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Routine and Active Metabolic Rates of Migrating Adult Wild Sockeye Salmon (*Oncorhynchus nerka* Walbaum) in Seawater and Freshwater

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ABSTRACT

We present the first data on the differences in routine and active metabolic rates for sexually maturing migratory adult sockeye salmon (*Oncorhynchus nerka*) that were intercepted in the ocean and then held in either seawater or freshwater. Routine and active oxygen uptake rates (M_{O_2}) were significantly higher (27%–72%) in seawater than in freshwater at all swimming speeds except those approaching critical swimming speed. During a 45-min recovery period, the declining postexercise oxygen uptake remained 58%–73% higher in seawater than in freshwater. When fish performed a second swim test, active metabolic rates again remained 28%–81% higher for fish in seawater except at the critical swimming speed. Despite their differences in metabolic rates, fish in both seawater and freshwater could repeat the swim test and reach a similar maximum oxygen uptake and critical swimming speed as in the first swim test, even without restoring routine metabolic rate between swim tests. Thus, elevated M_{O_2} related to either being in seawater as opposed to freshwater or not being fully recovered from previous exhaustive exercise did not present itself as a metabolic loading that limited either critical swimming performance or maximum M_{O_2}. The basis for the difference in metabolic rates of migratory sockeye salmon held in seawater and freshwater is uncertain, but it could include differences in states of nutrition, reproduction, and restlessness, as well as ionic differences. Regardless, this study elucidates some of the metabolic costs involved during the migration of adult salmon from seawater to freshwater, which may have applications for fisheries conservation and management models of energy use.

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Introduction

Many species of salmonids, including all Pacific salmon, undertake anadromous migrations between freshwater (FW) and seawater (SW). During the spawning migration, maturing adults depart SW for FW and undergo physiological changes to maintain osmotic and ionic balance. In SW, fish largely maintain hypoosmotic state by actively drinking and secreting ions from the intestine, kidney, and gill epithelia that have high Na^+/K^+-ATPase activities (see reviews by Evans 1984; Wendelaar Bonga 1997; Høgåsen 1998). For fish in FW, the hyperosmotic state requires ion uptake at the gills that is facilitated by a lower Na^+/K^+-ATPase activity than in SW as well as water excretion by the kidney. Ionic and osmotic balance is maintained during subfatigue swimming (Wood and Randall 1973*a*, 1973*b*), but maximum aerobic activity compromises the permeability of the gills, allowing passive changes in ion and water concentration (Schreck 1990; Thomas 1990; Wendelaar Bonga 1997).

The energetic cost of maintaining a hypoosmotic versus a hyperosmotic gradient to the environment has been estimated for salmonids, with varying results. For example, gill metabolism was estimated to be higher in FW (1.0%–3.9% of resting oxygen uptake [M_{O_2}]) than in SW (0.5%–2.4% of resting M_{O_2}) in both rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout (*Oncorhynchus clarki clarki*; Eddy 1982; Morgan and Iwama 1999). Conversely, a higher gill energy demand was found for SW than for FW in both rainbow trout (5.7% vs. 1.6% of resting M_{O_2}; Kirschner 1993, 1995) and Atlantic salmon (*Salmo salar*; 16% higher; McCormick et al. 1989). Measurements of whole-animal M_{O_2} in different salinities also reveal

variable results. For example, Rao (1968) found that at all swimming speeds juvenile rainbow trout Mo_2 values were higher in SW than in FW by 10% and 14% at 15° and 5°C, respectively. Similarly, routine Mo_2 of juvenile rainbow trout and chinook salmon (*Oncorhynchus tshawytscha*) in 50%–60% SW was 25%–30% higher than in FW (Morgan and Iwama 1991). However, the same authors subsequently reported no salinity effect on routine Mo_2 for juvenile coho salmon (*Oncorhynchus kisutch*; Morgan and Iwama 1998).

Currently there is no comparison of routine and active Mo_2 for adult wild salmon in SW and FW. In fact, the previous laboratory studies with juvenile fish sometimes used small fish (4–196 g) that had not smoltified to permit proper acclimation to SW. Here we have taken a different approach to these previous laboratory studies by intercepting migrating sockeye salmon (*Oncorhynchus nerka*) in the marine environment as they approached their natal river estuary, and then measuring Mo_2 during prolonged swimming performance trials with fish held in either SW or FW. In addition, a repeated-swimming trial was used to examine whether the ability to recover from exhaustion differed between SW and FW such that performance in the second swim test was affected.

