Exhaustive Exercise in "Wild" Atlantic Salmon (*Salmo salar*): Acid–Base Regulation and Blood Gas Transport

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Wild Atlantic salmon (*Salmo salar*) collected during their spawning migration were transported to the laboratory to investigate effects of exhaustive exercise on acid–base regulation and blood gas transport. Exhaustive exercise resulted in a very large extracellular acidosis which lasted for about 4 h. Blood lactate levels were extremely high and remained significantly elevated for at least 8 h after exercise. The degree of erythrocyte pH regulation was minimal and there was a significant fall in both erythrocyte pH and hemoglobin:oxygen carriage during the recovery period. Together, the significant decrease in erythrocyte pH and a significant reduction in the arterial partial pressure of oxygen resulted in a significant fall in arterial oxygen content immediately after exercise. Thereafter, arterial oxygen content was maintained by a significant increase in hematocrit and an increase in the arterial partial pressure of oxygen. Despite the extremely large lactacidosis in these wild fish, there were no mortalities during the recovery period. However, significant mortality has been reported in studies on domestic salmonids, and this suggests that wild salmonids may be better adapted for exhaustive exercise. This result supports the rationale of a "catch and release" recreational fishery for Atlantic salmon.

On a soumis en laboratoire des saumons de l'Atlantique (*Salmo salar*), recueillis durant leur migration de fraie, à un effort physique important pour en étudier les effets sur la régulation acido-basique et le transport sanguin des gas. L'effort a provoqué chez les saumons une acidose extracellulaire très marquée qui s'est prolongée durant environ 4 h durant la période de repos. Les taux de lactate sanguin étaient très élevés et sont restés supérieurs à la normale au moins 8 h après l'effort. Le degré de régulation du pH érythrocytaire de même que le taux de transport de l'oxygène par l'hémoglobine ont chuté de façon significative au cours de la période de récupération. Les baisses significatives du pH érythrocytaire et de la pression artérielle partielle de l'oxygène se sont traduites par une baisse significative de la concentration artérielle de l'oxygène immédiatement après l'effort. Par la suite, la concentration artérielle de l'oxygène a été maintenue par un accroissement significatif de l'hématocrite et une augmentation de la pression artérielle partielle de l'oxygène. Malgré la lacticémie très marquée observée chez ces poissons sauvages, aucun d'entre eux n'est mort durant la période de récupération. Par ailleurs, les résultats de plusieurs études ont fait état de taux de mortalité importants attribuables à l'effort chez des salmonidés domestiques, ce qui laisse entendre que les salmonidés sauvages résisteraient mieux à un effort important. L'idée d'une pêche récréative du saumon de l'Atlantique où les pêcheurs remettraient leurs prises à l'eau trouve appui dans les résultats de la présente étude.

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The Atlantic salmon (*Salmo salar*) is clearly the most valuable recreational species on the east coast of Canada as well as an important commercial species in some areas. Recent declines in the population of this species over the past several years, therefore, have resulted in legislative action by the federal government to preserve the stocks and attempt to return the population to previous levels. As part of this legislation, recreational fishermen must release all multi-sea winter salmon (i.e. over 63 cm in length) and all smaller salmon over and above the angler's daily or seasonal limit. Thus, a large number of these fish which are ascending rivers on their spawning migration are exercised to complete exhaustion by anglers and then released. Although a number of physiological studies have examined the effects of exhaustive exercise on acid–base balance and blood gas transport in salmonids, the vast majority of these studies have typically been carried out on hatchery stocks of easily domesticated species such as the rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*). To date, there has been no attempt to characterize the physiological disturbance associated with exhaustive muscular activity in *S. salar*. The degree of lactacidosis following exhaustive activity may be quite variable between salmonid species (Heisler 1984). Furthermore, the physiology of exercise-trained fish may be markedly different than that of untrained fish (Johnston 1982; Lackner et al. 1988). Clearly, this suggests that marked physiological differences probably also exist between hatchery and domestic fish stocks. Exercise data on other salmonid species, particularly domestic fish, therefore, may not be directly applicable to wild *S. salar* which is renowned for its capacity for burst activity. Finally, there are reports that exhaustive muscular activity may be associated with delayed mortality in fish (Black 1958; Wood et al. 1983). Thus, a description of the stress associated with exhaustive muscular activity in wild Atlantic salmon would clarify the effects of the present "catch

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and release'' fishery on the physiology of *S. salar*. On this background, the purpose of the present experiments was to characterize the physiological disturbance associated with exhaustive exercise in ''wild'' *S. salar* through a detailed examination of acid–base regulation and blood gas transport.

Materials and Methods

Animals and Rationale

Wild Atlantic Salmon (1.5 ± 0.4 kg; $N = 16$) were collected during their spawning migration in July from the fishladder on the Lahave River in Nova Scotia. The animals were transported to Dalhousie University in Halifax, N.S., where they were held in the aquatron facility at 18°C for at least 1 wk prior to the experiments. Under natural conditions, these animals do not feed during the spawning run, and therefore, no attempt was made to feed the salmon in captivity.

