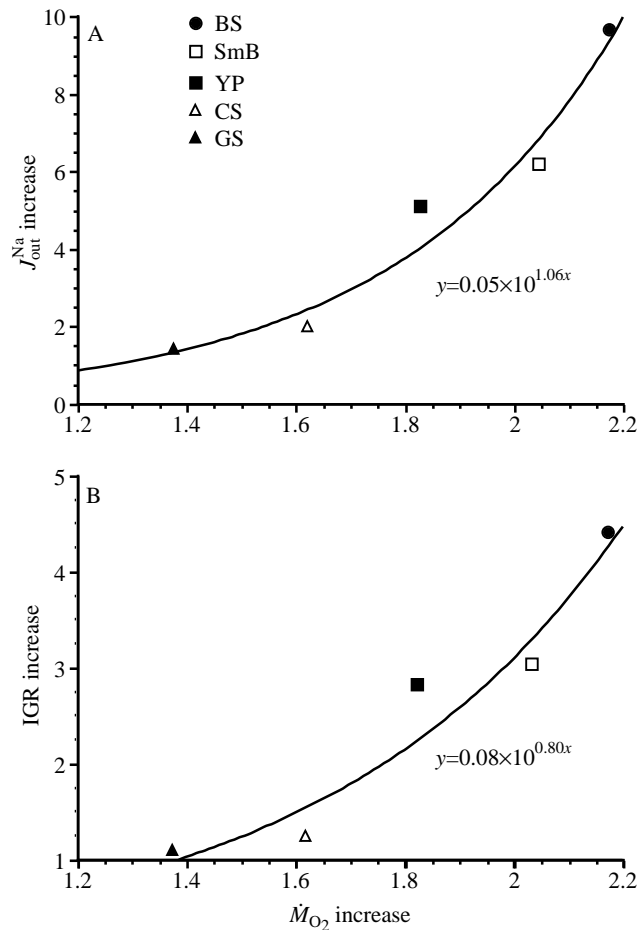


Fig. 1. (A) The relationship between the relative increase in sodium efflux (post-exercise J_{out}^{Na} /routine J_{out}^{Na}) and the relative increase in \dot{M}_{O_2} (post-exercise \dot{M}_{O_2} /routine \dot{M}_{O_2}) for five species of freshwater fish. (B) The relationship between the relative increase in ion/gas ratio (post-exercise IGR/routine IGR) and the relative increase in \dot{M}_{O_2} for five species of freshwater fish. BS, banded sunfish; YP, yellow perch; SmB, smallmouth bass; GS, golden shiner; CS, common shiner. All species were tested at 18 °C.

2 and 6 h. In the second category, as exemplified by the banded sunfish (Fig. 3B), J_{out}^{Na} did not differ from control values after 2 h. The smallmouth bass and yellow perch fell into this category.

Brief osmotic shock

A brief osmotic shock (5 min of exposure to 200 mmol l⁻¹ NaCl) during exercise had no effect on \dot{M}_{O_2} , relative to controls exercised in tap water, in the species tested (data not shown). In contrast, J_{out}^{Na} of every species, except the banded sunfish, was significantly elevated (Table 4). The two shiner species exhibited the greatest proportionate increases: J_{out}^{Na} of both species were about five times greater than control values. J_{out}^{Na} of smallmouth bass and yellow perch were about 2.5 times greater than control values.