Material and Methods

Fish Capture and Holding

Approximately 240 wild sockeye salmon were captured by a commercial seine vessel in marine waters of the Strait of Georgia (salinity = 33 ppm; surface temperature = 12.5°C), near the mouth of the Fraser River, British Columbia, Canada, on August 26–28, 2003. Fish were landed at the Department of Fisheries and Oceans West Vancouver Laboratory (WVL). Forty of these fish were used for this study. Twenty fish (62.5 ± 1.0 cm fork length; 2.8 ± 0.1 kg ± SEM) remained at WVL and were held for 1 mo in a 7,000-L aquarium containing aerated seawater (salinity = 33 ± 0.1 ppm; temperature = 12.5° ± 0.1°C). Twenty fish (61.0 ± 0.9 cm; 2.6 ± 0.2 kg) were transported to Cultus Lake Laboratory and held for 2 mo in a 20,000-L aquarium containing aerated freshwater (salinity = 0 ppm; temperature = 12.7° ± 0.3°C).

It was possible to perform only one repeat-swim test per day. Therefore, while fish were acclimating to FW, the swim tests were performed on the SW fish. This meant that SW fish were tested 3–6 wk earlier than fish in FW, and as a result, fish were in slightly different nutritional and reproductive states. Normally, wild sockeye cease feeding before they reach the Fraser River estuary and use their extensive energy stores to fuel routine metabolic costs, migration costs associated with an arduous upriver migration, and reproductive development. Our fish did not have to use energy for upriver migration, which would have preserved some of the energy stores (Patterson et al. 2004), but at the time of testing, SW fish would have had a slightly higher energy status and lower reproductive development than the fish held in FW. All procedures were approved by the University of British Columbia Animal Care Committee and the Department of Fisheries and Oceans Animal Care Committee.

Routine Blood Chemistry

Blood was sampled from subgroups of cannulated fish to assess routine ionic (plasma chloride, potassium, and sodium levels) and metabolic (plasma glucose, lactate, and cortisol levels) status for both SW and FW fish. Sockeye salmon were individually netted and anesthetized in a bath of 60 mg L^{-1} MS-222 and then placed on a surgery table, where the gills were continuously irrigated with 30 mg L^{-1} MS-222. A cannula filled with a 0.9% NaCl solution containing heparin (100 IU mL^{-1}) was inserted into the dorsal aorta using an internal trochar and anchored in place using 3-0 silk sutures. Fish were then placed into one of four 80-L isolation chambers with a water flow rate of 10 L min^{-1} to recover for 14–20 h. Four 1-mL blood samples were taken over a 2-d period. Samples were taken when fish were known to be at rest within the chambers. Each blood sample was centrifuged and the plasma stored at −80°C until the analyses were performed (as described in Farrell et al. 2001*a*). Samples of gill filaments were frozen for enzymatic analysis of Na^+/K^+-ATPase activity (as described by McCormick 1993). Following the procedures of Crossin and Hinch (2005), lipid percentage and gross somatic energy density were estimated using a commercially produced fat meter (Distell.com, Fauldhouse, UK).

Swimming Performance and Postexercise Blood Chemistry

A separate subgroup of fish was used for the swimming studies. Fish were netted individually and placed into a bath of 60 mg L^{-1} MS-222. Body measurements were taken before transferring fish to the swim tunnel (a 471-L Brett type as described in Farrell et al. [2003)] with a modified net plug to prevent drafting in the turbulent area near upstream end). A conditioning swim (Jain et al. 1997) was performed after a 2-h recovery period at a water velocity of 0.45 body lengths per second (bl s^{-1}) by increasing water velocity by 0.15 bl s^{-1} every 2 min until fish could no longer continue swimming against the current (i.e., resting for >20 s on the downstream grid). Fish then recovered overnight (14–16 h) at 0.45 bl s^{-1}. The next morning, duplicate routine oxygen uptake measurements were taken at 0.45 bl s^{-1} during a 1-h period by pumping water from the swim tunnel over an oxygen electrode (Mark IV Oxyguard; Point Four Systems, Richmond, British Columbia). A ramped critical swimming speed (U_{crit}) protocol (Jain et al. 1997, 1998) was then started by increasing water velocity by 0.15 bl s^{-1} every 5 min to approximately 50% of the maximum speed in the conditioning swim. Subsequently, water velocity was increased by 0.15 bl s^{-1} every 20 min until the fish fatigued.

