J. exp. Biol. 171, 301–314 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

SWIMMING PERFORMANCE AND HAEMATOLOGICAL VARIABLES IN SPLENECTOMIZED RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

BY P. GALLAUGHER, M. AXELSSON AND A. P. FARRELL

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Accepted 2 July 1992

Summary

Haematological variables were measured during aerobic swimming (45–55 % of U_{crit}) and at critical swimming velocity (U_{crit}) in acutely splenectomized and sham-operated rainbow trout. There was no correlation between haematocrit (Hct) and U_{crit} in either group of fish. The control values for the haematological variables did not differ significantly between the two groups of fish. Some haematological variables changed during aerobic swimming and at U_{crit}, but there were no significant differences between the two groups for any of the variables. Arterial blood oxygen tension was significantly reduced at U_{crit}. Arterial blood oxygen content (Ca_{O_2}) was maintained in sham-operated fish because the Hct increased significantly. However, in the splenectomized animals, Ca_{O_2} decreased (compared to control values) during aerobic swimming and at U_{crit} because the Hct did not change. Plasma concentrations of lactate and catecholamines were elevated only at U_{crit}. We provide evidence of a graded spleen contraction during aerobic swimming.

Introduction

One factor which may limit aerobic swimming performance of fish is the oxygen-carrying capacity of the blood. An earlier study with rainbow trout demonstrated that phenylhydrazine-induced anaemia or exposure to hypoxia reduced aerobic swimming performance (Jones, 1971). Moreover, this response was apparently correlated with temperature. Anaemic fish that were cold-acclimated (8–10°C) had a 40 % lower haematocrit (Hct) and a 34 % lower critical swimming velocity (U_{crit}) than non-anaemic fish, whereas warm-acclimated (21–23°C) anaemic fish showed a 67 % decrease in Hct and a 40 % decrease in U_{crit} compared to non-anaemic fish held at the same temperature. In addition, hypoxic exposure reduced swimming performance by 43 % in cold-acclimated fish but by only 30 % in the warm-acclimated group. In this case, the better swimming performance of the warm-acclimated fish was associated with an 8 % higher Hct. Although these results suggest that blood oxygen-carrying capacity may be an important factor in

Key words: haematocrit, splenectomy, oxygen, trout, aerobic exercise, *Oncorhynchus mykiss*.

determining swimming performance of trout, Jones (1971) reported that there was no significant relationship between Hct and U_{crit} in either warm- or cold-acclimated fish.

The conclusion of Jones (1971) is curious in view of the now well-established fact that the spleen contracts following exhaustive exercise in rainbow trout (Yamamoto *et al.* 1980; Yamamoto, 1989, 1991; Kita and Itazawa, 1989; Wells and Weber, 1990, 1991; Pearson and Stevens, 1991*a*). Furthermore, there is evidence of a graded release of red blood cells from the spleen during aerobic swimming in cannulated yellowtails, *Seriola quinqueradiata* (Yamamoto *et al.* 1980). These findings, at the very least, associate an increase in Hct, due to the transfusion of red blood cells from the spleen, with faster swimming speeds. In fact, Pearson and Stevens (1991*a*) recently demonstrated poorer swimming performance in splenectomized rainbow trout compared to sham-operated fish. In this case, splenectomized fish had a Hct value 21 % lower and a U_{crit} value 16 % lower than those of sham-operated fish. Thus, these observations do not substantiate the findings of Jones (1971).

Wells and Weber (1991) have taken a different approach to studying the relative importance of Hct and blood viscosity in terms of oxygen transport in fish. They defined a theoretical optimal Hct for rainbow trout and compared it with Hct values measured under several different conditions; i.e. acute and chronic hypoxic exposure and following strenuous exercise. An important observation in their study was that greater intraspecific variation in Hct values resulted from different blood sampling methods than from any of the experimental perturbations. In particular, significantly lower Hct values were reported for cannulated fish.

The difference in Hct values between cannulated and non-cannulated fish is probably related to handling stress, which causes an increase in Hct resulting from adrenergically mediated swelling of the erythrocytes, from fluid shifts between plasma and intracellular or interstitial compartments, or from the mobilization of erythrocytes from the spleen. This raises concern regarding the 'true' Hct values of non-cannulated fish. It is noteworthy that neither the study by Jones (1971) nor the study by Pearson and Stevens (1991*a*) used cannulated fish. Jones removed each fish from the swim tunnel, killed and measured it, and then sampled the blood by tail section. Similarly, Pearson and Stevens sampled blood from the caudal peduncle following removal of the spleen (if present) from fish which had been killed with a cephalic blow. Both of these sampling methods are likely to give an erroneous Hct value. In addition, Pearson and Stevens (1991*a*) did not obtain initial Hct values and, therefore, one cannot conclude that the reduced performance observed in splenectomized fish was due to the lower Hct at U_{crit} without eliminating the involvement of cell swelling or fluid shifts.

