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PROLONGED SWIMMING, RECOVERY AND REPEAT SWIMMING PERFORMANCE OF MATURE SOCKEYE SALMON *ONCORHYNCHUS NERKA* EXPOSED TO MODERATE HYPOXIA AND PENTACHLOROPHENOL

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Summary

Mature, wild sockeye salmon (*Oncorhynchus nerka*) demonstrated their remarkable stamina and recovery abilities by performing three consecutive critical swimming speed tests with only a 45 min interval for recovery between subsequent tests. Although the repeated swimming challenges were performed without a full recovery, normoxic fish swam just as well on the second swim, and the majority of fish swam only marginally more poorly on the third swim. In addition, metabolic loading in these fish, as measured by the rate of oxygen consumption, ventilation rate and plasma lactate levels during recovery, did not appear to be cumulative with successive swims. Fish, however, did not recover as well after a similar level of initial swimming performance under moderately hypoxic conditions (water P_{O_2}>100 mmHg; 1 mmHg=0.1333 kPa). Four out of the five fish did not swim again and their high plasma lactate levels indicated a greater anaerobic effort. In another group of fish, metabolic loading (elevated control rates of oxygen consumption) was induced with an overnight sublethal exposure to pentachlorophenol, but these fish swam as well as normoxic fish on the first swim, and five of the six fish swam for a third time at a marginally lower critical swimming speed. In contrast to expectations, pentachlorophenol pretreatment and moderate hypoxia were not additive in their effects. Instead, the effects resembled those of pentachlorophenol pretreatment alone. The results are discussed in terms of what aspects of fatigue might impair the repeat swimming performance of sockeye salmon.

Key words: prolonged swimming, critical swimming speed, oxygen consumption, plasma lactate, pentachlorophenol, hypoxia, recovery ratio, sockeye salmon, *Oncorhynchus nerka*.

Introduction

The ability of fish to exercise, recover and swim again without hindrance has important ecological ramifications. Even though fish apparently spend most of their time swimming at much slower velocities (Preide and Young, 1977; Tytler, 1978), critical swimming speed (U_{crit}; Brett, 1964) is a popular measure of swimming performance because it conveys important information regarding a fish's ability successfully to forage, to escape predation, to maintain position in a current (Beamish, 1978) and to migrate upstream. For example, the U_{crit} of sockeye salmon (*Oncorhynchus nerka*) has a temperature optimum (Brett, 1971) that corresponds with the ambient water temperature in the Fraser River, British Columbia, when they migrate through the Hell's Gate rapids (Farrell, 1997). When the river temperature is a few degrees above this optimum, mature sockeye salmon often fail to navigate the rapids and do not reach their spawning grounds. There are, however, many rapids in the Fraser Canyon and since repeat swimming performance, perhaps to exhaustion, is inevitable, a long recovery period could easily be a selective disadvantage.

Information on the recovery period needed for unhindered repeat U_{crit} performance is limited. Studies with single and repetitive bouts of exhaustive swimming suggest that recovery may take between 40 min and several hours (e.g. Black, 1955; Brett, 1964, 1965; Stevens and Black, 1966; Turner *et al.* 1983; Milligan and Wood, 1986; Scarabello *et al.* 1991, 1992; Arthur *et al.* 1992; Pagnotta *et al.* 1994; for a review, see Wood, 1991). For example, following a single 5 min exhaustive burst swim in rainbow trout (*Oncorhynchus mykiss*), the oxygen debt was repaid in 2–3 h (Scarabello *et al.* 1992). Similarly, following U_{crit} swimming, oxygen debt was repaid in 3.2 h in sockeye salmon and over a somewhat longer period in coho salmon (*Oncorhynchus kisutch*) (Brett, 1964). Furthermore, because burst exercising at intervals up to 1 h resulted in almost additive metabolic effects and culminated in a refusal to swim, Stevens and Black (1966) concluded that rainbow trout do not tolerate

frequent periods of exercise of short duration. However, because all accumulated lactate need not be oxidized before the fish can swim again (Hochachka, 1961), full metabolic recovery is not necessary for repeat swimming performance. In fact, a few studies indicate that salmonids can swim to the same U_{crit} after less than 2 h of recovery. For example, chinook salmon (*Oncorhynchus tshawytscha*) at 10 °C performed equally well when two U_{crit} tests were separated by 60 min (Randall *et al.* 1987), as did wild juvenile coho salmon using a 2 h recovery period (Brauner *et al.* 1994). Similarly, the recovery period was 70 min for hatchery-reared rainbow trout swimming in fresh water at 8–9 °C and only 40 min for preliminary experiments with mature, wild sockeye salmon swimming in fresh water at 19 °C (Jain *et al.* 1998). Peake *et al.* (1997) suggested that the recovery times and recovery speeds prior to U_{crit} testing and following handling stress in juvenile rainbow trout may have been too conservative.

The present study characterises for the first time the ability of mature, wild sockeye salmon to perform three consecutive swim challenges separated by short (45 min) recovery periods in a situation that mimicked certain aspects of their spawning migration (i.e. the transition to fresh water and a high water temperature). We also examined the degree to which metabolic loading affected recovery. To do this, fish were stressed with moderately hypoxic water during the first swim and/or with a sublethal exposure (12–14 h) to pentachlorophenol prior to the first swim.

Materials and methods

Fish capture and maintenance

On 9 and 16 July 1996, mature sockeye salmon (aged 4^+ to 5^+ years) were captured in Alberni Inlet on the west coast of Vancouver Island (British Columbia, Canada) by a commercial seiner and placed into the ship's seawater hold. At the Port Alberni wharf, these fish were transferred to an insulated fibreglass tank for transport by truck to the West Vancouver Laboratory (WVL), British Columbia (Department of Fisheries and Oceans, Canada). To reduce stress, individual fish were handled with a low-abrasion dipnet, and the transport tank contained oxygenated, chilled iso-osmotic water (2 parts sea water to 1 part fresh water; 6.4–9.8 °C) with 0.25 mg l^{-1} of the anaesthetic metomidate hydrochloride (Syndel Laboratories, Vancouver, British Columbia, Canada) and 500 g of the ammonia-binding clay Ammorex (Argent Chemical Laboratories, Redmond, WA, USA). At the WVL, fish were kept at a density of 2 kg m^{-3} or less in outdoor 3500 l round fibreglass tanks continuously supplied with air-equilibrated sea water (10.0–12.9 °C). As a therapeutic treatment for suspected seawater vibriosis as well as a prophylactic treatment against bacterial disease and fin rot, all fish were exposed for 1 h to Chloramine-T (*N*-sodium-*N*-chloro-*p*-toluenesulphonamide; 8.5 mg l^{-1}) in an aerated static bath. A second treatment was administered 24 h later. Fish were held for at least a further 3 weeks before experimentation at Simon Fraser University (SFU) and were not fed after capture.

