Sex differences in metabolic rate and swimming performance in pink salmon (*Oncorhynchus gorbuscha*): the effect of male secondary sexual traits

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Abstract – Do secondary sexual traits, such as large dorsal hump and hooked snout, decrease the swimming efficiency of male pink salmon during freshwater migration? This is the first study to address the effects of secondary sexual traits in pink salmon on oxygen uptake and swimming capacity. We conducted a laboratory experiment using a swimming respirometer and a field study using electromyogram (EMG) telemetry in the Shibetsu River, Hokkaido, Japan. We compared the relationship between MO_2 (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and swimming velocity U (m·s^{-1}) in male and female fish, and also investigated the effects of morphological traits (secondary sexual characters) on the relationship between MO_2 (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and swimming velocity U (m·s^{-1}). Additionally, we compared energy costs and swimming behaviour during upstream migration between male and female pink salmon. The laboratory experiment revealed that MO_2 exponentially increased with increasing U; this increase was described by $MO_2 = 167.9e^{1.23U}$ for males and $144.9e^{1.14U}$ for females. Linear mixed models found that hump height and the upper jaw length in males significantly and positively affected the relationship between MO_2 and U; no effect was found in females. The field study found that swimming velocity for both sexes estimated from EMG calibration was lower than optimal swimming velocity (U_{opt}) calculated from the laboratory experiment. We suggest that pink salmon in the Shibetsu River do not swim at the optimal swimming velocity because of the short migration distance involved (20 km).

Key words: oxygen uptake; respirometry; electromyogram telemetry

Introduction

Sexual dimorphism, which is thought to be a consequence of sexual selection (Andersson 1986, 1994; Parker 1992), has been well-studied in anadromous Pacific salmon (*Oncorhynchus* spp.). These salmonid fishes are born in freshwater streams and rivers, then migrate to the ocean where they feed, before returning to their natal streams for reproduction. During this latter period, male Pacific salmon develop secondary sexual traits such as bright colouration, hooked snout (kype), humped back and large sharp teeth (Groot & Margolis 1991).

Previous studies have investigated evolutionary explanations for the secondary sexual traits of salmon from the perspective of natural and sexual selection (e.g., Kinnison et al. 2003; Esteve 2005). Quinn & Foote (1994) found that male sockeye salmon (*O. nerka*) which were bigger and had a larger dorsal hump size had superior access to the most fecund females and had the best spawning sites. It has been hypothesised that the dorsal hump may serve as a shield against attack from competitors, as a means to block access to ovipositioning females by competing males, or as a display to other males and to females (Fleming & Gross 1994; Quinn & Foote 1994). The

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elongated snout with developed canine-like teeth in males functions as a powerful weapon in male-male competition (Gross 1984), and an enlarged body size is also an advantage (Fleming & Gross 1994). Thus, the large dorsal hump and elongated snout of the males are considered to play an important role in reproductive success.

Some secondary sexual characteristics may adversely affect survival. For example, bears feeding on salmon selectively attack the dorsal hump of male salmon in chum (*O. keta*), sockeye (*O. nerka*) and pink salmon (*O. gorbuscha*) (Ruggerone et al. 2000; Gende et al. 2001). Additionally, salmon with large humps may experience difficulty in accessing the spawning grounds in very shallow streams (Quinn & Foote 1994). It is also likely that bulky secondary sexual traits can increase resistance and drag, and thus hamper the swimming ability of the fish or greatly increase their energy consumption during upstream migration. There is some evidence that male salmon body shape is subjected to natural selection, as fish with a more fusiform body are likely to be more successful in migrating short distances in mountain streams (Hendry & Berg 1999; Crossin et al. 2003; Kinnison et al. 2003).

The spawning migration of salmonid species is physiologically as well as energetically challenging for the fish (Bernatchez & Dodson 1987; Hinch et al. 2006). In addition, salmonid species generally cease feeding prior to spawning migration and thus rely on stored energy during migration. Male and female salmon differ in their comparative energy use during upstream migration. Studies using electromyogram (EMG) telemetry showed that male sockeye salmon migrated less efficiently than females, perhaps due to shape differences arising from the development of a humped back and kype in males (Hinch & Rand 1998; Standen et al. 2002). Studies comparing somatic energy contents showed that male pink salmon expended slightly more energy than females between the time of entry into the mouth of the river and arrival at the spawning ground (Crossin et al. 2003).