In view of the uncertainties and contradictions of earlier studies, we have conducted a more comprehensive investigation into the contribution that red cell release from the spleen makes to aerobic performance of rainbow trout. We used blood samples from cannulated fish to determine haematological changes associated with increased swimming velocities. In contrast to the results of Pearson and

Stevens (1991*a*) and in support of those of Jones (1971), we were unable to demonstrate a significant relationship between Hct and U_{crit}.

Materials and methods

Experimental animals

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), were obtained from a local hatchery (West Creek Trout Farm, Aldergrove, BC) and held outdoors in a 200-l fibreglass tank for at least 1 month prior to experimentation. Aerated dechlorinated tap water flowed through the tank at 5 l min^{-1}, the temperature varying from 17 to 19°C. The mean body masses and lengths for both groups of fish were 530–738 g and 36–41 cm, respectively.

Surgical procedures

Fish were netted and immediately anaesthetized in a 1:10 000 solution of tricaine methanesulphonate MS222 (Sigma Chemicals, St Louis), buffered to pH 7.5 with sodium bicarbonate. Body dimensions were determined and the fish were transferred to an operating sling where their gills were irrigated with a buffered solution of 1:30 000 MS222 during surgery. A cannula was inserted into the dorsal aorta as described by Soivio *et al.* (1975) using polyethylene tubing (PE-50, Intramedic, Parsippany, NJ) filled with heparinized (50 i.u. ml^{-1}) saline. In one group of fish (splenectomized fish), a small abdominal incision was made, and blood vessels to the spleen were tied off before removal of the spleen. In another group (sham-operated fish), the same procedure was followed except that the spleen was not removed and the blood vessels to the spleen were not tied off. The two different surgical procedures were used alternately as fish were caught from the stock aquarium. Recovery from the anaesthetic was initiated by irrigating the gills with fresh water until body movements began. The fish were then allowed to recover and swim freely in oval aquaria for at least 48 h prior to experimentation.

Experimental protocol

Swimming trials

Fish were transferred from the holding aquarium to a swim tunnel similar to that described by Gehrke *et al.* (1990) and held with the water velocity at 10 cm s^{-1} for at least 4 h prior to swimming trials. The temperature of the water in the swim tunnel ranged from 18 to 19°C. A swimming step test was used to determine U_{crit} as follows. Water velocity was increased in increments of 10 cm s^{-1} and the fish swam for 10 min at each speed until fatigued. Fish were considered to be fatigued when they remained on an 8 V electrified grid at the rear of the tunnel for at least 10 s. The time swum at that water velocity was noted. Fatigue is a discrete event which occurs when the fish can no longer swim at the imposed water velocity. Therefore, to get a representative blood sample at U_{crit}, we quickly reduced the

swimming speed by one step so that the fish continued swimming while the blood was sampled. U_{crit} values were calculated after appropriate adjustment for the blocking effect of the fish (Bell and Terhune, 1970). Water velocity was calibrated prior to the swimming trials using a pitot tube (Gehrke *et al.* 1990). The oxygen tension of the water was never less than 19 kPa (140 mmHg).

Blood samples

Arterial blood samples (0.6 ml) were taken before the swim at $10\,cm\,s^{-1}$ (control), during aerobic swimming at $40\,cm\,s^{-1}$ (45–55 % of U_{crit}), and immediately after U_{crit} had been reached but while the fish was kept swimming at a velocity of approximately 90 % of U_{crit}. Sampling was completed within 30 s of the reduction in swimming speed. Blood samples were replaced with an equivalent volume of heparinized saline. The dorsal aorta cannula was connected to a pressure transducer (Narco, LDI5) and a chart recorder (Gould, RS3400) during the entire experiment to provide a continuous record of dorsal aortic blood pressure (P_{DA}). Heart rates (f_H) were determined from the P_{DA} traces.

Analytical techniques

From each blood sample, 200 μl was used to measure partial pressure of oxygen (Pa_{O_2}), 30 μl for oxygen content (Ca_{O_2}) determination, 60 μl for duplicate Hct samples, 20 μl for haemoglobin (Hb) determination and 50 μl for duplicate red blood cell (RBC) counts. The remainder of the blood was centrifuged and the plasma was analyzed for catecholamines. The blood used for the Pa_{O_2} measurement was centrifuged and the plasma was sampled for determinations of [lactate] and [protein].

Measurement of Pa_{O_2} was made using a Radiometer Copenhagen E5046 P_{O_2} electrode in a D616 cell regulated at 18°C and a PHA 930 P_{O_2} module in conjunction with a PHM 71 acid–base analyzer. The P_{O_2} electrode was calibrated using nitrogen-saturated and air-saturated water. Ca_{O_2} was measured using the method of Tucker (1967). Plasma catecholamine concentrations were determined using high pressure liquid chromatography (HPLC) with electrochemical detection following a modification of the method described by Woodward (1982). Plasma [protein] was determined using a Sigma diagnostics kit (procedure no. 541) for total protein; plasma [lactate] was determined using a Sigma diagnostics kit for pyruvate/lactate (procedure no. 826-UV); and [Hb] was determined using the cyanomethaemoglobin method (Sigma, no. 525A). Blood cell counts were made using a Neubauer chamber. The mean cell volume (MCV) was calculated by dividing Hct by the number of red blood cells. The mean cell haemoglobin concentration (MCHC) was calculated as [Hb]/Hct.

