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Author(s)	Miyoshi, Koji; Hayashida, Kazufumi; Sakashita, Taku; Fujii, Makoto; Nii, Hisaya; Nakao, Katsuya; Ueda, Hiroshi
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**Comparison of the swimming ability and upstream-migration
behavior between chum salmon and masu salmon**

**Koji Miyoshi, Kazufumi Hayashida, Taku Sakashita, Makoto Fujii, Hisaya Nii,
Katsuya Nakao, and Hiroshi Ueda**

Koji Miyoshi, Kazufumi Hayashida, and Hiroshi Ueda. Division of Biosphere Science,
Graduate School of Environmental Science, Hokkaido University, North 9 West 9, Kita-ku,
Sapporo, Hokkaido 060-0809, Japan.

Kazufumi Hayashida. Watershed Environmental Engineering Research Team, Civil
Engineering Research Institute for Cold Region, Hiragishi 1-3, Toyohira-ku, Sapporo,
Hokkaido 062-8602, Japan.

Taku Sakashita, Makoto Fujii, Hisaya Nii, and Katsuya Nakao. Hokkaido Aquaculture
Promotion Corporation, Yonesato 4-1-3-3, Shiroishi-ku, Sapporo, Hokkaido 003-0874, Japan.

Hiroshi Ueda. Field Science Center for Northern Biosphere, Hokkaido University, North 9
West 9, Kita-ku, Sapporo, Hokkaido 060-0809, Japan.

E-mail address of each author: K. Miyoshi: miyoshi0503@gmail.com, K. Hayashida:
hayashida0109@gmail.com, T. Sakashita: sakashita@saibai.or.jp, M. Fujii: fujii@saibai.or.jp,

20 H. Nii: nii-h3181@saibai.or.jp, K. Nakao: nakao@saibai.or.jp, and H. Ueda:

21 hueda@fsc.hokudai.ac.jp

22

23 **Koji Miyoshi's present address.** Abashiri Fisheries Research Institute, Fisheries Research

24 Department, Hokkaido Research Organization, Masu Ura 1-1, Abashiri, Hokkaido 099-3119,

25 Japan.

26

27 **Corresponding author:** Dr. Hiroshi Ueda, Field Science Center for Northern Biosphere,

28 Hokkaido University, North 9 West 9, Kita-ku, Sapporo, Hokkaido 060-0809, Japan.

29 Tel and Fax number: +81-11-706-2598, e-mail address: hueda@fsc.hokudai.ac.jp

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Abstract: The spawning ground of chum salmon (*Oncorhynchus keta*) is usually located farther downriver than that of masu salmon (*O. masou*) in Hokkaido, Japan. To compare the swimming abilities of these two species, the relationship between swimming speed and oxygen consumption was compared using a swim tunnel in the laboratory. Then, the upstream-migration behaviors of chum and masu salmon were compared using electromyogram (EMG) telemetry at fish passages in the Toyohira River, Hokkaido. In the laboratory study, the standard metabolic rate of masu salmon was lower and the critical swimming speed (U_{crit}) was faster than those of chum salmon. In the field study, the holding time needed to recover the swimming performance exceeding U_{crit} at the fish passages, and the trial number needed to pass the fish passages were significantly lower for masu salmon than chum salmon. These results revealed that masu salmon are more adaptable to extended swimming in high-water-velocity conditions than chum salmon and that masu salmon are better equipped for a long distance upstream to their spawning ground than chum salmon.

Keywords: swimming ability; upstream-migration behavior; standard metabolic rate; critical swimming speed; fish passage; chum salmon; masu salmon.

Introduction

Semelparous Pacific salmon must migrate upstream with limited energy stores to reach their spawning ground, and the metabolic energy use involved in swimming ability is important for their upstream migration. The relationships between metabolic traits and swimming performance in salmon have been investigated previously. Gamperl et al. (2002) showed that wild rainbow trout (*Oncorhynchus mykiss*) exhibit aerobic fitness of at least one-third greater than that of hatchery-raised individuals. Lee et al. (2003b) suggested that the heightened aerobic fitness might be related to natural experiences during exercise. Wagner et al. (2006) investigated the differences in routine and active metabolic rates for sexually maturing migratory adult sockeye salmon (*O. nerka*) that were intercepted in the ocean and then held in either seawater or freshwater, suggesting that some of the metabolic costs might change during the migration from seawater to freshwater.

The upstream migration of salmon is energetically demanding because the fish must pass a variety of natural and anthropogenic barriers, such as waterfalls, rapidly flowing water, weirs, hydroelectric facilities, and ground sills in their natal rivers (Pon et al. 2006; 2009a, b). Specifically anthropogenic barriers have brought serious problems during the upstream migration of anadromous salmon. The upstream

66 spawning migration of sockeye salmon in the Fraser River, British Columbia, Canada,
67 has been extensively examined (Hinch et al. 1996; Hinch and Rand 1998; Hinch and
68 Bratty 2000), and many physiological data have been reported: the average swimming
69 activity of sockeye salmon passing through Hell's Gate were higher than at any other
70 location in the Fraser Canyon and non-aerobic swimming performance exceeding
71 critical swimming speed (U_{crit}) had to be sustained over a long period of time. Roscoe
72 and Hinch (2010) reported that these barriers impede or hinder the spawning-migration
73 routes of salmon and result in reduced salmon populations. In response, a large number
74 of fish passages have been installed all over the world.

