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REGULATION OF BLOOD OXYGEN TRANSPORT AND RED CELL pH_i AFTER EXHAUSTIVE ACTIVITY IN RAINBOW TROUT (*SALMO GAIRDNERI*) AND STARRY FLOUNDER (*PLATICHTHYS STELLATUS*)

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SUMMARY

In vitro, exogenous adrenaline reduced the Bohr and Root shifts caused by elevated Pa_{CO_2} and depressed plasma pH in rainbow trout blood, but not in starry flounder blood. *In vivo* immediately after exercise, plasma adrenaline (Ad) and noradrenaline (NAd) increased about 12-fold in rainbow trout. Associated with this catecholamine mobilization was a significant haemoconcentration, red blood cell (RBC) swelling and a reduction in RBC [NTP]; the latter was larger than that explained by cell swelling alone, indicating metabolic degradation of nucleoside triphosphate (NTP). RBC intracellular pH (pH_i) fell only slightly after exercise (0·07 units) at 0 h, but was restored by 0·5 h in the face of a large plasma acidosis (0·4 units). $[O_2]/[Hb]$ fell significantly, but this decline may have been due in part to the significant reduction in Pa_{O_2}. The reduction in $[O_2]/[Hb]$ was less than predicted from *in vitro* O_2-dissociation curves at low (0·5 nmol l^{-1}) catecholamine levels, but similar to that predicted at high (90 nmol l^{-1}) catecholamine levels. In flounder, resting Ad and NAd levels were about 10 times those in trout and did not change significantly after exercise. As a consequence, there was no reduction in RBC [NTP], and RBC pH_i fell significantly (0·10 units) after exercise in the face of a large plasma acidosis (0·4 units) and remained depressed until 4 h, although RBC swelling did occur. These factors in addition to the increased Pa_{CO_2} may have contributed to the reduction in arterial $[O_2]/[Hb]$, in the face of a constant Pa_{O_2}. However, $[O_2]/[Hb]$ was restored to resting levels *prior* to the correction of RBC pH_i and Pa_{CO_2}. This, in conjunction with the observation that catecholamines did not affect the *in vitro* blood–O_2 dissociation curve, suggests that additional factors may be involved in regulating O_2 transport after exercise in flounder.

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INTRODUCTION

In both rainbow trout, *Salmo gairdneri* and starry flounder *Platichthys stellatus*, exhaustive exercise results in a severe reduction in plasma pH (0·5–0·6 units) due to metabolic and respiratory acidoses (Turner, Wood & Clark, 1983; Milligan & Wood, 1986, 1987*a*,*b*). However, in both species, red blood cell (RBC) pH_i underwent little change (Milligan & Wood, 1986,1987*b*). Studies with tonometered blood *in vitro* have shown that, for a comparable reduction in plasma pH, RBC pH_i falls and haemoglobin oxygen-affinity is decreased *via* Root and Bohr effects (Wood, Johansen & Weber, 1975; Boutilier, Iwama & Randall, 1986; Nikinmaa, 1986). However, *in vitro*, these effects can apparently be ameliorated by adrenaline (Ad) acting on β-adrenergic receptors on the red cells (see Nikinmaa, 1986, for a recent review). Exhaustive exercise has been reported to elevate plasma catecholamine levels greatly, at least in salmonids (see Table 2 for references). Nikinmaa, Cech & McEnroe (1984) and Primmett, Randall, Mazeaud & Boutilier (1986) have suggested that mobilization of catecholamines into the bloodstream plays a role in maintaining RBC pH_i, and therefore haemoglobin oxygen-affinity, *in vivo* after severe exercise in striped bass *Morone saxatilis* and rainbow trout, respectively. Adequate blood oxygen transport would be maintained during the post-exercise acidosis so that subsequent aerobic activity would not be compromised. Catecholamine mobilization has also been implicated in lactate retention in the muscle of flatfish (Wardle, 1978), although the evidence was indirect as no catecholamine measurements were made.

The present study investigates further the role of circulating catecholamines, as well as possible interactive factors, such as plasma cortisol and red cell nucleoside triphosphate levels, in the regulation of RBC pH_i and blood O_2 transport after exhaustive exercise. In addition, the effect of Ad on the *in vitro* blood–oxygen dissociation curves of trout and flounder was examined.

Rainbow trout and starry flounder represent extremes in terms of their capacity for aerobic activity, dependence upon aerobic metabolism, and ability to perform burst exercise. Salmonids are highly aerobic fish, capable of a maximum O_2 consumption of about 30 mmol kg^{-1} h^{-1} (Brett, 1972), compared with only 6–8 mmol kg^{-1} h^{-1} in flatfish (Duthie, 1982). In addition, salmonids are capable of sustained aerobic activity even after periods of exhaustive, burst exercise (see, for example, Primmett *et al.* 1986). As salmonids are pelagic in nature, their survival may depend upon an ability to continue swimming after glycolytic exhaustion. In contrast, flatfish are benthic in nature, and rely more heavily on camouflage (i.e. burying in sand) than on sustained swimming ability for survival. Furthermore, flounder are quite tolerant of anaemia, with exercise performance virtually unaffected at haematocrits below 1 % (Wood, McMahon & McDonald, 1979). Rainbow trout, however, do not generally survive, let alone swim, with haematocrits much less than 5 % (Wood, McDonald & McMahon, 1982), illustrating the greater dependence of trout on O_2 transport and aerobic metabolism. By examining these two very different species, we hoped to gain insight into both the mechanisms and the generality of any catecholamine-mediated protective effect on O_2 transport in fish.

MATERIALS AND METHODS

Experimental animals

Rainbow trout

Adult rainbow trout *Salmo gairdneri* (736 ± 40·3 g, mean ± 1 S.E.M., $N = 15$) of both sexes were obtained from Highland Springs Trout Farm, Holland Center, Ontario in November 1985. All animals were sexually mature and in breeding condition. Fish were held indoors in large fibreglass tanks supplied with continually flowing dechlorinated Hamilton city water at 15°C. Fish did not feed during holding. Room lights were left on continually to minimize any circadian rhythmicity in hormone levels.

For *in vitro* experiments, trout (356·8 ± 23·7 g, $N = 16$) were obtained from Spring Valley Trout Farm, Petersburg, Ontario in June 1986 and held as described.

Trout were anaesthetized in a 1:10 000 solution of MS 222 (Sigma) and the dorsal aorta was chronically cannulated as described previously (Milligan & Wood, 1986). Fish were allowed to recover for at least 48 h in 20-l darkened Lucite fish boxes continually supplied with well-aerated tap water ($P_{O_2} = 150$ mmHg; 1 mmHg = 133·3 Pa) at 15°C prior to experimentation.

Starry flounder

Adult starry flounder *Platichthys stellatus* (842 ± 77·9 g, $N = 14$) of both sexes were collected by otter trawl from East Sound, Orcas Island and Birch Bay, Washington in November and December 1984. All fish were sexually mature, and most were in breeding condition. Fish were held in large, circular tanks with sand-covered bottoms supplied with fresh running sea water (29‰) at seasonal temperature (9 ± 1°C) at Friday Harbor Laboratories, University of Washington. During holding, fish fed *ad libitum* on other small fishes and invertebrates present in the tank. Prior to experimentation, fish were acclimated to laboratory conditions indoors for 3–5 days in Plexiglas tanks with sand-covered bottoms, supplied with fresh sea water, and were not fed. Room lights were left on continually to minimize any circadian rhythmicity in hormone levels.

