

**ACID–BASE REGULATION FOLLOWING EXHAUSTIVE
EXERCISE: A COMPARISON BETWEEN FRESHWATER-
AND SEAWATER-ADAPTED RAINBOW TROUT
(*SALMO GAIRDNERI*)**

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Summary

Blood acid–base regulation following exhaustive exercise was investigated in freshwater- (FW) and seawater- (SW) adapted rainbow trout (*Salmo gairdneri*) of the same genetic stock. Following exhaustive exercise at 10°C, both FW and SW trout displayed a mixed respiratory and metabolic blood acidosis. However, in FW trout the acidosis was about double that of SW trout and arterial blood pH took twice as long to correct. These SW/FW differences were related to the relative amounts of net H⁺ equivalent excretion to the environmental water, SW trout excreting five times as much as FW trout. The greater H⁺ equivalent excretion in SW trout may be secondary to changes in the gills that accompany the adaptation from FW to SW. It may also be related to the higher concentrations of HCO₃⁻ as well as other exchangeable counter-ions (Na⁺ and Cl⁻) in the external medium in SW compared to FW.

Introduction

Exhaustive exercise in fish leads to a marked extracellular acidosis of respiratory and metabolic origin. The nature, severity and duration of this acid–base disturbance have been well characterized (see reviews by Wood & Perry, 1985; Heisler, 1986). Associated with this extracellular acidosis is net H⁺ equivalent excretion to the environmental water which is thought to occur as a result of combined NH₄⁺ and H⁺ equivalent excretion (or HCO₃⁻ and OH⁻ uptake) (Holeton & Heisler, 1983; Holeton *et al.* 1983; Milligan & Wood, 1986*a,b*). Net H⁺ efflux has also been demonstrated in fishes subjected to extracellular acidoses induced by environmental hypercapnia (Toews *et al.* 1983) and mineral acid infusion (McDonald *et al.* 1982; Evans, 1982; Tang *et al.* 1988), and is considered to

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be achieved primarily by branchial $\text{HCO}_3^-/\text{Cl}^-$ and/or $\text{H}^+(\text{NH}_4^+)/\text{Na}^+$ exchange mechanisms (Evans, 1986; Heisler, 1986).

Since transepithelial ion exchange mechanisms at the gill are central to the regulation of acid-base status in fish (Heisler, 1984), their utility in responding to an acidosis may be limited by the concentrations of the corresponding counter-ions in the environmental water. Certainly, marine fishes are not faced with such limitations. In addition, the branchial epithelium of marine fishes is decidedly more permeable to ions than that of freshwater fishes (Evans, 1979). Moreover, recent studies indicated that, following acid infusion, seawater-adapted rainbow trout were able to compensate their blood pH faster than freshwater-adapted trout (Boutilier *et al.* 1986; Tang *et al.* 1988).

Few studies on acid-base regulation in actively exercising fish have been carried out in which intraspecific comparisons could be made between freshwater and seawater animals. The marine fishes which have been studied are either inactive benthic forms which seldom perform exhaustive exercise (e.g. flounder; Wood & Milligan, 1987), or species for which no freshwater counterpart exists (e.g. dogfish; Holeyton & Heisler, 1983). In the present study, dealing with blood acid-base status and branchial ionic fluxes following exhaustive exercise, a comparison was made between freshwater- (FW) and seawater- (SW) adapted rainbow trout of the same genetic stock. Rainbow trout are particularly well suited for such comparison since they are active pelagic fish which can be readily adapted to either sea water or fresh water.

Materials and methods

Animals and preparation

Freshwater rainbow trout (*Salmo gairdneri*), weighing 170–300 g, were obtained from Merlin Fish Farms, Wentworth, Nova Scotia. After 1 week of acclimation to dechlorinated Halifax City tapwater, they were unselectively divided into two stocks. One stock remained in fresh water ($[\text{Na}^+] = 0.3 \text{ mequiv l}^{-1}$; $[\text{Cl}^-] = 0.2 \text{ mequiv l}^{-1}$; $[\text{HCO}_3^-] = 0.5 \text{ mmol l}^{-1}$; pH 7.5–7; 6–9°C) as a freshwater-adapted group. The other stock was adapted to filtered sea water (32‰; $[\text{Na}^+] \approx 470 \text{ mequiv l}^{-1}$; $[\text{Cl}^-] \approx 540 \text{ mequiv l}^{-1}$; $[\text{HCO}_3^-] = 2.2 \text{ mmol l}^{-1}$; pH 7.9; 8–10°C) supplied by the Aquatron Laboratory of Dalhousie University. To minimize stress, the turnover from fresh water to sea water was performed gradually and took 10 days to reach 100% seawater strength. Fish of both stocks were kept in 4 m³ fibreglass tanks supplied with a continuous flow of aerated water for at least 6 weeks before the experiment. They were fed daily with commercial trout pellets, but feeding was suspended 4 days prior to surgery.