The statistical significance of data was determined using Wilcoxon signed-ranks tests for paired samples within a test group and Mann–Whitney tests for unpaired samples between test groups. We report statistically significant differences at a level of $P<0.05$. At least 8 fish were used for each group.

Results

Effect of splenectomy

There was no significant difference between the U_{crit} values obtained for splenectomized and sham-operated fish (Fig. 1B). Although Hct values varied widely (22–40 % for sham-operated fish; 23–45 % for splenectomized fish), there was no significant correlation between U_{crit} and Hct for either of the groups. Under control conditions, f_H, Pa_{O_2}, Ca_{O_2}, [lactate], [protein], [catecholamine], Hct, MCV, MCHC and RBC counts were not significantly different in sham-operated compared with splenectomized fish (Figs 1A, 2A,B; Table 1). While there were significant changes from control values in some of these variables during sustained swimming (45–55 % of U_{crit}) and at U_{crit}, there were no significant differences between the two groups. Since there were no differences between the groups, the following description applies to both groups except where noted.

Changes associated with aerobic swimming

When fish were swimming at approximately half of their U_{crit}, both arterial blood pressure and heart rate were elevated significantly but there was no significant change in plasma lactate, protein or catecholamine concentrations. Hct and Ca_{O_2} were significantly lower than in the controls (Figs 1A, 2B). Pa_{O_2} was significantly decreased in sham-operated but not splenectomized fish (Fig. 2A).

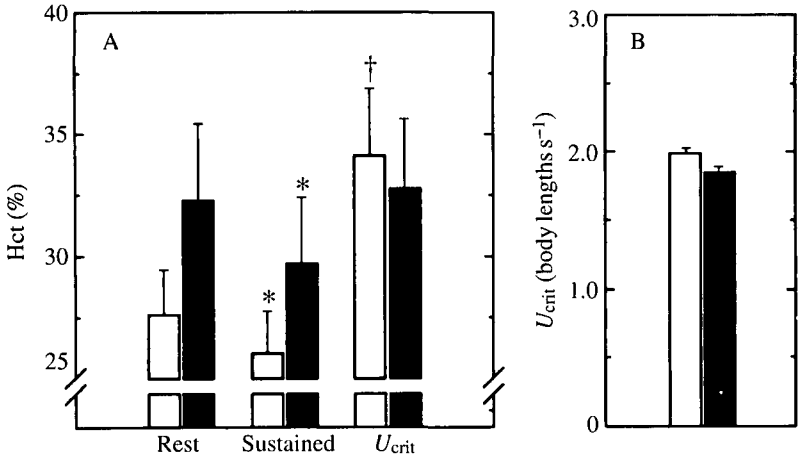

Fig. 1. (A) Haematocrit values (Hct) for sham-operated (open bars; N=8) and splenectomized (filled bars; N=11) rainbow trout under control conditions, during sustained aerobic swimming (45–55 % of U_{crit}) and at critical swimming speed (U_{crit}). Vertical bars indicate S.E.M. Significant differences from rest (P<0.05, paired test) are indicated by an asterisk for aerobic swimming and a dagger for U_{crit}. (B) U_{crit} values for sham-operated (open bars, N=11) and splenectomized fish (filled bars, N=11). Vertical bars indicate S.E.M.

