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James D. Kieffer [a], Andrew M. Rossiter [b], Christine A. Kieffer [b], Kevin Davidson [c] & Bruce L. Tufts [d]

[a] Department of Biology, University of New Brunswick, Saint John, New Brunswick, E2L 4L5, Canada

[b] Department of Biology, Queen's University at Kingston, Kingston, Ontario, K7L 3N6, Canada

[c] Canadian Wildlife Service, Sackville, New Brunswick, E4L 1G6, Canada

[d] Department of Biology, Queen's University at Kingston, Kingston, Ontario, K7L 3N6, Canada

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Physiology and Survival of Atlantic Salmon following Exhaustive Exercise in Hard and Softer Water: Implications for the Catch-and-Release Sport Fishery

JAMES D. KIEFFER*

Department of Biology, University of New Brunswick, Saint John, New Brunswick E2L 4L5, Canada

ANDREW M. ROSSITER AND CHRISTINE A. KIEFFER

Department of Biology, Queen's University at Kingston, Kingston, Ontario K7L 3N6, Canada

KEVIN DAVIDSON

Canadian Wildlife Service, Sackville, New Brunswick E4L 1G6, Canada

BRUCE L. TUFTS

Department of Biology, Queen's University at Kingston, Kingston, Ontario K7L 3N6, Canada

Abstract.—This study examined the influence of environmental water hardness (40 mg/L versus 100 mg/L $CaCO_3$) on the physiology and survival of exhaustively exercised Atlantic salmon *Salmo salar*. Cannulated Atlantic salmon provided blood samples at rest and at 0, 1, 2, 4, and 8 h postexercise, whereas white muscle samples were acquired from noncannulated Atlantic salmon at rest and at 0 and 4 h postexercise. Exhaustive exercise resulted in significant metabolic, acid–base, and electrolyte disturbances in the blood and white muscle of Atlantic salmon in hard (about 95 mg/L $CaCO_3$) water, although postexercise survival was 100%. For resting Atlantic salmon, the physiological status of those in softer (about 40 mg/L $CaCO_3$) water was similar to that of those in hard water. In softer water, however, exhaustive exercise caused a significantly greater elevation in postexercise blood lactate and a larger acid–base disturbance compared with fish in hard water. Postexercise survival of Atlantic salmon in softer water was directly related to environmental water hardness, and those that succumbed failed to exhibit any postexercise correction of their extracellular acid–base disturbance. Despite these pronounced effects of water hardness on the blood compartment following exercise, the postexercise status of the muscle was only marginally different between fish in hard and softer water.

In 1985 approximately 55,000 anglers spent an estimated Can$84 million dollars on fly fishing for Atlantic salmon *Salmo salar* in Canada's Atlantic provinces (Tuomi 1987). In recent years, however, the salmon stocks that are the core of this resource have declined precipitously (Watt 1987; Lacroix 1992). In fact, some rivers have lost their salmon runs altogether. These declines have been attributed to factors such as habitat damage (e.g., acid-rain, water quality changes), poaching, overharvesting, and decreases in sea survival (Watt 1989; Friedland and Reddin 1992; Ritter 1992). Considerable effort continues to be invested to sustain and enhance Atlantic salmon stocks. These endeavors include the river stocking programs by government fisheries agencies, habitat restoration projects (e.g., liming of acidic rivers by local salmon associations), and the voluntary release of caught fish by conservation-minded anglers.

The practice of releasing angled fish, (i.e., catch-and-release angling) has become a popular strategy to reduce the harvest of fish by recreational anglers, while still allowing anglers to enjoy their sport (Barnhart 1989). The conservation benefits of such angling have been recognized by fisheries mangers, and the practice is currently endorsed by many international and local angling associations (Anderson and Nehring 1984; Barnhart 1989). A mandatory catch-and-release policy was established in Canada's maritime provinces, stating that all multi-sea-winter Atlantic salmon (>63 cm long) must be released, along with any smaller

* Corresponding author: jkieffer@unbsj.ca

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adult salmon (grilse) caught in excess of daily or seasonal quotas. Where such laws do not exist, the voluntary release of all fish is often encouraged and practiced.

Several studies have shown that exhaustive exercise, such as that associated with angling (Kieffer et al. 1995), leads to severe physiological disturbances in fish (see Wood 1991; Booth et al. 1995; Kieffer et al. 1995; Milligan 1996). In some situations, these disturbances may be severe enough to cause delayed mortality (Beggs et al. 1980; Wood et al. 1983; Ferguson and Tufts 1992; Wilkie et al. 1996).

According to Lacroix (1992), water quality is of critical importance to the survival of Atlantic salmon. The issue of water hardness is highly relevant to Atlantic salmon stock management in Atlantic Canada and the northeastern United States because these areas generally have poorly buffered soil and the water tends to be acidic. In rainbow trout *Oncorhynchus mykiss*, water hardness has a significant effect on the physiological disturbance in blood following exhaustive exercise (Graham et al. 1982). To date, however, no studies have evaluated the importance of water hardness on the physiological response following exhaustive exercise in Atlantic salmon. In addition, information is scant regarding the relationship between water hardness, blood and muscle physiology, and survival following exercise in salmonids. Nonetheless, this information may be extremely useful for the effective management of species such as the Atlantic salmon, for which recreational fisheries are largely dependent upon catch-and-release as a conservation and enhancement tool.

On this background, the objective of our investigation was to examine the influence of water hardness (softer versus hard) on the postexercise physiology of blood and muscle tissue of Atlantic salmon, as well as the effects on survival. Given the known negative effects of softer water on the physiology noted for other salmonids (Graham et al. 1982), we hypothesized that there would be a greater physiological disturbance following exhaustive exercise in softer water fish compared with hard water. We further predicted that this greater physiological disturbance for Atlantic salmon in softer water would translate into greater postexercise mortality in this group.