75 The recent rapid development of radio telemetry technology and methods make it
76 possible to observe the upstream-migration behavior of salmon (Cooke et al. 2004).
77 Fish telemetry with integrated muscle electromyogram (EMG) has been used to
78 examine the upstream migration of sockeye salmon (Hinch et al. 1996; Hinch & Bratty,
79 2000; Pon et al. 2009a, b). EMG can be used directly as an indicator of the intensity of
80 fish muscle activity and to evaluate the efficiency of fish passage in relation to the
81 swimming performance of Pacific salmon (Roscoe and Hinch 2010; Makiguchi et al.
82 2011).

83 In Hokkaido, Japan, the spawning seasons of chum salmon (*O. keta*) and masu

salmon (*O. masou*) are nearly identical, but there are many differences in their upstream spawning migrations. Masu salmon enter their natal river between April and June and then migrate upstream to the spawning ground near the riverhead in September, possibly triggered by the swollen autumnal river (Kato 1991; Mayama 1992). In contrast, chum salmon begin upstream migration between September and October and quickly reach the spawning ground in the middle reaches of the river (Salo 1991). We hypothesized that differences in swimming ability might shape the different upstream-migration behaviors of chum and masu salmon; however, to date, no study has examined the relationship between swimming ability in the laboratory and upstream-migration behavior in the field using chum and masu salmon.

The purposes of this study were (1) to examine the relationship between swimming speed and oxygen consumption, and compare swimming abilities of chum and masu salmon in a swim tunnel in the laboratory using U_{crit} , standard metabolic rate (SMR), active metabolic rate (AMR), and aerobic metabolic scope (AMS); (2) to investigate the upstream-migration behaviors of chum and masu salmon in the field using EMG telemetry to compare their behaviors at the fish passages in protection beds and groundsills in the Toyohira River; and (3) to discuss their upstream spawning-migration behavior in terms of metabolic energy use and swimming ability.

Materials and Methods

1) Swimming abilities in the laboratory

Experimental animals

Adult chum salmon (3 males and 2 females; mean $\pm$ SE: fork length: 62.1 ± 4.9 cm; body weight: 1.9 ± 0.7 kg) and adult masu salmon (6 males and 5 females; fork length: 53.3 ± 13.4 cm; body weight: 1.7 ± 1.4 kg) were captured using a waterwheel located approximately 70 km from the Chitose River mouth in the upper reaches of the Ishikari River in western Hokkaido, Japan, during their upstream spawning migration (Fig 1A). Experiments were conducted at the Chitose Salmon Aquarium in September and December of 2010 and 2011. Fish were individually transferred to a compact water tank ($L \times W \times H = 1.8 \times 0.9 \times 0.6$ m) in an artificially flowing stream using water from the Chitose River.

Swim tunnel and swimming test protocol

A swim tunnel (West Japan Fluid Engineering Laboratory Co. Ltd, Nagasaki, Japan) was used to measure critical swimming speed (U_{crit}) and dissolved oxygen consumption. Water flow was generated using a voltage-controlled motor and propeller, with the voltage calibrated against the flow velocity. The swim tunnel was sealed with

an acrylic board so that no gas exchange occurred, and water from the Chitose River was pumped into it before the U_{crit} trial. The water temperature ranged from 12.0 to 13.5°C and did not vary more than 1°C during any experiments.

Fish were individually placed into the swimming chamber of the swim tunnel. In all cases, fish were acclimated for 1 hour at 0.25 body lengths (BL) s^{-1} to minimize handling effects before all U_{crit} trials. After each 15-min period, the water velocity was increased by an additional 0.25 BL s^{-1} and was maintained at the new speed for 15 min until the fish became fatigued or was unable to swim against the current. After the U_{crit} trial was completed, body mass and fork length were measured. Flow-velocity values were corrected to account for solid blocking effects (Gehrke et al. 1990), as described by Bell and Terhune (1970).

The U_{crit} (BL s^{-1}) was calculated with Brett's formula (1964):

$$U_{crit} = U_i + (t \cdot t_i^{-1}) \cdot U \quad (1)$$

where U_i (BL s^{-1}) is the highest velocity maintained for the entire swimming period; t_i is the prescribed swimming period (i.e., 15 min); t (min) is the amount of time spent at the fatigue velocity; and U is the velocity increment (i.e., 0.25 BL s^{-1}).