In the wild, Atlantic salmon are exercised to complete exhaustion by anglers, followed by a brief period of handling and their subsequent release. It was not practical to angle these fish in our laboratory setting, but we did make every attempt to reproduce the period of exhaustive exercise and brief handling characteristic of angled fish. In addition, all fish were fitted with a dorsal aortic cannula 48 h prior to the experiment which permitted nonstressful blood sampling of each fish prior to exercise and during the recovery period. This method has clear advantages over ''grab and stab'' techniques of blood sampling which cause the fish to struggle and can influence the acid–base variables followed in this type of study. Thus, our method should provide an accurate description of the magnitude of the disturbance caused by angling in a wild fish and the time course of recovery.

Surgery

To insert the dorsal aortic cannula, the salmon were first anaesthetized in an aerated and pH-balanced solution of tricaine methanesulfonate (MS-222) (66.7 mg MS-222·L^{-1} and 133.3 mg $NaHCO_3 \cdot L^{-1}$. The cannulation was then performed on a surgical table where a lighter dose of the aerated anaesthetic solution (50 mg MS-222·L^{-1} and 100 mg $NaHCO_3 \cdot L^{-1}$) was recirculated over the gills. A cannula of P.E. 50 tubing was implanted in the dorsal aorta using the technique of Smith and Bell (1964). Following surgery, the fish recovered for 48 h in light-proof perspex boxes.

Experimental Protocol

In in vivo experiments, following 48 h of recovery from surgery, a total of 1.5 mL of blood was taken into two 1-mL gas-tight Hamilton syringes. Whole-blood (extracellular) pH (pH_e), carbon dioxide content (C_{CO_2}), oxygen content (C_{O_2}), and the partial pressure of oxygen (P_{O_2}) were measured immediately using 300 μL of the sample. Duplicate hematocrit measurements were made on 150 μL of blood. The C_{CO_2} of true plasma was determined on a 50-μL sample of plasma from the hematocrit tubes. Two 100 μL fractions of the original sample were each added to 200 μL of chilled 8% perchloric acid (PCA) for the subsequent determination of whole-blood lactate and nucleotide triphosphate (NTP) concentrations, respectively. The samples for NTP were centrifuged and the supernatant was stored in liquid nitrogen until further analysis. The lactate samples were stored in the refrigerator (4°C). In addition, duplicate 25-μL samples of whole blood were each added to 5 mL of Drabkin's reagent for the determination of hemoglobin (Hb) concentration. The remaining sample was centrifuged in a 1.5-mL eppendorf tube. The supernatant and the buffy coat from the erythrocytes were removed and the erythrocyte pellet was immediately frozen in liquid nitrogen for the subsequent determination of erythrocyte pH (pH_i).

After the control sample was taken, the salmon was moved to a cylindrical tank (1.5-m in diameter) where it was exhaustively exercised by manual chasing. The exercise period was terminated after about 10 min when the salmon rolled on its side and would no longer respond to chasing. The exhausted fish was then returned to the perspex container and another 1.5-mL blood sample was removed. Analyses similar to those described for the control sample were performed on this blood sample. Samples were also taken at 0.5, 1, 2, 4, 8, and 24 h of recovery from exercise. Throughout the experiment, blood samples were replaced with a similar volume of Cortland saline. This protocol was repeated on six of the salmon. As a control, an identical sampling protocol was performed on an additional four salmon which were not exhaustively exercised.

Further analysis of the characteristics of wild Atlantic salmon blood was carried out in vitro. The first series of in vitro experiments was conducted to determine the in vitro gas transport properties of the blood. In these experiments, 3-mL blood samples were removed from resting cannulated salmon and equilibrated in an intermittently rotating glass tonometer. The blood was equilibrated for 30 min with a humidified 0.2% CO_2–air mixture at 18°C. Following equilibration, a 1-mL blood sample was removed with a gas-tight Hamilton syringe. pH_e, C_{CO_2}, C_{O_2}, and Hb concentration were determined immediately and the remainder of the sample was centrifuged. The resulting supernatant was discarded and the erythrocyte pellet was frozen in liquid nitrogen for the determination of pH_i. This procedure was also repeated on the same blood pool with humidified 1 and 3% CO_2–air mixtures.

Another series of in vitro experiments examined the effects of adrenergic stimulation on the pH gradient across the membrane of salmon red blood cells. This time, 4-mL blood samples were removed from resting cannulated salmon ($N = 6$) and equilibrated with a humidified 1% CO_2–air mixture in paired intermittently rotating tonometers at 18°C with 2mL of blood per tonometer. Following a 30-min equilibration period, a 1-mL blood sample was removed from each tonometer and analysed for pH_e and pH_i. At this point, 100 μL of either saline (sham) or isoproterenol (final concentration 10^{-5} M) in saline was added to each blood pool. The blood was then equilibrated for a further 15 min at which time the final 1 mL of blood was removed for the determination of pH_e and pH_i.

In the final series of in vitro experiments, we investigated the effects of adrenergic stimulation on the aerobic metabolism of salmon red blood cells. In this series, 3-mL blood samples were removed from resting cannulated salmon ($N = 6$) and equilibrated with a humidified 1% CO_2–air mixture in paired tonometers at 15°C with 1.5 mL of blood per tonometer. Following a further 10 min of equilibration, a 1-mL blood sample was removed from each tonometer with a 1-mL gas-tight Hamilton syringe. Determinations of C_{CO_2}, C_{O_2}, and Hb concentration were made immediately on 250 μL of blood. The remaining blood was equilibrated within the gas-tight syringe over a 4-h period at 15°C in a constant-temperature room. Samples from the syringe were also taken after 2 and 4 h for similar analyses. Together, these three samples were used to determine

erythrocyte oxygen consumption and carbon dioxide production rates. It should be noted that this final series was carried out at 15°C rather than 18°C to facilitate direct comparison of aerobic metabolism with data from other studies.