Methods

Experiments on hatchery-reared Atlantic salmon (300–500 g) were carried out at the Margaree Salmonid Enhancement Center, Cape Breton, Nova Scotia, Canada, during July–August 1995. The salmon had been raised in outdoor water flow-through holding ponds filled from the hatchery's normal Ingraham Brook water; this water was neutral (pH 6.7–7.2) and hard (total about 90–100 mg/L as $CaCO_3$; $[Ca^{2+}]$ = 32 mg/L; $[Mg^{2+}]$ = 0.3 milliequivalents, mEq; $[Na^{+}]$ = 3.7mEq; $[Cl^{-}]$ = 3.8mEq/L). Before the experiments, the fish were transferred to large, indoor water flow-through tanks containing water identical in chemical composition to that in the outdoor ponds. The fish were given at least 24 h to acclimate to the indoor tanks and were then divided into two treatment groups: hard water and softer water. The hard-water group was tested using the normal hatchery water; these fish remained in hard water. The softer water group were transferred from the hard water into an identical tank containing neutral (pH = 7.1 ± 0.02) water; the water hardness, however, was manipulated by varying the relative flow rates of hard and softer water that was produced using a commercial water conditioner (Culligan, Canada) which removed all Ca^{2+}. Hardness was checked several times each day with a total hardness direct reading kit (LaMotte, Chestertown, Maryland).

The fish in the softer water groups were given a 4-d period to acclimate to the softer water because McDonald et al. (1980) found that the blood ion and acid–base status of rainbow trout transferred from hard to softer water stabilized within 4 d. The Atlantic salmon in Canada's catch-and-release sport fishery are migrating fish, many of which experience large variations in water hardness over short periods as they move between seawater and freshwater with varying degrees of hardness.

Pilot Experiment on Water Softness and Mortality

A pilot experiment was carried out to determine the relationship between water hardness and survival following exercise. For this experiment, three ranges of water hardness were used: 0–12, 30–50, and 90–100 mg/L $CaCO_3$. Once at the desired water hardness, the fish were divided into two groups: cannulated (see blood experiments below) and uncannulated. Fish were exercised for 5 min, and returned to the holding boxes (see below for details). The percent survival was determined over the next 12 h. When mortality occurred, it normally happened within the first 2 h postexercise. Because the mortality rate of fish was excessive at the lowest water hardness (i.e., 0–12 mg/L $CaCO_3$), we selected 40 mg/L $CaCO_3$ ($[Ca^{2+}]$

about 14 mg/L) as the softer water used for our physiological studies. We used cannulated fish in this pilot study because we were going to use cannulated fish in our primary experiment. None of the fish in the pilot experiment were used for blood or muscle analysis.

Blood and Muscle Experiments

Following the acclimation periods, Atlantic salmon in each group were divided for use in either blood or muscle experiments. Fish were not fed for about 72 h before the experiments began to reduce any effects of feeding on metabolic rate (Kieffer and Tufts 1998). In all experiments, the water temperature was 13–17°C and the tanks were thoroughly aerated. Dissolved oxygen levels were checked daily with a portable dissolved oxygen meter (YSI, Yellow Springs, Ohio) to ascertain they remained close to saturation. The water was also checked periodically for ammonia levels, with a test kit (LaMotte), to ascertain that water ammonia levels were minimal (<1 mg/L).

Blood experiments.—The Atlantic salmon from each group that were chosen for the blood experiments were fitted with dorsal aortic cannulae (per Smith and Bell 1964). Each cannulated fish was then allowed to recover in a darkened flow-through Perspex box for at least 24 h. Following this recovery period, a resting blood sample (about 500 μL) was withdrawn from the fish, via the cannula, with a gas-tight Hamilton syringe. The fish was then transferred to a small, cylindrical tank and exhaustively exercised by manual chasing (i.e., tail grabbing) for 5 min (after the methods of Kieffer et al. 1994). This chasing regime resulted in sequential bursts of swimming, which quickly exhausted the fish (see review by Wood 1991). Exhaustion was indicated by rapid ventilation, cessation of response to the stimulation, and in some cases, loss of equilibrium. At the end of the exercise period, the fish was returned to its box, and another blood sample was immediately taken (time = 0 h). Blood samples were also taken at 1, 2, 4, and 8 h postexercise. All blood samples were replaced with an equal volume of heparinized saline. To determine the effects of repeated sampling, the sampling protocol was also performed on a control group of fish that was acclimated to hard water but not exercised. All cannulated fish were held for 24 h following exercise to monitor survival.

Approximately 150 μL of each blood sample was immediately used for duplicate hematocrit determination; the remaining blood was centrifuged (4 min at 15,000 rpm) to separate the plasma from the red blood cells. A total of approximately 100 μL of the plasma was then used for pH, total CO_2 content (C_{CO2}), and osmolarity measurements. We measured plasma pH with a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) set at 15°C, plasma C_{CO2} with a Corning model 965 CO_2 analyzer (CIBA Corning Canada, Inc.), and plasma osmolality with a model 3MO Plus micro-osmometer (Advanced Instruments, USA). The remaining plasma (about 200 μL) was added to two volumes of 8% perchloric acid (PCA) in labeled Eppendorf tubes. These tubes were shaken, and then frozen and stored under liquid nitrogen for later determination of plasma [lactate], [Na^+], [Cl^-], and [K^+].

Muscle experiments.—Using dip nets, we removed individual fish from their acclimation tank and transferred them to a smaller circular tank (1.5 m diameter) filled with either hard or softer water. Fish were then immediately exercised to exhaustion (except for controls or resting fish, which were not exercised) by manually chasing them for 5 min, as described above. Samples of white muscle were taken immediately following exercise (0 min of recovery) or, in other fish, after 4 h of recovery in blackened Perspex boxes. Fish sampled immediately following exercise (0 h) were placed directly into the anesthetic (tricaine methane-sulfonate [MS-222] buffered with $NaHC0_3$ [pH = 7; 0.25 mg/L]) before removal of muscle. To sample the fish at 4 h, flow to the Perspex box was first stopped and an MS-222 solution was added. The salmon were fully anesthetized after 2–3 min. We chose to anesthetize the fish before muscle sampling because this method has been shown to reduce any metabolic and acid–base changes associated with the handling of a conscious animal (see critiques by Tang and Boutilier 1991 and Wang et al. 1994b). Following anaesthetization, a sample of white muscle was removed from the epaxial musculature and well above the lateral band of red muscle. Samples were immediately freeze-clamped in precooled aluminum tongs and stored in liquid nitrogen. The time between removing the fish from the box and freeze-clamping the tissue was less than 10 s. Resting (control) values were obtained in a manner similar to that described above, except that individual fish were isolated in separate Perspex boxes for at least 24 h before sampling and were not exercised. The frozen muscle samples were later processed and analyzed for tissue water, metabolites (glycogen, adenosine triphosphate [ATP], phosphocreatine [Pcr], and lactate), and acid–base status (pH_i and C_{CO2}) .