To measure oxygen consumption (MO_2) during the acclimatization period and during the U_{crit} trials, the oxygen concentration in the swim tunnel was measured

throughout the trial at 1-min intervals using a multi-water-quality sensor probe (U-50; Horiba Ltd., Kyoto, Japan), which was housed inside the swim tunnel. Before the fish were introduced, air bubbles were removed from the swim tunnel. Prior to each U_{crit} trial, oxygen levels in the tunnel were reset by pumping fresh river water into the tunnel. The MO_2 per 15-min period for each fish was calculated by subtracting the quantity of oxygen at the end from the starting value. The MO_2 rate ($mg\ O_2\ kg^{-1}\ h^{-1}$) for individual fish during a velocity increment was calculated as:

$$MO_2 = [O_2] \cdot v / m \quad (2)$$

where the rate of oxygen concentration $[O_2]$ is measured in $mg\ O_2\ per\ l^{-1}\ h^{-1}$; v is the water volume of the swim chamber (l); and m is the body mass of the fish (kg). MO_2 was then standardized to a value corrected for a fish of 1 kg (MO_{2cor}), using the following allometric relationship derived by Schurmann and Steffensen (1997):

$$MO_{2cor} = MO_{2meas} \cdot (w \cdot w_{cor}^{-1})^{(1-A)} \quad (3)$$

where MO_{2meas} is the MO_2 measured during the trial; w is the mass of the fish; and w_{cor} is the standard body mass of 1 kg. A is the condition factor [weight (g) / length³(cm)•100], calculated using the mean fork length and bodyweight for each species.

Standard metabolic rate (SMR) is defined as the MO_2 at zero activity (Fry 1971;

Claireaux et al. 2006; Luna-Acosta et al. 2011). Because MO_2 was expected to increase exponentially with swimming speed (Brett 1964), a curve was fitted for each individual, according to the following equation:

$$MO_{2cor} = SMR \cdot \exp^{bU} \quad (4)$$

where MO_{2cor} is the oxygen consumption obtained from (3); SMR is the standard metabolic rate that corresponds to the intercept (i.e., the MO_2 when $U = 0 \text{ BL s}^{-1}$); U is the swimming speed (in BL s^{-1}); and b is a constant.

The active metabolic rate (AMR) of the fish was estimated as the maximum MO_2 recorded during the swimming test (i.e., when swimming speed was close to U_{crit}), and AMS (aerobic metabolic scope) was calculated as follows (Fry 1971):

$$AMS = AMR - SMR \quad (5)$$

The net cost of transport (COT_{net} ; $\text{mgO}_2 \text{ kg}^{-1} \text{ m}^{-1}$) was calculated as follows for each swimming speed:

$$COT_{net} = MO_{2net} / U \quad (6)$$

where U is the swimming speed (m h^{-1}) and MO_{2-net} is equal to the MO_2 at the considered swimming speed minus SMR. AMR, SMR, AMS and COT_{net} values were determined for each fish.

2) Upstream migration behavior in the field

Study area

The Toyohira River in Hokkaido, Japan, which flows through the city of Sapporo, is a rare rapid-flow urban river and an important spawning ground for chum and masu salmon. Six cross-river constructions (#1, #3-8), called groundsills, were constructed to prevent the lowering of the riverbed, and fish passages were built at each groundsill (Fig. 1B). A protection bed was also constructed at the groundsill #5, and a fish passage was installed in March 2010 (Fig. 2). Makiguchi et al. (2011) reported the effects of groundsills on the upstream migration of chum salmon in 2008 and 2009 using EMG telemetry; however, the Toyohira River environment changed significantly after their investigation; the fish passage was established in protection bed #5 in 2010, and a severe typhoon hit near Sapporo City in September 2011. In the protection bed #5, a large part of the passage was filled by a sandbar because of the change in water flow and current caused by the typhoon.

Experimental animals and transmitter attachment procedures

In the Toyohira River, adult chum and masu salmon migrate upstream from September to October. All chum and masu salmon used in this study were captured using a net 11.5-16.5 km from the mouth of the Toyohira River and transferred to

192 outdoor tanks at the Chitose Salmon Aquarium for calibration of the EMG signals to
193 swimming speeds. In 2010, 3 males (fork length, 56.4–74.1 cm; body weight 1.8–4.2
194 kg) and 3 females (fork length, 62.6–73.5 cm; body weight 2.7–3.9 kg) chum salmon,
195 and 3 males (fork length, 44.3–55.1 cm; body weight 0.9–1.8 kg) and 3 females (fork
196 length, 51.0–58.6 cm; body weight 1.5–2.5 kg) masu salmon were studied. In 2011, 1
197 male (fork length, 68.7 cm; body weight 3.3 kg) and 5 females (fork length, 54.6–69.5
198 cm; body weight 1.4–3.4 kg) chum salmon, and 2 males (fork length, 53.2–56.5 cm;
199 body weight 1.6–1.7 kg) and 1 female (fork length, 58.7 cm; body weight 2.6 kg) masu
200 salmon were studied.

201 Each fish was equipped with a cylindrical, epoxy-encased EMG transmitter
202 (CEMG-R11-35, Lotek Engineering Inc., Newmarket, Ontario, Canada; 18.3 g in air,
203 16.2 mm in diameter, and 53.0 mm in length) attached externally to the body surface
204 and positioned in front of the dorsal fin using the procedure developed by Makiguchi et
205 al. (2011). External attachment is suitable for short-term telemetry research, which
206 reduces handling stress for the fish (Bridger and Booth 2003; Makiguchi et al. 2007). To
207 attach the tag, experimental fish were anaesthetized using FA100 (eugenol; Tanabe
208 Seiyaku Co. Ltd, Osaka, Japan) at a concentration of 0.5 ml l⁻¹ in water from the
209 Chitose River and placed upright on a surgical table. Their gills were irrigated with

210 water containing diluted FA100 to maintain sedation during the attachment procedure.