Active Mo_2 was measured during every speed increment. Fish recovered for 45 min at 0.45 bl s^{-1}, and the extent to which routine Mo_2 was restored was ascertained by measuring Mo_2 during the final 15 min of the recovery period. A second ramped U_{crit} test was then performed and the measurements of active Mo_2 repeated. Mo_2 measurements were continued for a further 30 min at 0.45 bl s^{-1} to determine the rate of recovery from exhaustive exercise. At the end of the recovery period, fish were removed from the swimming chamber and killed in 140 mg L^{-1} MS-222. Blood samples were taken via caudal puncture and centrifuged to obtain plasma that was then stored at −80°C.

Data Analysis and Statistics

Sample sizes for routine blood measurements were reduced to $n = 6$ in SW and $n = 8$ in FW as a result of the failure of some cannulas. Postexercise blood samples were taken from only five fish of the 10 fish swum in FW, reducing sample size to $n = 5$. Finally, one fish used for swim testing in FW was excluded from the data set because it had became infected with a myxozoan kidney parasite. Values presented are means ± SEM, and statistical significance was assessed at $P < 0.05$ using a standard statistical program (JMP 4.0.4, SAS Institute).

The four routine blood samples from each fish were screened, and the sample with the lowest plasma lactate and cortisol values was used to best estimate resting condition. The routine and postexercise plasma data were analyzed by two-way ANOVA with salinity and exercise level as the two variables. U_{crit} value was calculated using the formula of Brett (1964) and corrected for solid blocking effects when necessary (i.e., >10% and >20% difference) according to Bell and Terhune (1970). Date for U_{crit}, Mo_2, and cost of transport (COT; $Mo_2/[U \times 60]$) for each swimming speed (U) were analyzed using two-way ANOVA to determine differences between salinity treatments, with salinity and swimming speed as the two independent variables. In all cases, salinity was the independent variable, and fish length, fish mass, and water temperature were tested for covariation and included if significant. A one-way repeated-measures ANOVA was used to determine differences in Mo_2 between swimming speeds for each treatment. Recovery ratios were calculated by dividing the results of the two swim tests (U_{crit2}/U_{crit1}) with the expectation that the recovery ratio for wild sockeye salmon is typically equal to 1.00 (Farrell et al. 2003; Lee et al. 2003*b*).

Results

As expected, metabolic rate increased progressively with swimming speed (Fig. 1*A*). For SW fish, active Mo_2 for the four highest swimming speeds was not significantly different from that measured at U_{crit} (Fig. 1*A*). For FW fish, active Mo_2 for the penultimate swimming speed was not significantly different from that measured at U_{crit} (Fig. 1*A*). Routine Mo_2 and all active Mo_2 values, except the for the two final velocity increments, were significantly higher in SW fish than in FW fish by between 27% and 72% (Fig. 1*A*). As a result, COT (mg O_2 kg^{-1} m^{-1}) at speeds less than U_{crit} was significantly higher in SW fish than in FW fish (Fig. 1*C*). Nevertheless, neither $Mo_{2_{max}}$ (15.3 ± 0.6 mg O_2 kg^{-1} min^{-1}) nor U_{crit1} (1.4 ± 0.1 bl s^{-1}) in SW fish was significantly different from the values measured in FW fish (13.9 ± 1.5 mg O_2 kg^{-1} min^{-1} and 1.6 ± 0.1 bl s^{-1}, respectively), and as a result, COT at U_{crit} was also similar (Fig. 1*C*).

Similar findings were made for the second swim test (Fig. 1*B*). Again, active Mo_2 values were significantly higher in SW fish than in FW fish, by between 28% and 81% for all velocity increments except for the highest one. During the final recovery period, Mo_2 again remained significantly higher, by 58%–73%, in SW fish than in FW fish. Thus, compared with FW fish, SW fish had elevated excess postexercise oxygen consumption (EPOC) even though they had swum twice to the same maximum Mo_2 and U_{crit} as FW fish.

An important difference between the first and second swim tests was that Mo_2 did not recover to the routine level in either SW fish or FW fish at the outset of the second swim test. Despite this metabolic loading at the start of the second swim test (Fig. 1*B*), fish in both SW and FW performed to the same levels for U_{crit} and $Mo_{2_{max}}$ as in the first swim, and so the recovery ratios for U_{crit} and $Mo_{2_{max}}$ did not differ significantly from 1.0 (Table 1). Thus, elevated Mo_2 related to either being in seawater as opposed to freshwater or not being fully recovered from previous exhaustive exercise did not present itself as a metabolic loading that limited either critical swimming performance or maximum Mo_2.