Analytical Techniques and Calculations

Blood.—We measured [Lactate] on thawed plasma by the enzymatic assay method of Lowry and Passonneau (1972). Plasma [Na^+] and [K^+] were measured with a Corning model 410C clinical flame photometer (CIBA Corning Canada, Inc.) and plasma [Cl^-] was determined with a CMT 10 chloride titrator (Radiometer, Copenhagen, Denmark). All plasma measurements were made in duplicate and averaged.

Arterial plasma CO_2 tension (Pa_{CO2}), plasma [HCO_3], and the change in the plasma metabolic proton load ([H_m^+]) were calculated according to Graham et al. (1982) by using a rearrangement of the Henderson–Hasselbach equation, for which we followed the equations of Boutilier et al. (1984) to calculate values for the CO_2 solubility in plasma (alpha CO_2) and apparent carbonic acid dissociation constant (pK′). The plasma nonbicarbonate buffering value (β) was calculated for each sample using hematocrits and the following equation for rainbow trout from Kieffer et al. (1994): $\beta = -0.45 \text{ hematocrit} + 0.53$.

Muscle.—White muscle tissue water was measured by placing 0.4–0.5 g of white muscle in dried, tared Eppendorf tubes and drying it at 80°C until a constant mass was obtained. The following formula was then used to calculate the tissue water:

$$\text{tissue water (\%)} = 100[(\text{wet mass} - \text{dry mass})/\text{wet mass})].$$

Another portion of each muscle sample was used to determine white muscle metabolite concentrations. Using a cooled mortar and pestle, this muscle was first ground to a fine powder under liquid nitrogen. Approximately 1.0 g of the muscle powder was added to 4.0 mL of an ice-cold 8% perchloric acid solution containing 1mM ethylenediamine tetraacetic acid (EDTA). The tissue mixture was vortexed for 10 s and then rotated for five minutes at 5°C to allow for acid extraction. Next, the slurry was transferred to Eppendorf tubes, centrifuged for 4 min at 5°C, and the resulting supernatant was collected and weighed in a tared tube. Finally, the extractions were neutralized with 2M KOH solution containing 0.4M imidazole and 0.4 M KCl and then divided into labeled tubes and frozen in liquid nitrogen.

Lactate, ATP and PCr concentrations were determined by enzymatic assay (Lowry and Passonneau 1972). Another portion of each muscle was used to measure glycogen by the method of Hassid and Abraham (1957). All assays used Sigma standards, and all measurements (made in duplicate) were corrected for both tissue water and the dilution factors associated with extraction and assaying.

The acid–base status of muscle was determined by the method of Portner et al. (1990). According to this method, approximately 200 mg of muscle that had been ground under liquid nitrogen was added to 400 μL of ice-cold metabolic inhibitor solution (150 mM potassium fluoride; 6 mM nitrilotriacetic acid). Another 400 μL of inhibitor was added and the mixture was quickly vortexed and centrifuged. The resulting supernatant was used for duplicate measures of pH (PHM 73 pH meter and micro-pH unit set at 15°C) and C_{CO2} (Corning model 965 CO_2 analyzer, CIBA Corning, Canada). The mean C_{CO2} values were expressed in terms of tissue water and were then corrected for extracellular fluid via the equations of Portner et al. (1990) and for extracellular and intracellular fluid volume ratios as calculated for trout by Milligan and Wood (1986). Next, white muscle P_{CO2} and [HCO_3] were calculated, as described above in the blood section, by using the corrected muscle C_{CO2} values and the alpha CO_2 and pK determined by Kieffer et al. (1994). Finally, white muscle [H_m^+] was calculated for each fish at 0 and 4 h postexercise, using the following formula from Milligan and Wood (1986):

$$[H_m^+] = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2),$$

where the subscript 1 refers to the mean resting value for each group, and 2 refers to the individual's postexercise values. The nonbicarbonate buffering capacity (β) for Atlantic salmon was previously determined to be −88 slykes (M. Wilkie, University of Toronto, personal communication).

Statistics

For the blood data, a repeated measures analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used to compare each group's postexercise values with their respective resting values. Unpaired *t*-tests were used to compare the means from softer water and hard water for each time. Unpaired *t*-tests were also used to compare the blood values for the surviving and dying fish in each group. For muscle tissues, we used one-way ANOVAs to compare mean metabolite values in each of the water types; where ANOVA indicated significance, a Scheffé *F*-test was used to determine significant differences between

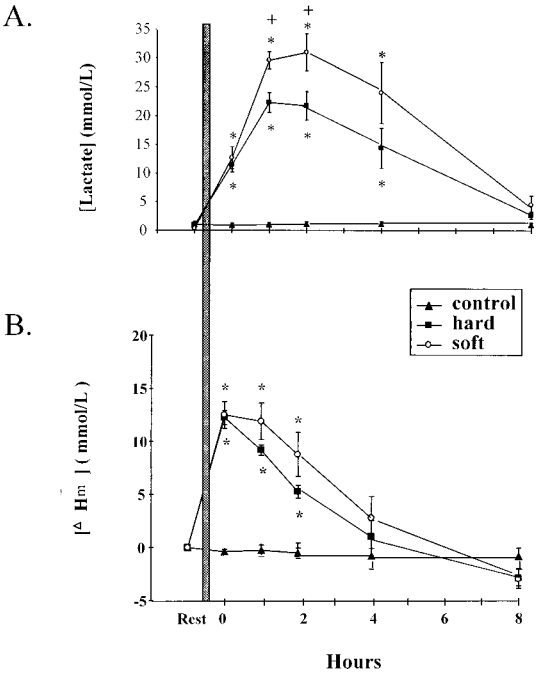

FIGURE 1.—Mean (±SE) arterial blood plasma **(A)** [lactate] and **(B)** [H_m^+] in cannulated Atlantic salmon recovering from 5 min of exhaustive exercise (stippled bar) in hard (N = 9) or softer (N = 7) water. Controls (hard water, N = 9) were resting fish under this sampling regime. All fish survived 24 h postexercise. Asterisks denote a significant difference ($P < 0.05$) between resting and postexercise values; plus signs indicate a significant difference ($P < 0.05$) between the fish in hard versus softer water for a given hour in the experiment.