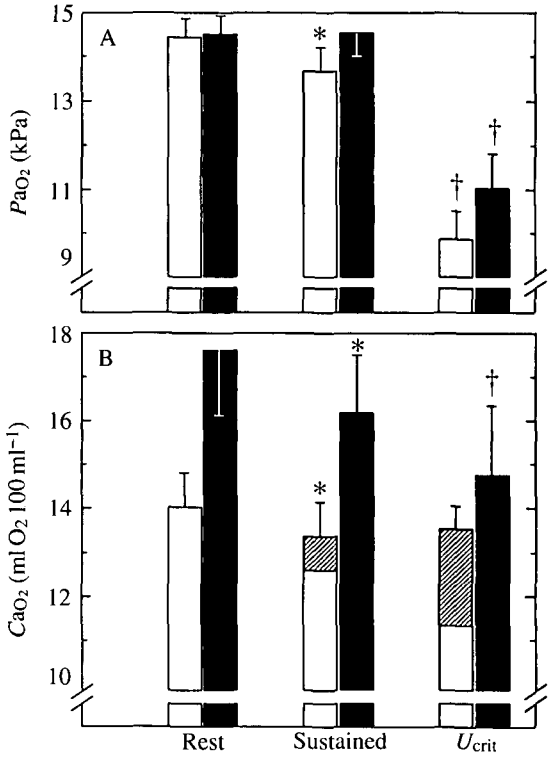

Fig. 2. (A) Arterial blood oxygen tension (Pa_{O_2}) of sham-operated (open bars, N=8) and splenectomized (filled bars, N=10) rainbow trout under control conditions, during sustained aerobic swimming (45–55 % of U_{crit}) and at critical swimming speed (U_{crit}). Vertical bars indicate S.E.M. Significant differences from the control value (P<0.05, paired test) are indicated by an asterisk for aerobic swimming and a dagger for U_{crit}. (B) Arterial blood oxygen content (Ca_{O_2}) of sham-operated (open bars, N=10) and splenectomized (filled bars, N=11) rainbow trout under control conditions, during sustained aerobic swimming (45–55 % of U_{crit}) and at critical swimming speed (U_{crit}). Significant differences from the control value (P<0.05, paired test) are indicated by an asterisk for aerobic swimming and a dagger for U_{crit}. Shaded area indicates the estimated change in Ca_{O_2} due to splenic blood cell transfusion (see Fig. 3 and text for explanation).

Changes associated with swimming at U_{crit}

When fish were swimming at U_{crit} there were significant changes in most of the haematological variables. Compared with control values, P_{DA} was elevated by 25 % and heart rate was elevated by 32–39 %. Plasma lactate and catecholamine concentrations were significantly increased (Table 2). Pa_{O_2} was significantly decreased compared with controls in both sham-operated and splenectomized fish (Fig. 2A). MCHC was decreased and MCV was increased, indicating significant erythrocyte swelling (Table 1). Compared with control values, Hct was increased by 6.7 % in sham-operated fish but was unchanged in the splenectomized group (Fig. 1). Although Ca_{O_2} was unchanged compared with control values in sham-

Table 1. *Recorded and calculated haematological variables in the sham-operated and splenectomized rainbow trout under control conditions, during sustained aerobic swimming (45–55 % U_{crit}) and at critical swimming speed (U_{crit})*

	Rest	45–55 % of U_{crit}	U_{crit}	*N*
Sham-operated				
P_{DA} (kPa)	4.2±0.1	4.4±0.2	5.6±0.3†	8
f_H (beats min^{-1})	64.8±4.7	82.5±5.6*	106.7±11.1†	8
10^{-6}×RBC (mm^{-3})	1.9±0.1	1.8±0.1*	2.2±0.1†	11
MCV (fl)	14.9±0.5	15.2±0.3	17.1±0.8†	10
MCHC (g l^{-1})	338±0.81	338±0.9	262±0.8†	10
[Lactate] (mmol l^{-1})	1.0±0.1	0.7±0.1*	3.0±0.3†	10
[Noradrenaline] (nmol l^{-1})	0.38±0.10	0.54±0.28	2.4±0.7†	10
[Adrenaline] (nmol l^{-1})	0.22±0.06	0.48±0.19	11.6±5.4†	10
Splenectomized				
P_{DA} (kPa)	4.67±0.2	5.1±0.2*	6.2±0.3†	11
f_H (beats min^{-1})	68.8±4.9	83.4±6.4*	101.1±4.7†	11
10^{-6}×RBC (mm^{-3})	2.2±0.2	1.9±0.1	2.0±0.2†	10
MCV (fl)	16.1±0.9	16.5±0.8	17.7±0.7†	10
MCHC (g l^{-1})	327±0.1	321±0.8	302±0.8†	10
[Lactate] (mmol l^{-1})	0.75±0.80	0.62±0.05	2.2±0.2†	10
[Noradrenaline] (nmol l^{-1})	0.23±0.04	0.27±0.04	2.8±0.5†	8
[Adrenaline] (nmol l^{-1})	0.32±0.11	0.49±0.22	7.0±1.2†	8

Values are mean±S.E.M.

*Significantly different from rest value ($P<0.05$, Wilcoxon signed-ranks test for paired samples).

†Significantly different from 45–55 % U_{crit} value ($P<0.05$, Wilcoxon signed-ranks test for paired samples).

P_{DA}, dorsal aortic pressure; f_H, heart rate; RBC, red blood cells; MCV, mean cell volume; MCHC, mean cell haemoglobin concentration.

operated fish, the Ca_{O_2} of splenectomized fish was even lower than that during sustained swimming (Fig. 2B).

Effect of blood sampling

During these experiments 1.2 ml of blood was removed from the fish and replaced with 1.2 ml of saline. To test the possibility that haemodilution may have occurred as a result of blood sampling, the sampling protocol was repeated on separate groups of sham-operated and splenectomized trout which were not exercised. The results showed that sampling had no significant effect on the Hct values of either group after removal of the first 0.6 ml blood sample [the absolute change in Hct was −0.67 % (S.E.M.=0.65) for nine sham-operated fish and −0.59 % (S.E.M.=0.34) for nine splenectomized fish]. After removal of the second 0.6 ml blood sample, the Hct of sham-operated fish again did not change significantly [the absolute change in Hct was +0.46 % (S.E.M.=0.65) for nine sham-operated fish]; however, there was a significant decrease in the Hct of

splenectomized fish [the absolute change in Hct was −1.20% (S.E.M.=0.45) for nine splenectomized fish]. In fact, when the blood removal procedure was extended by removing 0.5 ml of blood at 30 min intervals, more than 3 ml of blood had to be removed to produce a 4% decrease in Hct in sham-operated fish.