For the *in vitro* experiments, flounder (457·8 ± 12·4 g, $N = 11$) were obtained from Seacology, Inc. (Vancouver) in June 1986. They were held indoors for 2 weeks without feeding in a Plexiglas, sand-floored tank in a recirculating seawater (10°C, 32‰) facility at the University of Guelph.

Caudal artery catheters were surgically implanted as described previously (Milligan & Wood, 1987*a*) while the fish were anaesthetized in a 1:10 000 solution of MS 222. To prevent infection, the wound was dusted with the antibiotic oxytetracycline hydrochloride (Syndel Labs, Vancouver) prior to closure with silk sutures. Fish were then placed in 15-l plastic tubs fitted with black plastic mesh, supplied with fresh flowing sea water ($P_{O_2} = 156$ mmHg) at 9 ± 1°C, and allowed to recover for at least 72 h prior to experimentation.

Experimental protocol

In vivo

For each species, parallel experiments were performed on two groups, one of which was subjected to exercise (trout, $N = 8$; flounder, $N = 8$). The other group (trout, $N = 7$; flounder, $N = 6$) served as controls for handling and sampling effects. The controls were left at rest throughout but otherwise treated identically to the experimental group.

Trout were exercised by vigorously chasing them around a large circular tank (500 l) for 6 min, while flounder were chased for 10 min in a shallow rectangular tank (see Milligan & Wood, 1986, 1987*a*). At the end of exercise, fish were returned to their boxes.

Blood samples (trout, 950 μl; flounder, 1400 μl) were drawn into gas-tight Hamilton syringes from the dorsal aorta catheter of trout or the caudal artery catheter of flounder. Samples were taken prior to exercise (rest), immediately after exercise (0 h), and again at 0·5, 1, 2, 4, 8, 12 and 24 h. Samples were analysed for pH, haematocrit (Hct), [haemoglobin] ([Hb]), whole blood levels of lactate and nucleoside triphosphate (NTP), arterial oxygen tension (Pa_{O_2}), arterial oxygen content (Ca_{O_2}) and red blood cell (RBC) pH_i. Plasma was analysed for total CO_2 and levels of cortisol, adrenaline (Ad) and noradrenaline (NAd). In the flounder control group, only pH, whole blood [haemoglobin] and [NTP], RBC pH_i and plasma levels of cortisol, Ad and NAd were measured. Previous studies on flounder under control conditions have documented the effect of sampling on most of the other parameters (Milligan & Wood, 1987*a*). The volume of blood sampled was replaced with Cortland's saline (Wolf, 1963).

In vitro

For each set of blood–oxygen dissociation curves, blood (5–6 ml) was drawn from the dorsal aorta or caudal artery of 3–4 fish, pooled, heparinized (5000 i.u. ml^{-1} sodium heparin; Sigma) and 5 ml was transferred to each of four 50-ml tonometer vessels in a shaking water bath at either 10 °C (flounder) or 15 °C (trout). To two of the vessels, Ad [(−)epinephrine (+)bitartrate salt; Sigma] was added to a final concentration of 90–100 nmol l^{-1}, to simulate post-exercise levels (see Fig. 2). To prevent oxidation of both exogenous and endogenous catecholamines, the monoamine oxidase inhibitor pargyline (Sigma) was added to all vessels to a final concentration of 50 μmol l^{-1}. Blood was equilibrated to humidified gas mixtures containing either 2 or 8 mmHg P_{CO_2} in air or nitrogen, supplied by Wösthoff gas-mixing pumps. Dissociation curves were prepared using the mixing technique described by Haab, Piiper & Rahn (1960). Following an equilibration period of at least 1 h, samples (500 μl) were drawn from the CO_2/air- and CO_2/nitrogen-equilibrated vessels in proportional amounts to achieve from 0 to 100 % oxygenated blood. The samples were mixed with a metal bead in a gas-tight Hamilton syringe.

Blood samples were analysed for plasma pH (pH_e), RBC pH_i, whole blood [NTP], P_{O_2}, O_2 content, Hct and [Hb]. At the beginning and end of each experiment, blood was analysed for [lactate] and plasma for [Ad] and [NAd].

Analytical techniques, calculations and statistical analysis

Whole blood pH and red cell lysate pH (RBC pH_i) were determined on 40-μl samples injected into a Radiometer pH microelectrode (type E5021) maintained at either 10°C (flounder) or 15°C (trout) and linked to a Radiometer PHM 71 or 72 acid–base analyser. Red cell lysates were prepared by the freeze–thaw method as described by Milligan & Wood (1986). Plasma total CO_2 was measured on 50-μl samples using the method described by Cameron (1971) in the flounder studies and with a Corning model 965 CO_2 analyser in the trout studies. P_{CO_2} and [HCO_3^-] in blood and plasma were calculated using the Henderson–Hasselbalch equation, employing αCO_2 and pK′ values reported by Boutilier, Heming & Iwama (1984). Whole blood P_{O_2} was measured with a Radiometer P_{O_2} electrode (type E5036) maintained at experimental temperature. In the *in vivo* studies, blood oxygen content was determined with a Lex-O_2-Con analyser (Lexington Instruments) using a 50-μl sample and the recalibration procedure described by Wood *et al.* (1982). In the *in vitro* studies, blood oxygen content was measured on 50-μl samples using the method described by Tucker (1967) and Radiometer P_{O_2} electrodes. To adjust for differences in [Hb] and physically dissolved oxygen concentrations between fish, haemoglobin-bound O_2 per unit haemoglobin was calculated as:

$$[O_2]/[Hb] = (Ca_{O_2} - Pa_{O_2} \times \alpha_{O_2}) \times [Hb]^{-1},$$

where $[O_2]/[Hb]$ is in mmol g^{-1}, Ca_{O_2} in mmol l^{-1}, Pa_{O_2} in mmHg and [Hb] in g l^{-1}. α_{O_2} represents either the measured O_2 solubility coefficient in starry flounder blood plasma at 9°C, 2·048 μmol l^{-1} $mmHg^{-1}$ (Wood *et al.* 1982) or, for trout, the tabulated value at 15°C, 1·7745 μmol l^{-1} $mmHg^{-1}$ reported by Boutilier *et al.* (1984).

Haematocrit (by centrifugation), [haemoglobin] (as cyanmethaemoglobin) and their ratio (mean cell haemoglobin concentration) were measured according to Milligan & Wood (1987*a*). Whole blood [lactate] was determined enzymatically (L-lactate dehydrogenase/NADH) as described by Turner *et al.* (1983). Whole blood NTP levels were measured by fixing either 100 μl (trout) or 200 μl (flounder) of whole blood in an equal volume of ice-cold 12 % trichloracetic acid and freezing the slurry in liquid nitrogen. No more than 48 h passed before samples were thawed and immediately analysed. NTP levels were assayed using the phosphoglycerate phosphokinase/glyceraldehyde phosphate dehydrogenase enzyme system and Sigma reagents. Since NTP is almost entirely intracellular (Wood *et al.* 1975), levels were expressed both as content per unit haemoglobin (i.e. [NTP]/[Hb]) and as cellular concentration (i.e. [NTP]/Hct).

For measurement of cortisol, Ad and NAd, 400 μl of whole blood was centrifuged for 3 min at 9000 ***g***. Approximately 200 μl of plasma was drawn off, 10 μl of preservative was added (90 mg ml^{-1} EDTA and 60 mg ml^{-1} glutathione; Sigma), and samples were then immediately frozen in liquid nitrogen. The remaining red cell

pellet was used for measurement of red cell pH_i. Plasma samples were stored at −80°C for not longer than 60 days before analysis. Cortisol was measured in duplicate on 25 μl of plasma using a commercially available ^{125}I-radioimmunoassay kit (Corning Medical). Plasma Ad and NAd were also measured in duplicate on 50-μl samples using a commercially available ^{3}H-labelled radio-enzymatic assay (Cat-A-Kit, UpJohn Diagnostics).