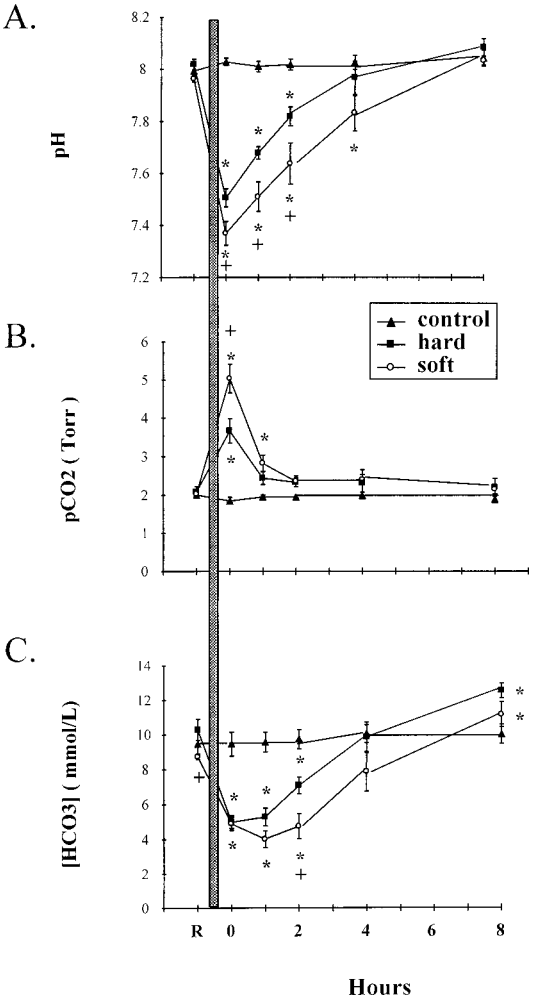

FIGURE 2.—Arterial blood plasma **(A)** pH, **(B)** arterial plasma CO_2 tension (Pa_{CO2}), and **(C)** [HCO_3^-] in cannulated Atlantic salmon recovering from 5 min of exhaustive exercise. Other details as in Figure 1.

resting values and postexercise values. At each sampling time, unpaired *t*-tests were used to compare the mean muscle values for fish from softer and hard water. Lastly, chi-square tests were used to determine whether a relationship existed between water hardness and mortality rates. For all tests, significance was set at $\alpha = 0.05$. Statistics were calculated with the Statview 512 + program or with Sigma-Stat (version 2.0).

Results

Blood

Exhaustive exercise caused significant increases in the plasma lactate concentration of Atlantic salmon in hard versus softer water. The plasma lactate loads were greatest around 1–2 h after exercise, and during this period the lactate load of the softer-water group's plasma was significantly (30–40%) greater than that of the hard-water group (Figure 1A). The plasma [H_m^+] also increased significantly in both groups after exercise. Both groups' [H_m^+] peaked at 0 h and declined to resting levels between 4 and 8 h (Figure 1B).

Exhaustive exercise caused the plasma pH of fish in the hard versus softer water to fall by 0.51 and 0.59 units, respectively (Figure 2A). Those in softer water also experienced a significantly greater postexercise elevation of plasma Pa_{CO2}; at 0 h the Pa_{CO2} elevation for those in softer water was almost two times greater than those in the hard water (Figure 2B). At rest, the plasma HCO_3^- concentration of the softer-water group was significantly lower than that of the hard-water group (Figure 2C). After exercise, both groups' plasma

TABLE 1.—Mean (±SE) arterial blood plasma osmolality, [Na^+], [Cl^-], [K^+], and hematocrit in cannulated Atlantic salmon recovering from 5 min of exhaustive exercise in hard ($N = 9$) or softer ($N = 7$) water. Controls (hard water, $N = 9$) represent resting fish under this sampling regime. All fish survived 24 h after exercise. Asterisks denote a significant difference ($P < 0.05$) between resting and postexercise values; plus signs indicate a significant difference ($P < 0.05$) between the hard and softer water groups for a given time.

	Rest	0 h[a]	1 h	2 h	4 h	8 h
Osmolality (mOsm)						
Control	304 ± 2	301 ± 1	299 ± 2	302 ± 1	303 ± 2	305 ± 2
Hard water	304 ± 2	334 ± 3*	338 ± 4*	327 ± 4*	314 ± 4*	289 ± 3
Softer water	306 ± 2	338 ± 3*	360 ± 6*+	344 ± 8*	324 ± 7*	301 ± 3
[Na^+] (milliequivalents [mEq]/L)						
Control	129.8 ± 5.9	139.0 ± 4.5	133.6 ± 2.4	131.8 ± 1.7	136.2 ± 7.3	136.2 ± 2.4
Hard water	146.3 ± 6.2	148.8 ± 6.6	147.0 ± 3.9	142.1 ± 3.8	136.6 ± 2.3	130.3 ± 4.9
Softer water	136.2 ± 3.3	151.2 ± 2.2	155.1 ± 2.0*	157.3 ± 2.3*+	153.6 ± 10.2*	139.3 ± 4.0
[Cl^-] (mEq/L)						
Control	114.4 ± 5.2	125.0 ± 2.5*	123.9 ± 2.6*	123.4 ± 2.7*	124.5 ± 2.4*	121.0 ± 2.9
Hard water	123.7 ± 3.0	127.9 ± 1.7	121.1 ± 2.4	117.6 ± 1.8	115.5 ± 2.5	115.7 ± 4.3
Softer water	127.9 ± 3.0	136.6 ± 2.2*+	125.4 ± 1.6	120.3 ± 1.5*	114.9 ± 1.6*	119.1 ± 3.0*
[K^+] (mEq/L)						
Control	2.3 ± 0.3	2.6 ± 0.2	2.5 ± 0.2	2.3 ± 0.1	2.5 ± 0.1	2.5 ± 0.4
Hard water	2.8 ± 0.3	4.8 ± 0.8*	4.8 ± 0.5*	4.6 ± 0.4*	4.1 ± 0.5	2.9 ± 0.2
Softer water	3.3 ± 0.3	4.7 ± 0.2	5.1 ± 0.2*	6.8 ± 0.5*+	6.3 ± 1.0*	3.5 ± 0.6
Hematocrit (%)						
Control	27.8 ± 1.4	25.8 ± 1.4	23.0 ± 1.4*	20.4 ± 1.3*	18.8 ± 1.1*	16.9 ± 1.2*
Hard water	29.1 ± 1.3	31.6 ± 1.9	28.8 ± 2.1	24.0 ± 1.8*	21.5 ± 1.8*	17.5 ± 1.5*
Softer water	28.2 ± 1.5	33.4 ± 1.7*	35.1 ± 2.4*	31.2 ± 2.0+	27.0 ± 2.4	20.0 ± 1.5*