The results of the plasma analysis are summarized in Table 2. As expected, all routine and postexercise plasma ion levels were significantly higher for fish in SW than in FW, with potassium being an exception. Although potassium levels were not significantly higher for fish in SW during routine activity, they did increase significantly after exercise. The routine plasma lactate and cortisol levels were unusually high in SW fish (see Farrell et al. 2001*b*) and were significantly higher than in FW fish. As expected, plasma lactate levels increased significantly after exercise for fish in FW, but not for fish in SW, where levels were already high. Plasma ion levels were well defended after exhaustive exercise in both treatments, with a significant increase occurring only in plasma potassium in SW. Unexpectedly, Na^+/K^+-ATPase activity was significantly lower in SW fish than in FW fish after exercise. The whole-body energy estimates for SW fish were significantly higher than for FW fish, but there was no difference in glucose concentrations between the two groups. This difference reflects the fact that measurements were made 3–6 wk earlier for fish in SW, and all of the fish had ceased to feed before capture in preparation for their river migration.

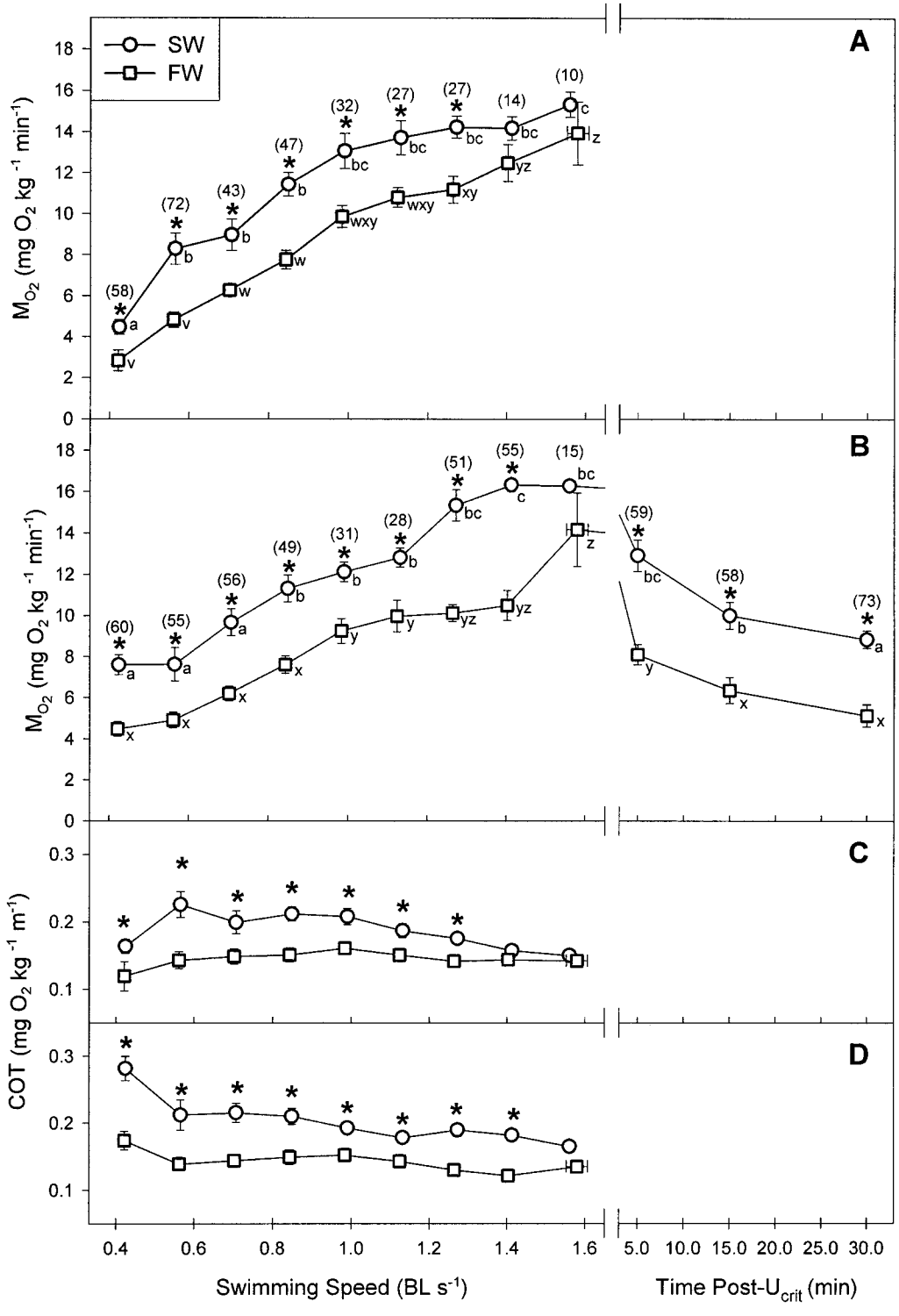

Figure 1. Relationships between the swimming speeds of sockeye salmon in seawater (SW; *circles*) and freshwater (FW; *squares*) and their Mo_2 and COT during initial (*A*, *C*) and repeated (*B*, *D*) U_{crit} tests. Mo_2 during recovery after the second U_{crit} also is presented in B. Routine and recovery measurements were taken at a swimming speed of 0.45 m s^{-1}, which allowed fish to rest on the bottom of the swim chamber and orient into the water current. Numbers in brackets represent percentage differences between comparable Mo_2 values for SW and FW fish. An asterisk indicates Mo_2 is significantly higher ($P < 0.05$) in sockeye swimming in SW. A dissimilar letter next to the symbol indicates a significant difference ($P < 0.05$) in Mo_2 among swimming speeds. Numbers in brackets represent percentage differences between Mo_2 values in SW and FW.

Table 1: Routine (RMR) and maximum (Mo_{2max}) metabolic rates, repeated maximum prolonged swimming performance (U_{crit}), and recovery ratios of sockeye salmon in freshwater and seawater