Means ± 1 S.E.M. (N) are reported throughout. Student's two-tailed t-test (paired design) was used to test for significant differences ($P < 0{\cdot}05$) within groups, using each fish as its own control. Regression lines were fitted by the least squares method, and the significance of the Pearson's correlation coefficient (r) was assessed. Hormone data were log-transformed prior to statistical analysis.

RESULTS

In vivo

Exhaustive exercise resulted in a pronounced extracellular acidosis in both starry flounder and rainbow trout (Fig. 1A,F). This acid–base disturbance was qualitatively similar in both species, and was associated with an increase in Pa_{CO_2} (Fig. 1C,H) and reduction in plasma [HCO_3^-] (Fig. 1B,G). The extracellular acidosis was rapidly corrected in rainbow trout, and recovery was completed by 4 h, whereas in starry flounder, the correction was not complete until 8 h (Fig. 1A,F). There was no extracellular acid–base disturbance in either the trout or flounder control groups (Fig. 1A,F).

At rest, red cell pH_i was virtually identical in trout and flounder, averaging 7·33 ± 0·01 ($N = 8$) and 7·30 ± 0·03 ($N = 8$), respectively (Fig. 1D,I). In both fish, RBC pH_i fell significantly immediately after exercise, though to a greater extent in flounder than in trout (0·10 *versus* 0·07 units). These intracellular pH depressions were very small relative to the extracellular pH depressions (about 0·4 units). The RBC acidosis was short-lived in trout, with RBC pH_i fully corrected by 0·5 h (Fig. 1D). In starry flounder, the RBC acidosis persisted through to 4 h, after which RBC pH_i returned to rest levels (Fig. 1I). Once RBC pH_i had been corrected, it remained constant until 24 h. RBC pH_i remained constant in the flounder control group (Fig. 1I), but in the trout control group, RBC pH_i was significantly depressed at 4 and 8 h, but had fully recovered by 12 h and was not different at 24 h (Fig. 1D).

Whole blood [lactate] increased in both species after exercise, though to very different extents. Peak levels in rainbow trout were about 10-fold greater than in flounder (8·82 ± 1·64 *versus* 0·86 ± 0·22 mmol l^{-1}, $N = 8$; Fig. 1E,J). In both fish, lactate appearance in the blood followed a similar time course, with peak levels attained 2–4 h into recovery and rest values restored by 12 h.

Exhaustive exercise in trout also resulted in a 12-fold increase in plasma levels of both adrenaline (Ad; rest = 2·2+1·6, −1·0, 0 h = 29·7+10·3, −7·7 nmol l^{-1}, $N = 8$) and noradrenaline (NAd; rest = 2·7+1·8, −1·1, 0 h = 36·4 + 8·4, −6·3 nmol l^{-1}, $N = 8$) (Fig. 2A,B). Levels peaked immediately after exercise (0 h), when the acidosis was most severe, and declined slowly, attaining rest levels by 4 h, in

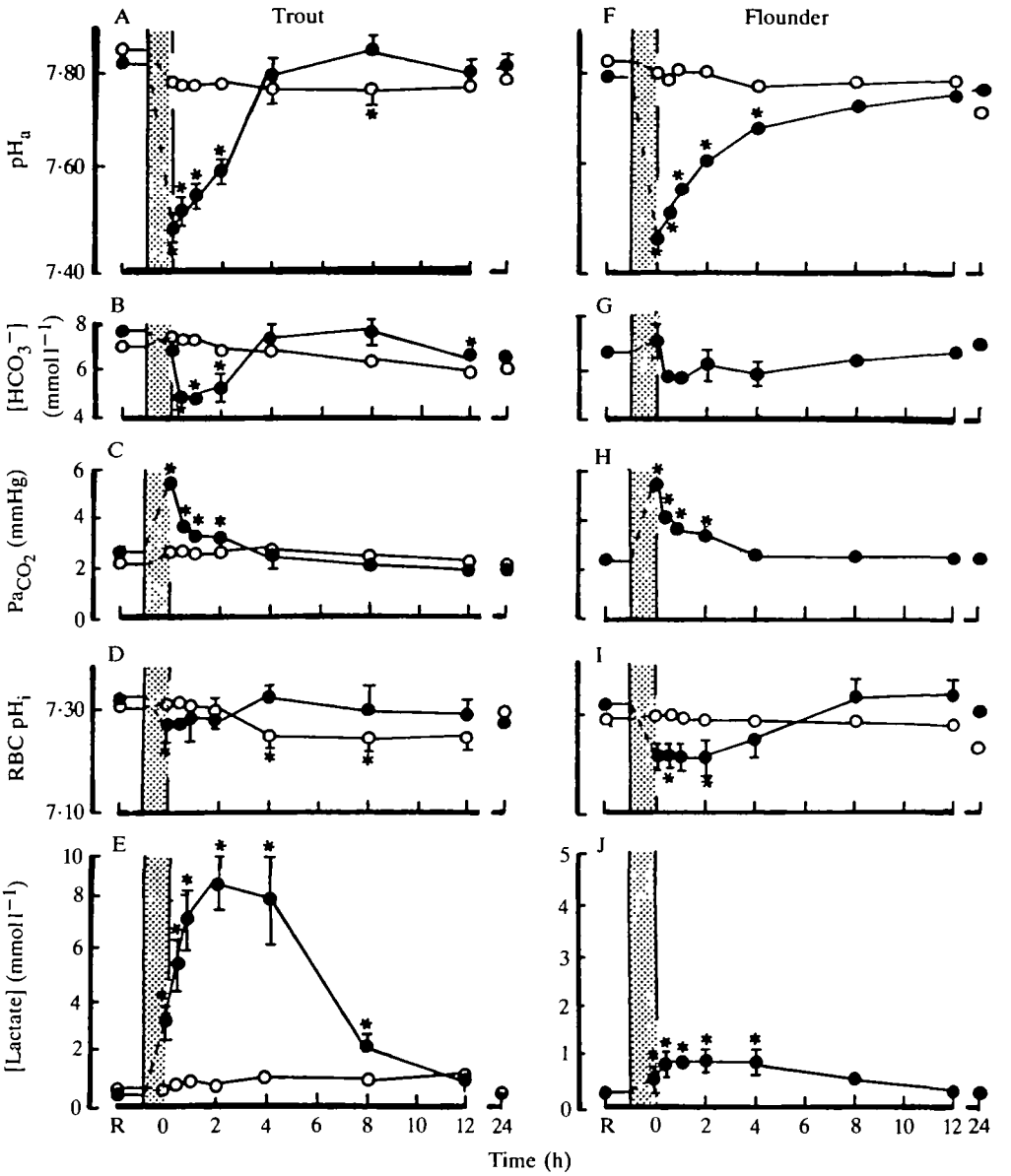

Fig. 1. Effect of exhaustive exercise on (A,F) arterial plasma pH_a, (B,G) $[HCO_3^-]$, (C,H) CO_2 tension (Pa_{CO_2}), (D,I) red blood cell pH_i (RBC pH_i) and (E,J) whole blood [lactate] in rainbow trout and starry flounder, respectively. Means ± 1 S.E.M. R indicates rest value, shaded vertical bar indicates period of exercise (6 min for trout and 10 min for flounder), 0 is immediately after exercise. ○, control group (trout, $N = 7$; flounder, $N = 6$); ●, exercise group (trout, flounder, $N = 8$). * indicates a significant difference ($P < 0{\cdot}05$) from the corresponding rest value.