Analyses

pH_e and pH_i were determined with a PHM 84 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) at 18°C. Erythrocyte samples were frozen and thawed twice in liquid nitrogen prior to the determination of pH according to the method of Zeidler and Kim (1977). The P_{O_2} of whole blood was measured with an E5046 oxygen electrode (Radiometer, Copenhagen, Denmark) at 18°C. Another E5046 oxygen electrode was used to determine the C_{O_2} of whole blood samples using the Tucker method (Tucker 1967). Analysis of Hb concentrations was performed by the Drabkin's method with Sigma reagents. Total C_{CO_2} of whole blood and plasma were measured with a Carle Series 100 gas chromatograph (Carle Instruments Inc., USA), as described by Boutilier et al. (1984). Arterial CO_2 tension (P_{aCO_2}) and plasma bicarbonate concentration ($[HCO_3^-]_{pl}$) were calculated via a rearrangement of the Henderson–Hasselbach equation, using measured values of pH_e and plasma C_{CO_2}. Constants for plasma pK′ and CO_2 solubility to be used in these calculations were obtained from Boutilier et al. (1984). The concentration of metabolic protons added to the whole blood ($[H^+]_m$) over any given time period (e.g. time 1 to 2) was calculated according to McDonald et al. (1989) using the following equation:

$$H^+{}_m = [HCO_3^-]_{wb,1} - [HCO_3^-]_{wb,2} - B(pH_{e,1} - pH_{e,2})$$

where $[HCO_3^-]_{wb}$ is the whole-blood bicarbonate concentration which was determined using the same calculation described for plasma bicarbonate, the only exception being that whole-blood C_{CO_2} was used rather than plasma C_{CO_2}. The nonbicarbonate buffer value of whole blood (B) was determined from the hemoglobin concentration ([Hb]) according to the following relationship:

$$B = (-1.895\ [\mathrm{Hb}]) + 2.251, \quad r = -1.0$$

which was experimentally determined in the second series of experiments. The concentration of lactate was measured using the L-lactate dehydrogenase method (Loomis 1961) using Sigma reagents. The amount of lactate added to the blood over any given time (t) was calculated according to the equation

$$\Delta La^- = [La^-]_t - [La^-]_R$$

where $[La^-]_R$ is the $[La^-]$ at rest.

The proton (H^+) deficit was then obtained by subtracting $[H^+]_m$ from the ΔLa^-.

Statistics

A paired Student t-test was used to compare the resting values with the postexercise values in the in vivo experiments and the pre- and posttreatment erythrocyte values in the in vitro experiments. An unpaired Student t-test was also used to compare the control salmon and the exercised salmon in the in vivo experiments. Finally, an analysis of covariance was used to compare the in vivo and in vitro slopes of the regression lines for pH_e and pH_i. In all cases, $p < 0.05$ was the accepted level of significance.

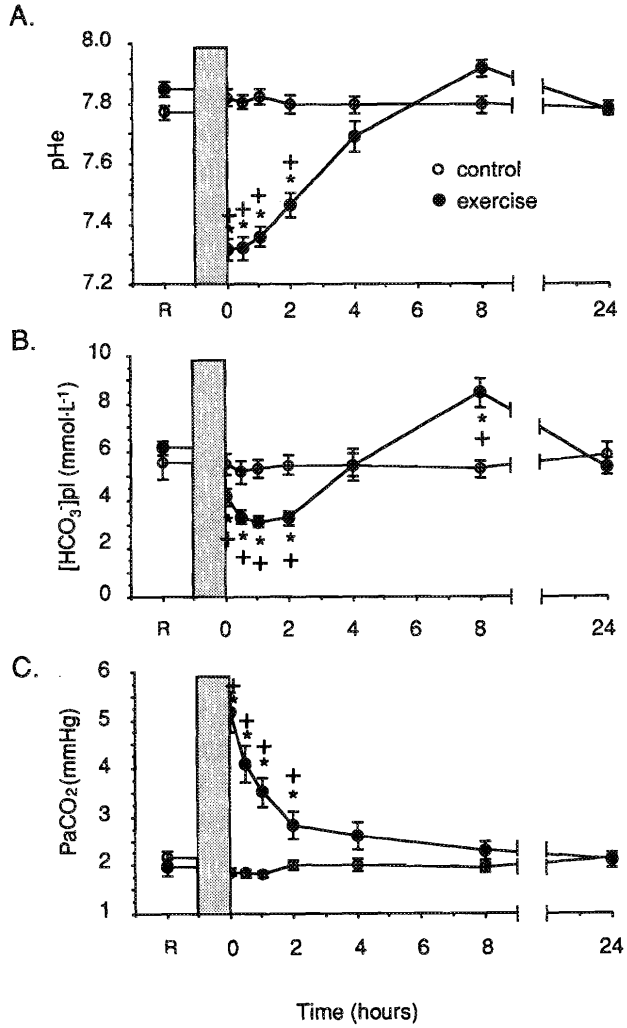

FIG. 1. (A) Extracellular pH (pH_e), (B) plasma bicarbonate concentration ($[HCO_3^-]_{pl}$), and (C) arterial CO_2 tension (P_{aCO_2}) at rest (R) and 0, 0.5, 1, 2, 4, 8, and 24 h following exhaustive exercise (shaded bar) in *S. salar*. Values are means ± standard error (control, $N = 4$; exercise, $N = 6$). Asterisk denotes significant ($p < 0.05$) difference from resting value; plus sign denotes significant ($p < 0.05$) difference from control value.