Groups of three or four fish were transported to SFU in a 1 m^3 insulated transport tank containing aerated iso-osmotic water. At SFU, fish were placed into a 1600 l outdoor annular fibreglass raceway (length 343 cm, depth 41 cm, width 46 cm) filled with iso-osmotic water that was gradually replaced with dechlorinated Vancouver municipal fresh water at a flow rate of 5 l min^{-1}. The salinity in the annular raceway was reduced to less than 3 ‰ over approximately 10–12 h. Ambient water temperature in the raceway ranged from 15.6 to 18.6 °C over the study period.

Cannulation and transfer to the swim-tunnel

Soon (24–36 h) after the salmon had been transported to SFU, they were briefly anaesthetized in MS-222 (0.2 g l^{-1}) buffered with $NaHCO_3$. Fish mass, fork length (BL), maximum width and maximum depth were measured and the fish were placed supine on a surgical table to allow for dorsal aortic cannulation (PE 50; Smith and Bell, 1967) while under anaesthesia (0.1 g l^{-1} buffered MS-222). Following the 3–5 min cannulation procedure, fish were either placed directly into a 130 l Brett-type swim-tunnel respirometer (see Gehrke *et al.* 1988), supplied with dechlorinated water at a rate of 3 l min^{-1}, or returned to the outdoor tank for up to 3 days before experimentation. When transferring fish from the outdoor tank to the respirometer, they were briefly and lightly anaesthetized with buffered MS-222.

Experimental protocols

Fish recovered for 30 min in the swim-tunnel before a brief swim to habituate them to increases in current velocity (Jain *et al.* 1997). In this initial swim, current velocity was gradually increased to 1 BL s^{-1} over 10–15 min and was then maintained at 0.4 BL s^{-1} overnight. A water P_{O_2} of 150 mmHg (20 kPa) was achieved in the respirometer by passing the incoming water through a gas-exchange column receiving a controlled mixture of air and oxygen. Water P_{O_2} was monitored overnight and during subsequent U_{crit} tests by continuously withdrawing water from the respirometer using a peristaltic pump (Masterflex, Chicago, IL, USA) and passing it through a thermostatted P_{O_2} electrode (Radiometer, model E5046-0, Copenhagen, Denmark).

After an overnight habituation for 12–14 h at 0.4 BL s^{-1}, the water temperature was raised from ambient to 19–20 °C over a 1 h period using a heat-exchange column of local construction. Three successive critical swimming speed tests (U_{crit1}, U_{crit2} and U_{crit3}) were then carried out for each fish at this temperature. These tests were separated by 45 min recovery periods during which water velocity was maintained at 0.4 BL s^{-1}. In our U_{crit} protocol, current velocity was gradually increased to 40 cm s^{-1} (approximately 0.6 BL s^{-1}) over 15 min. Thereafter, current velocity was increased by 12.5 cm s^{-1} (approximately 0.25 BL s^{-1}) every 15 min until the fish fatigued. This protocol produces U_{crit} values similar to those obtained using more standard protocols in which current velocity increments are constant throughout (Jain *et al.* 1997). Exhaustion was established by the inability of the fish to swim

away from the electrified (5 V) grid at the rear of the swim chamber after three short shocks. U_{crit} was calculated as described by Brett (1964). When necessary, U_{crit} values were corrected for the solid blocking effects (Bell and Terhune, 1970). At the end of the experiment, fish were humanely killed by an overdose of the fish anaesthetic and cervical dislocation. The ventricle was removed, blotted with a paper towel and weighed. Relative ventricular mass was calculated from the ventricular mass and fish mass.

Experimental groups

Twenty-one fish were randomly assigned to one of four experimental groups, and each group had similar morphometrics, haematocrit and leucocrit (Table 1). Normoxic fish (N=5) performed the three U_{crit} tests, as described above, under normoxic conditions. Hypoxic fish (N=5) were made moderately hypoxic (a water P_{O_2} of 100–106 mmHg=13.3–18.8 kPa) for the U_{crit1} test by reducing the rate of oxygenation in the gas-exchange column once the water temperature in the swim-tunnel had reached 19–20 °C. These fish also had their post-exercise $\dot{V}_{O_2}$ measured under hypoxic conditions. Thereafter, water P_{O_2} was increased to 150 mmHg within 5 min and maintained at a normoxic level for the remainder of the recovery period and during subsequent swim tests. The protocol used for normoxic fish pretreated with pentachlorophenol (PCP) (N=6) was identical to that used for normoxic fish except that the fish were pre-exposed overnight to a sublethal concentration of PCP (13.7±1.0 μg l^{-1}; mean ± S.E.M.) in the water (Webb and Brett, 1973; NRCC, 1982). The median lethal concentration, killing 50 % of juvenile sockeye salmon in 96 h, is 63 μg l^{-1} (Webb and Brett, 1973). Also, the half-time for PCP disappearance from the blood of rainbow trout after an intraarterial injection is about 5 h (Chris Kennedy, personal communication). Toxicant was flushed from the respirometer for at least 1 h prior to increasing the water temperature in the swim-tunnel. The protocol used with hypoxic fish pretreated with PCP (N=5) was identical to that for hypoxic fish except that the fish were also pre-exposed to PCP (13.7±1.3 μg l^{-1}; mean ± S.E.M.) as described above.

Toxicant preparation and measurement

A stock solution of sodium pentachlorophenate (23 mg l^{-1}) was prepared by dissolving 2.0 g of NaOH and 46.6 mg of PCP in 2 l of double-distilled water (Webb and Brett, 1973). This solution was diluted 1:19 with dechlorinated water just prior to use and was delivered to the swim-tunnel using a pre-calibrated peristaltic pump at a rate of 10 ml min^{-1} to bring the PCP water concentration in the swim-tunnel to a nominal 20 μg l^{-1} within 15 min. Pumping rate was then adjusted to 2 ml min^{-1} for 12–14 h to maintain the PCP concentration. The actual PCP concentration reported above was based on 1 l water samples taken at the end of each exposure period. These samples were stored in the dark at 4 °C until they were analyzed at the Analytical Chemistry Laboratory (Organics), Pacific Environmental Science Centre, Environment Canada, North Vancouver, British Columbia.