Factors contributing to observed changes in haematocrit

The results presented in Fig. 3 are based on the assumption that observed changes in Hct are due to a combination of four separate changes: transfusion of cells from the spleen, erythrocyte swelling, the blood sampling procedure, and shifts of fluid between the red blood cells and the plasma. The values reported for Hct and for the change due to sampling are measured values. The values for erythrocyte swelling were calculated from the MCV and MCHC, and the percentage difference in these values was compared to control values. Since the splenectomized group cannot use the spleen transfusion route to change Hct, the change in Hct due to fluid shift for splenectomized fish was deduced as follows:

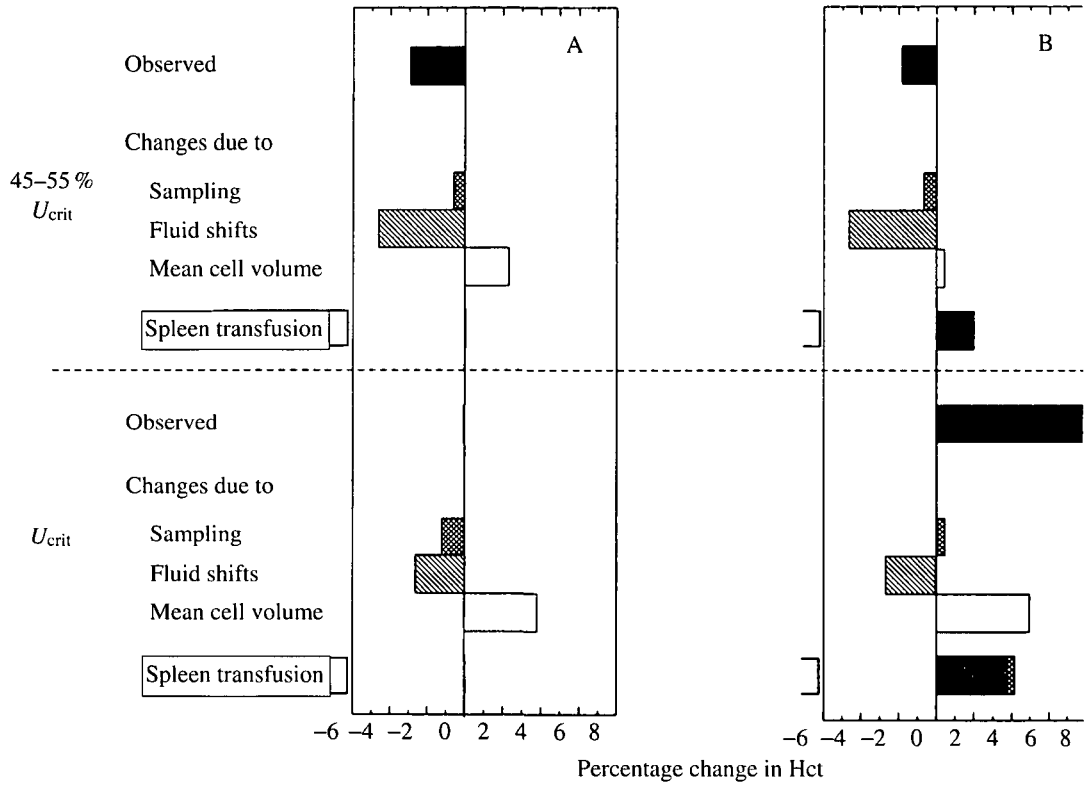

Fig. 3. Estimates of the relative contribution of blood sampling, fluid shifts, cell swelling and splenic blood transfusion to the observed changes in haematocrit (Hct) during swimming in (A) splenectomized and (B) sham-operated rainbow trout (see text for details of the calculations).

change in fluid volume=(observed Hct+change due to sampling−change due to cell swelling). Then, by assuming that the fluid shifts in sham-operated fish were equal to those of splenectomized fish, excepting minor differences due to changes resulting from sampling, we were able to estimate the change due to spleen transfusion in the sham-operated fish by comparing the two groups of fish. With this value we were able to show the proportion of Ca_{O_2} due to spleen transfusion in the sham-operated fish as swimming intensity increased (hatched area in Fig. 2B).

Discussion

Contrary to the results of Pearson and Stevens (1991*a*) we found no difference in the average U_{crit} values of splenectomized and sham-operated fish, indicating that aerobic swimming performance was not affected by spleen removal. Moreover, we were unable to establish a positive correlation between Hct and U_{crit} in either group of fish, supporting the results of Jones (1971).