One week before surgical operation, fish were transferred to a 560-l living stream tank (Frigid Unit Inc., USA). The water in the tank was automatically recirculated, aerated and thermostatted to $10 \pm 0.2^\circ\text{C}$. Following anaesthetization in a 1:10 000 seawater solution (pH 7.0) of MS-222 (Sigma) or a 1:10 000 freshwater solution of MS-222 buffered to pH 7.0 with NaHCO_3 , catheters (Clay-

Adams PE50) were surgically implanted into the dorsal aorta using the method of Smith & Bell (1964). Catheters were filled with heparinized (20 i.u. ml⁻¹) Cortland saline (Wolf, 1963) for FW fish, or marine teleost saline (Hoar & Hickman, 1983) for SW fish. Following surgery, fish were placed into 2.8-l darkened Lucite flux boxes supplied with flowing aerated water from a thermostatted living stream tank (10 ± 0.2°C) and allowed to recover for at least 48 h prior to experimentation. The flux boxes were designed so that vigorous aeration of an outer chamber provided convective mixing of water throughout an inner animal chamber (see McDonald & Rogano, 1986, for details).

Experimental protocols

Freshwater trout

Post-exercise changes in arterial acid–base status were measured in 11 fish. The experiment began by closing the inflow and outflow of the flux boxes so that the system contained a fish within a standardized volume of water (approximately 12 l kg⁻¹ fish). The temperature was maintained by bathing the box with thermostatted water (10 ± 0.2°C). Control blood samples were drawn anaerobically from the dorsal aorta catheter for analysis of true plasma pH (pHe), and whole blood total CO₂ and lactate concentrations (see below for analytical procedures). Each fish was then subjected to exhaustive exercise; manual chasing around a circular tank (90 litres, diameter 70 cm) for 10 min. At this point it was incapable of further burst performance but still able to swim slowly around the tank. Each fish was then immediately returned to its individual box and monitored for the following 4 h. Blood samples for pHe, whole blood total CO₂ and lactate were taken at 10, 40, 70, 130 and 250 min post-exercise. The volume of blood sampled was replaced with heparinized (20 i.u. ml⁻¹) saline.

For measurement of the pre- and post-exercise changes in net H⁺ equivalent flux with the environment, water samples (18 ml) were taken over the 1-h period just before exercise (resting flux) and at 0, 1, 2 and 4 h post-exercise. These water samples were subsequently measured for titratable alkalinity and ammonia concentrations. These measurements, together with the above sampling schedule, allowed us to calculate the net H⁺ flux, in terms of μmol kg⁻¹ h⁻¹, during the periods before exercise and over the 0–1, 1–2 and 2–4 h post-exercise periods (see below for analytical procedures and calculations).

Seawater trout

A genetically identical group of seven SW trout was treated to the same experimental protocol and acid–base measurements as the FW trout described above. Net H⁺ equivalent flux was determined using a similar protocol to that of SW trout, except that total CO₂ (at a fixed P_{CO₂}) was measured instead of titratable alkalinity.

Analytical procedures

Arterial pH was determined using a microcapillary pH electrode (Radiometer

G279/G2) coupled with a PHM84 pH meter. The total CO₂ of anaerobically obtained whole blood was measured on 50 µl samples using a gas chromatography method (Boutilier *et al.* 1985) in SW fish, and using a total CO₂ analyser (Corning model 905) for FW fish. Measurements of pHe and whole blood total CO₂ were used to calculate arterial CO₂ tension (Pa_{CO₂}) and whole blood bicarbonate concentration ([HCO₃⁻]_{wb}) using experimentally determined pK' and CO₂ solubility coefficients for trout blood (Boutilier *et al.* 1984). This method (i.e. using whole blood instead of true plasma) will result in slight errors; Pa_{CO₂} will be consistently underestimated by 5–10% and [HCO₃⁻]_{wb} overestimated by less than 0.5%. For the purposes of calculating the metabolic proton load (below), such differences are minor. Whole blood lactate concentrations were analysed using the L-lactate dehydrogenase/NADH method (Loomis, 1961) using Sigma reagents.