$\dot{V}_{O_2}$ *and other physiological measurements*

$\dot{V}_{O_2}$ was measured on seven occasions during the experiment. Control (rest) $\dot{V}_{O_2}$ was measured prior to U_{crit1} while the fish was swimming at a current velocity of 0.4 BL s^{-1} at 19–20 °C. Post-exercise $\dot{V}_{O_2}$ was measured during the 10 min period immediately following each of the three U_{crit} tests during which the current velocity was 0.4 BL s^{-1}. Recovery $\dot{V}_{O_2}$ was measured during the last 10 min of the 45 min recovery period following each of the three swim tests. $\dot{V}_{O_2}$ was calculated from the measured decrease in water P_{O_2} over an 8–10 min period with the respirometer closed. Duplicate water samples were taken from the swim-tunnel at the beginning and end of the measurement period and injected into a second Radiometer P_{O_2} electrode (model E5046-0, Copenhagen, Denmark) to measure the decrease in water P_{O_2}. At the end of each $\dot{V}_{O_2}$ measurement, the water P_{O_2} was rapidly

Table 1. *Morphometric and haematological variables in the different test groups of mature sockeye salmon* Oncorhynchus nerka

Test group		Fork length (m)	Body mass (kg)	Ventricular mass (g)	Relative ventricular mass (%)	Leucocrit (%)	Haematocrit (%)
Normoxic	Mean	0.56	1.94	2.58	0.13	1.77	31.7
	S.E.M.	0.02	0.18	0.29	0.01	0.25	3.5
	N	5	5	5	5	5	5
PCP-treated	Mean	0.57	1.86	2.27	0.13	1.38	28.8
normoxic	S.E.M.	0.01	0.14	0.11	0.01	0.14	3.4
	N	6	6	6	6	5	5
Hypoxic	Mean	0.53	1.56	2.10	0.14	1.24	33.8
	S.E.M.	0.01	0.08	0.10	0.01	0.12	4.5
	N	5	5	5	5	5	5
PCP-treated	Mean	0.56	1.94	2.58	0.13	1.77	31.7
hypoxic	S.E.M.	0.02	0.18	0.29	0.01	0.25	3.5
	N	5	5	5	5	5	5

PCP, pentachlorophenol.

restored (over a 5 min period) by flushing the swim-tunnel with oxygenated water.

Ventilation rate (min^{-1}) was recorded towards the end of the $\dot{V}_{O_2}$ measurement by counting opercular movements for 45 s. Blood samples (1.0 ml) were withdrawn from the dorsal aorta to measure haematocrit, leucocrit, arterial blood P_{O_2} (Pa_{O_2}) and plasma lactate concentration. Haematocrit and leucocrit were determined on 20 µl blood samples following centrifugation at 10 000 ***g*** for 2 min. Pa_{O_2} was measured using a thermostatted Radiometer P_{O_2} electrode (model E5046-0, Copenhagen, Denmark) connected to a Gould chart recorder (model 2200, Cleveland, OH, USA). Plasma, obtained by centrifugation of whole blood at 10 000 ***g*** for 1 min, was quickly frozen in liquid nitrogen and stored at –80 °C. Plasma lactate concentrations were determined on duplicate 10 µl plasma samples using colorimetry (Sigma Diagnostics, St Louis, MO, USA). Following centrifugation, the remaining red blood cells were resuspended in 0.8 ml of saline and returned to the fish through the dorsal aortic cannula.

Data analysis

The recovery ratio for U_{crit} was calculated by dividing either U_{crit2} or U_{crit3} by U_{crit1}. Mean values are presented with the standard error of the mean (S.E.M.). Statistical comparisons were performed using a software package (SYSTAT Inc.). A one-way repeated-measures analysis of variance (ANOVA) was used for comparisons involving repeated sampling, and a one-way ANOVA was used for comparisons among test groups. Multiple comparisons were made using Fisher's least-squares difference (LSD). Linear regression analysis was used for the correlations. $P<0.05$ was used to establish statistical significance.

Results

Normoxic fish

After overnight habituation to the swim-tunnel, control $\dot{V}_{O_2}$ was 2.10±0.19 ml O_2 kg^{-1} min^{-1} and ventilation rate was 69.8±6.9 min^{-1} (Figs 1, 2). Plasma lactate concentration was 1.0±0.5 mmol l^{-1} and Pa_{O_2} was 110±6 mmHg (Figs 3, 4). U_{crit1} for normoxic fish was 1.44±0.09 BL s^{-1} (Fig. 5).

Following the first swim, post-exercise $\dot{V}_{O_2}$, ventilation rate and plasma lactate concentration were all significantly elevated two- to fourfold (Figs 1–3). Pa_{O_2} did not change significantly (Fig. 4). At the end of the 45 min recovery period, $\dot{V}_{O_2}$ and ventilation rate had returned to their control values (Figs 1, 2). However, Pa_{O_2} was significantly elevated compared with the control value (Fig. 4), and the plasma lactate level tended to be higher at 4.4±1.3 mmol l^{-1} (but $P>0.05$) (Fig. 3). All five fish swam equally well after the 45 min recovery period. U_{crit2} was not significantly different from U_{crit1} (Fig. 5), and the recovery ratio of 0.97±0.02 was not significantly different from unity.

Variables measured during the second post-exercise recovery period showed similar patterns to those observed following the first swim (Figs 1–5). An exception to this generalization was a significant post-exercise decrease in Pa_{O_2} compared with the value during the first recovery period (Fig. 4). Also, the plasma lactate levels were unchanged compared with the first recovery period, and the high variance, mainly because one fish had an especially high (>8 mmol l^{-1}) lactate value, obscured a statistical difference compared with control values.

Three out of the five normoxic fish swam for a third time. Individual recovery ratios were 0.93, 0.95 and 0.67 for U_{crit3}, and the mean value of 0.85±0.09 was significantly different from unity. Interestingly, those fish that swam a third time had

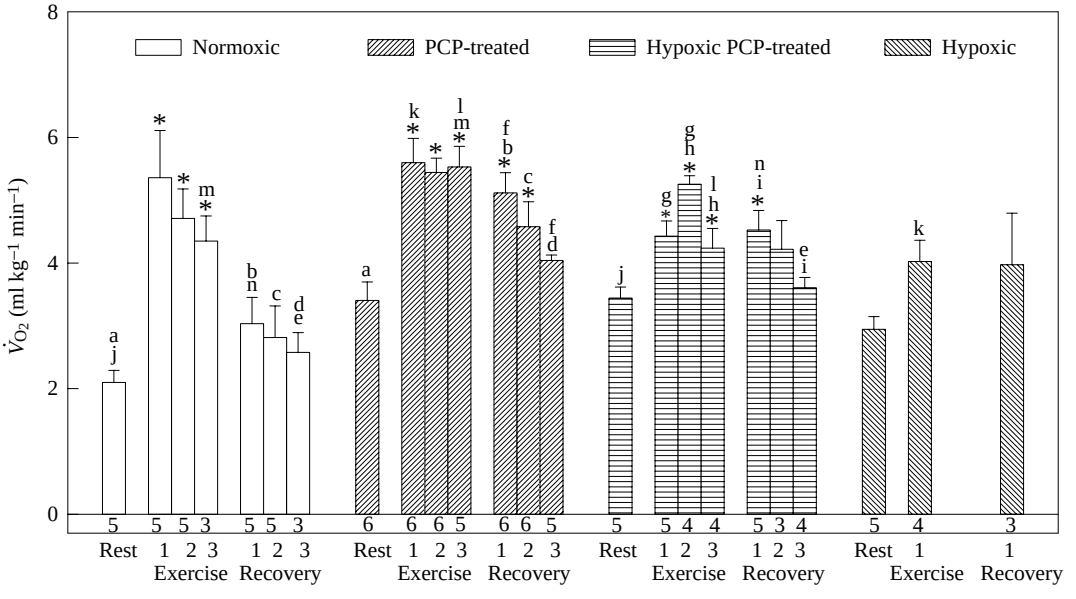

Fig. 1. The rate of oxygen uptake ($\dot{V}_{O_2}$) of mature sockeye salmon (*Oncorhynchus nerka*) prior to, during and after, three consecutive swimming challenges separated by 45 min recovery periods. The results for the four treatment groups are presented separately. Rest, exercise and recovery refer to the value prior to the first swim, the value immediately post-exercise and the value at the end of the 45 min recovery period, respectively. Numbers (1, 2 and 3) denote the first, second and third swims. The numbers located immediately under each column are the numbers of fish tested, and the vertical bars represent S.E.M. An asterisk denotes a significant difference ($P<0.05$) from the rest value within a test group. Similar letters denote a significant difference ($P<0.05$) between comparable values among test groups. PCP, pentachlorophenol.