In reviewing the work of Pearson and Stevens (1991*a*), we note that, while the splenectomized fish clearly did not perform as well as the sham-operated group, we question the validity of their conclusions about a correlation between lower U_{crit} values and reduced Hct and [Hb] in the splenectomized fish. They were unable to establish a relationship between Hct and U_{crit} within each group of fish, and thus the significance of their regression for the Hct and U_{crit} relationship for the combined data sets is questionable, particularly given the large degree of overlap of individual Hct values for the two experimental groups. Furthermore, the [Hb] and U_{crit} regression is only significant at the 10% level. [Hb] is a more accurate measure of blood oxygen-carrying capacity than is Hct as it does not reflect changes caused by stress-induced cell swelling. Thus, the relationship between oxygen-carrying capacity of the blood and aerobic performance of the two groups of fish in the study by Pearson and Stevens (1991*a*) was only significant at the 10% level. Finally, since Pearson and Stevens did not report initial Hct values for either group of fish, we cannot be sure that the better performance in the sham-operated fish was the result of an increased Hct due to addition of cells from the spleen. It is possible that the two groups of fish had a differential response to handling stress.

There are several observations which may explain some of the differences between the results of the present study and those of Pearson and Stevens (1991*a*). Our study was conducted at 18°C but their fish were swimming at 8.2°C. The results of Jones (1971) and others have shown temperature-related differences in swimming performance of rainbow trout. Moreover, Pearson and Stevens assessed the chronic effects of splenectomy on swimming performance 6 months after spleen removal, whereas we used acute splenectomy (48 h recovery) to assess the role of spleen transfusion during aerobic swimming.

The lack of difference in haematological values between the sham-operated and splenectomized groups of fish in the present study is in agreement with results of other studies, where the shorter-term effects of splenectomy were assessed.

Johansson-Sjöbeck (1979) reported no significant differences in Hct, [Hb] or MCV in unoperated compared with splenectomized groups of eels after 1 week. In addition, Perry and Kinkead (1989) observed no differences in Hct and [Hb] between splenectomized and sham-operated rainbow trout 2 months after surgery.

Control Hct values observed here (27.7±1.84 %, S.E.M., for sham-operated fish, N=12; 32.6±2.76 %, S.E.M., for splenectomized fish, N=11) are similar to those of other studies using cannulated rainbow trout. In an analysis of Hct data from a number of these studies, Wells and Weber (1991) reported control Hct values ranging from 17 to 30 %. Compared with the Hct values reported by Pearson and Stevens (1991*a*) for non-cannulated rainbow trout at U_{crit}, our Hct values were similar for splenectomized fish (32.5±2.94 %, S.E.M., N=11 *vs* 33.1±1.9 %, S.E.M.) but lower for sham-operated fish (34.4±2.7 %, S.E.M. *vs* 42.0±1.5 %, S.E.M.). Additionally, in support of the observations of Wells and Weber (1991), we found that the variability of Hct values within groups exceeded that caused by experimental manipulation, i.e. alteration of swimming velocity. This variability as well as the lack of correlation between U_{crit} and Hct in both groups of fish suggests that individual differences in Hct are compensated for by a number of interacting adjustments in the cardiovascular system during swimming.

As in other studies (Butler *et al.* 1992; Nielsen and Lykkeboe, 1992; Thomas *et al.* 1987), there was a significant increase in Hct at U_{crit} in sham-operated fish. The absence of this increase in splenectomized fish indicates that the increase in Hct is due to a transfusion of cells from the spleen. The higher red blood cell count in sham-operated but not in splenectomized fish supports this observation. Other investigators have reported splenic constriction following exhaustive (anaerobic) exercise in a number of different fish species (Yamamoto *et al.* 1980; Yamamoto, 1989, 1991) including rainbow trout (Milligan and Wood, 1987; Kita and Itazawa, 1989; Wells and Weber, 1990, 1991; Pearson and Stevens, 1991*a*,*b*).

The observation that the aerobic swimming performance of splenectomized fish was not compromised by spleen removal leads us to question the view that the dominant role of the spleen transfusion response (increasing Hct) is to enhance aerobic swimming performance. Instead, we propose the following relationships for Hct and critical swimming speed in rainbow trout. Very low Hct values (below around 20 %) reduce aerobic swimming performance in rainbow trout, presumably as a result of reduced blood oxygen-carrying capacity caused by a lower blood [Hb] (Jones, 1971). In contrast, the elevated blood viscosity associated with very high Hct values may impede blood oxygen transport, thereby reducing swimming performance in the way suggested by Wells and Weber (1991), who put forward an optimal Hct concept. We propose that there are lower and upper 'threshold Hcts' between which there is a weak relationship between U_{crit} and Hct, and that the 'plateau' between the lower and upper thresholds probably spans the normal range for Hct in rainbow trout (approximately 25–40 %). Thus, rather than a discrete peak for the 'optimal' Hct (Wells and Weber, 1991), we propose a broader 'optimal plateau'. This weak relationship between U_{crit} and physiological Hct values may account for the differing conclusions of earlier studies. There are a

number of possible reasons why a weak relationship exists. Foremost, it might reflect the involvement of anaerobic metabolism when measuring U_{crit} or other inadequacies of the U_{crit} measurement. Therefore, a more objective measurement of aerobic swimming performance, such as maximal oxygen uptake, may be more revealing. Nevertheless, splenic contraction does occur during swimming and, consequently, we must look for benefits other than increased U_{crit} which may explain the functional advantage of increased Hct within the 'optimal plateau'. Mobilization of cells from the spleen during exercise may benefit cardiovascular function by, for example, augmenting blood volume and cardiac output or by altering blood flow distribution, but these changes may facilitate metabolic/physiological processes other than muscle contraction associated with swimming. This idea is supported by the observations of Primmett *et al.* (1986), who noted that aerobic swimming performance in rainbow trout was not compromised during recovery from burst swimming, and by our observations that the splenectomized fish were visibly more fatigued after swimming at U_{crit}. The increased Hb levels could also help to buffer the exercise-induced blood acidosis, thus facilitating recovery following strenuous swimming.