The concentration of metabolic protons added to the whole blood (ΔHm⁺) over any period (1 to 2) was calculated by the equation of McDonald *et al.* (1980a):

$$\Delta Hm^+ = [HCO_3^-]_{wb,1} - [HCO_3^-]_{wb,2} - \beta(pHe_1 - pHe_2),$$

where β, the non-bicarbonate buffer capacity of whole blood, is estimated from the haematocrit values at time 2 using the relationship determined by McDonald *et al.* (1980b):

$$\beta = -24.6 \times \text{haematocrit} - 3.97.$$

Water [ammonia] was measured using a micromodification of the salicylate–hypochlorite reaction (McDonald & Wood, 1981). In the experiment with SW trout, the total CO₂ content of water was determined using a gas chromatography technique (Tang *et al.* 1988) and a Carle series 100 chromatograph (Carle Instruments Inc., USA). In the experiment with FW trout, titratable alkalinity was measured as described by McDonald & Wood (1981). The net fluxes of ammonia, bicarbonate and titratable acidity were calculated from the changes in their respective concentrations in water. The titratable alkalinity method (see McDonald & Wood, 1981) and the gas chromatography method (see Tang *et al.* 1988) can be applied equally (with corresponding ammonia measurements) for estimating the *net acidic equivalent flux*. For consistency, bicarbonate flux and titratable alkalinity flux are termed *apparent base flux* in the following text. It should be pointed out that the present procedure can neither separate H⁺ uptake from HCO₃⁻ excretion (or *vice versa*) nor distinguish between NH₃ and NH₄⁺ movements. Rather, it gives a valid measure of *net acidic equivalent flux*.

Statistical analysis

Mean values ± 1 s.e.m. are reported throughout. Results were analysed statistically using paired or unpaired Student's *t*-tests between sample means, 5% being taken as the fiducial limit of significance.

Results

Blood acid–base status following exercise

Exhaustive exercise in FW trout caused an acute extracellular acidosis (Fig. 1), the magnitude and duration of which were similar to that of other analogous studies (see reviews by Wood & Perry, 1985; Heisler, 1986). In contrast to their FW counterparts, the same exercise protocol in SW trout produced a markedly smaller blood acid–base disturbance. Ten minutes after exercise, changes in blood acid–base variables were approximately half those of FW trout (Fig. 1). Subsequent restoration to pre-exercise levels in SW trout took about half as long as in their FW counterparts; i.e. time to full recovery for pH_e , 130 min (SW) vs 250 min (FW); $[\text{HCO}_3^-]_{\text{wb}}$, 130 min (SW) vs 250 min (FW); Pa_{CO_2} , 70 min (SW) vs 130 min (FW).

Blood lactate concentrations and metabolic proton load

In both FW and SW trout, whole blood lactate concentrations rose rapidly from resting values of about 0.5 mmol l^{-1} to approximately 8 mmol l^{-1} at 10 min and about 10 mmol l^{-1} at 40 min post-exercise (Fig. 1D). Thereafter, lactate levels in SW trout continued to increase, albeit slowly. In contrast, lactate levels in FW trout rose to a peak at 70 min, and slowly declined thereafter. As a result, by 130 min following exercise, lactate concentrations in SW trout were significantly higher than those in their FW counterparts (Fig. 1D).

The calculated blood metabolic proton load (ΔHm^+), blood lactate load ($\Delta\text{lactate}$) and the 'H⁺ deficit' ($\Delta\text{lactate} - \Delta\text{Hm}^+$, signs considered) following exercise are illustrated in Fig. 2. At rest, ΔHm^+ is equal to zero by definition. Differences in the levels of blood $\Delta\text{lactate}$ and ΔHm^+ at any time resulted in a large H⁺ deficit, especially in SW trout (Fig. 2B). In FW trout, a negative H⁺ deficit developed immediately following exercise. Thereafter, the H⁺ deficit increased in a sigmoidal fashion. A negative H⁺ deficit was never observed in the SW animals. Instead, a positive H⁺ deficit developed immediately after exercise, and increased in a sigmoidal fashion to values which were markedly higher than in FW trout (Fig. 2B).

H⁺ equivalent excretion to environmental water

During the period prior to exercise, net ammonia flux and apparent net base flux in FW trout were higher than the corresponding measurements in SW trout; however, the net H⁺ exchange with the environment was not significantly different between the two groups (Fig. 3); i.e. $-74 \pm 92 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ (FW) and $-31 \pm 50 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ (SW). During the period of exercise, it was not possible to follow H⁺ equivalent excretion owing to the short time (10 min) and the large volume of water required to exercise the fish.

During the first hour after exercise in FW trout, there was a small but significant increase in net H⁺ equivalent excretion ($212 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) due to an approximate doubling of ammonia excretion without much change in apparent base flux.