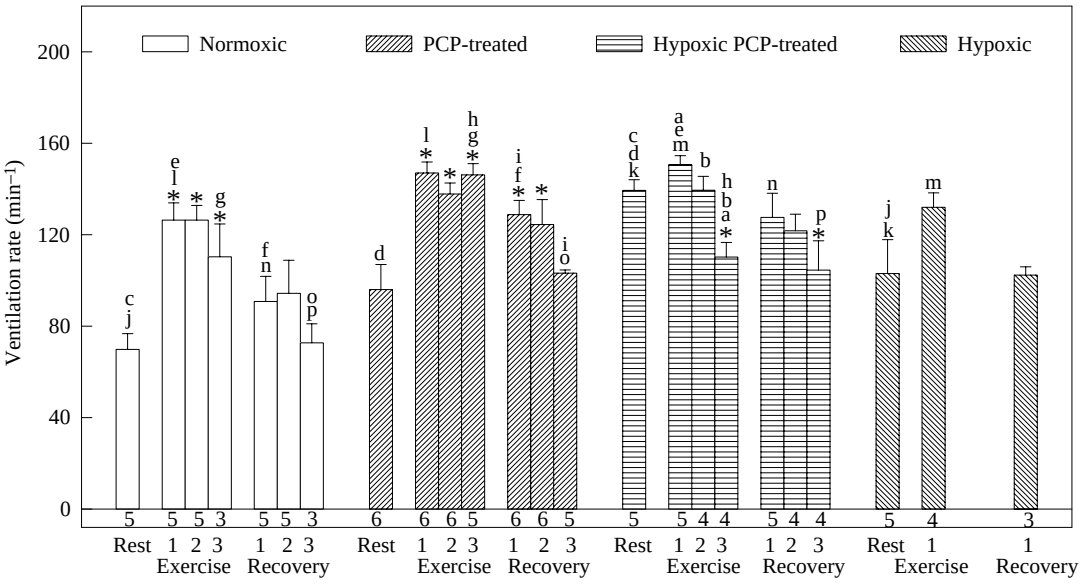

Fig. 2. The ventilation rate of mature sockeye salmon (*Oncorhynchus nerka*) before and following three consecutive swimming challenges separated by 45 min recovery periods. See Fig. 1 and the text for other details.

post-exercise and recovery plasma lactate levels that were less than 2.0 mmol l^{-1} during the second recovery period, whereas individuals with higher plasma lactate values did not swim for a third time (Fig. 3).

Normoxic fish pre-treated with PCP

After overnight exposure to PCP, control $\dot{V}_{O_2}$ in clean, normoxic water (3.41±0.29 ml O_2 kg^{-1} min^{-1}) was elevated by 50 % compared with that of normoxic fish (Fig. 1), but plasma lactate level, Pa_{O_2} and ventilation rate were unchanged (Figs 2–4). Despite this metabolic load, PCP-treated fish swam just as well as normoxic fish. U_{crit1} was 1.28±0.11 BL s^{-1} (Fig. 5). Moreover, all six PCP-treated fish swam a second time following a 45 min recovery period. Although U_{crit2} (1.19±0.11 BL s^{-1}) was not significantly different from U_{crit1} (Fig. 5), the recovery ratio of 0.93±0.04 for U_{crit2} was significantly different from unity. Thus, the recovery ratio proved to be a more sensitive index of the subtle impairment on repeat swimming performance of PCP treatment.

Many of the variables measured in PCP-treated fish during the first recovery period changed in a manner qualitatively and quantitatively similar to that already described for normoxic fish. However, there were important differences, and these all point to U_{crit1} producing a greater metabolic loading in PCP-treated fish. Notably, ventilation rate, $\dot{V}_{O_2}$ and plasma lactate level remained significantly elevated during the recovery period compared with the control values, unlike values in the normoxic fish (Figs 1–3). Surprisingly, therefore, a greater proportion of PCP-treated fish (five out of six) swam for a third time compared with normoxic fish, and U_{crit3} was not significantly different from U_{crit1}. Nonetheless, the individual recovery ratios for U_{crit3} were

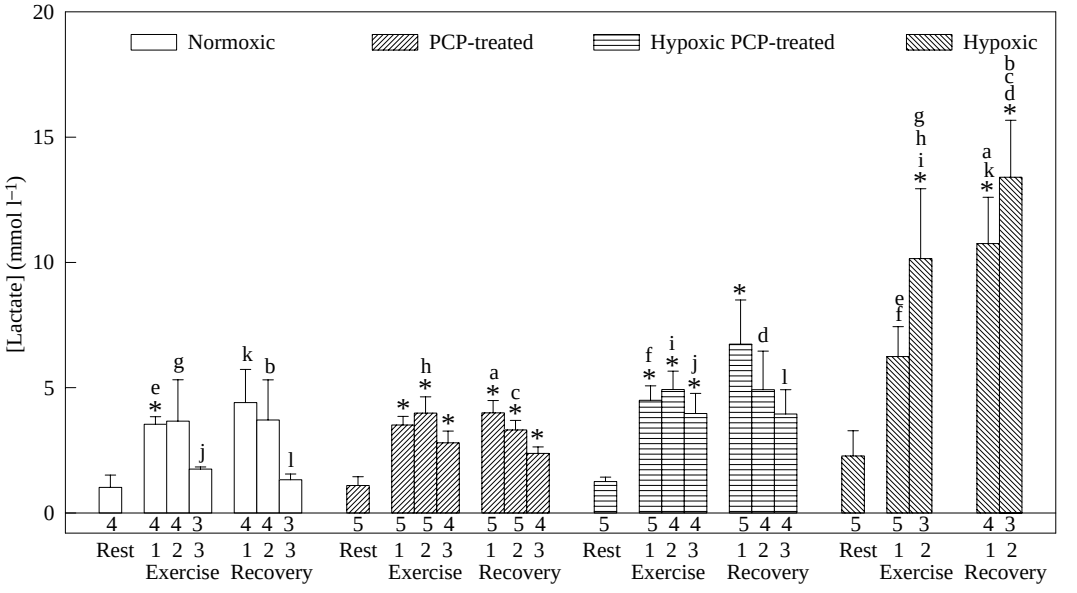

Fig. 3. The plasma lactate levels of mature sockeye salmon (*Oncorhynchus nerka*) before and following three consecutive swimming challenges separated by 45 min recovery periods. See Fig. 1 and the text for other details.