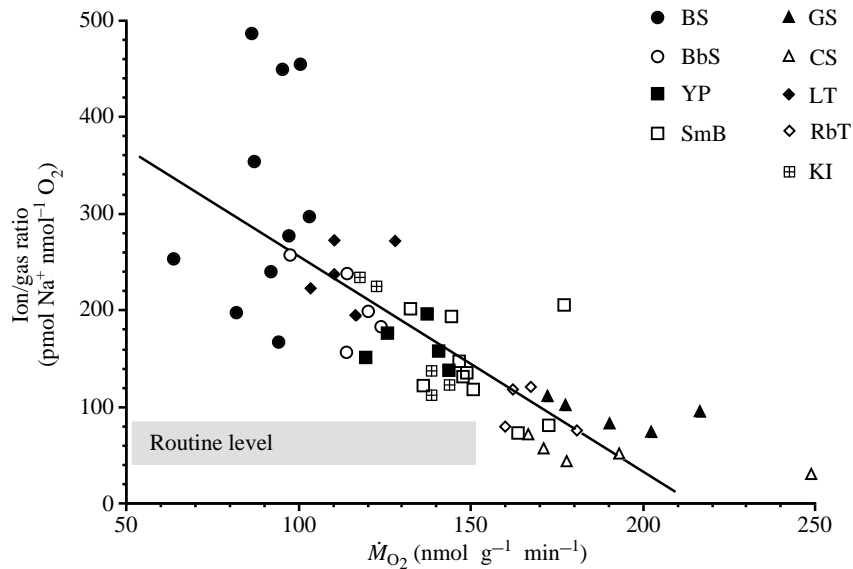


Fig. 2. The relationship between post-exercise ion/gas ratio and \dot{M}_{O_2} for nine species of fish. BS, banded sunfish; BbS, black banded sunfish; YP, yellow perch; SmB, smallmouth bass; GS, golden shiner; CS, common shiner; KI, killifish; LT, lake trout; RbT, rainbow trout. Rainbow trout and lake trout were tested at 10 °C. All other species were tested at 18 °C. $IGR = 465.3 - 2.1\dot{M}_{O_2}$; $r^2 = 0.58$; $P < 0.001$.

Discussion

Routine levels

For the warm-water species tested in this study (common and golden shiners, smallmouth bass, yellow perch and banded sunfish), the routine IGRs at 18 °C were similar (given that we only had single estimates for each species and the magnitude of rise after exhaustive exercise) and averaged about $61.6 \text{ pmol Na}^+ \text{ nmol}^{-1} O_2$ (Table 1). This uniformity is remarkable given the almost fourfold range in routine \dot{M}_{O_2} values in these species (Table 1). Why is the routine IGR not even lower, since it can be argued that fish would benefit from minimizing ion losses given that any ion lost must be replaced (an energy-requiring process) in order to maintain equilibrium? We do find lower values for IGR when, for example, animals are recovering from exercise in 'Na⁺-free' water (see smallmouth bass in Table 1), indicating that the potential exists for further restricting ion losses without compromising gas exchange. Thus, it is clear that ion losses are not minimized at rest. We suggest that, while further reductions in the IGR might seem to be energetically beneficial, they may actually compromise other gill functions such as acid–base regulation and nitrogenous waste excretion. McDonald *et al.* (1991a) have recently argued that a certain degree of ionic permeability has been incorporated into the design of the gills of freshwater fish in order to produce higher rates of ion uptake and, consequently, Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchange. This ionic permeability then either creates the conditions for higher active excretion of acid–base equivalents or plays a direct role in transferring acid–base equivalents by permitting their outward leak along