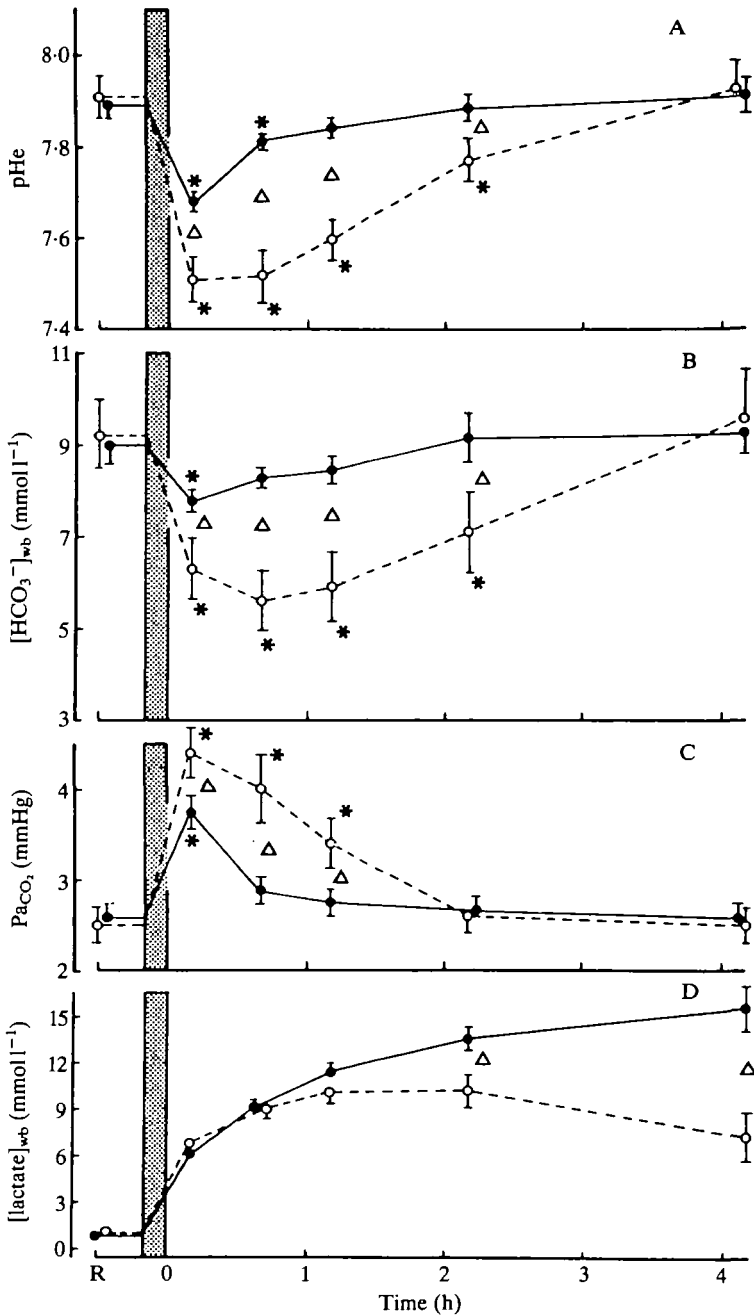


Fig. 1. Effects of 10 min of exhaustive exercise on (A) arterial plasma pH (pHe); (B) whole blood bicarbonate concentration ($[\text{HCO}_3^-]_{\text{wb}}$); (C) arterial CO_2 tension (Pa_{CO_2}) (1 mmHg = 133.3 Pa); and (D) whole blood lactate concentration ($[\text{lactate}]_{\text{wb}}$) in freshwater (FW) trout (○, $N = 11$) and seawater (SW) trout (●, $N = 7$); R, rest; bar indicates period of exercise; 0, immediately after exercise; an asterisk indicates a significant difference ($P < 0.05$) from rest; Δ indicates a significant difference ($P < 0.05$) between FW and SW trout.