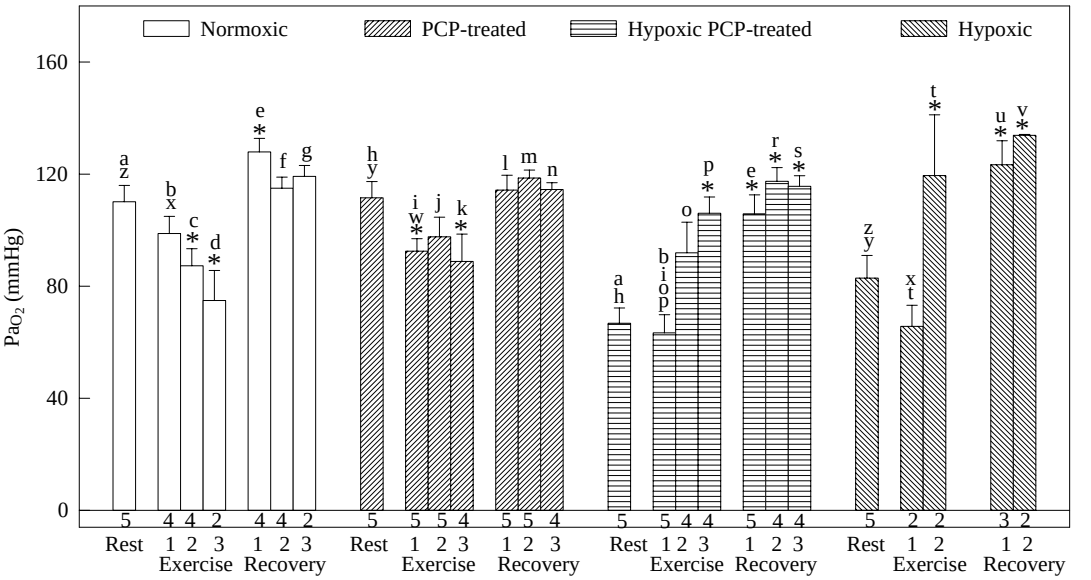

Fig. 4. The arterial blood partial pressure of oxygen (Pa_{O_2}) (1 mmHg=0.1333 kPa) of mature sockeye salmon (*Oncorhynchus nerka*) before and following three consecutive swimming challenges separated by 45 min recovery periods. See Fig. 1 and the text for other details.

0.98, 0.97, 0.96, 0.88 and 0.80 and the group mean was 0.92±0.03, a value significantly lower than unity. Consequently, the significant metabolic load of sublethal PCP pretreatment had only a marginal negative effect on repeat swimming performance of mature sockeye salmon.

Fish performing the initial swim under moderately hypoxic conditions

Compared with normoxic fish, exposure to moderate hypoxia (water P_{O_2}=100–106 mmHg) prior to the first swim significantly decreased Pa_{O_2} (83±8 mmHg) and increased ventilation rate (103±14 min^{-1}) (Figs 2, 4) without affecting control $\dot{V}_{O_2}$, control plasma lactate level and U_{crit1} (1.19±0.13 BL s^{-1}) (Figs 1, 3, 5). The swimming effort under moderate hypoxia elevated plasma lactate levels both immediately post-exercise (6.24 mmol l^{-1}) and after the 45 min recovery period (10.8 mmol l^{-1}) compared with that of normoxic fish (Fig. 3). Furthermore, repeat swimming ability was very poor. Four out of five fish did not swim again in normoxic water (Fig. 5). Plasma lactate level was followed in two of these fish for the next 45 min and remained significantly elevated (between 7.8 mmol l^{-1} and 17.8 mmol l^{-1}) despite a normal Pa_{O_2}. One fish in this group did swim three times, and U_{crit2} and U_{crit3} in normoxic water were higher than U_{crit1} in hypoxic water (the recovery ratios were 1.32 and 1.29 for U_{crit2} and U_{crit3}, respectively). This fish turned out to be the best individual swimmer of all the 21 fish tested (Fig. 5).

PCP-treated fish performing the initial swim under moderately hypoxic conditions

Because hypoxia severely impaired recovery, and because PCP pretreatment reduced the recovery ratio, the expectation was that these negative effects would be additive. This was not true. In fact, it appeared that PCP pretreatment

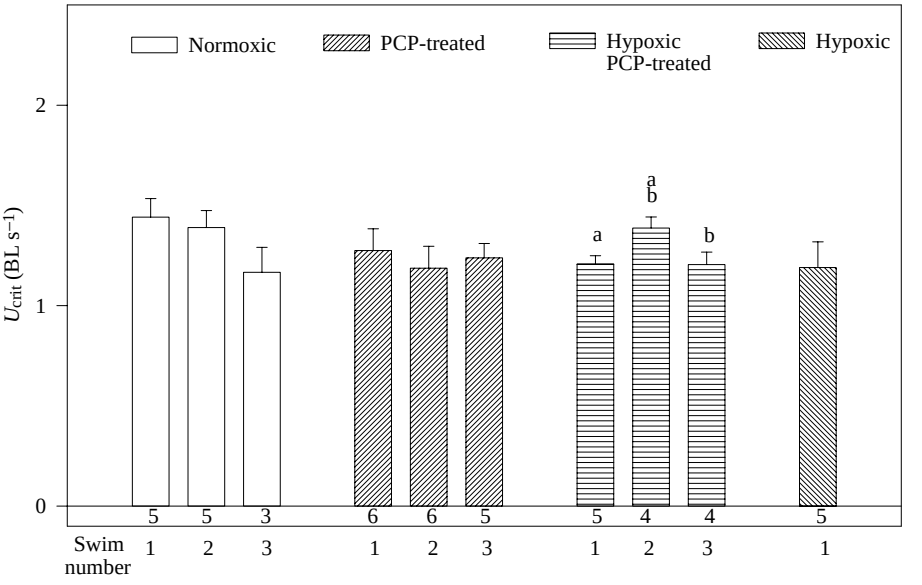

Fig. 5. The swimming performance (U_{crit}) of mature sockeye salmon (*Oncorhynchus nerka*) for three consecutive swimming challenges separated by 45 min recovery periods. See Fig. 1 and the text for other details. Similar letters denote a significant difference (P<0.05) within test groups. BL, fork length.

ameliorated the effect of swimming in moderate hypoxia. Four out of five PCP-treated hypoxic fish swam three times, and plasma lactate levels were no more elevated compared with the post-exercise and recovery values for the normoxic and normoxic PCP-treated fish (Fig. 3). These findings clearly contrast with those for fish exposed to hypoxia alone. However, this level of performance was not maintained for the third swim. U_{crit3} under normoxic conditions ($1.21\pm0.06\,BL\,s^{-1}$) was significantly lower than U_{crit2} ($1.35\pm0.06\,BL\,s^{-1}$) and was no better than U_{crit1} under hypoxic conditions ($1.21\pm0.04\,BL\,s^{-1}$).