Results

Exhaustive exercise in *S. salar* results in a large immediate drop in pH_e from the resting value of 7.848 ± 0.026 to 7.316 ± 0.035 (Fig. 1A). The pH_e remained significantly reduced until the 4-h sample. A similar pattern was also observed in $[HCO_3^-]_{pl}$ which fell significantly from 6.18 ± 0.28 to 4.15 ± 0.34 mmol·L^{-1} immediately after exercise (Fig. 1B). The lowest $[HCO_3^-]_{pl}$ value of 3.13 ± 0.24 mmol·L^{-1} was observed 1 h into the recovery period, and by the 4-h sample, $[HCO_3^-]_{pl}$ had risen to a value which was not significantly different from the resting value. This was followed by an overcompensation in $[HCO_3^-]_{pl}$ at 8 h to 8.46 ± 0.61 mmol·L^{-1}, but at 24 h the value had returned to normal. P_{aCO_2} increased significantly following the exercise period (Fig. 1C). The greatest increase was observed immediately following exercise when P_{aCO_2} had risen from 1.97 ± 0.17 mmHg (1 mmHg = 133.322 Pa) at rest to 5.19 ± 0.41 mmHg. P_{aCO_2} remained significantly elevated until the 4-h recovery sample.

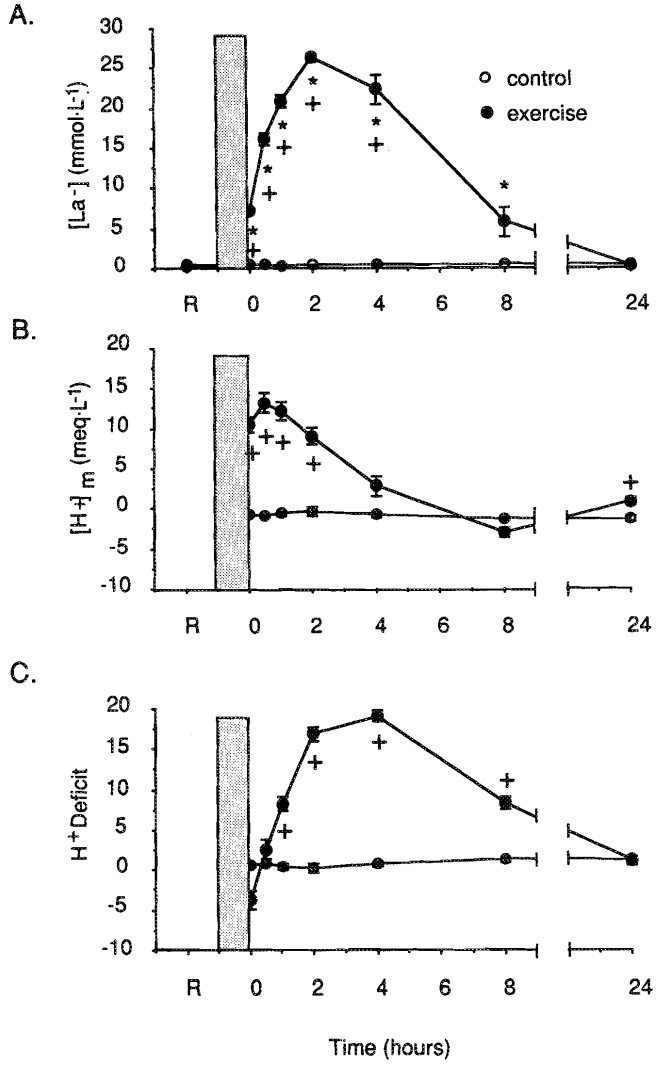

FIG. 2. (A) Lactate concentration ($[La^-]$), (B) metabolic proton load ($[H^+]_m$), and (C) proton deficit at rest (R) and 0, 0.5, 1, 2, 4, 8, and 24 h following exhaustive exercise (shaded bar) in *S. salar*. Values are means ± standard error (control, $N = 4$; exercise, $N = 6$). Asterisk denotes significant ($p < 0.05$) difference from resting value; plus sign denotes significant ($p < 0.05$) difference from control value.