211 Two stainless-steel needles large enough hold the restraining nylon ties were pushed

212 through the dorsal muscle to secure the EMG transmitter and sutured into place using

213 nylon ties and epoxy resin. Silicon pads were attached to minimize abrasion. The nylon

214 ties were passed through the needles and tied on the opposite side (Bridger and Booth

215 2003). The EMG transmitters consisted of an epoxy-coated transmitter package with a

216 pair of Teflon-coated electrodes with brass muscle-anchoring tips (dimension 5×1

217 mm). The EMG electrodes were inserted subcutaneously using a hypodermic needle at a

218 ratio to the body length of approximately 0.7 on the left side of the fish. The electrodes

219 detected electrical potentials within the axial dark muscle, which contained red muscle

220 tissue. The amplitude and frequency of these pulses was directly correlated to the level

221 of muscle activity. Paired electrode tips were positioned approximately 1 cm apart and

222 secured in the lateral red muscle toward the rear of the fish. These muscles are primarily

223 used in steady, non-bursting, aerobic swimming activity (Beddow and McKinley 1999).

224 EMG signals are generally related to swimming speed (Økland et al. 1997). The

225 electrodes were sutured to avoid entangling vegetation or other environmental structures.

226 The CEMG model was equipped with a differential muscle probe, a signal conditioning

227 circuit, a digitizer, a microcontroller, and a radio transmitter. The voltage corresponding

to muscle activity was sampled at 2-s intervals. Individual samples were summed and stored temporarily. At the end of the interval, the mean value was calculated, and an activity level (EMG signal) ranging from 0 to 50 (no units) was assigned, and transmitted to a radio receiver (model SRX_600; Lotek Engineering Inc.). The attachment procedures usually required approximately 5 min to complete.

Calibration of EMG signals to swimming speeds

EMG signals were calibrated to swimming speed in the swim tunnel using procedures developed by Makiguchi et al. (2011). The Chitose River water was poured into the swim tunnel before each trial. The water temperature was set between 11.5 and 14.5°C, which was similar to the water temperature at the capture point in the Toyohira River. Experimental fish were individually placed into the swimming section of the swim tunnel, and 10 EMG signals at 0 m s⁻¹ were measured using the EMG radio receiver when the fish maintained a holding position. Next, the trial was started at 0.3 m s⁻¹, and the water velocity was increased incrementally by 0.3 m s⁻¹, with 10 EMG signals measured at each increment. Fish readily swam against the current and rarely came in contact with the grid at the back of the swim chamber. The trial ended at 1.2 m s⁻¹. We used the average EMG output at each velocity for each fish to calibrate transmitter output to swimming speed. We evaluated the relationship between the mean

EMG output and water velocity using a linear regression analysis.

Field Study

Few studies report that fish with EMG transmitters were calibrated in respirometers prior to their release. In September and October of 2010 and 2011, chum and masu salmon used in the EMG-calibration experiment were individually released in the study area and tracked on foot during upstream migration using a hand-held directional Yagi antenna (Table 1). The position of each individual was determined using EMG-transmitter signals received by three SRX_600 radio receivers. The radio receivers recorded EMG signals at 2-s intervals. Migration time in each segment was measured for each fish by subtracting the time on reaching the exit from the time at entry. The chum and masu salmon were often observed to have stopped swimming during their upstream migration, and more than 3 min of swimming cessation during upstream migration was defined as holding behavior. The calculation of swimming time did not include holding time. EMG signals were converted to swimming speed using the equations established from the EMG calibration for each fish. After tracking, water depth was measured at 0.2-m intervals in the water column, and water velocity was measured at 2–5-m intervals along the tracking area. All tagged fish were released below protection bed #5, and the tagged fish were tracked intermittently for

approximately 2–5 days. The number of times the fish exceeded U_{crit} and the time spent swimming over U_{crit} were calculated from the time-course trajectory of swim speed, using the U_{crit} calculated in the laboratory for both species.

Statistical analyses

An analysis of covariance (ANCOVA) was used to compare the swimming abilities of chum and masu salmon in the laboratory, and separate predictive relationships were required for body length and body mass. ANCOVA was also used to compare the swimming abilities of male and female in masu salmon in the laboratory. If the ANCOVA was not be applicable in the statistical analysis, Student's t-test was used.

Statistical comparisons of the swimming performance of chum and masu salmon in the field were analyzed using Student's t-test. The results were expressed as the mean $\pm$ SE, and data were considered significant when $P < 0.05$.

For the relationship between swimming speed and MO_{2cor} , regression analysis of exponential equations using a parametric analysis of variance (ANOVA) were used. A value of $P < 0.05$ was considered statistically significant.