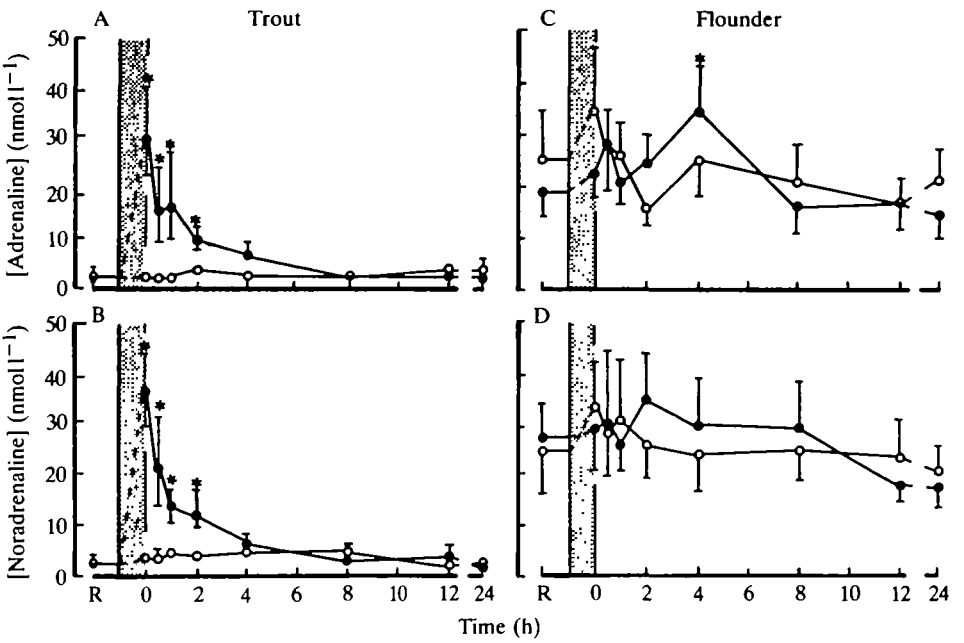

Fig. 2. Plasma levels of (A,C) adrenaline and (B,D) noradrenaline prior to and following exhaustive exercise in rainbow trout and starry flounder, respectively. Other details as in the legend of Fig. 1.

concert with the restoration of pH_a. The response of starry flounder, however, was very different. Resting levels of Ad (19·1+6·5, −4·9 nmol l^{-1}, $N = 8$) and NAd (27·5+6·4, −6·9 nmol l^{-1}, $N = 8$) were about 10 times those in trout (Fig. 2C,D) and did not change much after exercise, except for a small, but significant increase in Ad at 4 h (Fig. 2C). Sampling in itself did not mobilize catecholamines as plasma levels of Ad and NAd remained constant in the control groups (Fig. 2A–D).

Associated with the post-exercise acidosis and catecholamine mobilization in trout was an almost 60 % increase in haematocrit (Fig. 3A), a 25 % increase in [Hb] (Fig. 3B) and a 20 % reduction in mean cell [haemoglobin] (MCHC, Fig. 3C), the latter indicative of red cell swelling. These parameters had returned to rest levels by 2–4 h. However, MCHC continued to increase, so that by 8–12 h, it was significantly elevated over rest levels, but had returned to pre-exercise levels by 24 h. Similar relative trends, though of smaller absolute magnitude, occurred in starry flounder (Fig. 3F), but no secondary rise in MCHC was observed (Fig. 3F). MCHC did decline significantly, however, suggesting red cell swelling had occurred (Fig. 3F). The diluting effect of repetitive sampling became apparent at 4–8 h in both trout (Fig. 3A,B) and flounder (Fig. 3D,E), when haematocrit and [Hb] declined significantly.

At rest, arterial oxygen tension (Pa_{O_2}) in trout (93·4 ± 9·4 mmHg, $N = 8$; Fig. 4A) was about double that in flounder (44·2 ± 7·1 mmHg, $N = 8$; Fig. 4D), and arterial blood oxygen content (Ca_{O_2}) in trout was almost triple that of flounder (5·2 ± 0·3

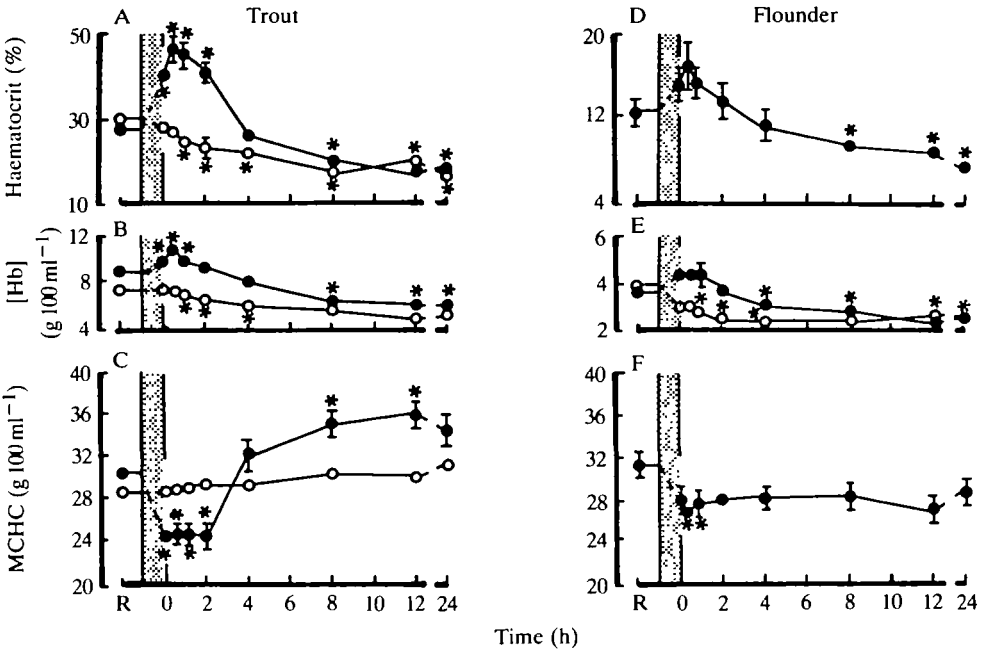

Fig. 3. Haematological changes associated with exhaustive exercise in rainbow trout and starry flounder. (A,D) Haematocrit, (B,E) whole blood [haemoglobin] ([Hb]) and (C,F) mean cell [haemoglobin] (MCHC). Other details as in the legend of Fig. 1.

versus $1{\cdot}9 \pm 0{\cdot}2$ mmol l^{-1}, $N = 8$; Fig. 4B,E). The latter was due in part to the difference in [Hb] (Fig. 3B,E), although haemoglobin-bound O_2 content per unit haemoglobin ($[O_2]/[Hb]$) was also higher in trout than flounder ($0{\cdot}056 \pm 0{\cdot}001$ *versus* $0{\cdot}048 \pm 0{\cdot}003$ μmol g^{-1}, $N = 8$; Fig. 4C,F). Pa_{O_2} showed small fluctuations in the trout control group, with significant increases at 4 h and 8–24 h.