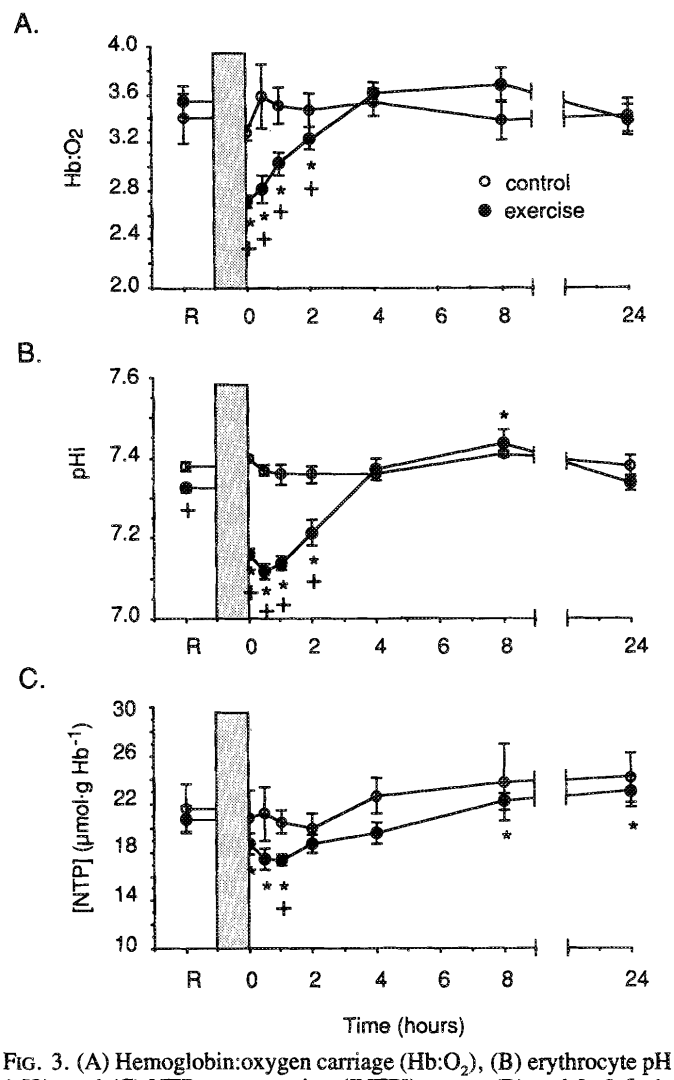

FIG. 3. (A) Hemoglobin:oxygen carriage ($Hb:O_2$), (B) erythrocyte pH (pH_i), and (C) NTP concentration ([NTP]) at rest (R) and 0, 0.5, 1, 2, 4, 8, and 24 h following exhaustive exercise (shaded bar) in *S. salar*. Values are means ± standard error (control, $N = 4$; exercise, $N = 6$). Asterisk denotes significant ($p < 0.05$) difference from resting value; plus sign denotes significant ($p < 0.05$) difference from control value.

Whole blood lactate concentrations $[La^-]$ reached peak levels 2 h after exercise (26.3 ± 0.5 mmol·L^{-1}) and remained significantly higher than the resting value for 8 h (Fig. 2A). $[H^+]_m$, however, peaked at 13.2 ± 1.3 meq·L^{-1} after only 0.5 h and had returned to levels which were no longer significantly different than rest by 4 h (Fig. 2B). Figure 2C plots the proton deficit which is the calculated difference between ΔLa^- and $[H^+]_m$. Immediately following the exercise period, $[H^+]_m$ exceeds ΔLa^- and the proton deficit, therefore, is negative. After 1 h, however, this relationship is reversed. The largest proton deficit of 19.1 ± 0.9 mmol·L^{-1} is observed after 4 h when the $[H^+]_m$ has fallen close to the resting value, but ΔLa^- is still high.

Exhaustive exercise in *S. salar* is associated with a substantial (25%) drop in hemoglobin:oxygen ($Hb:O_2$) carriage (Fig. 3A). Immediately following the exercise period, the significant fall in P_{O_2} could contribute to the change in $Hb:O_2$ (Table 1). The P_{O_2} quickly returns to normal, however, and by 1 h has significantly increased. Thus, the significant decrease in $Hb:O_2$ for the first 2 h of the recovery period is mainly due to the fall in pH_i and the associated reduction in $Hb:O_2$ via the Root effect (Fig. 4). The largest drop in pH_i of 0.210 pH unit occurs after 0.5 h and pH_i remains significantly below the resting value until the 4-h sample (Fig. 3B). At 8 h, there is a significant increase in pH_i, but by 24 h, pH_i had again returned to normal. Concurrent with the fall in pH_i, there is also a significant decrease in erythrocyte NTP levels, which may partially ameliorate the deleterious effects of pH_i on $Hb:O_2$ affinity. Despite the fall in $Hb:O_2$ carriage, arterial oxygen content is only significantly reduced immediately following exercise. By 0.5 h of recovery, there is a significant increase in hematocrit, and oxygen content is thereby preserved. It should be noted that there was no significant difference in mean cellular Hb concentration after exercise, and the increase in hematocrit is therefore probably due to an increase in the number of circulating erythrocytes. A significant reduction in oxygen content is also observed after 8 and 24 h in both the control and exercise animals. This reduction can be attributed to repetitive blood

TABLE 1. Effect of exhaustive exercise on hematocrit, Po_2, and O_2 content (CaO_2) in *S. salar* at 18°C. Values are means ± standard error (control, N = 4; Exercise, N = 6). Asterisk denotes significant ($p < 0.05$) difference from resting value; plus sign denotes significant ($p < 0.05$) difference from control value.