	Freshwater	Seawater
RMR_1 (mg O_2 kg^{-1} min^{-1})	2.8 ± .5	4.4 ± .3*
RMR_2 (mg O_2 kg^{-1} min^{-1})	4.5 ± .35	8.1 ± .7*
Mo_{2max1} (mg O_2 kg^{-1} min^{-1})	13.9 ± 1.5	15.3 ± .6
Mo_{2max2} (mg O_2 kg^{-1} min^{-1})	14.2 ± 1.8	16.3 ± .1
Mo_{2max2}/Mo_{2max1}	1.0 ± .1	1.0 ± .1
U_{crit1} (bl s^{-1})	1.6 ± .1	1.4 ± .1
U_{crit2} (bl s^{-1})	1.6 ± .1	1.4 ± .1
U_{crit2}/U_{crit1}	1.0 ± .1	1.0 ± .1

Note. Sample sizes were $n = 9$ in freshwater and $n = 10$ in seawater. Fish were swim tested in two consecutive swim trials separated by a 45-min recovery period. Full recovery is indicated when $x_2/x_1 = 1.0$. Recovery ratios were not significantly different at either salinity. Values are presented as mean ± SEM.

* Significant difference ($P < 0.05$) between sockeye salmon swimming in freshwater and seawater.

Discussion

This study is the first to show that adult sockeye salmon have significantly higher routine and active metabolic rates in SW than in FW. To the best of our knowledge, these are also the first measurements of U_{crit} and Mo_2 for migrating adult sockeye salmon in SW. While our results confirm some of the earlier findings for juvenile fishes that the metabolic cost of life is higher in SW than in FW, these are the first data for maturing wild fish during a natural migration from SW to FW. While some of the Mo_2 differences we measured between fish in SW and FW are probably related to differences in ionoregulatory costs, as in earlier studies, we caution that this study was performed on migrating and maturing fish intercepted just before their normal entry into the Fraser River. In addition, the measurements were made on FW fish at least 3 wk later than those on SW fish. As a result, differences between the SW and FW fish in their respective states of nutrition, reproduction, and ionic balance need to be considered as potential confounding factors, while other physiological changes such as morphological transformation and absorption of the alimentary tract (Brett 1995; Kiessling et al. 2004) may be less important. Therefore, unlike in the earlier laboratory studies on juvenile fishes, the differences in Mo_2 we observed for migratory sockeye salmon held in SW and FW may not be entirely results of the ionic differences in their environment.