To date, the relative energy use (oxygen uptake) of the sexes in anadromous salmonid species and the effect of secondary sexual traits on swimming performance have not been investigated in detail. Mature adult pink salmon return to freshwater streams to spawn after a 2 year feeding migration in the ocean (Williams 1991). When they return from the ocean, the fish are mature and display dramatic sexual dimorphism. As mentioned earlier, the most prominent dimorphic features are the elongated snout and large dorsal hump in the males. The aims of this study was to determine the relationships between oxygen uptake rate and swimming velocity differ in the two sexes

(using a swimming respirometer) and to examine the effects of secondary sexual morphological traits (hump height, body depth, upper jaw length, caudal depth and total length) on these relationships. This latter experiment was performed in the laboratory. However, we also compared swimming performance between the sexes (using EMG telemetry) in a field study in the Shibetsu River, Hokkaido, Japan.

Materials and methods

Fish collection

Sexually mature male and female pink salmon were collected in September 2004, 2007 and 2008 using an 'Urai', a fishing weir for capturing adult salmon, in the Shibetsu River estuary (43°43′N, 145°7′E, Hokkaido, Japan; Fig. 1). The fish were transferred to outdoor tanks (10.0 × 2.5 × 1.1 m) that were maintained with a natural photoperiod at the Hokkaido Fish Hatchery Doto Research Branch for 2 days until the experiment was started.

Swimming respirometry

To measure oxygen uptake during swimming, we used a PT-110R swimming respirometer (West Japan Fluid Engineering Laboratory Co. Ltd, Nagasaki, Japan); the respirometer was 110 cm long, 30 cm wide and 30 cm deep. The experiment was conducted at the Hokkaido Fish Hatchery Doto Research Branch (Figure S1). The tank in the swimming section of the respirometer held 400 l water; water flow was generated by a voltage-controlled motor and propeller, where the voltage was calibrated against water velocity. The swimming respirometer was sealed using an acrylic board so that no gas exchanged occurred. Dissolved oxygen concentration in the chamber was measured using a U-21XD multi water quality sensor probe with automatic temperature compensation (HORIBA Ltd, Kyoto, Japan) housed outside the chamber in a flow-through channel. The water for the experiment was obtained from the Shibetsu River. Prior to introducing the fish, the respirometer was run to remove any bubbles. The fish were placed individually into the swimming section of the chamber. Initial and final water temperatures were monitored for each trial. If the water temperature rose more than 2 °C during the course of a trial, the data on oxygen uptake were excluded. Mean water temperature values ± standard deviations were 13.8 ± 0.22 °C and 14.9 ± 0.53 °C at the beginning and end of the trials, respectively, in 2007, and 12.2 ± 0.53 °C, and 12.9 ± 0.57 °C, respectively, in 2008. Background dissolved oxygen concentration with no fish in the swim chamber was
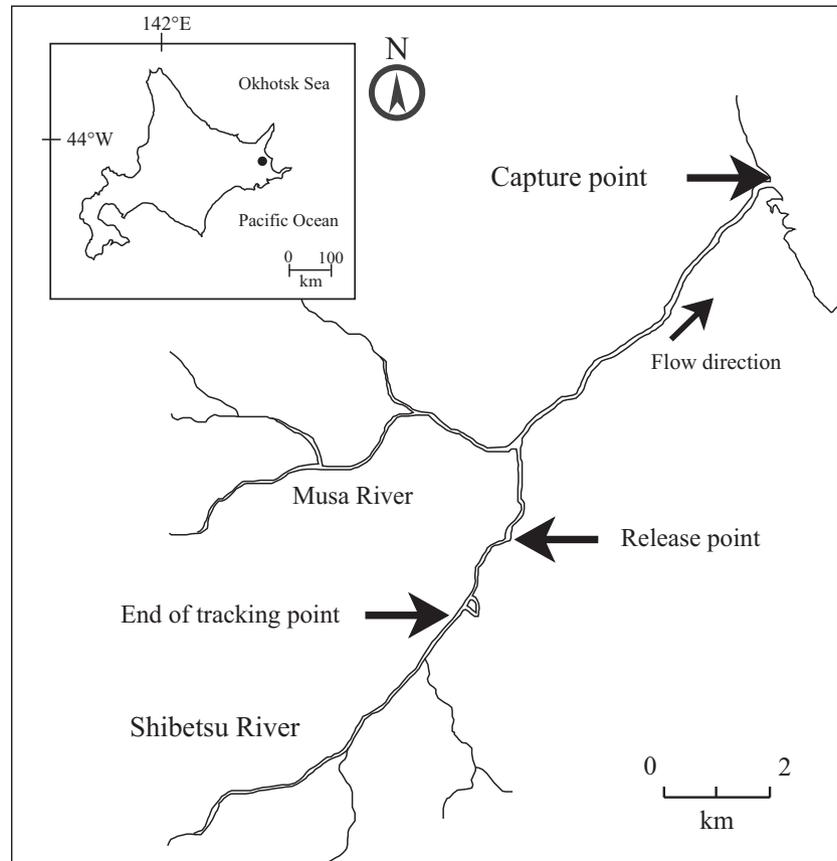
Fig. 1. Field study area showing the site where the pink salmon used in this study were caught and the site where the tagged fish were released.