	Rest	0 h	0.5 h	1 h	2 h	4 h	8 h	24 h
Hematocrit (%)								
Control	25.4 ± 3.8	25.2 ± 3.5	24.5 ± 4.7	25.9 ± 3.4	24.2 ± 3.5	21.6 ± 3.5*	20.6 ± 4.8*	19.4 ± 3.4*
Exercise	29.5 ± 2.7	32.7 ± 1.9	38.2 ± 2.1*	37.9 ± 1.4*+	35.6 ± 1.6+	29.9 ± 1.9	21.4 ± 1.9*	20.0 ± 3.0*
Po_2 (mmHg)								
Control	87.2 ± 5.4	86.7 ± 4.9	87.8 ± 3.9	87.9 ± 3.6	90.9 ± 4.7	92.4 ± 4.4	88.0 ± 6.0	81.9 ± 6.1
Exercise	89.4 ± 2.5	77.3 ± 4.0*	84.9 ± 7.3	98.6 ± 3.7*	92.4 ± 4.8	87.5 ± 4.6	95.8 ± 2.3	94.7 ± 4.1
CaO_2 (vol %)								
Control	10.4 ± 1.6	9.7 ± 1.2	10.5 ± 2.4	10.1 ± 1.4	9.8 ± 1.5	8.7 ± 1.4*	8.2 ± 2.0*	8.2 ± 1.4*
Exercise	12.0 ± 0.6	9.7 ± 0.6*	11.6 ± 0.7	12.0 ± 0.6	11.8 ± 0.5	11.5 ± 0.6	9.4 ± 0.7*	8.4 ± 1.1*

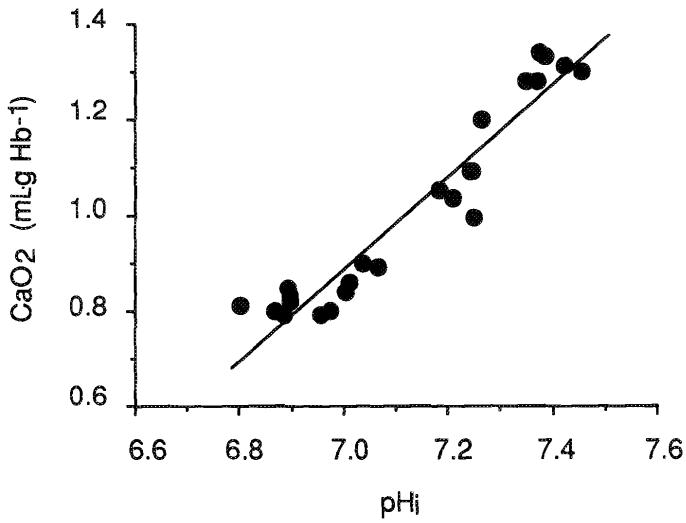

FIG. 4. Relationship between arterial oxygen content (Cao_2) and erythrocyte pH (pH_i) for the blood of *S. salar*. The regression equation describing the relationship is $Cao_2 = (0.952)pH_i - 5.77$, $r^2 = 0.917$.

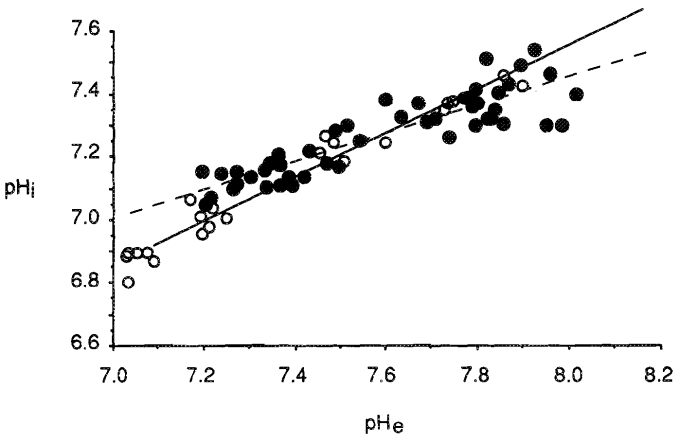

FIG. 5. Extracellular pH (pH_e) versus erythrocyte pH (pH_i) for blood from *S. salar* in vitro (○) and in vivo (●). The regression equation for the in vitro values is $pH_i = (0.696)pH_e + 1.985$, $r^2 = 0.96$ and the equation for the in vivo values is $pH_i = (0.443)pH_e + 3.904$, $r^2 = 0.77$. The lines are significantly ($p < 0.05$) different.

sampling, since it is clearly associated with a significant decrease in hematocrit during the latter stages of the experiment.

The slope of the relationship between pH_e and pH_i in vivo is significantly different from that in vitro (Fig. 5). Thus, below

TABLE 2. Effect of isoproterenol on pH_e, pH_i, and the pH gradient (ΔpH) across the membrane of *S. salar* erythrocytes at 18°C. Values are means ± standard error ($N = 6$). Asterisk denote significant ($p < 0.05$) difference from control value.

	Control	Treatment
Sham		
pH_e	7.411 ± 0.016	7.497 ±0.022*
pH_i	7.214 ± 0.013	7.238 ± 0.007
ΔpH	0.196 ± 0.027	0.260 ± 0.025*
Isoproterenol		
pH_e	7.473 ± 0.023	7.339 ± 0.015*
pH_i	7.234 ± 0.011	7.262 ± 0.011
ΔpH	0.239 ± 0.028	0.076 ± 0.014*

pH_e of 7.6, the pH_i measured in vivo is higher than that measured in vitro. At a pH_e of 7.3, characteristic of the exhausted salmon, this difference in pH_i is equivalent to 0.072 pH unit.