[a] Immediately after exercise.

HCO_3^- fell significantly, and at 2 h postexercise the plasma HCO_3^- concentration of the softer-water group was again significantly lower than that of the hard-water group. By 8 h postexercise, the plasma HCO_3^- concentrations of both exercised groups significantly exceeded the resting levels. The hard-water control (not exercised) group did not show changes in any of the above plasma variables throughout the entire 8 h sampling regime (Figures 1, 2).

Resting plasma osmolality did not differ significantly between the hard-water and softer-water group, nor did major ion concentrations (Na^+, Cl^-, K^+) or blood hematocrits (Table 1). However, postexercise plasma osmolality in the softer-water group versus hard-water groups differed significantly, partly because of the significant increase in the softer-water groups' plasma Na and Cl concentrations following exercise. In the hard-water group, no significant changes were detected in the plasma Na^+ and Cl^- concentrations following exercise (Table 1). Plasma K^+ after exercise increased significantly for fish in both hard and softer water, but those in softer water experienced a greater and more prolonged postexercise plasma K^+ elevation than did those in hard water. Both the control and hard-water groups exhibited significant decreases in their hematocrits over the 8 h following exercise. These decreases in hematocrit were due to the continual removal of blood from the fish. Hematocrit values increased significantly following exercise for fish in soft water, and at 2 h postexercise were significantly greater than for fish in hard water. By 8 h, the softer-water group's hematocrit fell below resting levels.

Muscle

Fish in hard and softer water had mean resting tissue water content values of 70.6% (SE = 1.0%) and 71.4 ± 1.1%, respectively. The tissue water values immediately after exercise (i.e., 0 h) were similar to those at rest, but by 4 h the hard- and softer-water values had increased to 76 ± 0.5% and 73.2 ± 1.0%, respectively. The increase observed in the hard-water fish was significant. Because the tissue water levels varied among groups of fish, the muscle metabolite concentrations were expressed in terms of tissue water to account for these changes.

The Atlantic salmon in hard and softer water had similar resting levels of white muscle ATP, PCr, and glycogen (Figure 3). Exercise reduced

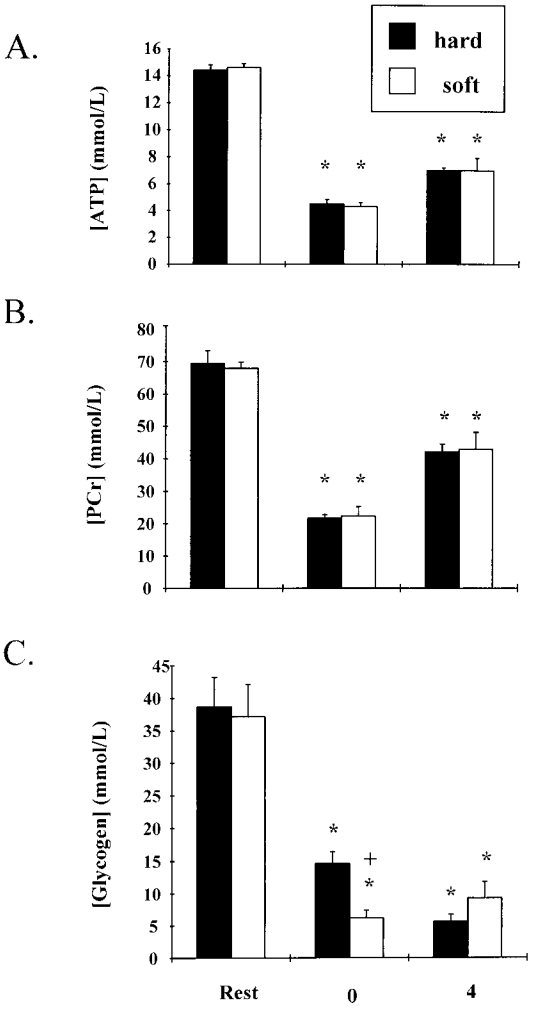

FIGURE 3.—Mean (+SE) white muscle **(A)** adenosine triphosphate [ATP], **(B)** phosphocreatine [PCr], and **(C)** glycogen in Atlantic salmon at rest (before exercise), immediately following 5 min of exhaustive exercise and after 4 h of recovery from exhaustive exercise; salmon were acclimated to either hard or softer water. Asterisks denote a significant difference ($P < 0.05$) between resting and postexercise values; plus signs indicate a significant difference ($P < 0.05$) for fish in hard versus softer water for a given hour in the experiment. Sample sizes were 9 (resting), 6 (at 0 h postexercise), and 6 (at 4 h postexercise) for the hard-water group and 8 (resting), 13 (0 h), and 12 fish (4 h) for the softer-water group.

both groups' ATP and PCr concentrations to approximately 30% of resting values. After 4 h of recovery, both groups' ATP concentrations recovered to approximately 48% of resting levels while their PCr concentrations were restored to approximately 62% of resting levels. The 4 h ATP and PCr values for both groups, however, were still significantly less than their respective resting levels. Exercise significantly reduced the hard- and softer-water groups' glycogen concentrations to 38% and 17% of their respective resting values. Glycogen concentrations were still significantly lower than resting levels for both the hard and softer water fish after 4 h of recovery.