Immediately after exercise, trout Pa_{O_2} fell significantly, by about 35 %, although it returned to a level not significantly different from rest at 0·5 h (Fig. 4A). Ca_{O_2} in trout fell in concert with Pa_{O_2} immediately after exercise, but had returned to rest levels by 0·5 h (Fig. 4B). Thereafter, it remained constant until 8 h, when the diluting effect of sampling on [Hb] became apparent (Figs 3B, 4B). Pa_{O_2} in flounder was not significantly affected by exercise. Similarly, Ca_{O_2} in flounder remained fairly constant after exercise until 4 h, when it fell significantly, reflecting the effect of sampling on [Hb] (Figs 3E, 4E). When post-exercise variations in [Hb] were taken into account, $[O_2]/[Hb]$ fell by about 20–25 % in both trout (Fig. 4C) and flounder (Fig. 4F). This decline persisted for about 1 h in both species; thereafter $[O_2]/[Hb]$ returned to rest levels, with a slight increase evident in trout at 8 h. In the trout control group, $[O_2]/[Hb]$ remained constant throughout the experimental period (Fig. 4C).

After exercise, there was an apparent 'metabolic' degradation of NTP in trout, indicated by the significant decline in [NTP]/[Hb] by about 20 % (Fig. 5A). The diluting effect of red cell swelling (Fig. 3C) compounded this metabolic reduction,

so that the actual reduction in mean cellular [NTP] was about 35 % (Fig. 5B). Red cell NTP levels were fully restored by 4 h into recovery. In flounder, there was no apparent 'metabolic' degradation of red cell NTP; [NTP]/[Hb] remained constant after exercise, except for a significant increase at 12 and 24 h (Fig. 5C). While mean cellular [NTP] tended to decline, reflecting the red cell swelling, the changes were not significant (Fig. 5D). In trout and flounder control groups [NTP]/[Hb] tended to increase, although the changes were significant only in the trout group at 4, 8 and 24 h (Fig. 5A).

As with catecholamines (Fig. 2), resting levels of plasma cortisol (28·5+14·3, −9·3 ng ml^{-1}, $N=8$) in trout were lower than those in flounder (101·5+35·9, −34·0 ng ml^{-1}, $N=8$, Fig. 6A,B). Cortisol levels tended to increase after exercise in flounder, although the changes were not significant; in the control group, cortisol remained constant (Fig. 6B), except for a small, but significant, increase at 8 h. However in trout, plasma [cortisol] increased 3- to 4-fold in both the exercise and control groups (Fig. 6A). The changes followed similar, although not identical, patterns in both groups, making it difficult to discern any definite exercise effect.

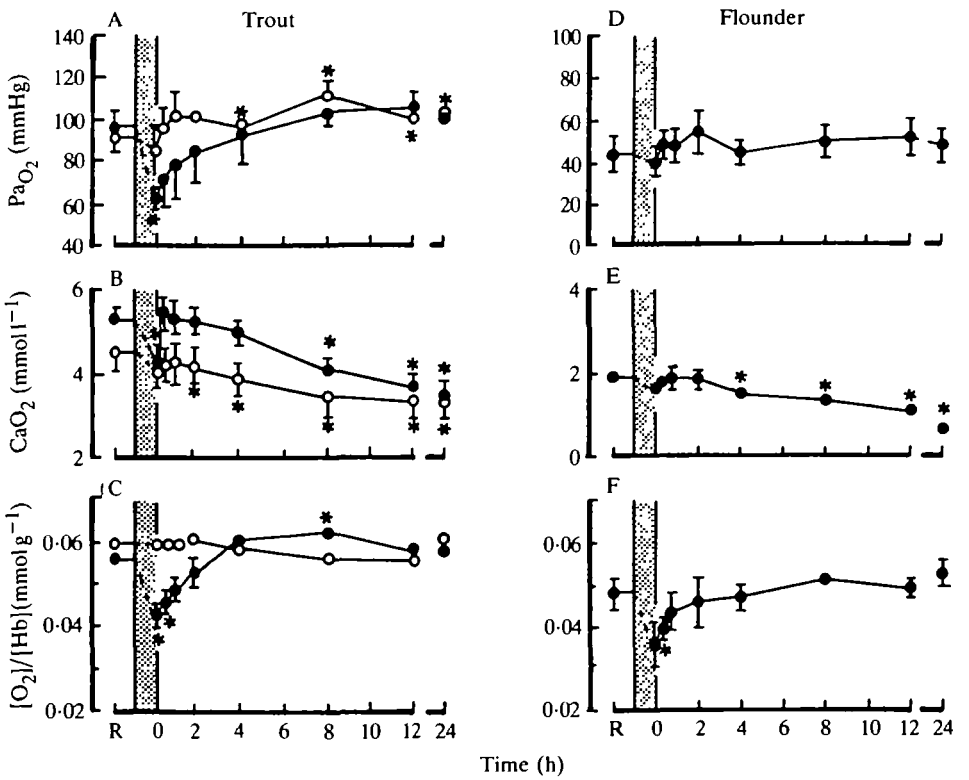

Fig. 4. The effects of exhaustive exercise on (A,D) arterial blood oxygen tension (Pa_{O_2}), (B,E) blood oxygen content (Ca_{O_2}) and (C,F) haemoglobin-bound O_2 per unit haemoglobin ($[O_2]/[Hb]$) in rainbow trout and starry flounder, respectively. Other details as in the legend of Fig. 1.

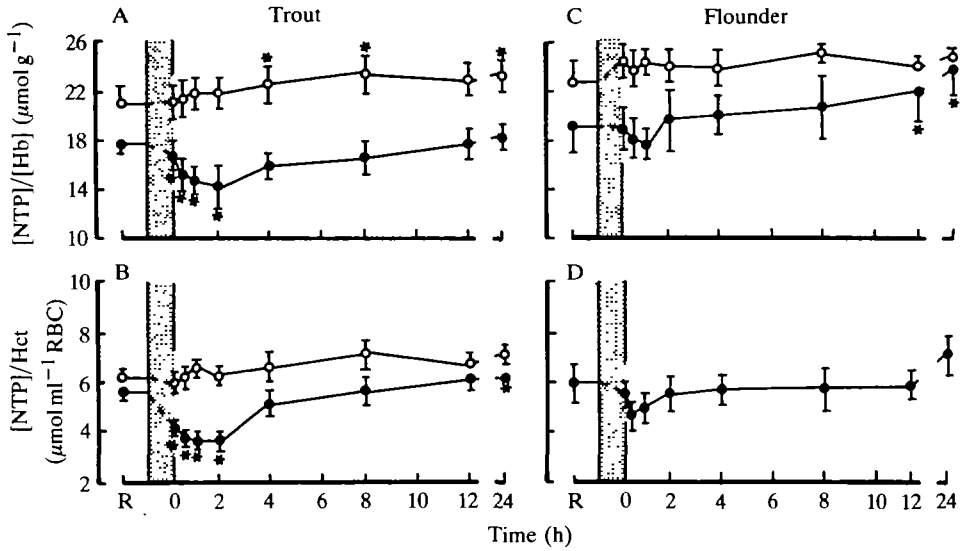

Fig. 5. Changes in (A,C) blood nucleoside triphosphate (NTP) content per unit haemoglobin ([NTP]/[Hb]) and (B,D) cellular NTP levels ([NTP]/Hct) after exhaustive exercise in rainbow trout and starry flounder, respectively. Other details as in the legend of Fig. 1.