The erythrocytes of *S. salar* are clearly sensitive to adrenergic stimulation in vitro. Addition of isoproterenol results in a significant decrease in the pH gradient across the erythrocyte membrane, indicating transfer of protons from the intracellular to the extracellular compartment (Table 2). Isoproterenol addition also increases the aerobic respiration of the erythrocytes causing a 52% increase in oxygen consumption and a 94% increase in CO_2 production (Fig. 6).

Discussion

Burst activity in salmonids is associated with a marked acidosis due to the large production and release of protons into the extracellular fluid from the poorly perfused white muscle. In wild *S. salar*, this acidosis is most severe during the 2 h immediately following exercise, and by 4 h the pH_e returns very close to normal (Fig. 1). The duration of this extracellular recovery period is very similar to that of burst-exercised rainbow trout (Holeton et al. 1983; Turner et al. 1983; Milligan and Wood 1986a, 1986b; Primmett et al. 1986; Tang et al. 1989; McDonald et al. 1989). However, the magnitude of the disturbance is markedly greater in *S. salar*. As well, the whole-blood lactate concentrations reach much higher values than those frequently reported for other salmonid species (Black 1957a, 1957b; Holeton et al. 1983; Turner et al. 1983; Primmett et al. 1986; Tang et al. 1989; McDonald et al. 1989). Only one study by Black (1957c) on *Oncorhynchus nerka* has reported higher extracellular lactate concentrations, although it should be pointed out that 26% of the fish in this study died

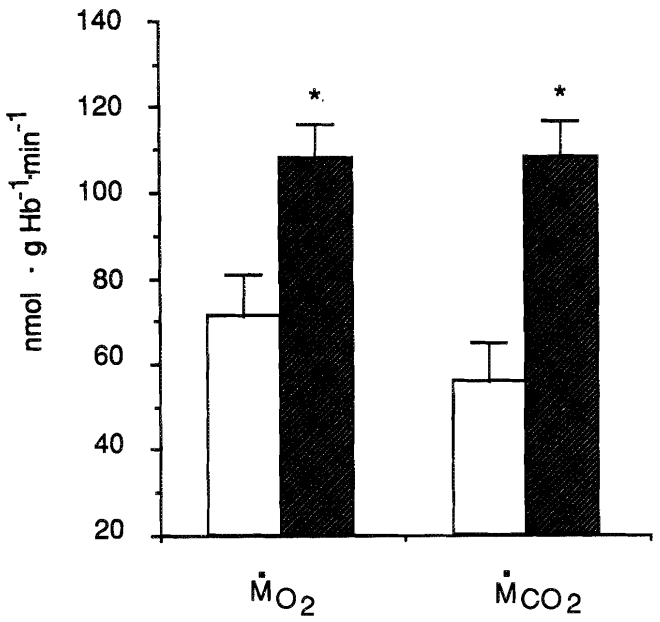

FIG. 6. Oxygen consumption ($\dot{M}O_2$) and carbon dioxide production ($\dot{M}CO_2$) of *S. salar* erythrocytes in vitro under control conditions (open bars) and following addition of isoproterenol (shaded bars). Values are means ± standard error ($N = 6$). Asterisk denotes significant ($p < 0.05$) difference from control value.

during the recovery period. The reason for this variability in reported peak lactate concentrations does not appear to be entirely due to differences in experimental protocol. Indeed, pronounced differences in peak extracellular lactate concentrations following exercise are clearly apparent when broad comparisons are made between fish species (Heisler 1984). Thus, it is reasonable to assume that this variability may also occur to a certain extent within the salmonids.

Oncorhynchus mykiss is the only other salmonid in which $[H^+]_m$ has been determined following exhaustive activity (Milligan and Wood 1986a, 1986b; Tang et al. 1989, McDonald et al. 1989). In comparison, the blood $[H^+]_m$ in the present study is about 50% higher than in the freshwater rainbow trout which is further evidence of a much greater lactacidosis in the Atlantic salmon (Fig. 2B). These metabolic protons, however, disappear from the blood quickly in comparison with extracellular lactate, and therefore, there is a very large proton deficit in our experiments (Fig. 2C). To our knowledge, this is the largest proton deficit ever reported in a fish.