Differences between the lactate concentrations, $[H_m^+]$ or pH_i values in the white muscle were not significant between the hard- and softer-water groups at rest (Figure 4). Immediately following exercise, large increases in the white muscle lactate concentrations were evident in all fish. Immediately after exhaustive exercise, those in hard water had significantly more lactate in their white muscle than did those in softer water (Figure 4A). At 4 h postexercise, white muscle lactate loads were reduced by only 29% in the hard and 36% in the softer-water groups. Accompanying the postexercise increases in lactate were large increases in white muscle $[H_m^+]$, which similar to lactate, were also significantly greater in the hard-water fish. By 4 h, the $[H_m^+]$ for the hard- and softer-water groups had diminished by 14% and 35%, respectively (Figure 4B). Finally, exercise caused the white muscle pH_i in the hard- and softer-water groups to fall by 0.77 and 0.68 pH units, respectively (Figure 4C). Despite some recovery, the white muscle pH_i values of both groups were still significantly lower than resting levels at 4 h.

Mortality

Table 2 summarizes the mortality that occurred during these experiments. All hard-water fish survived following exhaustive exercise. As the water was softened to 40 mg/L $CaCO_3$, a significant increase in postexercise mortality became apparent in both the cannulated and noncannulated fish (chi-square: $P < 0.05$). Further depression of the water hardness to 0–12 mg/L $CaCO_3$ increased mortality rates to around 50%.

At 1 and 2 h postexercise, mortalities in softer water had greater plasma $[H_m^+]$ than their surviving counterparts (Table 3). Plasma HCO_3^- concentrations among softer water mortalities were also significantly lower than those among the softer water survivors, both at rest and immediately following exercise. Generally, mortalities also failed to demonstrate any recovery of plasma pH after exercise and exhibited a more pronounced and prolonged disturbance in plasma osmolality. Because a terminal sampling method was used to sample the white muscle, no comparisons could

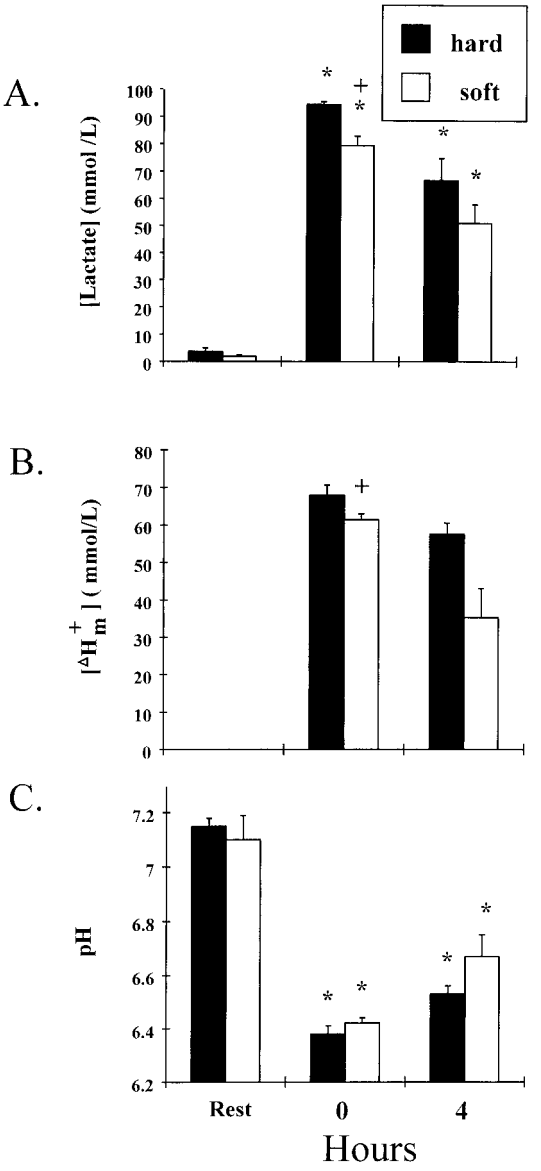

FIGURE 4.—Mean (+SE) white muscle **(A)** [lactate], **(B)** [H_m^+], and **(C)** pH in Atlantic salmon at rest (before exercise), immediately following 5 min of exhaustive exercise, and after 4 h of recovery from exhaustive exercise; salmon were acclimated to either hard or softer water. Asterisks denote a significant difference ($P < 0.05$) between resting and postexercise values; plus signs indicate a significant difference ($P < 0.05$) for fish in hard versus softer water for a given hour in the experiment. Sample sizes as noted in Figure 3.

TABLE 2.—Summary of Atlantic salmon mortality following 5 min of exhaustive exercise in water of three different hardness ranges. Tests included cannulated fish survival that were monitored for 24 h postexercise and noncannulated fish survival that were monitored for 4 h postexercise. Chi-square tests indicated a significant ($P < 0.05$) effect of water hardness on mortality for both cannulated and noncannulated fish.

Total hardness (mg/L $CaCO_3$)	pH	Number of fish		Mortality (%)
		Exercised	Died	
Cannulated (monitored 24 h)				
0–12	7.0–7.1	4	2	50
30–48	7.1–7.5	14	6	43
90–100	6.7–7.2	10	0	0
Noncannulated (monitored 4 h)				
0–12	7.0–7.8	15	9	60
30–50	7.1–7.4	11	2	18
90–100	6.8	6	0	0

be made for the white muscle variables between survivors and nonsurvivors after exhaustive exercise.

Discussion

The results of this study clearly show that water quality has profound effects on both the postexercise physiology and survival of Atlantic salmon. The blood and muscle physiology of salmonids exercising in hard water having a neutral pH have previously been examined in detail by other investigators (e.g., Milligan and Wood 1986; Tang and Boutilier 1991; Wood 1991; Ferguson et al. 1993; Kieffer et al. 1994; Wang et al. 1994a; McDonald et al. 1998). This discussion will therefore focus on the impact of softer water on these responses.