Results

The statistical analysis by ANCOVA showed no significant parallelism of the

regression line for body length ($P = 0.217 > 0.05$) or body mass ($P = 0.234 > 0.05$) to compare the swimming abilities of chum and masu salmon in the laboratory. In the test of parallelism on regression line in the swimming performance (e.g. U_{crit}) in the laboratory study, U_{crit} of male and female masu salmon was not significant ($P = 0.895 > 0.05$). Student's t-test also revealed no significant differences ($P > 0.05$) in the swimming abilities in the laboratory or upstream-migration behavior in the field between females and males of chum and masu salmon.

Swimming abilities in the laboratory

The U_{crit} values for the chum and masu salmon were 1.42 BL s^{-1} and 1.89 BL s^{-1} , respectively, and masu salmon were significantly faster than chum salmon ($P < 0.01$; Fig. 3). The chum and masu salmon SMRs were $243.6 \text{ mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$ and $112.3 \text{ mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$, respectively, and the masu salmon's SMR was significantly lower than that of chum salmon ($P < 0.01$; Fig. 3). The chum and masu salmon AMRs were $369.0 \text{ mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$ and $391.9 \text{ mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$, respectively. The chum and masu salmon AMSs were $174.1 \text{ mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$ and $272.8 \text{ mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$, respectively. The differences in AMR and AMS values between the 2 salmon species were not significant (Fig. 3).

During the swimming test, the rate of oxygen consumption (MO_2) tended to increase proportionally with swimming speed. No increases in MO_2 were observed at

low swimming speeds in masu salmon (Fig. 4A). Figure 4B shows the relationship between the MO_{2cor} and swimming speed on an exponentially curve. The exponential curves satisfactorily fitted the data for chum and masu salmon, and a significant difference was found between chum and masu salmon ($P < 0.05$). The COT_{net} was also influenced by swimming speed. It is worth noting that the lowest COT_{net} was found at 0.5 and 1.0 BL s^{-1} , which were considered the optimal swimming speeds in chum salmon and masu salmon, respectively (Fig. 5). Although the COT_{net} of masu salmon tended to be lower than for chum salmon, there was no significant difference between the two species ($P > 0.05$).

From the EMG data, the muscle-activity levels were significantly correlated with swimming speed according to simple regression analysis for all chum and masu salmon (average of 12 chum salmon in 2010 and 2011, $r^2=0.92$, $P<0.05$; average of 9 masu salmon in 2010 and 2011, $r^2=0.97$, $P<0.05$). A simple regression analysis was used to convert EMG signals to swimming speeds in the field study by avoiding EMG values above 40 showing no change in swimming speed. In the plots of all fishes, $U=0.043EMG-0.0138$ for the chum salmon (Fig. 6A) and $U=0.049EMG-0.0487$ for the masu salmon (Fig. 6B), where U is swimming speed (BL s^{-1}) and EMG is the EMG values.

Upstream-migration behavior in the field

In 2010 and 2011, EMG data for all chum and masu salmon were collected around the protection bed and groundsill #5. All tagged fish migrated upstream or downstream using the fish passages on the day of release, and none of the fish that entered fish passages at the protection bed and groundsill #5 made downstream migrations. Table 1 shows the percentage of passing fish. The passage rate for both chum and masu salmon at each structure dropped in 2011. At the fish passages of the ground sill #5, the passage rate for masu salmon did not change, but only one fish was observed passing successfully in 2011.

Figures 7 shows the water velocity and passage path at the fish passages of the protection bed and groundsill #5 in 2010 (A) and 2011 (B). In 2011, 60-80% of the fish passage area at the protection bed #5 was filled up by a sandbar because a severe typhoon hit Sapporo City in September 2011. As a result, the water velocity was greater in 2011 than in 2010. In 2010, all fish passed through the fish passages at the protection bed and groundsill #5, but the fish only passed along the sandbar at the protection bed #5.

Table 2 shows the swimming speed and approach time at the fish passages of the protection bed and groundsill #5 in 2010 and 2011. At the groundsill #5, chum and masu

salmon showed no difference in swimming speed and approach time in either 2010 or 2011.

Fig. 8 shows the time-course trajectory of swimming speeds of representative chum salmon (A, C) and masu salmon (B, D) through the fish passages. Non-aerobic swimming performance exceeding U_{crit} was observed in both species at each step in the fish passages. In the pools of the fish passages, each fish swam relatively slowly, approaching the optimal swimming speed calculated in the laboratory. No significant difference was found the approaching time between chum and masu salmon at each step ($P > 0.05$).

The holding time at each step through the fish passage at the protection bed and groundsill #5 was significantly shorter for masu salmon than chum salmon in 2010 ($P < 0.01$; Fig. 9A), and the search time at each step through the fish passage at the protection bed and groundsill #5 was significantly longer for masu salmon than chum salmon in 2010 ($P < 0.01$) and 2011 ($P < 0.05$; Fig. 9B). There was also a significant difference in the search time for chum salmon between 2010 and 2011 ($P < 0.01$; Fig. 9B). Fig. 9C shows the number of trial times for each step through fish passage at the protection bed and groundsill #5. The number of trial times for the protection bed and groundsill #5 in 2010 was significantly shorter for masu salmon than chum salmon ($P <$

0.01). The number of times that fish were observed swimming at speeds greater than U_{crit} at the protection bed and groundsill #5 was significantly lower for masu salmon than chum salmon in 2010 (Fig. 9D). In addition, the time over U_{crit} for the protection bed and groundsill #5 was significantly longer for masu salmon than chum salmon in both 2010 and 2011 ($P < 0.01$; Fig. 9E).