In vitro

Both trout and flounder whole blood *in vitro* showed pronounced Root and Bohr effects when P_{CO_2} was increased from 2 to 8 mmHg (Fig. 7A,B), at low (endogenous) catecholamine levels. In trout, P_{50} increased from 19 to 23 mmHg and maximal oxygen saturation fell by about 26 %. In flounder, P_{50} increased from 7 to 12 mmHg and maximal oxygen saturation fell by about 15 % (Fig. 7B). At higher catecholamine levels, similar to those observed after exercise (Fig. 2A), the Root and Bohr effects in trout blood were virtually abolished; maximum oxygen saturation was only marginally reduced (1 %) at the higher P_{CO_2} and P_{50} increased only slightly (from 19 to 20 mmHg). Increasing catecholamine levels did not have a comparable effect on flounder blood. In the presence of 98 nmol l^{-1} catecholamines, maximum oxygen saturation was still reduced (by about 15 %), and P_{50} still increased (from 6 to 10 mmHg) at the higher P_{CO_2} (Fig. 7B). Catecholamines did not alter the relationship between RBC pH_i and pH_e at high or low P_{CO_2} for either trout or flounder blood.

NTP levels in trout blood tended to decrease as oxygen saturation decreased at both CO_2 tensions; the effect was greater at the higher catecholamine levels (Table 1). However, in flounder blood, NTP levels were affected neither by the degree of oxygen saturation nor by the catecholamine level (Table 1). In both trout and flounder blood *in vitro*, lactate (0·5–1·5 mmol l^{-1} and 0·2–0·5 mmol l^{-1}, respectively) and NTP levels remained constant with time during tonometry.

DISCUSSION

Control experiments

Since a number of blood samples, each of considerable volume, were required from individual fish in this study, it was anticipated that sampling itself might induce significant changes in some parameters. The control experiments showed that, in general, these effects were not large, apart from those directly reflecting loss of red cells from the circulation (Figs 3, 4B), and did not confound the patterns seen in the experimental groups. An important exception was plasma cortisol in trout, where changes in the control group were as large as in the experimental group (Fig. 6A). Interestingly, this was not observed in plasma catecholamines (Fig. 2), which are

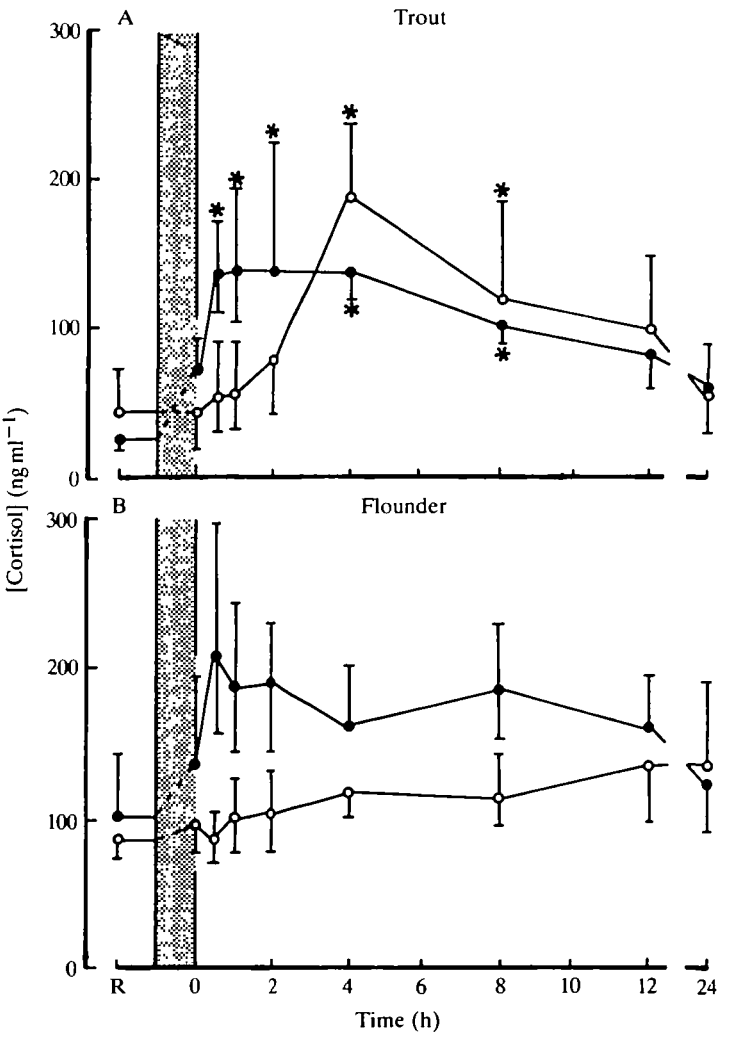

Fig. 6. Plasma [cortisol] in (A) rainbow trout and (B) starry flounder prior to and following exhaustive exercise. Other details as in the legend of Fig. 1.

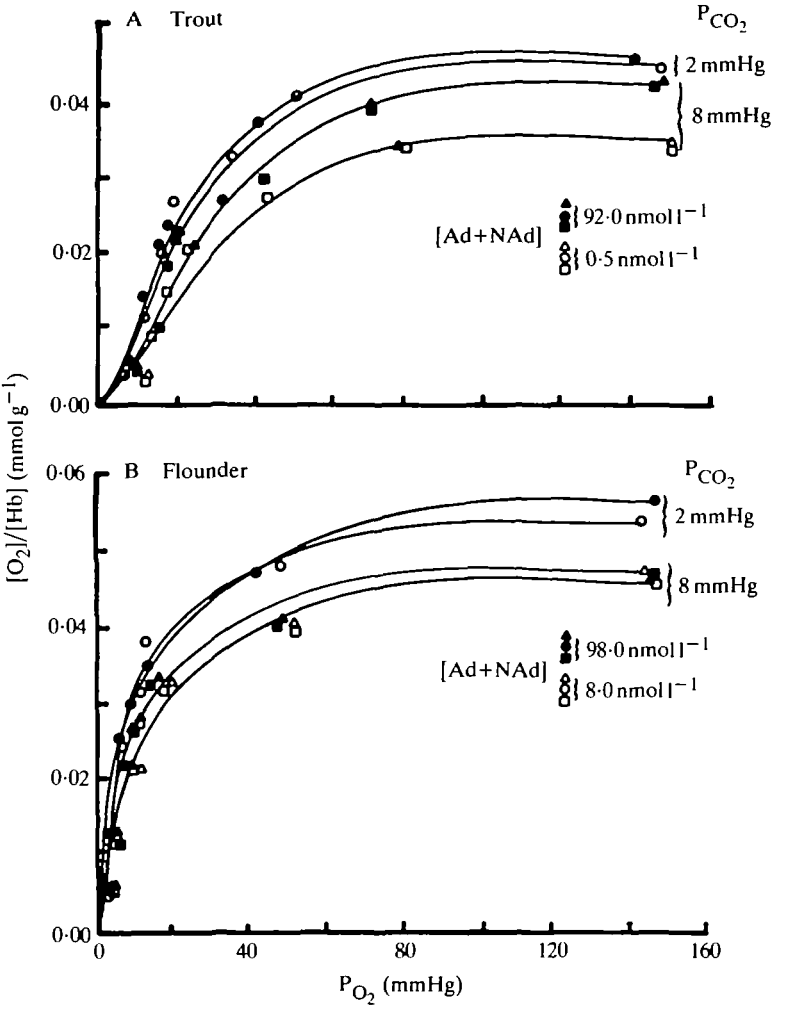

Fig. 7. *In vitro* haemoglobin–oxygen dissociations curves for (A) rainbow trout and (B) starry flounder at 15°C and 10°C, respectively, in the presence (closed symbols) and absence (open symbols) of added exogenous adrenaline. Total measured catecholamine levels are shown. Differently shaped symbols signify three separate experiments.

often considered a sensitive indicator of stress in fish (Mazeaud, Mazeaud & Donaldson, 1977).