The differences in routine and active Mo_2 between SW fish and FW sockeye salmon ranged from 27% to 72% and were generally higher than those reported for juvenile fish (10%–30%; Rao 1968; Morgan and Iwama 1991). An important finding is that this metabolic difference was repeatable in a second swim test and also occurred during EPOC. Also, the difference in Mo_2 did not affect the ability of fish to recover sufficiently to perform the repeated-swim test. Nevertheless, the metabolic difference did decrease with increasing swimming speed and was not statistically resolved at or near U_{crit}. This finding agrees with earlier work on adult Atlantic salmon, since neither U_{crit} nor maximum cardiac output (an indicator of Mo_2) differed significantly when fish were swum in SW and FW (Wagner et al. 2004). Thus, it appears as if the metabolic difference between FW and SW sockeye salmon could be deferred during high-speed swimming, but only temporarily because it reappeared during recovery. Deferring metabolic costs could have different consequences for the physiology of fish, depending on origin of the metabolic cost. For example, if an ionoregulatory cost were deferred, it might result in a postexercise ionic imbalance. In contrast, if a cost associated with gonadal development were deferred, it might cause a minor and undetectable delay in gonadal maturation, given that maturation occurs over several months. Likewise, if metabolic rate were elevated because of stress, this would be reflected in unusual plasma biochemistry, and the cost of recovery could be deferred. However, if the difference reflected a poor nutritional status, it is likely that routine metabolic rate would be suppressed and swimming performance compromised. Each of these possibilities is considered below. An additional issue is whether the metabolic difference between FW and SW fish was completely deferred at U_{crit} or reduced to a large degree. This may be a fine distinction that requires further study because while there is no doubt that the difference was reduced and that there was no statistical difference between maximum Mo_2 for FW fish and SW sockeye salmon, it is important to note that the maximum Mo_2 values for the first and second swims in SW remained 9% and 13% higher than in FW—values that are within the range of difference reported as significant for FW and SW juvenile rainbow trout (Rao 1968).

If the elevated routine Mo_2 in SW fish is a result of an ionoregulatory cost and is deferred at U_{crit}, as our data suggest, the prediction is that the ionic disruption after exercise would be greater in SW fish than FW fish. However, our data do not support this prediction. While routine plasma ion levels in FW fish were similar to those reported previously for FW salmonids (Eddy and Bath 1979; Maxime et al. 1990; Morgan and Iwama 1991, 1998; Talbot 1992), postexercise values did not change significantly for fish in either FW or SW. The two exceptions were postexercise plasma potassium in SW fish and plasma cortisol, which is expected to be generally elevated for maturing salmon during river migration (Robertson et al. 1961). Ion levels were generally higher in SW and lower in FW than those of Atlantic salmon (Wagner et al. 2004), but plasma osmolality remained balanced and within the range of coho salmon migrating between SW and FW (Uchida et al. 1997). Caution is needed, however, in making these comparisons. For example, the 30-min recovery period before sampling blood allowed for further ion fluctuations compared with sampling immediately

Table 2: Routine and postexercise blood and tissue variables of sockeye salmon in freshwater and seawater

Blood Variable	Routine Measurement[a]	Postexercise Measurement[b]
Sodium (mmol L^{-1}):		
Freshwater	153.7 ± 1.6	162.6 ± 4.7
Seawater	204.4 ± 9.7*	209.5 ± 6.4*
Chloride (mmol L^{-1}):		
Freshwater	124.2 ± 3.6	117.0 ± 3.5
Seawater	168.6 ± 8.3*	176.8 ± 4.7*
Potassium (mmol L^{-1}):		
Freshwater	2.6 ± .5	1.4 ± .1
Seawater	4.1 ± 1.2	6.2 ± .9*,**
Osmolality (mOsm kg^{-1}):		
Freshwater	288.8 ± 4.8	326.8 ± 23.3
Seawater	419.9 ± 21.7*	405.8 ± 13.7*
Glucose (mmol L^{-1}):		
Freshwater	6.4 ± .4	7.0 ± .7
Seawater	6.4 ± .1	6.3 ± .6
Lactate (mmol L^{-1}):		
Freshwater	2.0 ± .9	6.1 ± 1.2**
Seawater	9.6 ± 3.6*	9.9 ± 2.4*
Cortisol (mmol L^{-1}):		
Freshwater	125.4 ± 17.9	228.9 ± 82.0
Seawater	354.21 ± 95.1*	357.0 ± 77.8
Na^+/K^+ ATPase (μmol ADP mg protein^{-1} h^{-1}):		
Freshwater	1.8 ± .1	1.4 ± .3
Seawater		.7 ± .1*
Energy (MJ kg^{-1}):		
Freshwater	6.8 ± .5	5.9 ± .3
Seawater		7.8 ± .3*

Note. Sample sizes were $n = 8$ in freshwater and $n = 6$ in seawater for routine measurements and $n = 5$ in freshwater and $n = 10$ in seawater for postexercise measurements. Postexercise measurements were taken after the final 30-min recovery period.

[a] Samples taken from cannulated fish in 80-L isolation chambers.

[b] Samples taken by caudal puncture after 30-min recovery from a U_{crit} test.

* Significant difference ($P < 0.05$) between freshwater and seawater.