Discussion

Swimming ability, measured using a swim tunnel in the laboratory, and upstream spawning-migration behavior, measured using EMG telemetry at the fish passages in the Toyohira River, were compared between chum and masu salmon. Masu salmon are better adapted to swimming in a high-water-velocity area for an extended periods of time than chum salmon, and this adaptability could make masu salmon more successful than chum salmon in completing a migration to their spawning ground in the upper reaches of the river.

Both the mean MO_2 throughout the swimming test and the COT_{net} tended to be lower in masu salmon than chum salmon. This pattern was particularly obvious above 1 $BL\ s^{-1}$, where the COT_{net} increased with speed in a parallel in the two species until 2 $BL\ s^{-1}$ (Fig. 5). Excess post-exercise oxygen consumption (EPOC) is estimated as the time

required to return to their normal level of oxygen consumption (recovery time) and the total oxygen cost of swimming to U_{crit} (Brett 1964; Lee et al. 2003b). Lee et al. (2003b) and Farrell (2007) suggested that increase in the COT rate was estimated 20-50 % lower than it was without EPOC. Although we did not examine EPOC in this study, the COT_{net} increased in parallel with increasing the swimming speed over $1\text{ BL}^{-1}\text{ s}$ in chum and masu salmon.

Lee et al. (2003a) also showed that the MO_2 routine, with properties similar to the SMR, was no different between coho and sockeye salmon captured around the same time. Both U_{crit} and the optimal swimming speed were significantly faster for masu salmon than chum salmon, and the SMR of masu salmon was significantly lower than that of chum salmon in this study. Masu salmon spawning grounds are usually located in the upper reaches of rivers (Kato 1991). Therefore, it is likely that masu salmon must pass through more cross-river constructs with high-water-velocity than chum salmon. In high-water-flow areas, masu salmon may swim use aerobic swimming more successfully than chum salmon. As masu salmon stay in the river longer time than chum salmon, the metabolic rate of masu salmon is lower than chum salmon. Therefore, it was reasonable to assume that the basal metabolism of the masu salmon was lower than chum salmon, and there was difference in slope of MO_{2cor} between chum and masu

salmon. Lee et al. (2003a) showed that salmon, migrating for long-distance, the attained a significantly higher U_{crit} at and were more efficient swimmers at U_{crit} because of a lower MO_{2max} compared with other salmon migrating for a comparatively short distance. Masu salmon have higher U_{crit} and lower SMR than chum salmon. However, AMR was similar between chum and masu salmon in this study presumably because that small sample sizes may have prevented detecting differences.

It is well known that water temperature influences the metabolism and swimming performance of salmonids (Lee et al. 2003a; Farrell 2007). This study was carried out at the water temperature of the Chitose River in autumn. It should be mentioned that a significant change might be observed in the metabolism and swimming performance of masu salmon at a higher water temperature (as in summer, before masu salmon start their upstream migration). Lee et al. (2003a) investigated relationships between swimming performance and metabolism for sockeye salmon of different stocks in the swim test and found that fishes captured in the upper reaches of the river migrates much longer distances and negotiate more severe hydraulic challenges than the coastal fish. Compared to chum salmon, masu salmon have their spawning grounds farther upriver and remain in the river longer time; therefore, masu salmon are better adapted to swim for extended time in an area of high-water-velocity.

The MO_2 of both species tended to plateau before reaching U_{crit} (Fig.4A), and the slopes of the COT_{net} shifted between 1.75 BL s^{-1} and 2 BL s^{-1} in both species (Fig.5), the slopes of the EMG shifted between 1.5 BL s^{-1} and 2 BL s^{-1} in both species (Fig. 6). The amplitude and frequency of these pulses was directly correlated to the level of red-muscle activity (Beddow and McKinley 1999; Økland et al. 1997), indicating that the shift in muscle performance from white muscle to red muscle occurred between 1.5 BL s^{-1} and 2 BL s^{-1} ; this result suggests that supplementary swimming effort when approaching U_{crit} was sustained by anaerobic metabolism. Farrell (2007) indicated that the oxygen requirements should either plateau or decrease at nearly U_{crit} because of the activation of white muscle (based on electro-myography recordings) and glycolysis (based on glycogen depletion and lactate accumulation in white muscle); however, Farrell (2007) also explained that white-muscle contraction has a significant aerobic component that drives an increase in MO_2 near U_{crit} . White muscle has aerobic capacity and is activated at speeds below U_{crit} . Therefore, the metabolic cost of swimming increases exponentially with velocity (Fig. 4B).