Rainbow trout

In rainbow trout, the post-exercise acidosis in the extracellular compartment was qualitatively similar to that previously described for the species (Milligan & Wood, 1986; Turner *et al.* 1983) and the same explanations probably apply. Despite this pronounced extracellular acidosis, red cell pH_i was virtually unaffected, except for the small, but significant, decline immediately after exercise. Given the reduction in pH_e at time 0, based on the relationship between RBC pH_i and pH_e for trout blood

Table 1. *The effect of catecholamines, P_{CO_2} and haemoglobin oxygen saturation on nucleoside triphosphate levels ([NTP]) in trout and flounder whole blood* in vitro

Percentage saturation	[NTP] (μmol g^{-1} haemoglobin) 100	80	60	50	40	20	10	0
Trout*								
P_{CO_2} = 2 mmHg								
[Ad+NAd] = 0·5	19·9	19·0	18·9	18·3	18·1	17·8	17·1	16·8
[Ad+NAd] = 92	20·3	19·8	18·4	17·9	16·8	15·9	15·0	14·6
P_{CO_2} = 8 mmHg								
[Ad+NAd] = 0·5	18·7	17·8	17·1	16·8	16·2	15·9	15·7	15·1
[Ad+NAd] = 92	16·7	15·6	15·0	14·5	13·9	12·6	12·0	11·8
Flounder*								
P_{CO_2} = 2 mmHg								
[Ad+NAd] = 8	21·4	21·2	20·6	21·8	19·5	20·6	22·0	21·9
[Ad+NAd] = 98	20·7	20·9	21·4	22·0	20·1	21·7	20·8	20·9
P_{CO_2} = 8 mmHg								
[Ad+NAd] = 8	19·5	20·1	19·7	22·0	21·5	21·7	19·8	20·4
[Ad+NAd] = 98	20·1	18·7	18·9	19·2	19·7	20·3	21·4	19·9

* Catecholamine values are in nmol l^{-1}.
Ad, adrenaline; NAd, noradrenaline.

determined *in vitro* ($pH_i = 0·73 \times pH_e + 1·74$; Milligan & Wood, 1985), RBC pH_i should have fallen by about 0·23 pH units. However, *in vivo*, RBC pH_i fell by only 0·07 pH units. The ability of trout red cells to regulate pH_i better *in vivo* than *in vitro* is illustrated in Fig. 8A, which shows the relationship between pH_e and RBC pH_i *in vivo* and *in vitro*, with the former having a slope much less than the latter (0·20 *versus* 0·73).

This almost perfect regulation of RBC pH_i *in vivo* can probably be attributed to the significant elevation in circulating levels of Ad and NAd during the acidotic period (see Fig. 2A,B). The post-exercise levels of plasma Ad and NAd in trout in the present study are in agreement with those recently reported for this species (Table 2), although the early very high values of Nakano & Tomlinson (1967) have never been confirmed. A number of *in vitro* studies have demonstrated that in the presence of Ad, trout red cells are able to regulate pH_i better in the face of pH_e changes (Nikinmaa, 1986). The mechanism is thought to involve β-adrenergic stimulation of Na^+/acidic-equivalent exchange in excess of Cl^-/basic-equivalent exchange in the cell membrane, resulting in a net efflux of acidic equivalents, net influxes of Na^+, Cl^- and water, and cell swelling (Nikinmaa, 1986). However, in winter trout (fish obtained from water temperatures below 5 °C), Nikinmaa & Jensen (1987) were unable to demonstrate a similar β-adrenergic phenomenon. Erythrocyte swelling also occurs passively, in response to an increase in P_{CO_2}. Cell swelling will tend to dilute the fixed negative charges in the cell (e.g. haemoglobin, NTP). This will also raise RBC pH_i passively by a shift in the Donnan ratio for H^+ (Nikinmaa, 1986). In the present study, red cell swelling did occur as shown by the significant decline in MCHC (Fig. 3C). Release of immature erythrocytes from the spleen in

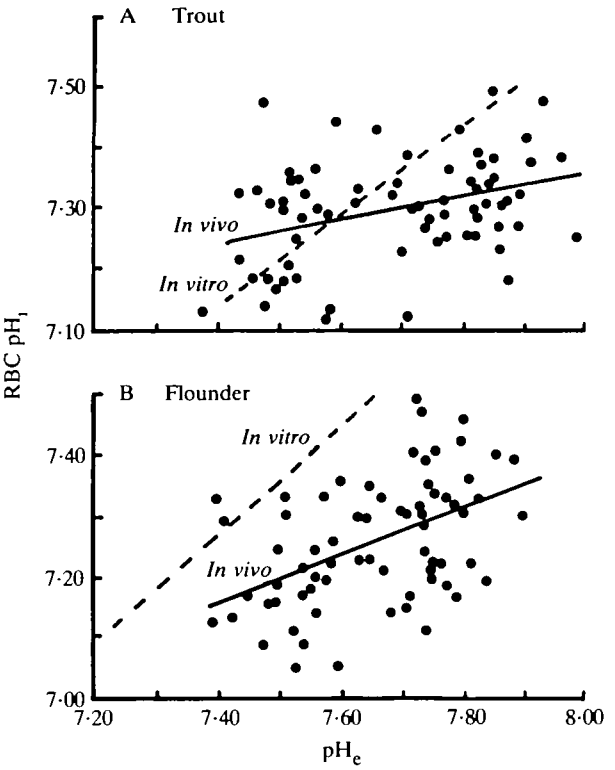

Fig. 8. The *in vivo* relationship (solid line) between red cell pH_i and pH_e in (A) rainbow trout and (B) starry flounder after exercise. The regression equations are: rainbow trout: $pH_i = 0{\cdot}20pH_e + 5{\cdot}78$ $r = 0{\cdot}35$, $N = 72$ ($P < 0{\cdot}01$); starry flounder: $pH_i = 0{\cdot}39pH_e + 4{\cdot}31$, $r = 0{\cdot}48$, $N = 69$ ($P < 0{\cdot}01$). The dotted lines show the *in vitro* relationships between pH_i and pH_e for rainbow trout. [$pH_i = 0{\cdot}73pH_e + 1{\cdot}74$, $r = 0{\cdot}90$, $N = 88$ ($P < 0{\cdot}001$)]; and starry flounder [$pH_i = 0{\cdot}91pH_e + 0{\cdot}54$, $r = 0{\cdot}72$, $N = 28$ ($P < 0{\cdot}01$)]. The *in vitro* relationships were determined in the absence of exogenous catecholamines.

response to adrenergic stimulation may also have contributed to the decline in MCHC (Yamamoto, Itazawa & Kobayashi, 1980).

The exercise-induced catecholamine surge may also have caused the significant reduction in cellular NTP concentrations (Fig. 5B). In addition to the dilution of cellular NTP by cell swelling, there was evidence of a metabolic degradation of red cell NTP stores because [NTP]/[Hb] also declined (Fig. 5A). In trout red cells *in vitro*, Nikinmaa (1986) has demonstrated that Ad induces a metabolic reduction in cellular NTP, perhaps by an inhibition of synthesis and/or a stimulation of consumption. This effect could be blocked by the β-agonist, propranolol (Nikinmaa, 1986). A similar action of catecholamines on red cell [NTP]/[Hb] was observed in trout whole blood *in vitro* in the present study (Table 1). Release of immature erythrocytes from the spleen may also have contributed to the reduction in [NTP]/[Hb] *in vivo*, as immature red cells contain less NTP than do their mature counterparts (Lane, Rolfe & Nelson, 1981).