Our estimates of factors contributing to changes in Hct (Fig. 3) provide evidence of a graded spleen contraction. This result supports the findings of Yamamoto *et al.* (1980), who reported evidence of a graded splenic release of erythrocytes in cannulated yellowtails (*Seriola quinqueradiata*) during aerobic swimming in a water course (cruising speeds of 0.36 and 1.03 body lengths s^{-1}).

The contraction of the fish spleen is known to be under adrenergic nervous control and (or) the control of circulating catecholamines (Nilsson and Grove, 1974; see Nilsson, 1983). Moreover, a dose–response relationship between blood adrenaline levels and [Hb] has been demonstrated in trout (see Perry and Wood, 1989). Recently, Perry and Kinkead (1989) have shown that injections of physiological concentrations of catecholamines induce changes in Hct, [Hb] and Ca_{O_2} (similar to those observed in this study) in sham-operated but not in splenectomized fish. Thus, the elevated levels of plasma catecholamines observed in our study could have contributed to splenic contraction at U_{crit} but not at 45–55 % of U_{crit}. The finding that plasma catecholamine levels are not elevated during aerobic swimming in rainbow trout has been reported previously (Ristori and Laurent, 1985).

Part of the change in Hct at U_{crit} for sham-operated fish was attributed to erythrocyte swelling (Table 1, Fig. 3) which probably resulted from the stimulatory effect of elevated plasma catecholamine concentrations on erythrocyte pH regulation. This response is known to produce red blood cell swelling in a number of fish species, including rainbow trout (see Nikimaa, 1986); the putative function being to maintain blood oxygen-carrying capacity in the face of a blood acidosis (see Perry and Wood, 1989). The elevated levels of lactate at U_{crit} are similar to those reported by Primmett *et al.* (1986) and, although blood pH was not measured in the present study, one would expect it to decrease to the same extent as that reported earlier.

The decreased Hct observed in both groups during aerobic swimming (45–55 % of U_{crit}) has been described previously but not accounted for (Kiceniuk and Jones, 1977). We have shown that this was not the result of haemodilution due to blood sampling, but to plasma fluid shifts at the onset of swimming, possibly resulting from an increase in the perfused gill surface area and (or) an increase in the gill permeability to water (Wood and Randall, 1973). In the latter study, fish swimming at $32\,cm\,s^{-1}$ had a significant uptake of water (increased body mass) with a gradual reversal only after swimming at the same velocity for 30 min. The present study suggests that the shift of fluid into the plasma during aerobic swimming had reversed before the sampling at U_{crit} (see Fig. 3).

Pa_{O_2} values decreased concomitantly with increased swimming velocity. This pattern is the same as the results of Thomas *et al.* (1987), who reported a mean decrease of 45 mmHg Pa_{O_2} (6 kPa) at velocities close to U_{crit}, but contradicts the findings of Kiceniuk and Jones (1977) and Stevens and Randall (1967). Despite the apparent blood acidosis and the reduced arterial oxygen tension, the Ca_{O_2} at U_{crit} was unchanged in sham-operated fish. One factor which contributed to this was the mobilization of cells from the spleen. The significant reduction in Ca_{O_2} during aerobic swimming (45–55 % of U_{crit}) in sham-operated fish means that the decreases due to desaturation of Hb and to haemodilution were greater than the increase due to the spleen transfusion effect. The decreased Ca_{O_2} in splenectomized fish reflects the inability of this group to compensate for loss of blood cells due to sampling as well as Hb desaturation and fluid shifts.

Our study supports the hypothesis that transfusion of red blood cells from the spleen during exercise in fish plays a role in maintaining blood oxygen-carrying capacity in the face of exercise-induced physiological disturbances (e.g. blood acid–base balance or reduced oxygen-carrying capacity resulting from the Root shift that accompanies the acidosis). However, aerobic swimming performance of splenectomized fish is as good as that of sham-operated fish, so the spleen transfusion is not translated into improved aerobic swimming performance as measured by U_{crit}. Moreover, there was no correlation between the haematocrit and the critical swimming velocity of either group of fish. Thus, there must be an alternative role for the transfusion of cells during exercise in fish, perhaps one related to the recovery process.

This study was supported by an NSERC operating grant to A.F. M.A. received support from the Swedish Science Council. We are indebted to Helgi Thoransen-sen for fruitful discussion of the manuscript.