In the field study, the searching time was significantly longer for masu salmon than chum salmon, and non-aerobic swimming performance exceeding U_{crit} was necessary for both two species at each step in the fish passages because they had to swim at high

flow rate (exceeding $2\text{m}^{-1}\text{ s}$). It is likely that masu salmon could search long periods of time in the each pool in order to find easy routes for the reduction of energy loss. Gowans et al. (1999) reported that migrating Atlantic salmon (*Salmo salar*) sometimes failed to locate the entrance to a pool of fish passage and appeared to be attracted by tailrace and turbine flows. Makiguchi et al. (2011) indicated that it was important for the successful migration that salmon find passage upstream rapidly and easily without wandering. Our results suggested that masu salmon search approach routes, reducing energy expended in approaching the fish passages compared to chum salmon.

Hinch and Bratty (2000) tracked adult sockeye salmon during upstream migration using EMG telemetry at Hell's Gate in the Fraser River and reported that swimming speed patterns alternated at different time scales between relatively fast and slow speeds. In our study, the search time was significantly longer for masu salmon than chum salmon, but the number of times over U_{crit} at each fish passages were significantly lower for masu salmon than chum salmon. Masu salmon could select slow and rapid swimming, depending on the river obstacle, and chum and masu salmon searched for routes of approach at speeds approximating the optimal swimming speed. Hinch and Rand (2000) reported that successful sockeye salmon swam at the Hell's Gate, and migrated according to an optimal swimming speed model range in whole of Fraser

River, which migrants minimized transport costs per unit distance. Our study also showed that successful chum and masu salmon used the energy-saving swimming at fish passages. In fact the fish passages were organized by some steps to secure recovering time in the Toyohira River, successful chum and masu salmon could almost use optimal swimming speed except over the U_{crit} swimming at each step.

Chum salmon, however, had longer holding times and shorter search times than masu salmon at each fish passage. Because the holding time occurred when each fish crossed to the next step in the fish passage, this finding indicated that the holding time occurred before or after the burst speed over U_{crit} . During upstream migration, chum salmon had a consistent holding behavior after swimming at high speed over U_{crit} , indicating that they swim at speeds greater than U_{crit} prior to holding (Makiguchi et al., 2007). Chum salmon showed the more trial than masu salmon at each fish passage, indicating that chum salmon swim over U_{crit} .

Lee et al. (2003b) reported that routine oxygen consumption was restored within approximately 1 h in adult sockeye salmon in the EPOC test. In our study, if the holding time was defined as the recovery time after excess swimming, chum salmon spent approximately 15-20 minutes in recovery at each steps. These observations may show that chum salmon suffered an energy loss at the fish passages; extended periods of

swimming at fast speeds may deplete the limited energy reserves of fish and leave little energy for additional upriver progress (Rand and Hinch 1998). It is also possible that the sustained and repeated bouts of anaerobic activity (e.g., swimming at speeds over U_{crit} for prolonged periods of time) could lead to mortality by causing lethal levels of blood lactate to accumulate, resulting in metabolic acidosis (Hinch and Bratty, 2000). It is possible that the loss of stored energy decreased the passing rate of chum salmon at the groundsill #5 (see Table 2). Moreover, swimming over U_{crit} caused the fish fatigue and stress. Webb (1971) reported that anaerobic metabolism occurred during swimming at 80% of U_{crit} . Chum salmon were considered more stressed than masu salmon because the U_{crit} of chum salmon were lower than that of masu salmon.

In contrast, masu salmon required only approximately 10 minutes of recovery time, suggesting two possibilities. First, masu salmon may be resilient to exceeding U_{crit} . Second, no more than 10 minutes was required to pass through the protection bed and groundsill #5 in the Toyohira River. For example, the total length of the fish passage of the protection bed and groundsill #5 was less than 50 m (8 pools; see Fig. 2), but the vertical-slot fishway at the Seton River and the Fraser River, British Columbia, is more than 100 m long (Hinch et al. 1996; Pon et al. 2009a). Nevertheless, more information is needed regarding energy cost and metabolism (e.g., EPOC) and spawning behavior in

480 masu and chum salmon.

481 Percentages of fish passing the fish passage of the protection bed #5 were 67 % and
482 30 % in chum and masu salmon, respectively. It was unclear if the differences between
483 successful and unsuccessful passages were due to swimming speed and approach time;
484 however, it was likely that unsuccessful individuals gave up quickly at the fish passages
485 or required a very long time to find passing routes. No significant difference was
486 observed between 2010 and 2011 in migration behavior at each fish passage, except in
487 the search time of chum salmon. The mean search time of masu salmon was lower in
488 2011 than 2010, when the routes had been filled up by a sandbar during the typhoon.

489 In conclusion, comparisons between chum salmon and masu salmon of both
490 swimming ability in the laboratory swim tunnel and upstream-migration behavior using
491 EMG telemetry at fish passages in the Toyohira River provided strong evidence that
492 masu salmon were better adapted than chum salmon to swimming for an extended
493 periods of time in areas of high-water-velocity. Masu salmon were better able to migrate
494 upstream to their spawning grounds in the upper reaches of the river than chum salmon.
495 In this study, swimming performance did not vary significantly with body length or
496 mass; however, these differences should be analyzed by further morphological and
497 genetic studies.