Recently, a great deal of attention has been paid to the importance of pH_i regulation in fish following stresses such as exhaustive activity when there are large perturbations in pH_e (Nikinmaa et al. 1984; Primmett et al. 1986; Milligan and Wood 1986a, 1986b; McDonald et al. 1989). Adrenergic regulation of pH_i under these circumstances can offset the reduction in oxygen carrying capacity associated with the Root effect. The magnitude of this pH_i regulation, however, is quite variable, even within a singles species, and can range from an entire lack of pH regulation (Nikinmaa and Jensen 1986) to complete regulation (Primmett et al. 1986; Nikinmaa and Jensen 1986). The significant fall in pH_i of 0.21 pH unit 30 min after exercise persisted until the 4-h sample when pH_e had recovered to almost normal values (Fig. 3B). *Salmo salar* blood has a significant Root effect, and this fall in pH_i was, therefore, associated with a simultaneous decrease in $Hb:O_2$ carriage (Fig. 3A and 4). When the relationship between pH_e and pH_i observed in vivo was compared with that in vitro, it is clear that there was a minimal, but significant, degree of pH_i regulation despite the fall in pH_i (Fig. 5). At a postexercise pH_e of 7.3, the pH_i in vivo was, in fact, 0.072 pH unit higher than that observed in vitro. Thus, from the Root effect data, it is possible to calculate that this degree of pH_i regulation would result in only a 7% increase in arterial blood $Hb:O_2$ carriage; the latter fell by about 25% following exercise. This decrease in arterial blood $Hb:O_2$ carriage may, in fact, have been even greater had there not been a significant drop in erythrocyte NTP levels at this time which would tend to increase the affinity of Hb for oxygen (Fig. 3C). The reason for the minimal degree of adrenergic pH_i regulation in the present study is not readily apparent. The erythrocytes are clearly sensitive to adrenergic stimulation in vitro (Table 2; Fig. 6). It is possible, however, that several factors might be contributing to the minimal response in vivo. One explanation could be the relative age of the circulating erythrocytes in migrating salmon. During their spawning migration, Atlantic salmon do not feed, and this would be expected to increase the mean age of the circulating erythrocytes, since erythropoietic activity is reduced during periods of starvation (Lane and Tharp 1980). The spleen may release a certain number of younger cells into the circulation following exercise (Wells and Weber 1990). The mean age of the entire circulating population of erythrocytes in migrating salmon, however, is probably older than in feeding fish. Aging in nucleated erythrocytes is also associated with a decrease in oxygen consumption (Tipton 1933). Thus, further evidence of a preponderance of "older" erythrocytes is provided by the observation that resting levels of erythrocyte oxygen consumption in the present experiments are less than half the value for erythrocytes from well-fed domestic *S. salar* (Ferguson and Boutilier 1988). According to Cossins and Kilbey (1989), the Na^+/H^+ exchange activity which drives the pH regulatory response is reduced in older erythrocytes. Together, this evidence suggests that the adrenergic pH response may be reduced in the erythrocytes of migrating salmon due to the greater mean age of the circulating erythrocytes in these starving animals. A further contributing factor to the apparently minimal pH response may also be the severe acidosis following exercise in these animals. Borgese et al. (1987) have demonstrated that a large concentration of external protons may inhibit the activity of the Na^+/H^+ exchanger. Regardless, the present results demonstrate that adrenergic regulation of pH_i probably provides only a minimal benefit to oxygen transport following exhaustive activity in migrating wild Atlantic salmon.

Despite the fall in $Hb:O_2$ carriage in exhausted salmon, arterial oxygen content was constant during the recovery period (Table 1). This regulation was not apparent immediately following the exercise period when the fall in $Hb:O_2$ carriage combined with a drop in P_{O_2} to reduce the content of oxygen. After 30 min, however, there was an increase (30%) in hematocrit, and P_{O_2} had returned to normal. Together these changes combined to offset the reduction in arterial $Hb:O_2$ carriage and preserve arterial oxygen content during the recovery period. Although a decrease in plasma volume after exercise will contribute somewhat to the increase in hematocrit, adrenergic stimulation of the spleen can substantially increase the number of circulating erythrocytes (Nilsson and Grove 1974). Our results suggest that the contribution of the spleen towards the regulation of oxygen content is probably more important than the regulation of pH_i following exhaustive exercise in migrating Atlantic salmon.

There are several reports of delayed mortality associated with exhaustive muscular activity in fish (Black 1957c, 1958; Beggs et al. 1980; Wood et al. 1983). Clearly, a significant mortality in exhausted fish could have important implications for the management of a "catch and release" fishery such as that now in existence for Atlantic salmon. Early reports suggested that this delayed mortality was due to an excessive lactacidosis in the blood, but according to Wood et al. (1983), the probable cause of delayed mortality is the intracellular acidosis associated with the exercise. In this regard, it is important to note that there were no mortalities amongst the wild Atlantic salmon in the present experiments even though the extracellular metabolic acidosis was much greater than that reported in studies where significant delayed mortality was observed. Thus, the present study supports the view that the degree of extracellular acidosis is probably not the cause of death in exhausted fish. Furthermore, on the basis of the present results, we submit that wild fish may not be as prone to delayed mortality following exhaustive exercise as hatchery stocks due to differences in both physiology and selective pressures between hatchery and wild stocks which probably impart a greater level of overall fitness for exhaustive activity amongst the wild species.

In summary, there is a very large metabolic acidosis associated with exhaustive muscular activity in wild Atlantic salmon which persists for at least 2 h into the recovery period. Extracellular lactate concentration levels are also higher than those commonly observed in salmonids, and removal of extracellular lactate is much slower than the recovery from the extracellular acidosis. Thus, we report a larger proton deficit than previously described in salmonids. There is a fall in arterial oxygen content immediately following exercise due to a significant fall in both P_{O_2} and pH_i which together decrease arterial Hb:O_2 carriage. Thereafter, oxygen content is preserved despite the fall in pH_i by a large increase in hematocrit and an increase in P_{O_2}. Even though there was a large metabolic acidosis in these fish, there were no mortalities observed in this study. This supports the view that the extracellular lactacidosis is probably not the cause of delayed mortality in exhausted fish. Furthermore, the absence of mortalities suggests a higher overall level of fitness for burst activity in wild *S. salar* than in domestic salmonids. Finally, the present results support the rationale for a "catch and release" recreational fishery for wild Atlantic salmon.

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