measured repeatedly and found to be negligible (<1%). The rate of oxygen uptake was determined during the measuring period as the decline in PO_2 in the chamber.

Experimental procedure for the swimming respirometry experiment

To measure oxygen uptake (MO_2) in relation to swimming velocity, 20 males were sampled in 2007 (fork length, 46.1–64.9 cm; body mass, 0.95–3.2 kg; hump height, 5.6–10.0 cm; body depth, 9.4–19.4 cm; upper jaw length, 7.4–9.4 cm; caudal length, 2.9–4.2 cm; total length, 48.2–66.5 cm);12 males (fork length: 47.5–64.5 cm; body mass: 0.97–3.1 kg; hump height, 6.0–11.8 cm; body depth, 10.7–20.7 cm; upper jaw length, 7.4–10.3 cm; caudal length, 3.2–4.1 cm; total length, 51.0–68.1 cm) and 12 females (fork length. 45.5–55.0 cm; body mass, 0.95–1.8 kg; hump height, 3.5–5.0 cm; body depth. 9.5–12.3 cm; upper jaw length, 4.9–6.0 cm; caudal length. 3.2–3.9 cm; total length, 49.0–58.5 cm) were sampled in 2008. The fish were anaesthetised using FA100 (eugenol [4-allyl-2-methoxyphenol], 107 mg·ml^{-1}; Tanabe Seiyaku Co. Ltd, Osaka, Japan) at a concentration of 0.5 ml·l^{-1} in Shibetsu River water (Makiguchi et al. 2008b, 2011). While anaesthetised, the fish were weighed and measured for body width. Next, a cylindrical epoxy-encased EMG transmitter, which weighed 33.0 g in air and was 16.0 mm in diameter and 87.0 mm in length (CEMG-R16-50; Lotek Engineering Inc., Newmarket, ON, Canada), was attached externally and positioned anterior to the dorsal fin to equalise the drag effects of tagging in the telemetry study. After tagging, experimental fish were placed individually into the swimming section of the chamber. Different experimental protocols were used for MO_2 measurements in 2007 and 2008. In 2007, each fish was introduced into the swimming respirometer 1 h before the experiment for acclimation to a velocity of 0.3 m·s^{-1} and to minimise handling effects (Jain et al. 1998). After the acclimation period, the water flow was increased to 0.75 m·s^{-1} and the fish were made to swim for 30 min. At the completion of each 30 min period, the water flow was increased by an additional 0.25 m·s^{-1} and was maintained at the new velocity for 30 min or until the fish became fatigued and was unable to swim against the current. In 2008, each fish was introduced into the swimming respirometer 1 h before the experiment for acclimation at a flow rate of 0.5 body lengths per second (BL·s^{-1}). After recovery, the flow was increased to 0.75 BL·s^{-1} and the fish was made to swim for

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30 min. The flow rate was then increased by 0.25 $BL \cdot s^{-1}$ every 15 min until the fish fatigued. Fish were defined to be fatigued when they rested or were pushed to the downstream screen and remained there for more than 10 s. During the trials the dissolved oxygen concentration (mg $O_2 \cdot l^{-1}$) was recorded every 5 min with a precision of ± 0.01 mg $O_2 \cdot l^{-1}$. At the end of each increase in flow rate, if the air-saturation was more than 70% and water temperature had not increased by more than 2 °C compared to the beginning of the experiment, then the flow rate was increased to the next increment and data collection resumed. MO_2 in mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ for an individual fish during a velocity increment was calculated as:

$$MO_2 = [O_2]v/m$$

where the oxygen concentration $[O_2]$ is measured in mg $O_2 \cdot l^{-1} \cdot h^{-1}$, v is the water volume of the swim chamber (l), and m is the body mass of the fish (kg). The relationship between individual values of MO_2 and swimming velocity (U) was described using the exponential function:

$$MO_2 = ae^{bU}$$

where a is the estimate of the standard metabolic rate (the minimum maintenance MO_2 at rest, i.e. MO_2 at zero velocity), U is the swimming velocity, and b is a constant (Lee et al. 2003; Behrens et al. 2006). The relationship between the cost of transport (COT) and U was described by an exponential function in which MO_2 was expressed as a function of U in kilometers per hour:

$$COT(U) = ae^{bU}U^{-1}$$

The expression for COT (U) was derived, and the value when this derivative was equal to zero was taken as the optimal swimming velocity (U_{opt}) in which the cost of transport was at a minimum (COT_{min}). U_{opt} was then determined as the value of U where the first derivative of this equation was equal to zero:

$$U_{opt} = 1/b$$

COT_{min} was obtained by inserting U_{opt} into COT (U). U_{crit} for each individual was calculated as shown below.

Step-test protocol for U_{crit}

Critical swimming velocity (U_{crit}) is defined as the highest swimming velocity that a fish can maintain for a period equal in magnitude to the time interval used in the test (Brett 1964, 1967; Peake et al. 1997).

The U_{crit} in $m \cdot s^{-1}$ or $BL \cdot s^{-1}$, was calculated as described by (Brett 1965):

$$U_{crit} = U_p + (T_p T_i^{-1}) \times U_i$$

where U_p is the velocity at which the fish last swam for the full period, U_i is the velocity increment (0.25 $m \cdot s^{-1}$ or 0.5 $BL \cdot s^{-1}$), T_p is the duration in minutes that the fish was able to swim against the water velocity that produced fatigue, and T_i is the time between velocity increments (15 or 30 min).

Water velocities were corrected for solid blocking effects as described by Bell & Terhune (1970). Solid blocking effects were calculated as:

$$Vc = Vm(1 + E_s)$$

where Vc is the corrected water velocity, Vm is the measured water velocity determined in the absence of a fish in the swim chamber and E_s in the fractional error due to the solid blocking effect of the fish. E_s was calculated as described by Bell & Terhune (1970), assuming the cross-sectional area and length of a fish to be best represented by an elliptical cylinder:

$$E_s = TL(A0/At)1.5$$

where T is a dimensionless factor depending on flume cross-sectional shape, L is the shape factor for the fish (described by $0.5 \times$ (fork length/fish depth)), $A0$ is the maximum cross-sectional area of the fish (described by $0.25 \times$ fish depth $\times$ fish width) and At is the cross-sectional area of the cylindrical swim section. For any sectional shape, $T = 0.8$ to one decimal place (Bell & Terhune (1970). U_{crit} was calculated, after correction for blocking effects, in $m \cdot s^{-1}$ and $BL \cdot s^{-1}$.

After the experiments were completed, the left side of each fish was photographed using a digital camera with a scale for measuring the secondary sexual traits.

Data analysis

To examine the effect of sex differences on the relationship between MO_2 and swimming velocity, we used a linear mixed model. The identity of the fish used in each trial was treated as a random effect on the slope and intercept in the models to avoid pseudo replication due to repeated measurement of the same individuals in the data. We used a likelihood ratio test to compare models retaining and excluding the explanatory variable (such as swimming velocity, sex, hump height, snout length, and so on), and to calculate P values of linear mixed models (Faraway 2006). We used maximum likelihood estimates for

the calculation of the likelihood ratio test and also for Akaike's Information Criterion (AIC) values. We report χ^2 values of the likelihood ratio test between the fitted model and the null model (that contains no explanatory variables) in the results and 95% confidence intervals for model parameters obtained by simulating 10,000 response vectors via the bootstrapping method. The calculated models were fitted with the lmer function from the package lme4 (Bates et al. 2011) in R 3.2.1 software (Team 2015). We used linear mixed models to test whether water temperature differences in 2007, which ranged from 13.8 ± 0.22 °C to 14.9 ± 0.53 °C, and 2008, which ranged from 12.2 ± 0.53 °C to 12.9 ± 0.57 °C, affected the relationships between MO_2 and swimming velocity. A previous study found that MO_2 of pink salmon exponentially increased with increasing water temperature (MacNutt et al. 2006). In this study, there were no significant effects of temperature differences on the relationship between MO_2 and swimming velocity ($P = 0.354$ and $\chi^2 = 0.858$ at the start of trial and $P = 0.433$ and $\chi^2 = 0.614$ at the end of trial). Therefore, the MO_2 data of 2007 and 2008 were pooled and used to examine the effects of sex differences. U_{crit} ($m \cdot s^{-1}$ and $BL \cdot s^{-1}$) was also compared between sexes using a linear mixed model, where 'Year' was treated as a random effect in the models.