** Significant difference ($P < 0.05$) between routine and postexercise values.

after exercise (Wendelaar Bonga 1997; Farrell et al. 2001*a*). Also, fish performed two exhaustive swims rather than one. Finally, there are inherent dangers in comparing values for blood sampled by cannulas and by caudal puncture in anesthetized fish. Even so, recent work (Jain and Farrell 2003) on cannulated rainbow trout and using a similar repeat-swimming protocol found that plasma variables for the second swim rarely differed between exhaustion and a 40-min recovery. In fact, these values were sometimes no different than those observed after the first recovery period that preceded the second swim. Thus, it seems that the plasma variables can reach a plateau in repeat U_{crit} tests, and it is unlikely that the stress associated with anesthesia and caudal puncture would have had large additive effects. Despite these caveats, the blood and tissues analyses were revealing in other ways.

Salmon are programmed to return to FW to spawn, and the fact that gill Na^+/K^+-ATPase activity in SW fish was below the normal range for wild sockeye salmon during their return ocean migration (1.5–4.0 μmol ADP mg protein^{-1} h^{-1}, mean $\approx$ 3.0; M. Shrimpton, unpublished data) points to the fish already having undergone substantial transformation in preparation for entry into FW. A decline in the gill Na^+/K^+-ATPase activity to a level below that of fish held in FW has been also reported for coho salmon held in SW during their spawning migration (Uchida et al. 1997). Thus, it is possible that the SW fish used here, because of their particular phase of migration, were in a nonsteady state for ionoregulation, and this nonsteady state resulted in a large difference in routine Mo_2 compared with FW fish. If this is the case, then plasma ions might be already disrupted to some extent before exercise, making the compar-

ison between routine and postexercise ion levels unreliable in the context in which it was used above. Consequently, the elevated routine M_{O_2} in SW fish may reflect a true metabolic cost of migratory salmon as they make their normal transition between SW and FW, although it exceeds the metabolic cost reported for juvenile salmonids properly acclimated to SW.

Sockeye salmon in SW had elevated routine cortisol and lactate levels. Moreover, while plasma lactate increased significantly after exercise in FW fish, it did not increase in SW fish but remained at the elevated level of 10 mmol L^{-1}. The most likely explanation for these observations is that SW fish were more restless than FW fish. Thus, despite the fact that blood was sampled four times in individual fish that were overtly quiet and only the lowest plasma lactate value was selected for each individual, the elevated plasma lactate level probably indicates that SW fish had not completely recovered from prior activity, a state that could also result in an elevated M_{O_2}. Consequently, restless behavior in SW fish may have contributed to the elevated routine M_{O_2}. Uchida et al. (1997) speculated that the loss of hypoosmoregulatory ability and increased hyperosmoregulatory ability of migrating salmon motivated them to enter FW and would account for their restlessness. They found the switch in osmoregulatory ability was a result of the respective loss of gill filament chloride cells and concomitant increase in the number of gill lamellar chloride cells. Plasma osmolality of chum salmon captured during their spawning migration also rose above 400 mOsm kg^{-1} after several days in captivity in SW (Uchida et al. 1997). The routine plasma osmolality in SW sockeye salmon was similarly above 400 mOsm kg^{-1} (Table 2). EPOC can be deferred at U_{crit} (as shown here and in previous studies of repeat swimming). Thus, it seems likely that elevated M_{O_2} caused by restlessness could also be deferred at U_{crit}.

For adult pink salmon nearing their spawning grounds, it has been shown that swimming performance and maximum metabolic rate decline in association with energy depletion and completion of gonadal development (Williams et al. 1986). Starvation also has been shown to cause muscle breakdown and to compromise metabolic rates, muscle enzyme activity, and swimming performance of other fish species with different life histories (Johnston 1981; Yang and Somero 1993; Martínez et al. 2004). Clearly, FW fish had no trouble achieving the same maximum swimming performance as SW fish in this study. The similarity in performance seems to rule out the possibility of severe differences in nutritional state and reproductive development contributing to the observed metabolic differences. Our data also agree with earlier measurements of $M_{O_{2max}}$ at U_{crit} in wild adult sockeye salmon (Brett and Glass 1973; Farrell et al. 2003; Lee et al. 2003*b*) and the time course for EPOC (Lee et al. 2003*a*). For example, the maximum M_{O_2} value obtained for individual FW fish could be repeated with a short recovery period, and the group mean value was similar to the 15.1 mg O_2 kg^{-1} min^{-1} value reported for field measurements on a stock of sockeye salmon that had migrated 400 km upriver (Lee et al. 2003*b*). Thus, the similarity of maximum M_{O_2} for FW fish and SW fish suggests that the 24% lower whole-body energy status in FW fish (Table 2) did not compromise maximum M_{O_2} and indicates FW fish were not at the limits of their energy reserves. Fat stores of sockeye salmon typically are not depleted until the fish have reached the spawning grounds, although different stocks have different fat stores in proportion to their migration distance (Brett 1995; Crossin et al. 2004). The fish in this study were starved for the approximate duration of their natural migration to the Adams River (normally a 400-km river migration lasting 3–6 wk) but were not required to expend energy for upriver swimming. Yang and Somero (1993) found 13–16 wk of starvation caused a 35%–40 % decline in M_{O_2} for scorpaenid fish, but this length of starvation was three to four times longer than in this study. Nevertheless, we cannot exclude the possibility that the 24% lower energy of FW fish had some contribution to the lower M_{O_2} values compared with SW fish.