A comparison of the metabolic, acid–base, and ion variables in the blood of hard- and softer-water fish suggests that a reduction in the hardness of the environmental water had minimal effect on the physiology of Atlantic salmon under resting conditions (Figures 1, 2; Table 1). If the reduction in environmental hardness had caused any adverse effects in the blood variables of these fish, the 4-d acclimation period was of sufficient duration for these effects to be corrected. Among the blood variables measured, only plasma [HCO_3] differed between the two groups under resting conditions (significantly lower in the softer water group; Figure 2). Graham et al. (1982) also reported low plasma HCO_3 levels in resting rainbow trout that had a 5-d acclimation period following transfer from hard to softer water. The reason for this effect is unknown, but it may be a direct effect of the

TABLE 3.—Comparisons of blood variable means (±SE) in dying and surviving Atlantic salmon in softer water (sample size, N, = 7; N for dying fish is in parentheses after each value) during the 4 h following 5 min of exhaustive exercise; values for hard-water survivors are included in the table for reference. Asterisks denote a significant different ($P < 0.05$) between postexercise and resting values; no statistics could be performed on the dying fish because of the decreased sample sizes as individuals died. Plus signs indicate a significant difference ($P < 0.05$) between surviving and dying fish for a given time.

	Rest	0 h[a]	1 h	2 h	4 h
			$[H_m^+]$ (mmol/L)		
Hard survivors	0	12.3 ± 0.7*	9.2 ± 0.5*	5.2 ± 0.6*	1.0 ± 1.5
Soft survivors		12.5 ± 1.3	11.9 ± 1.7*	8.8 ± 2.1*	2.8 ± 2.0
Soft died		11.5 ± 0.8 (5)	13.5 ± 2.3 (3)	14.5 ± 4.1 (3)	9.1 (1)
			pH_a		
Hard survivors	8.02 ± 0.02	7.51 ± 0.04*	7.68 ± 0.028*	7.82 ± 0.04*	7.97 ± 0.06
Soft survivors	7.96 ± 0.01	7.37 ± 0.05*+	7.51 ± 0.06*+	7.64 ± 0.08*+	7.83 ± 0.07*
Soft died	7.93 ± 0.04 (5)	7.38 ± 0.03 (5)	7.34 ± 0.07 (3)	7.34 ± 0.14 (3)	7.6 (1)
			P_{CO2} (Torr)		
Hard survivors	2.1 ± 0.1	3.7 ± 0.3*	2.4 ± 0.2	2.3 ± 0.1	2.3 ± 0.3
Soft survivors	2.0 ± 0.1	5.0 ± 0.4*+	2.8 ± 0.2*	2.4 ± 0.1	2.4 ± 0.1
Soft died	2.1 ± 0.3 (5)	3.69 ± 0.4 (5)+	3.3 ± 0.3 (3)	2.1 ± 0.6 (3)	2.1 (1)
			$[HCO_3]$ (mmol/L)		
Hard survivors	10.3 ± 0.6	5.0 ± 0.4*	5.3 ± 0.5*	7.1 ± 0.5*	9.8 ± 0.9
Soft survivors	8.7 ± 0.2	4.9 ± 0.4*	4.0 ± 0.5	4.8 ± 0.7*+	7.9 ± 1.1
Soft died	8.2 ± 0.1 (5)+	3.6 ± 0.2 (5)+	3.2 ± 0.7 (3)	2.3 ± 0.9 (3)	3.7 (1)
			Osmolality (mOsm)		
Hard survivors	304 ± 2	334 ± 3*	338 ± 4*	327 ± 4*	314 ± 4*
Soft survivors	306 ± 2	338 ± 3*	360 ± 6*+	344 ± 8*	324 ± 7*
Soft died	308 ± 1 (5)	338 ± 4 (5)	373 ± 8 (3)	382 ± 7 (3)+	368 (1)
			Hematocrit (%)		
Hard survivors	29.1 ± 1.3	31.6 ± 1.9	28.8 ± 2.1	24.0 ± 1.8*	21.5 ± 1.8*
Soft survivors	28.2 ± 1.5	33.4 ± 1.7*	35.1 ± 2.4*	31.2 ± 2.0+	27.0 ± 2.4
Soft died	23.5 ± 3.7 (5)	29.4 ± 3.2 (5)	34.0 ± 6.7 (3)	33.5 ± 8.3 (3)	26.5 (1)

[a] Immediately after exercise.

fish's acclimation to softer water. For example, a reduction in environmental $[Ca^{2+}]$ causes an increase in the passive branchial efflux of electrolytes from a fish. To compensate for these lost electrolytes, fish in softer water increase their uptake of Na^+ and Cl^- from the environmental water (Cuthbertz and Maetz 1972; Eddy 1975). Thus, the reduced $[HCO_3^-]$ in the softer-water group's plasma may have resulted from increased branchial exchange of Cl^- and HCO_3^- (Cl^- influx, HCO_3 efflux) during softer water acclimation.

Large metabolic, acid–base and electrolyte disturbances in the blood of Atlantic salmon in both hard and softer water occurred following exhaustive exercise. In many cases, these disturbances were significantly greater in the softer-water fish (Figures 1, 2; Table 1).

Severe extracellular acidoses were evident in both the softer- and hard-water fish's plasma immediately after exercise. The softer-water group had a significantly greater respiratory acidosis (increased P_{CO2}) compared with the hard water group, which contributed to a greater depression in plasma pH immediately after exercise (Figure 2A). During the postexercise recovery period, the two groups' P_{CO2} values were similar, suggesting that the greater acidosis that persisted in the plasma of the softer-water group was due to a larger metabolic acidosis that occurred in the blood of these fish (Figure 2). During the period that lactate peaked in the plasma (1–2 h after exercise), the lactate load in the softer-water group's plasma was roughly 40% greater than that of the hard-water group (Figure 1A). Thus, the softer-water group's greater plasma H_m^+ load appeared to result from a greater release of both lactate and protons from the white muscle of this group following exercise. In contrast to our results, Graham et al. (1982) did not observe, during the first 2 h postexercise, any differences in plasma levels of pH, HCO_3, lactate, or H_m^+ between rainbow trout acclimated to hard versus softer water. In fact, beyond 2 h postexercise,

they reported a more rapid return of plasma lactate levels in softer water and suggested that the acclimation to softer water may have in some way stimulated the processes that clears lactate from blood.