ImageJ (Schneider et al. 2012) was used to analyse length measurements; hump height (perpendicular length from lateral line to the apex of the hump), body depth (perpendicular length from the apex of the hump to the bottom of the abdomen), upper 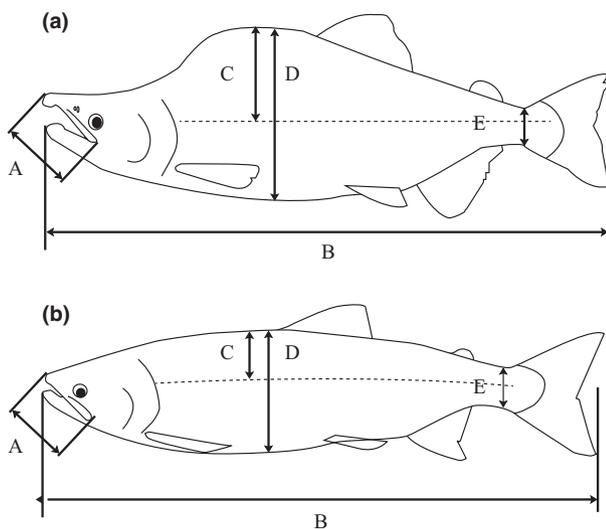jaw length and caudal depth were measured from photographs (Fig. 2). Data on morphological traits were log_{10} transformed for statistical analysis.

Telemetry study

The swimming behaviour of pink salmon during upstream migration was analysed using EMG telemetry; five males were analysed in 2004 (fork length, 51.0–59.7 cm; weight, 1.55–2.32 kg), 10 males in 2007 (fork length, 45.3–58.3 cm; weight, 1.01–2.26 kg) in 2007, and seven males and eight females in 2008 (male fork length, 45.4–60.4 cm; weight, 0.78–2.60 kg; female fork length, 49.2–57.9 cm; weight, 1.39–2.29 kg). Each fish was equipped with a cylindrical, epoxy-encased EMG transmitter (CEMG-R16-50; Lotek Engineering Inc.) that weighed 33.0 g in air, and was 16.0 mm in diameter and 87.0 mm in length. The transmitter was attached externally on the body anterior to the dorsal fin, as described by Makiguchi et al. (2008b, 2011)). External attachment of a transmitter has been reported to be suitable for short-term studies as it reduces handling stress and is less time-consuming (Bridger & Booth 2003). Once the attachment location was determined, experimental fish were anaesthetised using FA100 (0.5 $ml \cdot l^{-1}$) and placed on the surgical table. A pair of Teflon-coated electrodes with brass muscle-anchoring tips (dimension 5×1 mm) of the EMG transmitters were inserted under the surface of the skin using a hypodermic needle at a point approximately two-thirds of the length of the body toward the tail on the left side (Beddow & McKinley 1999). The electrodes detect the electro-potential within the axial dark muscle, with the amplitude and frequency of these pulses being directly correlated with the level of muscle activity. The paired electrode tips were positioned approximately 10 mm apart. The electrodes were sutured to the fish to avoid entangles with vegetation and/or other structures in the environment. An aminoglycoside antibiotic (Akiyama Seisakujyo Co. Ltd, Tokyo, Japan) was applied to the skin around the punctures and stitches to prevent infections. The CEMG model is equipped with a differential muscle probe, a signal conditioning circuit, a digitiser, a microcontroller and a radio transmitter. The voltage corresponding to muscle activity was sampled from the beginning to the end of the experiment every 3 s. The mean value from the samples was calculated, assigned an activity level (EMG signal) ranging from 0 to 50 (no units) and transmitted to a radio receiver (model SRX_600; Lotek Engineering Inc.). Following an hour of recovery, the EMG signals from the fish tagged with EMG transmitters were converted to swimming velocity following the method of Makiguchi et al. (2007, 2008b) who used

Fig. 2. Morphological measurement from male (a) and female (b) pink salmon. A, upper jaw length; B, total length; C, hump height; D, body depth; E, caudal peduncle depth.