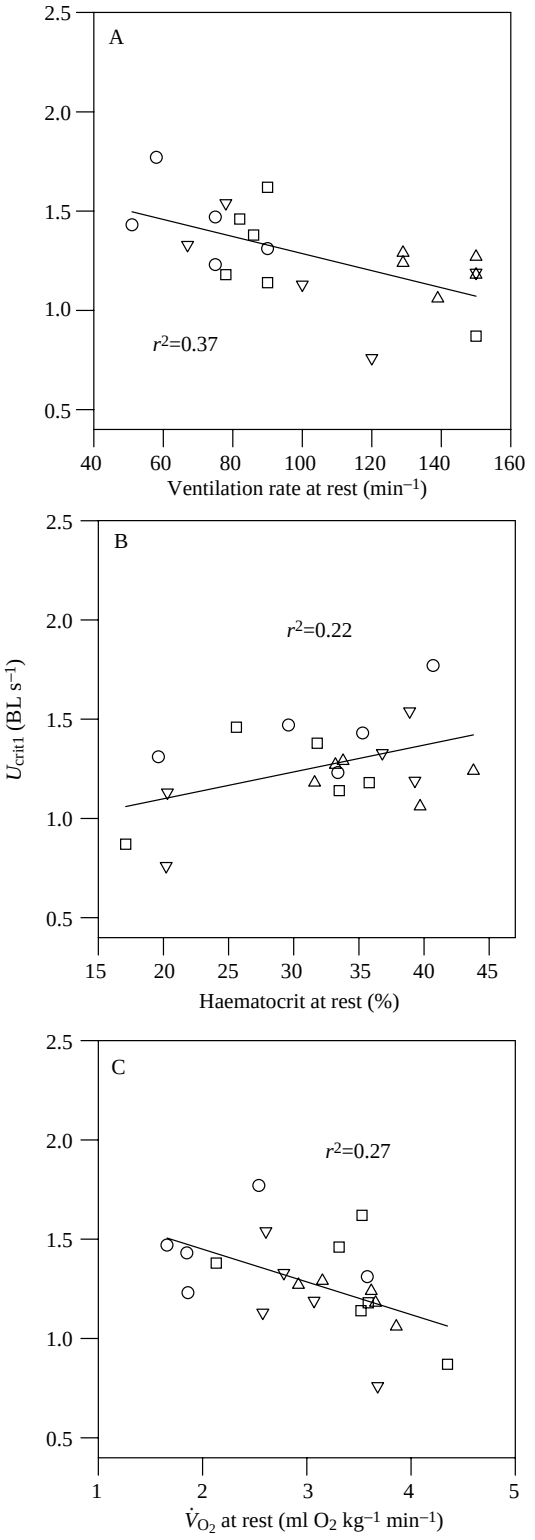

Fig. 6. Regression analyses for some variables that were found to be significantly correlated with U_{crit1} in mature sockeye salmon (*Oncorhynchus nerka*). Data from all four test groups were combined for these analyses. ○, normoxic; □, PCP-treated; △, hypoxic PCP-treated; ▽, hypoxic; PCP, pentachlorophenol.

Regression analyses

The limited supply of wild, mature sockeye salmon may have diminished the power of certain statistical comparisons among treatment groups. Therefore, data were pooled for regression analysis, the results of which are summarised in Table 2. The significant correlations between control lactate level and $\dot{V}_{O_2}$ and between ventilation rate and $\dot{V}_{O_2}$ were perhaps not surprising for some of the test groups. However, the finding that U_{crit1} was correlated both with plasma lactate level (for all hypoxic fish) and with haematocrit (for normoxic, hypoxic and PCP-treated fish) was important, as was the unexpectedly strong correlation coefficient for the regression between ventilation rate and $\dot{V}_{O_2}$ for all but the hypoxic grouping (Table 2). A number of significant regressions were found by pooling all treatment groups. As might be expected, ventilation rate was

Table 2. *Summary of the regression analyses for control variables measured in mature sockeye salmon* Oncorhynchus nerka

Variables	Group	*P* value	r^2 value
Lactate level *versus* U_{crit1}	All normoxic	NS	–
	All hypoxic	0.043	0.21
	All PCP-treated	NS	–
	All non-PCP-treated	NS	–
Lactate level *versus* $\dot{V}_{O_2}$	All normoxic	0.0001	0.40
	All hypoxix	NS	–
	All PCP-treated	0.006	0.26
	All non-PCP-treated	0.007	0.31
Lactate level *versus* ventilation rate	All normoxic	0.004	0.28
	All hypoxic	NS	–
	All PCP-treated	NS	–
	All non-PCP-treated	0.007	0.31
Lactate level *versus* Pa_{O_2}	All normoxic	NS	–
	All hypoxic	0.0004	0.44
	All PCP-treated	NS	–
	All non-PCP-treated	0.038	0.19
Haematocrit *versus* U_{crit1}	All normoxic	0.045	0.16
	All hypoxic	0.016	0.28
	All PCP-treated	0.0004	0.55
	All non-PCP-treated	NS	–
Ventilation rate *versus* $\dot{V}_{O_2}$	All normoxic	0.0001	0.67
	All hypoxic	NS	–
	All PCP-treated	0.015	0.18
	All non-PCP-treated	0.008	0.28

NS, not significant; $P>0.05$.

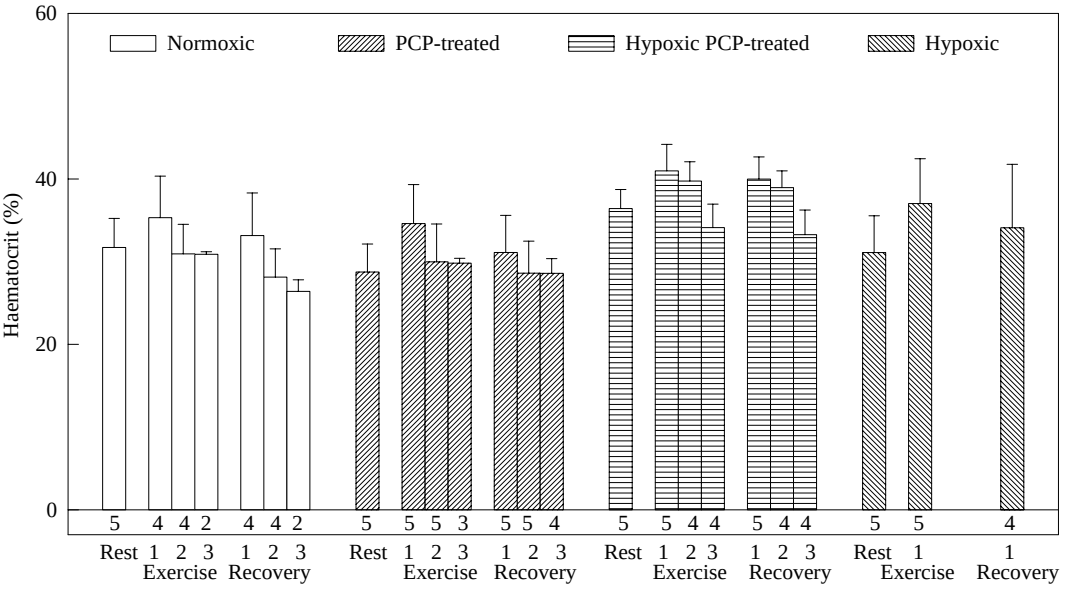

Fig. 7. The haematocrit of mature sockeye salmon (*Oncorhynchus nerka*) before and following three consecutive swimming challenges separated by 45 min recovery periods. See Fig. 1 and the text for other details.