498

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505

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Figure Captions

Figure 1. A map of the study site showing the experimental site in the Chitose Salmon Aquarium near the Chitose River (A), the positions of the groundsills, and chum and masu salmon release point in the Toyohira River (B)

Figure 2. A diagram of the fish passage of the protection bed #5 (a) and fish passage of the groundsill #5 (b). The dotted line represents the water's edge for a typical water level in 2010. The number of circles in the fish passage is the pool number.

Figure 3. Average ($\pm$ SE) standard metabolic rate (SMR), active metabolic rate (AMR), aerobic metabolic scope (AMS), and critical swimming speed (U_{crit}) for chum salmon (white column) and masu salmon (black column). The left Y axis corresponds to oxygen consumption (MO_2 in $mgO_2\ h^{-1}\ kg^{-1}$) related to SMR, AMR, and AMS. The right Y axis corresponds to swimming speed ($BL\ s^{-1}$) for U_{crit} . The asterisk indicates significant differences by Student's t-test ($P < 0.01$).

Figure 4. (A) The relationship between the rate of oxygen consumption (MO_2) and swimming speed ($BL\ s^{-1}$) for chum salmon (solid line) and masu salmon (dotted line). (B) The relationship between the MO_{2cor} and swimming speed ($BL\ s^{-1}$) for chum salmon (solid line) and masu salmon (dotted line). The relationship is exponential; for chum salmon, $MO_{2cor} = 224.39e^{0.409U}$ ($P < 0.05$; $r^2 = 0.952$); for

masu salmon, $MO_{2cor} = 112.51 e^{0.734U}$ ($P < 0.05$; $r^2 = 0.903$).

Figure 5. The relationships between net cost of transport (COT_{net}) and swimming speed ($BL\ s^{-1}$) for chum salmon (solid line) and masu salmon (dotted line). The COT_{net} varied for chum salmon according to the polynomial equation $COT_{net} = 3.55 e^{-1} + 4.25 e^{-3} + 0.19 U^2 - U$ ($r^2=0.911$) and for masu salmon according to the polynomial equation $COT_{net} = 3.91 e^{-1} + 5.25 e^{-3} + 0.18 U^2 - U$ ($r^2=0.920$).

Figure 6. The relationships between electromyogram (EMG) signals and swimming speed ($BL\ s^{-1}$) for chum salmon (A: n=12) and masu salmon (B: n=9) in all fish in the field study in 2010 and 2011. The standard error of each EMG value is expressed.

Figure 7. The swimming path (red dotted line) of passing chum and masu salmon and water velocity at the fish passage for the protection bed and groundsill #5 in 2010 (A) and 2011(B).

Figure 8. Time-series plots of swimming speeds of representative chum salmon in 2010(A) and 2011(C), and masu salmon in 2010 (B) and 2011(D). The dotted line represents the mean critical swimming speed (U_{crit} ; $1.42\ BL^{-1}\ s$ or $1.89\ BL^{-1}\ s$) and optimal swimming speed (U_{opt} ; $0.5\ BL^{-1}\ s$ or $1.0\ BL^{-1}\ s$) in chum salmon and masu salmon, respectively. The number of circles in the fish passage is the pool number

in Fig. 2.

Figure 9. The holding time (A), searching time (B), number of trials (C), number of times over U_{crit} (D) and over U_{crit} time (E) at each fish passage step in the fish passage of the protection bed and groundsill #5 for chum salmon (white column) and masu salmon (black column) in 2010 and 2011. The asterisk indicates significant differences by Student's t-test ($P < 0.01, 0.05$). The number of fish is provided in parentheses.

Table. 1

Year	Species	Releasing point	Releasing date	Number of experimental fish (female)	Number of upstream migrating fish (female)		Number of passing fish (female)		Percentage of passing fish at the fishway	
					#5 protection bed	#5 ground sill	#5 protection bed	#5 ground sill	#5 protection bed	#5 ground sill
2010	chum	#5 protection bed	2010/10/6	6(3)	4(2)	4(2)	4(2)	2(2)	100	50
	masu		2010/9/26	6(3)	6(3)	6(3)	6(3)	6(3)	100	100
2011	chum		2011/10/4	6(5)	6(5)	4(3)	4(3)	1	67	25
	masu		2011/9/25	3(1)	3(1)	1	1	1	33	100

* Data in parentheses are the number of female fish

Table. 2

Year	Species	Swimming speed (BL/s)*		Approaching time (min)*	
		#5 Protection bed	#5 ground sill	#5 Protection bed	#5 ground sill
2010	chum	0.87 ± 7.3 (4)	1.33 (2)	38.2 ± 28.0 (4)	45.1 (2)
	masu	1.13 ± 6.6 (6)	1.28 ± 6.5 (6)	25.3 ± 19.2 (6)	58.9 ± 37.1 (6)
2011	chum	1.14 ± 2.6 (4)	1.34 (1)	25.3 ± 16.5 (4)	16.5 (1)
	masu	1.38 ± 4.3 (3)	1.24 (1)	37.2 ± 4.3 (3)	16.0 (1)

* Values represent the mean ± standard error
** Data in parentheses are the number of female

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig.7

Fig.8

Fig.9