While we can eliminate the potential confounding effect of completed gonadal development in FW fish, we cannot completely eliminate the potential confounding effect of the difference in reproductive status. This status would have been more advanced in FW than in SW fish, but it would not have been completed since Patterson et al. (2004) have shown that holding adult sockeye salmon in static water delays gonadal maturation. We are unaware of any direct estimates of the additive metabolic cost to routine metabolism of converting somatic energy into gonads. For gonadal development to be a confounding effect in this study, the cost of early gonadal development (i.e., in SW) would have to be greater than that for late gonadal development (i.e., in FW). Even so, it seems likely that energy allocation to gonadal development could easily and relatively quickly be deferred during swimming, thereby allowing for normal U_{crit} and maximum M_{O_2} values in SW fish.

This study cannot distinguish between a deferred metabolic loading and an increased reliance on anaerobic swimming near U_{crit}. Differences are already recognized for anaerobic contributions to swimming among different stocks of sockeye salmon. For example, a more distinct plateau in active M_{O_2} and an elevated EPOC exist for Gates Creek sockeye salmon as compared with both Weaver Creek sockeye salmon and Chehalis Creek coho salmon (Lee et al. 2003*a*). Therefore, the fact that fish in SW achieved the same maximum M_{O_2} at U_{crit} despite a metabolic loading also could be explained by these fish relying on anaerobic metabolism at the higher swimming speeds to a greater degree than fish in FW. However, to resolve such a difference would require a more detailed examination of EPOC in SW and FW fish than that performed here.

The experiments of this study point to the metabolic costs for adult sockeye salmon returning to the Fraser River being reduced substantially once they have entered and fully adjusted to FW. Importantly, these costs were diminished as fish approached U_{crit} and maximum M_{O_2}, and as a result SW fish

could achieve the same U_{crit} and Mo_2 as FW fish. Moreover, fish in both SW and FW started the second swim test with an elevated Mo_2, which also did not impair either U_{crit} or maximum Mo_2. This means that as migrating adult salmon approach U_{crit}, they can apparently defer certain forms of metabolic loading, even those that show all the hallmarks of stress, in order to reach maximum Mo_2. Nonetheless, these metabolic costs do not remain deferred during recovery, as evidenced by the elevated EPOC for fish in SW compared with fish in FW. About 4% of this difference during swimming could be simply a result of the higher viscosity of SW (1.30 cP at 30 ppm, 12°C) compared with FW (1.24 cP at 0 ppm, 12°C; Dorsey 1968). Beyond this, we cannot be sure of the exact source for the observed differences in routine Mo_2, active Mo_2, and EPOC for fish in SW compared with fish in FW. In addition to true ionoregulatory costs associated with the FW and SW environments, our data also implicate four important potential costs that we cannot distinguish between: energy, reproduction, non–steady state ionoregulation, and restlessness. In future studies on wild salmon in SW, the problem of restlessness might be avoided by intercepting fish much earlier in their ocean migration.

The results of this study may prove to be important for fisheries conservation and management because they can be used to update current models of energy use by salmon during their adult migration. Elevated routine and active Mo_2 values in wild sockeye salmon in SW could potentially be compensated for by access to an environment that has a much higher primary productivity (>300 g C m^{-2} yr^{-1}, based on SeaWiFS global primary productivity estimates) in their northwest Pacific feeding grounds, as compared with FW, and so growth rate remains higher at higher salinities (Bœuf and Payan 2001). The result also raises questions about why some returning stocks of Fraser River sockeye salmon quickly enter the river to begin their spawning migration while others hold for weeks outside of the estuary, if this strategy is energetically more costly. While these findings elucidate some of the metabolic costs involved for the travel of salmon between SW and FW, the ecological and energetic implications require evaluation with further study.

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