positively correlated with $\dot{V}_{O_2}$ at rest (r^2=0.37), on first recovery (r^2=0.37) and on second recovery (r^2=0.54). Also, given the high recovery ratios for most fish, the correlations between U_{crit1} and U_{crit2} (r^2=0.25) and between U_{crit2} and U_{crit3} (r^2=0.49) were not surprising. Likewise, the negative correlation between Pa_{O_2} and ventilation rate at rest (r^2=0.50) was expected. Some predictive correlations were also revealed. For example, U_{crit1} was negatively correlated with both ventilation rate at rest and $\dot{V}_{O_2}$ at rest (Fig. 6), suggesting that the effect of metabolic loading on U_{crit} was too subtle to be revealed by comparisons of mean values. Similarly, haematocrit at rest was positively correlated with U_{crit1} (Fig. 6), even though mean haematocrit values were unchanged (Fig. 7). A positive relationship between haematocrit and U_{crit} has been demonstrated previously by some (Gallaugher *et al.* 1995) but not all researchers (Jones, 1971).

Discussion

The primary goal of this investigation was to examine the repeat swimming performance of mature, wild sockeye salmon subjected to three consecutive U_{crit} tests separated by 45 min recovery periods. The results show rather convincingly that normoxic sockeye salmon can repeat the same U_{crit} performance with only a short recovery period and that the metabolic loading associated with PCP pretreatment had no significant effect on repeat U_{crit} performance. These findings are consistent with earlier studies using either similar or longer recovery periods and with either a similar (rainbow trout and sockeye salmon, Jain *et al.* 1998) or a different (chinook salmon, Randall *et al.* 1987; coho salmon, Brauner *et al.* 1994) protocol. Collectively, these results show the remarkable ability of healthy salmonids to repeat their swimming performance. A novel finding is that 60 % of the normoxic fish and 80 % of the normoxic PCP-treated fish swam for a third time at 92–93 % of their normal U_{crit}.

New information was provided on the recovery of $\dot{V}_{O_2}$, ventilation rate and plasma lactate level following repeat swimming. Our data clearly show that complete recovery of these metabolic indicators was not necessary for repeat U_{crit} performance. Normoxic fish repeated their swimming performance with an elevated plasma lactate level, and PCP-pretreated fish repeated their swimming performance with an elevated plasma lactate level, ventilation rate and $\dot{V}_{O_2}$. Stevens and Black (1966) reported that 5 min bouts of burst swimming every hour ultimately prevented subsequent swimming in salmonids, and Scarabello *et al.* (1992) reported that as long as a full metabolic correction from a first bout of exercise had occurred, a second bout was better tolerated. Collectively, these studies point to two important concepts: (a) metabolic disturbances associated with swimming to fatigue must reach a threshold level before having a negative impact on repeat U_{crit} swimming; and (b) repeat swimming can lead to training effects. These two concepts are now discussed within the context of the present study.

Swimming to U_{crit} in salmonids involves aerobic metabolism at lower speed and increasingly more anaerobic metabolism as the fish nears U_{crit} (Jones, 1981; Day and Butler, 1996). Plasma lactate level is an indicator of the anaerobic swimming effort (Black, 1955) and, as the following discussion shows, could be useful to indicate a level of fatigue that inhibits repeat swimming. Although swimming to U_{crit1} elevated plasma lactate level, the second and third swims to at least 90 % of U_{crit1} produced no further accumulation of plasma lactate in three of the groups tested. A similar finding has been reported for a second swim in sockeye salmon (Jain *et al.* 1998). These data suggest that the anaerobic contribution to repeat U_{crit} swimming did not increase, otherwise plasma lactate level would have been higher for subsequent swims. The present data cannot establish why lactate did not accumulate in the plasma, but possibilities include a greater reliance on aerobic swimming and using lactate as a fuel during

the lower speeds of repeat swimming. Instead, an important observation is that repeat swimmers generally maintained plasma lactate level at less than 5 mmol l^{-1}, whereas plasma lactate levels were twice as high when sockeye salmon swam in moderately hypoxic water. This greater anaerobic swimming effort for U_{crit1} under hypoxic conditions resulted in an extremely poor repeat swimming ability. Since most of this group refused to swim again even at low swimming speeds, it is likely that aerobic muscle activity was impaired. The refusal of sockeye salmon to swim was characterised by plasma lactate levels above 10 mmol l^{-1} and, similarly, rainbow trout refused further repetitive burst swimming when plasma lactate level had reached 13 mmol l^{-1} (muscle lactate level was 52 mmol l^{-1}) (Stevens and Black, 1966) Thus, given that these two studies used different types of swimming tests, it appears that a plasma lactate level above 10 mmol l^{-1} may be indicative of a level of fatigue that prohibits repeat swimming within an hour.

The proximate cause of fatigue in fish awaits further study. Indeed, the degree to which metabolic and psychogenic factors lead to fatigue still needs some resolution. However, because our fish swam after a 45 min recovery, and because muscle glycogen level, pH and lactate level recover at a slower rate following exhaustive burst exercise (Milligan and Wood, 1986; Scarabello *et al.* 1992), these metabolic parameters are poor candidates. In contrast, muscle creatine phosphate and ATP levels may be more important because they can recover from a 70 % reduction within 1 h post-fatigue. Elevated ammonia levels are also associated with reduced U_{crit} performance in brown trout (*Salmo trutta*) (Beaumont *et al.* 1995; Day and Butler, 1996), but we know little of the recovery rate for ammonia other than that the post-exercise increase in ammonia excretion lasts for 2–4 h (Scarabello *et al.* 1992).

Interestingly, our fish either refused to swim for a third time or swam approximately 10–20 % slower for U_{crit3}. Thus, there may be two metabolic thresholds for repeat swimming rather than one. A higher threshold, indicated by plasma lactate levels greater than 10 mmol l^{-1}, could signal the fatigue that prevents repeat swimming. A lower metabolic threshold might inhibit anaerobic swimming, explaining why U_{crit} decreased by approximately 10–20 % and why plasma lactate did not accumulate beyond 3–4 mmol l^{-1} with repeat U_{crit} swimming. Inhibition of white muscle recruitment during swimming is not a novel suggestion since it has been shown that acidic water reduces the U_{crit} of brown trout in this manner (Day and Butler, 1996).