the same swim chamber for a respirometry experiment. Water from the Shibetsu River was pumped into the chamber before each trial. The water velocity (up to 120 cm·s^{-1}) at selected motor frequencies was determined using an impeller connected to a precalibrated frequency counter. Experimental fish were placed individually into the swim chamber and 10 EMG signals at 0 cm·s^{-1} were measured when the fish maintained a holding position. After this period, the experiment was initiated at a water velocity of 30 cm·s^{-1}, which was increased incrementally by 30 cm·s^{-1} to a maximum of 120 cm·s^{-1}, with 10 EMG signals measured at each increment. Fish readily swam against the current and rarely came into contact with the grid at the back of the swim chamber. The relationship between swimming velocity and the EMG signal output from tagged fish was plotted, and a simple linear regression was used to estimate swimming velocities of free swimming fish from the EMG signals (McFarlane et al. 2004). On completion of the experiment, the fish were transferred to a live-box and allowed to recover at the release point of the Shibetsu River for at least 24 h prior to release. The tracking area in the Shibetsu River covers a reach between 7.25 and 9.0 km from the river mouth and is 1.2 km long (Fig. 1; Makiguchi et al. 2007, 2008a).

After the tagged fish were released, we tracked them along the river upstream to the end of the tracking point using a hand-held directional Yagi antenna. EMG radio receivers (model SRX_600; Lotek Engineering Inc.) recorded EMG signals at 3–5 s intervals. Generally, we could visually observe the position of the fish in the river.

Migration time through the tracking area was monitored and measured for each fish, and the ground speed calculated by dividing the distance travelled by the time taken. The swimming efficiency index (SEI) was calculated by dividing each individual's ground speed by its swimming velocity; such data are useful for estimating an individual's energy utilisation strategy during upstream migration (Hinch & Rand 2000). EMG signals were converted to swimming velocity using the equations established from EMG calibration (Makiguchi et al. 2008b). Correlation and regression analyses were performed between EMG signals and swimming velocities. We used a linear mixed model to examine the effects of sex differences on the swimming velocity estimated from EMG signals in m·s^{-1} and BL·s^{-1}, ground speed in m·s^{-1} and BL·s^{-1}, and SEI. Furthermore, we also estimated MO_2 and COT of the fish tracked in the river for both sexes using the model obtained in the swimming respirometry experiment. We treated 'year' as a random effect on the slope in the model.

Results

Swimming respirometry experiment

Based on the linear mixed model, we found that when MO_2 (mg O$_2$·kg^{-1}·h^{-1}) was plotted against swimming velocity (m·s^{-1}), the data showed a best fit to an exponential curve (Fig. 3; Table 1). Both swimming velocity and sex were contributory variables for MO_2. Likelihood ratio tests showed that the model including both swimming velocity and sex effects was significantly better than that including only swimming velocity ($\chi^2 = 6.97$, $P < 0.001$). Thus the sex of the fish was an important parameter. The relationship between MO_2 and swimming velocity could be explained by the equations, $MO_2 = 167.9e^{1.23U}$ and $MO_2 = 144.9e^{1.14U}$ for males and females, respectively, where the constant $a = 167.9$ (144.9) is an estimate of the weight-specific standard metabolic rate (mg O$_2$·kg^{-1}·h^{-1}: Fig. 3). The slope of the relationship between MO_2 and swimming velocity was 1.27 times higher in males than in females. U_{crit} (m·s^{-1}) differed significantly between the sexes (1.12 ± 0.11 m·s^{-1} for males and 0.96 ± 0.17 m·s^{-1} for females, $\chi^2 = 6.11$, $P < 0.01$), while U_{crit} (BL·s^{-1}) showed no significant sex difference (2.10 ± 0.27 BL·s^{-1} for males and 1.91 ± 0.33 BL·s^{-1} for females, $\chi^2 = 0.639$, $P = 0.424$). We also examined the effects of morphological traits (hump height, body depth, upper jaw length, caudal depth and total length) on the relationship between MO_2 and swimming velocity for each sex using linear mixed models (Table 2; Figure S2). Models containing hump height, (swimming velocity + hump height) and upper
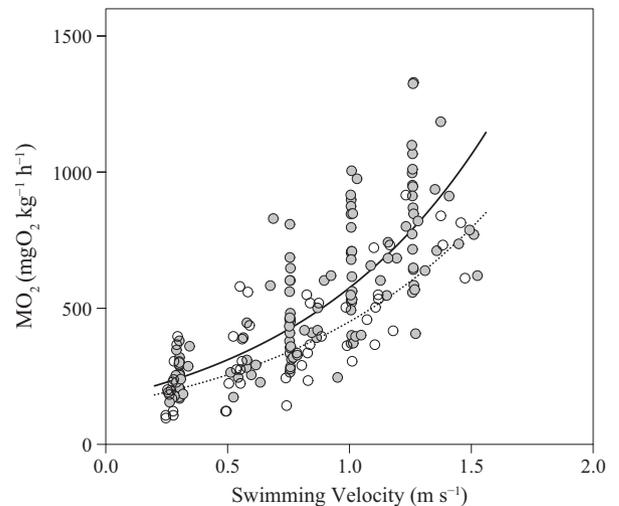