Table 2. *Plasma levels of catecholamines prior to and following exercise in various fish species*

Species	Rest		Post-exercise		Source
	Ad	NAd	Ad	NAd	
Salmo gairdneri (rainbow trout)	4·5	6·9	36·8	40·3	1
	25	20	1130	310	5*b*
	1·5	1·1	7·8	4·5	8*b*
	0·9	0·7	38	27	7*a*
	4	12	12	23	2*a*
			190	85	2*b*
	1·4	10·2	0·3	2·5	3*d*
			14·4	22·8	3*e*
			212·0	85·0	3*f*
Platichthys stellatus (starry flounder)	23·6	30·9	25·4	34·9	1
Scyliorhinus canicula (dogfish)	5	18	95	95	2*b*
			20	34	2*c*
	5·9	14	19·3	32·5	3*c*
			96·3	96·5	3*f*
Squalus acanthias (spiny dogfish)	5	8	22	38	6*b*
Petromyzon marinus (lamprey)	21	7	12	7	4*c*

Ad, adrenaline; NAd, noradrenaline. All values in nmol l^{-1}.

1, Present study; 2, Butler (1986); 3, Butler, Metcalfe & Ginley (1986); 4, Dashow, Epple & Nibbio (1982); 5, Nakano & Tomlinson (1967); 6, Opdyke, Carroll & Keller (1982); 7, Primmett, Randall, Mazeaud & Boutilier (1986); 8, Ristori & Laurent (1985).

a, Levels immediately after fish had swum to exhaustion in a water tunnel.

b, Levels immediately after 'violent' exercise (e.g. tail grasping).

c, Levels in spontaneously active fish.

d, Levels in fish swimming at 1 body length s^{-1}.

e, Levels in fish swimming at 2 body lengths s^{-1}.

f, Levels immediately after burst swimming.

Nucleoside triphosphates, which in trout and flounder are more than 90% ATP (Wood *et al.* 1975), are negative allosteric modifiers of haemoglobin oxygen-affinity (Wood *et al.* 1975), while reductions in red cell pH_i will similarly reduce oxygenation of haemoglobin *via* Root and Bohr shifts (Wood *et al.* 1975; Nikinmaa, 1986). Therefore, by minimizing the change in RBC pH_i and reducing red cell NTP levels, the post-exercise surge of circulating catecholamines in trout probably exerted a protective effect on blood oxygen transport during the period of acidosis. The catecholamine effect was not entirely successful, however, as haemoglobin-bound O_2 still fell significantly after exercise (Fig. 4C). Nonetheless, considering the post-exercise reduction in Pa_{O_2} (Fig. 4A) and rise in Pa_{CO_2} (Fig. 1C), the fall in haemoglobin-bound oxygen *in vivo* (23%) was less than predicted from *in vitro* oxygen dissociation curves (32%) (see Fig. 7A) at low catecholamine levels. However, at catecholamine levels similar to those observed *in vivo* post-exercise, the

predicted fall in oxygen saturation (20 %) was similar to the observed fall, suggesting that catecholamines did exert a protective effect on blood oxygen transport. A similar effect of Ad on the haemoglobin–oxygen dissociation curve has been demonstrated in trout red cells suspended in saline *in vitro* (Nikinmaa, 1986).

The post-exercise reduction in Pa_{O_2} (Fig. 4A) was similar to that reported by Primmett *et al.* (1986) who attributed the decline in Pa_{O_2} to a measured reduction in ventilatory frequency. However, other studies on rainbow trout have reported either constant or elevated Pa_{O_2} after exercise (Kiceniuk & Jones, 1977; Holeton, Neumann & Heisler, 1983). Reasons for these differences are not immediately clear.

Starry flounder

The exercise-induced extracellular acid–base disturbance in starry flounder was virtually identical to that previously described, (Milligan & Wood, 1987*a*) and the same explanations probably apply. In comparison with trout, pH_a was depressed and Pa_{CO_2} elevated to the same extent, although $[HCO_3^-]$ declined and blood [lactate] rose to a lesser extent.

Circulating levels of Ad and NAd in resting starry flounder were about 10 times greater than in trout, and generally higher than those reported for other fish species (Table 2). Circulating catecholamine levels have not been measured previously in flatfish. Perhaps these sluggish animals require higher maintenance levels than do more active species, which may be related to the observation that hearts of pleuronectid flatfish lack adrenergic innervation (Santer, 1985), but operate with relatively high cardiac stroke volume at rest (Wood *et al.* 1979).

In contrast to the rainbow trout, circulating levels of Ad and NAd did not increase significantly after exercise in starry flounder (Fig. 2C,D). Perhaps as a consequence of this lack of catecholamine mobilization, flounder red cell pH_i was less well regulated after exercise than was trout red cell pH_i. The slope of the *in vivo* relationship between RBC pH_i and pH_e in flounder was about double that in trout (0·39 *versus* 0·20, Fig. 8A,B), reflecting the better regulation of trout RBC pH_i *in vivo*. However, the *in vivo* slope for flounder blood was less than the *in vitro* slope (0·39 *versus* 0·91; Fig. 8B) and the absolute levels of the *in vivo* pH_i values were somewhat lower (Fig. 8B), suggesting that some RBC pH_i regulation did occur *in vivo*. The lack of a post-exercise catecholamine surge may also explain why there was no evidence of metabolic degradation of red cell NTP, or significant dilution of cellular NTP stores (Fig. 5C,D). However, *in vitro* these parameters were also insensitive to the presence of catecholamines (Table 1).

Even though Pa_{O_2} remained constant in flounder, the amount of oxygen bound to haemoglobin fell by about the same amount as in trout (23 %) immediately after exercise (Fig. 4). The fall in RBC pH_i, increase in Pa_{CO_2}, and the lack of a compensatory reduction in cellular NTP levels were, no doubt, contributing factors. The *in vivo* reduction in oxygenation of haemoglobin (23 %) was similar to that predicted from *in vitro* haemoglobin–oxygen dissociation curves, at both low (19 %) and high (18 %) catecholamine levels (Fig. 7B). Furthermore, the virtual lack of effect of catecholamines on the *in vitro* blood oxygen-dissociation curve at high CO_2

tensions argues against a role for catecholamines in regulating oxygen transport after exercise in flounder. However, the observation that haemoglobin oxygenation was restored to rest levels 2–3 h *prior* to the correction of red cell pH_i and reduction in Pa_{CO_2} suggests that factors other than those measured in the present study may be involved in regulating oxygen transport in flounder.

In summary, this study has indicated that in rainbow trout catecholamines released into the circulation after exhaustive exercise help sustain O_2 transport during the associated plasma acidosis. After a period of intense activity in this active, pelagic fish, O_2 transport to the aerobic muscles would be maintained. Therefore the capacity for continued swimming following glycolytic exhaustion of the white muscle would not be compromised. In contrast, catecholamines did not appear to play a comparable role in starry flounder, perhaps reflecting the difference between trout and flounder in terms of their dependence on sustained swimming ability. Nonetheless, there was evidence of some protection of blood O_2 transport in flounder, suggesting other unmeasured factors were influencing haemoglobin oxygen-affinity after exhaustive exercise.

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