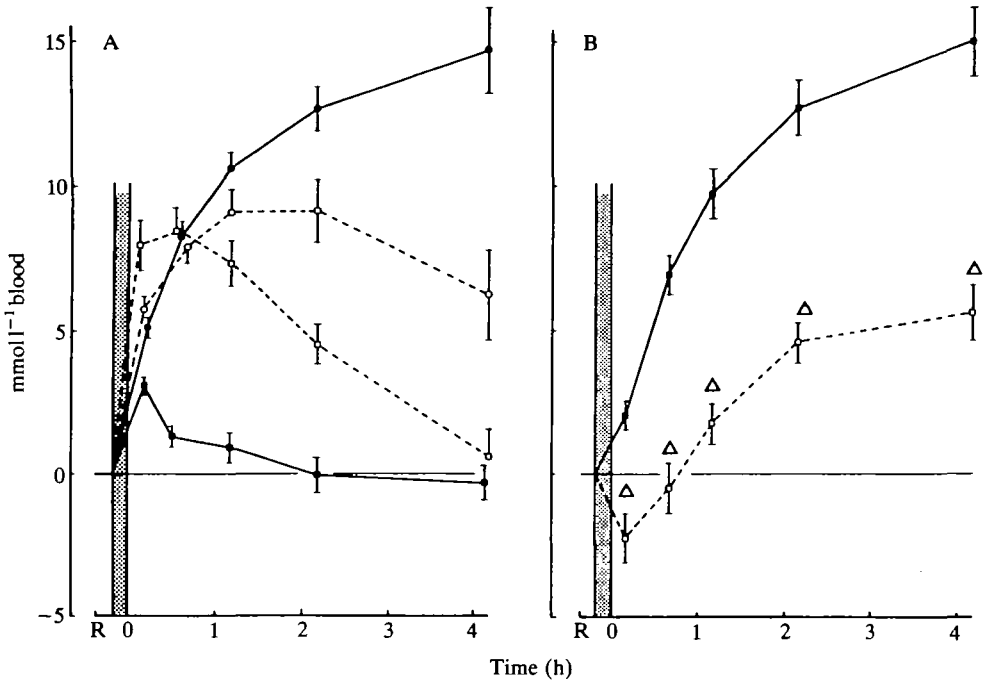


Fig. 2. Changes in (A) blood lactate load (Δ lactate, circles) and blood metabolic acid load (Δ Hm⁺, squares), and (B) blood H⁺ deficit (Δ lactate - Δ Hm⁺, signs considered) following exhaustive exercise in FW (open symbols) and SW (closed symbols) rainbow trout. Other details as in Fig. 1.

Ammonia excretion continued at this elevated level for 1–2 h and thereafter declined towards resting levels over the 2–4 h period of recovery. Apparent base excretion was unchanged over the first hour following exercise, but decreased slightly during the 1–2 and 2–4 h periods (Fig. 3). As a result, net H⁺ excretion was maintained at 225–344 μ mol kg⁻¹ h⁻¹ during these periods.

In SW trout, net ammonia excretion significantly increased by a factor of 1.5 during the first hour following exercise, and remained at this level over the following experimental periods (Fig. 3). The exercise-induced increase in ammonia excretion in SW trout was smaller than in FW trout. However, the apparent base flux in SW trout was markedly changed from a pre-exercise net excretion to a net uptake. As a result of the significant increase in both ammonia excretion and apparent base uptake, the net H⁺ excretion in SW trout was approximately five times that of FW trout over the 4-h period following exercise (Fig. 3).

Discussion

The changes in blood acid–base status observed following exhaustive exercise in both FW and SW trout were similar to those reported for other teleosts (see Wood