Fig. 3. Oxygen uptake (MO_2; mg O$_2$·kg^{-1}·h^{-1}) of male ($N = 32$; closed circles) and female ($N = 12$; open circles) pink salmon as a function of swimming velocity U (m·s^{-1}). MO_2 is strongly related to swimming velocity: $MO_2 = 167.9e^{1.23U}$ for males (solid line) and $MO_2 = 144.9e^{1.14U}$ for females (dotted line).

Table 1. The linear mixed model was used to test the effects of dependent variables on the MO_2 (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) in pink salmon. A likelihood ratio test was used to the significance of the fixed effects compared with null model.

Paramters	Estimates				CI for a coefficient			
	AIC	ΔAIC	Log-likelihood	Coefficient	Lower	Upper	SE	P-value
(1) Swimming velocity + Sex								
Swimming velocity	25.6	0.0	−5.8	1.20	1.09	1.31	0.06	<0.001
Sex (male)				1.27	1.06	1.52	0.09	
(2) Swimming velocity								
Swimming velocity	30.6	5.0	−9.3	1.20	1.09	1.32	0.06	<0.001
(3) Null model	136.1	110.5	−63.0	–	–	–	–	–

AIC, Akaike's information criteria; ΔAIC, difference for model relative to smallest AIC in the model set; CI, 95 % confidence interval for a coefficient; SE, standard error of a coefficient.
Coefficient in Sex (male) shows ratio compared to female.

jaw length (swimming velocity + upper jaw length) identified a significant and positive effect between MO_2 and swimming velocity in males but not in females (Table 2).

COT was calculated as $MO_2 \cdot U^{-1}$ at each measured swimming velocity for both sexes (Fig. 4). The relationship between COT and swimming velocity was characteristically U-shaped and could be described by COT $(U) = (167.9e^{1.23U})U^{-1}$ for males, and COT $(U) = (144.9e^{1.14U})U^{-1}$ for females, where U_{opt} was 0.81 m·s^{-1} and 0.88 m·s^{-1}, and 1.44 BL·s^{-1} and 1.64 BL·s^{-1} for males and females, respectively. The minimum value of COT was 561 mg $O_2 \cdot kg^{-1} \cdot km^{-1}$ and 449 mg $O_2 \cdot kg^{-1} \cdot km^{-1}$ for males and females, respectively.

Telemetry study

Muscle activity levels were positively correlated with swimming velocity (22 males, $r^2 = 0.93 \pm 0.07$; eight females, $r^2 = 0.95 \pm 0.05$), and were used to convert the EMG signals of each fish in the field study to swimming velocity. Unfortunately, nine males and five females migrated downstream of the tracking area and, therefore, we were able to collect EMG data from only thirteen males and three females through the tracking area. Linear mixed model analysis indicated that the sex of the fish had no effect on dependent variables except for MO_2 (Table 3).

Discussion

Our analysis using the swimming respirometer clearly showed differences in metabolic rate among male and female pink salmon in the laboratory. The experiment identified a significant increase in MO_2 with increased swimming velocity; the coefficient of the slope of the relationship between MO_2 and swimming velocity in males was 1.27-fold higher than in females, showing that males had a greater increase in MO_2 with increasing swimming velocity. A previous study in which the U_{crit} in pink salmon was examined, reported that energy use in males was 1.15-fold higher in males than females over the same distance (Williams & Brett 1987). Our estimates were consistent with this study.

The male secondary sexual traits hump height and upper jaw length in males had a significant and positive effect on the relationship between MO_2 and swimming velocity. Hump height and upper jaw length in males adversely affected MO_2 with increasing swimming velocity and resulted in a less efficient swimming performance; thus, the morphology of males affected the efficiency of their migration after the development of the secondary sexual characters. We suggest that these secondary sexual traits make males less streamlined and increase their drag, resulting in less efficient upstream swimming.