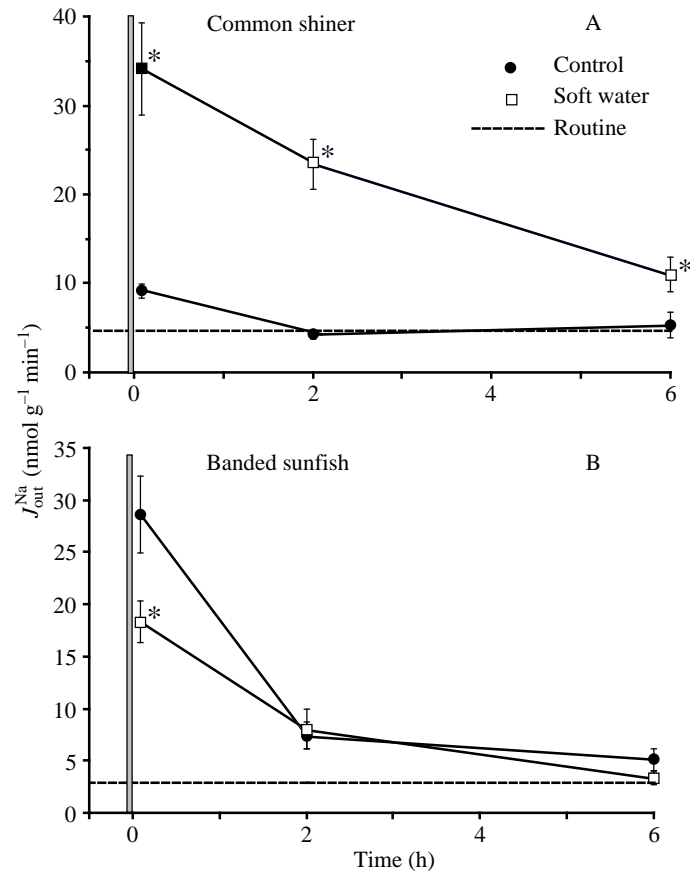


Fig. 3. Recovery of Na^+ efflux (J_{out}^{Na}) of the common shiner (A) ($N=5$ for both treatments; wet mass 11.5 ± 0.6 g) and banded sunfish (B) ($N=10$ for both treatments; wet mass 4.8 ± 0.2 g) from 5 min of enforced exercise. The shaded column indicates the exercise period. Control fish were exercised and allowed to recover in tap water ($1 \text{ mmol l}^{-1} \text{ Ca}^{2+}$). Fish exposed to soft water were exercised in tap water and allowed to recover in water containing $0.03 \text{ mmol l}^{-1} \text{ Ca}^{2+}$. Values are means \pm S.E.M. Asterisks indicate a significant difference from control values ($P < 0.05$, ANOVA and Sheffe multiple comparison test).

diffusion gradients. A similar argument applies to the excretion of nitrogenous wastes since, under routine circumstances, a large fraction of the total ammonia excretion appears to take place by $\text{Na}^+/\text{NH}_4^+$ exchange (McDonald *et al.* 1989).

Post-exercise IGR and O_2 uptake

In our earlier study on gas and ion exchange in the rainbow trout (Gonzalez and McDonald, 1992), we showed that IGR rose above routine levels after exhaustive exercise and proposed that this was caused by elevation of intralamellar blood pressure. The elevated pressure (brought about by an increased cardiac output and changes in pre- and postbranchial vascular resistance; Farrell, 1980; Pettersson, 1983) would either distort and widen tight junctional seals, making them more permeable to ions, or cause

ion losses *via* glomerulus-type ultrafiltration through the tight junctions. In either case, the result would be an increased ion permeability of the gills relative to gas permeability, i.e. an increase in the IGR.

In the present study, we found that the greater the increase in \dot{M}_{O_2} relative to routine levels for a given species, the greater the increase in the relative rate of ion loss (Fig. 1A,B). For example, the IGR of banded sunfish, whose post-exercise \dot{M}_{O_2} rose 2.2 times above their routine levels after exercise, rose more than fourfold. In contrast, the IGR of golden shiners, whose post-exercise \dot{M}_{O_2} rose only 1.4 times above their routine levels, remained virtually unchanged. These results are consistent with the notion of increased ion loss due to elevated blood pressure. Elevations in \dot{M}_{O_2} above routine levels, which are achieved by further increases in intralamellar blood pressure, lead to distortion of tight junctions and/or ultrafiltration and increasing ion losses.

Golden and common shiners had the highest post-exercise \dot{M}_{O_2} values of all the species tested (more than double that of the banded sunfish; Fig. 2) yet, by virtue of their high routine \dot{M}_{O_2} values, they experienced the least relative increase in \dot{M}_{O_2} (1.4 and 1.6 times routine values, respectively). Thus shiners, naturally active stream-dwellers, are able to achieve higher rates of oxygen consumption without the high rates of ion loss. To put it another way, they can achieve high rates of oxygen uptake before ion losses become prohibitive. This would seem to be an important mechanism for maintaining ion balance in a species that is frequently exercising exhaustively. In contrast, the moderate post-exercise \dot{M}_{O_2} of the sunfish was achieved through a greater elevation (2.2 times) of the routine level, and was considerably more costly in terms of ion balance.

Equally significant is the observation that a single straight line can be fitted to the post-exercise IGR and \dot{M}_{O_2} data from nine different species (two salmonid species at 10 °C, the remaining species at 18 °C). This observation, coupled with the previous one, strongly suggests that, although each species has a unique solution to the ionoregulatory problem facing it, those solutions are part of a continuum. The fact that values for all species fit on the same line indicates similar branchial adjustments (increased lamellar pressure) by which increases in \dot{M}_{O_2} are achieved. However, the wide variability in routine and post-exercise \dot{M}_{O_2} points to interspecific differences in some component of the O_2 uptake system. Diffusive uptake of O_2 is a function of branchial surface area and P_{O_2} gradient across the gill as well as of O_2 permeability of the branchial membrane. Of these three variables, the one most likely to differ among the species is the last one. Although there is a general trend for greater lamellar surface area in more active fish species (Hughes, 1984), previous studies have shown that banded sunfish, yellow perch, smallmouth bass and common shiner do not differ significantly in total lamellar surface area or diffusion distance (McDonald *et al.* 1991b). It does not seem likely that the P_{O_2} of blood entering the gills, which is typically below 5 kPa, could differ sufficiently among the species to explain the large differences in rate of uptake. It seems more likely that there is a trend for increased branchial O_2 permeability from banded sunfish to shiners. High O_2 permeability would allow the shiners to achieve higher rates of oxygen consumption without the high ion loss rates resulting from elevated blood pressure.