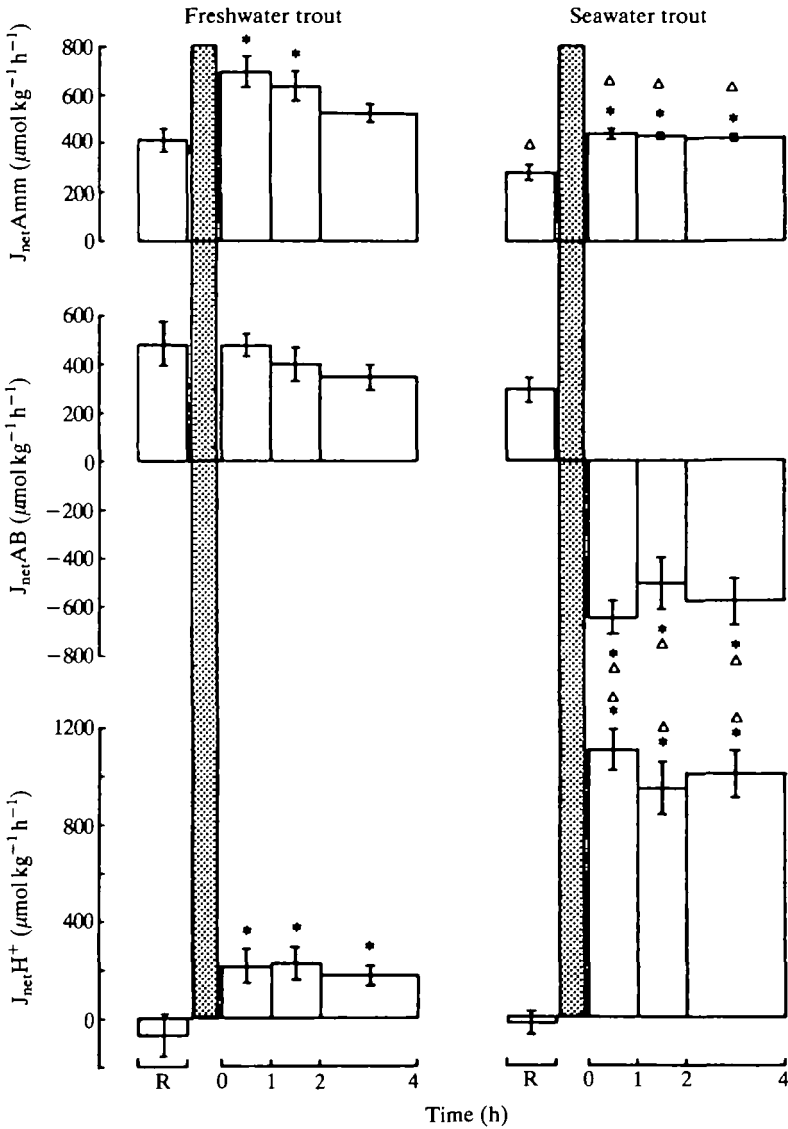


Fig. 3. Net ammonia ($J_{\text{net}}\text{Amm}$), apparent base ($J_{\text{net}}\text{AB}$) and proton equivalent ($J_{\text{net}}\text{H}^+$) fluxes in FW and SW trout following exhaustive exercise. Δ indicates a significant difference ($P < 0.05$) from corresponding freshwater-adapted animals. Other details as in Fig. 1.

& Perry, 1985; Heisler, 1986). The initial decreases in arterial pH were the result of both 'respiratory' and 'metabolic' contributions (Fig. 1). In both groups, the increase in Pa_{CO_2} can be attributed to a greater aerobic metabolism in addition to the buffering of metabolic protons by bicarbonate. It is clear from these data that SW trout are more proficient than FW trout at overcoming extracellular acid-base

disturbances caused by exhaustive exercise (Fig. 1). Since both groups of animals were from the same genetic stock and underwent the same exercise protocol, any differences in the magnitude of the acid–base disturbance and in its time course to recovery must be attributable to modulation of internal structures and/or regulatory mechanisms by the different properties of environmental water.

Different amounts of work and therefore of total lactate production could account for some of the differences between the FW and SW trout. Although the extent of work was not directly quantified, all animals were subjected to exactly 10 min of manual chasing, by which time all had reached a stage where it was impossible to elicit any further (anaerobic) burst performance. Moreover, if the large acidosis in FW animals was simply a reflection of greater lactate production, this was not obvious from the measurements of lactate concentrations in blood (Fig. 1). Another possibility for differences in the magnitudes of the acidoses could arise if there were large differences in the apparent buffering capacity between FW and SW animals. It is unlikely that such differences can be attributed to unique blood buffering capacities, since the resting bicarbonate concentrations and haematocrits were not significantly different between FW and SW animals. Although the intracellular compartments are undoubtedly important in buffering the endogenous acidosis of exhaustive exercise (Milligan & Wood, 1986*a,b*; Heisler, 1986), there is no reason to suspect that these compartments behave so differently between the two groups of animals. Certainly, further work is needed to establish whether intraspecific differences in scope for activity, buffering capacity, and the like, can arise as a result of acclimation to different environmental salinities.

It is clear from our data that the greater excretion of H^+ equivalents in SW trout (Fig. 3) is reflected by a more rapid clearance of the metabolic proton load (Fig. 1) as well as a greater H^+ deficit (Fig. 2). Thus, 'storage' of protons in the external environment may play a more important role in facilitating pHe recovery in SW than in FW trout. Assuming that the pHi and tissue buffering capacity measurements of similarly treated FW rainbow trout (Milligan & Wood, 1986*a,b*) are comparable to those in these experiments (i.e. that there are no large FW to SW differences in total lactate production or internal buffering), it is possible to calculate what fraction of the net whole body H^+ production (due to glycolysis and ATP degradation) would be 'buffered' by cumulative flux of acidic equivalents to the environment. Such analysis shows that less than 10% of the total load is transferred to the environment in FW compared with nearly 40% in SW. This comparatively greater H^+ excretion in SW animals (Fig. 3) must, at least in part, account for the more rapid recovery of pHe in SW than in FW trout (Fig. 1). The basis for such FW and SW differences in net H^+ equivalent excretion between animal and environment almost certainly resides with the gills. Although the principal sites for ion and acid–base regulation in fish include both gills and kidneys, the latter usually contribute relatively little to the overall transfer of acid–base relevant ions. Indeed, the maximum contribution of the kidney in FW fishes is usually less than 6% under various stress situations (see review by Heisler,

1984). Moreover, in SW fishes the contribution is even less (<1%) owing in large part to the comparatively small output of urine in these animals (Evans, 1982; McDonald *et al.* 1982; Heisler, 1980).