Training effects are reported for both short-term (Farlinger and Beamish, 1977) and long-term (e.g. Pearson *et al.* 1990; Farrell *et al.* 1990; Thorarensen *et al.* 1993) studies and even include, in the long term, an improved ability to defend against the osmotic imbalance (Gallaugher, 1994) associated with swimming (Wood, 1991). In the short term, Scarabello *et al.* (1992) suggested that the faster rates of recovery of lactate, creatine phosphate and respiratory gases for a second exhaustive burst exercise bout, and possibly the fact that the fish were less psychologically disturbed through learning, were training effects. Other short-term training effects are clearly possible. One possibility is that metabolic recovery and repayment of the O_2 debt could occur at low swimming speeds during subsequent exercise bouts. In this case, $\dot{V}_{O_2}$ for a given swimming speed would be higher for a repeat swim compared with that for an initial swim, a possibility that could be tested experimentally. A second possibility is that the locomotory muscles could operate more efficiently for repeat swimming (i.e. a reduction in the skeletal muscle O_2 requirement for the same work output) and thus compensate for a metabolic load accumulated with U_{crit1}. Unfortunately, this possibility will be difficult to distinguish experimentally from a third possibility, namely that the metabolic requirements of non-locomotory tissues can be either reduced or temporarily suspended during swimming and recovery (‘multitasking’) so as to divert blood flow and O_2 to locomotory muscles. This possibility has good experimental support. Chinook salmon decrease their blood flow to the gut as swimming speed and $\dot{V}_{O_2}$ increase, and trained fish are better able to maintain gut blood flow during swimming than untrained fish (Thorarensen *et al.* 1993). Clearly, salmonids must, and do, make decisions about how to multitask their O_2 requirements with exercise. How they do so, what the limits are and how training influences these demands will require much more research.

Implicit in the above discussion is the idea that maximum $\dot{V}_{O_2}$ is attained at U_{crit}. If this were not the case, then a higher $\dot{V}_{O_2}$ at U_{crit2} compared with U_{crit1} might preclude the need for full metabolic recovery. Many studies have suggested that active metabolic rate in salmonids provides a reliable measure of maximum $\dot{V}_{O_2}$ (e.g. Brett, 1971; Beamish, 1978; Thorarensen *et al.* 1993; Gallaugher *et al.* 1995), but perhaps the best experimental evidence for this comes from the finding that post-prandial swimming in salmonids lowered U_{crit} without affecting $\dot{V}_{O_2}$ (Thorarensen, 1994; Alsop and Wood, 1997). Conversely, therefore, post-prandial metabolism can compromise the ability of locomotory muscle to ‘steal’ O_2 from other tissues but, because $\dot{V}_{O_2}$ does not increase beyond the level normally encountered at U_{crit}, swimming can also compromise the efficiency and speed of digestion (see Jobling, 1981). $\dot{V}_{O_2}$ was not measured at U_{crit} in the present study, but had $\dot{V}_{O_2}$ for U_{crit2} been elevated, it is likely that the second post-exercise $\dot{V}_{O_2}$ would also have been elevated; this was not the case. Furthermore, Jain *et al.* (1998) observed a similar $\dot{V}_{O_2}$ at U_{crit2} compared with that at U_{crit1} in an earlier study with mature sockeye salmon. However, in non-salmonid species such as the Atlantic cod (*Gadus morhua*) and large-mouth bass (*Micropterus salmoides*), post-prandial $\dot{V}_{O_2}$ and post-exercise $\dot{V}_{O_2}$ may be better indicators of their metabolic scope (Beamish, 1974; Jobling, 1981; Soofiani and Priede, 1985; Reidy *et al.* 1995). There is also evidence that, under certain circumstances, $\dot{V}_{O_2}$ and U_{crit} may not remain tightly coupled in salmonids (i.e. that $\dot{V}_{O_2}$ at U_{crit} is not equal to $\dot{V}_{O_2max}$). Although parallel effects of haematocrit on maximum $\dot{V}_{O_2}$ and U_{crit} are observed in anaemic and normocythaemic rainbow trout, this was not the case when the fish were made polycythaemic (Gallaugher *et al.* 1995): U_{crit} increased slightly with haematocrit, whereas maximum $\dot{V}_{O_2}$ reached a plateau.

PCP, a mitochondrial uncoupling agent (NRCC, 1982), was expected to create a metabolic load and limit U_{crit}. Previously, Beamish (1978) has discussed how environmental challenges can load and limit metabolism. In fact, elevated temperature, hypoxia, disease and toxicant exposure have all been shown to decrease U_{crit} (Brett and Glass, 1973; Waiwood and Beamish, 1978; Thomas and Rice, 1987; Butler and Day, 1993; Nikl and Farrell, 1993; Beaumont *et al.* 1995; for a review, see Fry, 1971). Therefore, we have no explanation for why although control $\dot{V}_{O_2}$ increased by 50 % during PCP pretreatment (a) U_{crit1} was unchanged, (b) a higher proportion of normoxic PCP-treated fish performed repeat swimming compared with normoxic fish, and (c) the negative impact of an initial hypoxic swim was ameliorated. Although PCP can be removed from fish placed in clean water, the half-time for clearing PCP from the plasma of rainbow trout suggests that PCP effects would be present for at least the first two swim challenges. Previously, Holmberg and Saunders (1979) found that a 4 day treatment with 0.1 mg l^{-1} PCP elevated $\dot{V}_{O_2}$ by approximately twofold in American eels (*Anguilla rostrata*) and elevated the O_2 cost of slow-speed (35 cm s^{-1}) swimming (U_{crit} was not tested). In juvenile sockeye salmon, sublethal PCP exposure did not reduce U_{crit}, but reduced growth rate and food conversion efficiency (Webb and Brett, 1973), presumably as a result of the elevated resting metabolic rate.

As with previous studies, the moderate hypoxia experiments illustrated that repeat swimming performance is a valuable and sensitive measure of physiological impairment. Diseased mature sockeye salmon had a recovery ratio of 0.6 instead of 1.0, and pretreatment with a resin acid significantly reduced the recovery ratio to 0.92 and elevated control $\dot{V}_{O_2}$ (Jain *et al.* 1998). Also, we have estimated recovery ratios from the data of Brauner *et al.* (1994) for juvenile coho salmon following acute transfer to sea water. The recovery ratios were 0.96 and 0.94, respectively, for both wild and hatchery-reared fish tested in fresh water and were reduced to 0.86 and 0.82, respectively, following acute transfer to sea water. Thus, it appears that the metabolic loading associated with transfer to salt water was sufficiently large to impair recovery performance.

In conclusion, sockeye salmon performed three consecutive U_{crit} tests separated by short recovery intervals. A full metabolic recovery was not necessary for repeat U_{crit} swimming performance. Although plasma lactate levels appeared to be a useful index of repeat swimming capabilities, more studies are need to explain more fully the remarkable repeat swimming capability of sockeye salmon.

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