The lack of control of ion losses associated with elevated O_2 uptake by banded sunfish and other less active species is somewhat surprising. Limiting the increase in IGR could

be achieved either by strengthening the tight junctions, so that they are more resistant to strain, or by reducing the blood pressure elevations that cause the distortion. While limiting blood pressure elevations appears to be out of the question, strengthening of tight junctions seems likely. Our data indicate that banded sunfish, bass and perch do indeed have tight junctions resistant to disturbance, while those of shiners are susceptible. The brief osmotic shock and soft water exposure, which increase electrolyte permeability of the gills either through cellular shrinkage (brief osmotic shock) or by leaching Ca^{2+} from tight junctional proteins (Gonzalez and McDonald, 1992), had greatest effect on $J_{\text{out}}^{\text{Na}}$ of the shiner species (Table 4). In comparison, smallmouth bass and yellow perch showed less of a response to brief osmotic shock and no response to soft water. Banded sunfish, in particular, exhibited a remarkable ability to control tightly branchial ion permeability, immediately reducing $J_{\text{out}}^{\text{Na}}$ upon exposure to soft water (Table 4). In previous studies, these species have repeatedly demonstrated the ability to limit branchial electrolyte permeability during exposure to such conditions as low pH (Gonzalez and Dunson, 1987, 1989a,b; Freda and McDonald, 1988). It is safe, therefore, to conclude that the tight junctions of the sunfish, bass and perch are very resistant to disruptive forces, while those of the shiners are much less so. Nonetheless, banded sunfish, perch and bass all experience elevated IGRs after exercise relative to the shiners, and banded sunfish had by far the highest IGR. Given their demonstrated ability to control branchial ion permeability in less active fish, why do they allow $J_{\text{out}}^{\text{Na}}$ to rise at all?

We can suggest two possible explanations, one physiological and one ecological. As discussed previously, diffusive efflux may play either a direct or an indirect role in acid–base regulation. If the inactive species experienced a larger acid–base disturbance with exercise than the more active species, then the requirements for acid–base regulation might outweigh the benefits of limiting ion losses. In contrast, ion losses might not be controlled by such species, simply because there is no need. These species, which inhabit weedy ponds and bogs, are much less likely to experience regular bouts of burst activity that would result in significant ion losses. It seems possible that ion losses are not controlled during exercise because they are not likely to lead to sizable ion depletion. Given their demonstrated ability to control branchial ion permeability, if ion losses continued we would expect them to reduce ion permeability as the smallmouth bass did during recovery in ‘ Na^+ -free’ water (Table 1).

The much greater effect of temperature on $J_{\text{out}}^{\text{Na}}$ than on $\dot{M}\text{O}_2$ of rainbow and lake trout was striking (Table 3). The observation that in both species $J_{\text{out}}^{\text{Na}}$ jumped by almost 150 % ($\dot{M}\text{O}_2$ rose by only 20–25 %) as temperature rose from 10 to 18 °C suggests a common underlying cause. Clearly temperature is having an enormous influence on branchial ion permeability. The nature of this influence is not known, but there are a number of possibilities. For instance, both salmonids are considered to be cold-water species, and 18 °C is above their preferred temperature range. Perhaps, at this high temperature the gill epithelial membrane was disrupted in some way and, consequently, so were the tight junctions. Temperature has well-known effects on cell membrane fluidity (see review by Hazel, 1989), and fluidity of the cell membrane has been shown to influence the activity of membrane-bound enzymes (Cossins and Bowler, 1987). Perhaps changes in branchial cell fluidity affect the membrane-bound tight junctions deleteriously. Alternatively, the

structure of the tight junctional proteins may be directly affected by the temperature increase.

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