Differences in branchial H^+ equivalent transfer between animal and environment could occur for several reasons, including dynamic modulation of active ionic exchange mechanisms, changes in passive permeability and/or the availability of external ions. The gills of FW fishes are important sites of ionic and acid-base regulation owing to the coupling of Na^+ uptake to acidic equivalent (H^+ or NH_4^+) excretion and Cl^- uptake to basic equivalent (HCO_3^- or OH^-) excretion (see reviews by Heisler, 1984, 1986; Wood & Perry, 1985). Recent studies have indicated that these same ionic exchange mechanisms may also occur in the branchial epithelium of some SW fishes, even with the obligatory NaCl load that these hyporegulating animals must already endure (Evans, 1986; Heisler, 1986).

Dynamic modulation of Na^+ and Cl^- net fluxes can be achieved not only by branchial ionic exchange mechanisms, but also by passive diffusion of Na^+ and Cl^- as a function of their respective electrochemical gradients and the transepithelial permeabilities. Certainly, the branchial epithelium of SW teleosts is regarded as being much more permeable to ions than that of FW teleosts (reviewed by Evans, 1979). For example, the unidirectional efflux of Na^+ in SW rainbow trout was found to be 10 times that of their FW counterparts (Greenwald *et al.* 1974), and similar differences in flux rates (for both Na^+ and Cl^-) were also found when comparing SW and FW Atlantic salmon, *Salmo salar* (Potts *et al.* 1970). Larger efflux rates for Na^+ and Cl^- in sea water may indicate a greater potential for modulation of net acidic equivalent flux, and could serve to explain why proton equivalent excretion in SW trout is five times that of the FW animals (Fig. 3).

The greater acidic equivalent flux in SW trout may also be correlated with the greater availability of counter-ions (including HCO_3^-) in the external medium. For example, recent studies on rainbow trout have shown that net H^+ excretion can be increased by approximately 50% by raising the external concentrations of NaCl from 0.3 to 1.3 mmol l⁻¹ (D. G. McDonald, Y. Tang & R. G. Boutilier, unpublished results). Furthermore, in hypercapnic-exposed rainbow trout, extracellular pH compensation was facilitated when animals were exposed either to bicarbonate-enriched fresh water (Perry *et al.* 1981) or to progressive increases in external salinity at constant [HCO_3^-] (G. K. Iwama & N. Heisler, personal communication). In the present study, our sea water contained more bicarbonate than our fresh water (4.4 times that in FW) and could, therefore, have provided the SW animals with a greater capacity for Cl^-/HCO_3^- exchange. The greater acidic equivalent net flux in SW animals in the present investigation might also be related in part to the slightly more favourable conditions set up by the pH of sea water (7.9) compared with fresh water (7.6).

In conclusion, the more rapid recovery of acid-base status following exhaustive exercise in SW than in FW trout appears to be facilitated by greater branchial H^+ equivalent excretion to the environmental water. It is not yet possible to determine

whether the basis for these differences resides with changes in gill epithelium function or with the water composition itself.