References

BELL, W. H. AND TERHUNE, L. D. B. (1970). Water tunnel for fisheries research. *Fish. Res. Bd Can. Tech. Rep.* **195**, 1–69.

BUTLER, P. J., DAY, N. AND NAMBA, K. (1992). Interactive effects of seasonal temperature and

low pH on resting oxygen uptake and swimming performance of adult brown trout *Salmo trutta*. *J. exp. Biol.* **165**, 195–212.

GEHRKE, P. C., FIDLER, L. E., MENSE, D. C. AND RANDALL, D. J. (1990). A respirometer with controlled water quality and computerized data acquisition for experiments with swimming fish. *Fish Physiol. Biochem.* **8**, 61–67.

JOHANSSON-SJÖBECK, M. L. (1979). The effects of splenectomy on the hematology and on the activity of delta-amino-levulinic acid dehydratase (ALA-D) in hematopoietic tissues of the European eel, *Anguilla anguilla L. Comp. Biochem. Physiol.* **63**A, 333–338.

JONES, D. R. (1971). The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*) J. *exp. Biol.* **55**, 541–551.

KICENIUK, J. W. AND JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

KITA, J. AND ITAZAWA, Y. (1989). Release of erythrocytes from the spleen during exercise and splenic constriction by adrenaline infusion in the rainbow trout. *Jap. J. Ichthyol.* **36**, 48–52.

MILLIGAN, C. L. AND WOOD, C. M. (1987). Regulation of blood oxygen transport and red cell pHi after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **133**, 263–282.

NIELSEN, O. B. AND LYKKEBOE, G. (1992). Changes in plasma and erythrocyte K^+ during hypercapnia and different grades of exercise in trout. *J. appl. Physiol.* **72**, 1285–1290.

NIKIMAA, M. (1986). Control of red cell pH in teleost fishes. *Ann. Zool. Fenn.* **23**, 223–235.

NILSSON, S. (1983). *Autonomic Nerve Function in the Vertebrates.* Berlin, Heidelberg, New York: Springer Verlag. 253pp.

NILSSON, S. AND GROVE, D. J. (1974). Adrenergic and cholinergic innervation of the spleen of the cod: *Gadus morhua*. *Eur. J. Pharmac.* **28**, 135–143.

PEARSON, M. P. AND STEVENS, E. D. (1991*a*). Splenectomy impairs aerobic swim performance in trout. *Can. J. Zool.* **69**, 2089–2092.

PEARSON, M. P. AND STEVENS, E. D. (1991*b*). Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem.* **9**, 39–50.

PERRY, S. F. AND KINKEAD, R. (1989). The role of catecholamines in regulating arterial oxygen content during acute hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **77**, 365–378.

PERRY, S. F. AND WOOD, C. M. (1989). Control and coordination of gas transfer in fishes. *Can. J. Zool.* **67**, 2961–2970.

PRIMMETT, D., RANDALL, D. J., MAZEAUD, M. AND BOUTILIER, R. G. (1986). The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. *J. exp. Biol.* **122**, 139–148.

RISTORI, M. T. AND LAURENT, P. (1985). Plasma catecholamines and glucose during moderate exercise in the trout: comparison with bursts of violent activity. *Expl Biol.* **44**, 246–253.

SOIVIO, A., NYHOLM, K. AND WESTMAN, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *J. exp. Biol.* **62**, 207–217.

STEVENS, E. D. AND RANDALL, D. J. (1967). Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. exp. Biol.* **46**, 329–337.

THOMAS, S., POUPIN, J., LYKKEBOE, G. AND JOHANSEN, K. (1987). Effects of graded exercise on blood gas tensions and acid–base characteristics of rainbow trout. *Respir. Physiol.* **68**, 85–97.

TUCKER, V. A. (1967). A method for oxygen content and dissociation curves on microliter blood samples. *J. appl. Physiol.* **23**, 407–410.

WELLS, R. M. G. AND WEBER, R. E. (1990). The spleen in hypoxic and exercised rainbow trout. *J. exp. Biol.* **150**, 461–466.

WELLS, R. M. G. AND WEBER, R. E. (1991). Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements? *J. Fish Biol.* **38**, 53–65.

WOOD, C. M. AND RANDALL, D. J. (1973). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **82**, 257–276.

WOODWARD, J. (1982). Plasma catecholamines in resting trout, *Salmo gairdneri* Richardson by high pressure liquid chromatography. *J. Fish Biol.* **21**, 429–432.

YAMAMOTO, K. I. (1989). Contraction of spleen in three perciformes and two tetraodontiformes during severe exercise. *Comp. Biochem. Physiol.* **94**A, 633–634.

YAMAMOTO, K. (1991). Increase of arterial O_2 content in exercised yellowtail (*Seriola quinqueradiata*). *Comp. Biochem. Physiol.* **98**A, 43–46.

YAMAMOTO, K. I., ITAZAWA, Y. AND KOBAYASHI, H. (1980). Supply of erythrocytes into the circulating blood from the spleen of exercised fish. *Comp. Biochem. Physiol.* **65**A, 5–11.