The reason for the greater lactate load in plasma among our softer-water Atlantic salmon during recovery from exercise is not clear. A plausible explanation could be that the softer-water group exercised more vigorously and thereby produced a greater lactic acid load in their white muscle. Greater intracellular lactacidosis would provide greater intracellular to extracellular gradient for lactate and H_m^+ diffusion (Kieffer and Tufts 1996). This explanation would also account for the more severe and prolonged plasma acidosis observed in the plasma of the softer-water fish following exercise. However, this hypothesis can be ruled out because the postexercise muscle lactate load of the softer-water fish was not greater than the lactate load of the hard-water fish.

Our data indicate that lowering the hardness of the environmental water affected neither the concentrations of primary endogenous fuels (i.e., ATP, PCr, and glycogen), nor the acid–base status of the salmon's white muscle under resting conditions (Figures 3, 4). For the most part, the hard- and softer-water groups also exhibited similar reductions in their white muscle fuels during exercise. During the postexercise recovery period, however, the white muscle lactate and metabolic proton loads, as well as the pH depression, were significantly greater in the hard- versus softer-water group (Figure 4A, B). These results indicate that the anaerobic capacity of the hard-water group was not less than that of the softer-water group—and may have even been somewhat greater. Thus, factors other than a greater intracellular lactacidosis in the softer-water group's muscles must have caused their greater hematological disturbance.

Environmental calcium concentration is known to influence branchial ionoregulatory processes (McDonald et al. 1980). In our study, environmental calcium may also be affecting the mechanisms involved in proton excretion across the gills of salmon following exercise. However, differences in branchial ionoregulatory processes would not explain the differences in plasma lactate observed between hard- and soft-water groups. Thus, it is possible that the physiological basis for these differences may also involve relative differences at the level of the mechanisms involved in the retention and release of lactic acid from the muscle to the blood (See Wang et al. 1994a). Elucidation of the mechanistic basis for these differences will therefore require further study.

The large white muscle lactate load produced by burst exercise creates an osmotic gradient that causes water to move from the plasma into the muscle of fish (Milligan and Wood 1986). The resulting loss of water from the plasma may cause hemoconcentration of plasma electrolytes (Turner et al. 1983a, 1983b; Wood 1991), but this may also be offset by a net loss of these ions across the gills (Kieffer and Tufts 1996). Despite the significant postexercise increase in the white muscle tissue water of the hard-water fish, increases in the plasma Na and Cl concentrations were not significant in that group (Table 1). In contrast, the softer-water fish did experience significant plasma Na and Cl elevations following exercise, probably due a shift of water from the plasma into the red blood cells or into tissues other than white muscle (Table 1; Wang et al. 1994a). The eventual decline in the plasma Cl concentrations of the softer-water group may have been due to electroneutrality constraints during the period of high plasma lactate loading (Graham et al. 1982), or to Cl^- loss across the gill following exercise (Kieffer and Tufts 1996). Both hard- and softer-water fish exhibited increases in plasma osmolarity following exercise and again, the disturbance was greater in the softer-water fish (Table 1). The significant rise in the softer-water group's hematocrit after exercise provides further evidence of greater hemoconcentration in softer-water versus hard-water fish (Table 1).

Survival Following Exhaustive Exercise

The 100% survival of Atlantic salmon that were exhaustively exercised in hard water indicates that exhaustive exercise (e.g., angling) under ideal environmental conditions probably does not lead to significant mortality. The mortality that occurred in the softer-water group, however, does indicate that the composition of the environmental water may have a significant effect on the survival of exhaustively exercised Atlantic salmon (Tables 2, 3).

Mortality of the exercised salmon was related to the water hardness. In very soft water (0–12 mg/L $CaCO_3$), the postexercise mortality was 60% for noncannulated and 50% for cannulated fish. In moderately soft water (30–50 mg/L $CaCO_3$) postexercise mortality decreased to 18% for noncannulated and 43% cannulated fish (Table 2). The difference in the mortality rates between noncannulated and cannulated fish in moderately softer water could be related to the increased suscepti-

bility of cannulated fish to mortality (Ferguson and Tufts 1992).

The Atlantic salmon that did not survive in softer water exhibited an acid–base disturbance in their plasma, which initially was comparable to that of surviving fish (Table 3). Unlike the survivors, however, the mortalities did not show any correction of this disturbance. The failure of the mortalities to correct the acidosis may have been due in part to their lower initial HCO_3 concentration (Table 3). In other words, the mortalities were not well-poised to buffer the acidosis resulting from the exercise bout. Wood et al. (1983) suggested that mortality in rainbow trout following exhaustive exercise may be caused by an extreme intracellular acidosis. Although the results from the present blood experiment do suggest that nonsurviving fish had a greater extracellular acidosis, the results from the muscle experiments do not appear to fit with the intracellular acidosis hypothesis of Wood et al. (1983). Elucidation of the mechanism(s) responsible for the delayed mortality of exhaustively exercised fish (e.g., our salmon in softer water) may therefore require further study.

Management Implications

The results of this study clearly indicate that environmental water quality has an important influence on the magnitude of the physiological disturbance and on the survival of Atlantic salmon following exhaustive exercise. More specifically, Atlantic salmon in softer water had a greater physiological disturbance and higher mortality than salmon in hard water. In terms of the recreational fishery for Atlantic salmon, these findings may be particularly relevant to situations where Atlantic salmon are exhaustively angled and released in relatively soft freshwater; for example, during the transition to freshwater, they may be more susceptible to delayed mortality after exhaustive angling. In this regard, it is noteworthy that Brobbel et al. (1996) found that both the physiological disturbance and delayed mortality after angling were greater in ''bright'' salmon that had recently entered freshwater compared with fully acclimated ''kelts'' that had spent the winter in freshwater.

For our study, we did not attempt to provide a detailed comparison of our water composition with those of Atlantic salmon rivers in eastern North America. It is also important to consider that the physiological responses of hatchery salmon under laboratory conditions may be somewhat different than those of salmon angled in the wild. Thus, our survival results cannot be directly extrapolated to catch-and-release recreational fisheries for Atlantic salmon. In view of these findings, however, further studies on the effects of water quality and freshwater acclimation on the physiology and survival of wild salmon angled under more natural conditions may be warranted.

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