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References

- BOUTILIER, R. G., HEMING, T. A. & IWAMA, G. K. (1984). Physico-chemical parameters for use in fish respiratory physiology. In *Fish Physiology*, vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 401–430. New York: Academic Press.
- BOUTILIER, R. G., IWAMA, G. K., HEMING, T. A. & RANDALL, D. J. (1985). The apparent pK of carbonic acid in rainbow trout blood plasma between 5 and 15°C. *Respir. Physiol.* **61**, 237–254.
- BOUTILIER, R. G., IWAMA, G. K. & RANDALL, D. J. (1986). The promotion of catecholamine release in rainbow trout, *Salmo gairdneri*, by acute acidosis: interactions between red cell pH and haemoglobin oxygen-carrying capacity. *J. exp. Biol.* **123**, 145–158.
- EVANS, D. H. (1979). Fish. In *Comparative Physiology of Osmoregulation in Animals* (ed. G. M. O. Maloij), pp. 305–390. New York: Academic Press.
- EVANS, D. H. (1982). Mechanisms of acid extrusion by two marine fishes: the teleost, *Opsanus beta*, and the elasmobranch, *Squalus acanthias*. *J. exp. Biol.* **97**, 289–299.
- EVANS, D. H. (1986). The role of branchial and dermal epithelia in acid–base regulation in aquatic vertebrates. In *Acid–Base Regulation in Animals* (ed. N. Heisler), pp. 139–172. Amsterdam: Elsevier.
- GREENWALD, L., KIRSCHNER, L. B. & SANDERS, M. (1974). Sodium efflux and potential differences across the irrigated gill of sea water-adapted rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.* **64**, 135–147.
- HEISLER, N. (1980). Mechanisms of acid–base adjustment in dogfish (*Scyliorhinus stellaris*) subjected to long-term temperature acclimation. *J. exp. Biol.* **85**, 89–98.
- HEISLER, N. (1984). Acid–base regulation in fishes. In *Fish Physiology*, vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 315–401. New York: Academic Press.
- HEISLER, N. (1986). Acid–base regulation in fishes. In *Acid–Base Regulation in Animals* (ed. N. Heisler), pp. 397–450. Amsterdam: Elsevier.
- HOAR, W. S. & HICKMAN, C. P., JR (1983). *A Laboratory Companion for General and Comparative Physiology*, 3rd edn, p. 307. New Jersey: Prentice-Hall.
- HOLETON, G. F. & HEISLER, N. (1983). Contribution of net ion transfer mechanisms to acid–base regulation after exhaustive activity in the larger spotted dogfish (*Scyliorhinus stellaris*). *J. exp. Biol.* **103**, 31–46.
- HOLETON, G. F., NEUMANN, P. & HEISLER, N. (1983). Branchial ion exchange and acid–base regulation after strenuous exercise in the rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **51**, 303–318.
- LOOMIS, M. E. (1961). An enzymatic fluorometric method for the determination of lactic acid in serum. *J. Lab. clin. Med.* **57**, 966–972.
- MCDONALD, D. G., BOUTILIER, R. G. & TOEWS, D. P. (1980a). The effects of enforced activity on ventilation, circulation and blood acid–base balance in the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 273–287.
- MCDONALD, D. G., HÖBE, H. & WOOD, C. M. (1980b). The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *J. exp. Biol.* **88**, 109–131.
- MCDONALD, D. G. & ROGANO, M. S. (1986). Ion regulation by the rainbow trout in ion poor water. *Physiol. Zool.* **59**, 318–331.
- MCDONALD, D. G., WALKER, R. L., WILKES, P. R. H. & WOOD, C. M. (1982). Acid excretion in the marine teleost *Parophrys vetulus*. *J. exp. Biol.* **98**, 403–414.

- MCDONALD, D. G. & WOOD, C. M. (1981). Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. *J. exp. Biol.* **93**, 101–118.
- MILLIGAN, C. L. & WOOD, C. M. (1986a). Intracellular and extracellular acid–base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 93–121.
- MILLIGAN, C. L. & WOOD, C. M. (1986b). Tissue intracellular acid–base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 123–144.
- PERRY, S. F., HASWELL, M. S., RANDALL, D. J. & FARRELL, A. P. (1981). Branchial ionic uptake and acid–base regulation in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **92**, 289–303.
- POTTS, W. T. W., FOSTER, M. A. & STATHER, J. W. (1970). Salt and water balance in salmon smolts. *J. exp. Biol.* **52**, 553–564.
- SMITH, L. S. & BELL, G. R. (1964). A technique for prolonged blood sampling in free swimming salmon. *J. Fish. Res. Bd Can.* **21**, 711–717.
- TANG, Y., NOLAN, S. & BOUTILIER, R. G. (1988). Acid–base regulation following acute acidosis in seawater-adapted rainbow trout, *Salmo gairdneri*: a possible role for catecholamines. *J. exp. Biol.* **134**, 297–312.
- TOEWS, D. P., HOLETON, G. F. & HEISLER, N. (1983). Regulation of the acid–base status during environmental hypercapnia in the marine teleost fish *Conger conger*. *J. exp. Biol.* **107**, 9–20.
- WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progve Fish Cult.* **25**, 135–140.
- WOOD, C. M. & MILLIGAN, C. L. (1987). Effects of strenuous activity on intracellular and extracellular acid–base status and H^+ exchange with the environment in the inactive, benthic starry flounder *Platichthys stellatus*. *Physiol. Zool.* **60**, 37–53.
- WOOD, C. M. & PERRY, S. F. (1985). Respiratory, circulatory, and metabolic adjustments to exercise in fish. In *Circulation, Respiration, and Metabolism* (ed. R. Gilles), pp. 1–22. Berlin, Heidelberg: Springer-Verlag.