Although the U_{crit} (BL·s^{-1}; a relative measure of U_{crit}) did not differ between sexes, the U_{crit} (m·s^{-1}) was significantly higher in males than in females. We hypothesise that the sex difference in U_{crit} (m·s^{-1}) resulted from the larger body size of males. The MO_2 estimated at each U_{crit} (m·s^{-1}) was larger in males than in females (666 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ for males vs. 433 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ for females). These results suggested that swimming at a sub-maximum aerobic swimming velocity was energetically more expensive for males during their upstream spawning migration. The result of COT also supported this conclusion. The COT_{min}, which was COT calculated at U_{opt}, was larger in males than females (561 mg $O_2 \cdot kg^{-1} \cdot km^{-1}$ for males vs. 449 mg $O_2 \cdot kg^{-1} \cdot km^{-1}$ for females). These results also suggested that males consume more energy during swimming at U_{opt} and swam less efficiently than females.

The results of the telemetry study showed that the swimming velocity, ground speed, SEI and COT did

Models	Estimates				CI for a coefficient			
	AIC	ΔAIC	Log-likelihood	Coefficient	Lower	Upper	SE	P-value
Male								
(1) Swimming velocity								
Swimming velocity	−11.03	4.9	11.52	1.23	1.14	1.32	0.05	–
(2) Swimming velocity + Hump height								
Swimming velocity	−15.33	0.6	14.66	1.23	1.14	1.32	0.05	**0.012**
Hump height				1.36	0.32	2.41	0.53	
(3) Swimming velocity +Body depth								
Swimming velocity	−9.13	6.8	11.57	1.23	1.14	1.32	0.05	0.754
Body depth				−0.19	−1.43	1.00	0.60	
(4) Swimming velocity + Upper jaw length								
Swimming velocity	−15.89	0.0	14.95	1.23	1.14	1.32	0.05	**0.009**
Upperjaw				3.34	0.93	5.78	1.24	
(5) Swimming velocity + Caudal depth								
Swimming velocity	−9.17	6.7	11.59	1.23	1.14	1.32	0.05	0.710
Caudal depth				0.49	−1.96	3.01	1.26	
(6) Swimming velocity + Total length								
Swimming velocity	−9.32	6.6	11.66	1.23	1.14	1.32	0.05	0.592
Total length				−0.66	−3.09	1.70	1.18	
Female								
(1) Swimming velocity								
Swimming velocity	26.95	1.2	−7.47	1.14	0.80	1.49	0.17	–
(2) Swimming velocity + Hump height								
Swimming velocity	28.77	3.0	−7.38	1.13	0.78	1.46	0.17	0.673
Hump height				0.85	−2.59	4.22	1.64	
(3) Swimming velocity +Body depth								
Swimming velocity	28.11	2.4	−7.05	1.12	0.78	1.47	0.17	0.360
Body depth				2.18	−2.20	6.45	2.08	
(4) Swimming velocity + Upper jaw length								
Swimming velocity	25.93	0.2	−5.97	1.12	0.77	1.48	0.17	0.083
Upper jaw				6.27	−0.90	13.75	2.89	
(5) Swimming velocity + Caudal depth								
Swimming velocity	27.53	1.8	−6.77	1.11	0.77	1.46	0.17	0.235
Caudal depth				4.17	−1.18	9.56	2.57	
(6) Swimming velocity + Total length								
Swimming velocity	25.72	0.0	−5.86	1.10	0.75	1.46	0.17	0.073
Total length				6.52	−0.70	13.26	2.63	

AIC, Akaike's information criteria; ΔAIC, difference for model relative to smallest AIC in the model set; CI, 95 % confidence interval for a coefficient; SE, standard error of a coefficient. Values in bold indicates significant.

not differ between the sexes, although the estimated levels of MO_2 from swimming velocity using the EMG calibration equation was larger in males than in females. These results suggested that there were sex-related differences in swimming behaviour of pink salmon in the river.

Although we did not find a sex difference in SEI, which is a surrogate for the efficiency of swimming during upstream migration, we did find that the swimming performance of males was less efficient than that of females in the swimming respirometer experiment. This contradiction between the field study and the laboratory experiment suggests that pink salmon choose different energy conserving pathways in the two sexes during migration behaviour. However, we need to be cautious in drawing conclusions from our telemetry study because of the small

sample size; further investigations will be necessary in future to confirm our initial results.

The results of the telemetry study also showed that the swimming velocities ($m \cdot s^{-1}$) of both sexes (0.65 $m \cdot s^{-1}$ for males and 0.56 $m \cdot s^{-1}$ for females) were lower than U_{opt} (0.81 $m \cdot s^{-1}$ for males and 0.88 $m \cdot s^{-1}$ for females) calculated in the swimming respiratory experiment; these differences indicate that pink salmon in the Shibetsu River did not swim at optimal velocities. There are two explanations for this result. First, the pink salmon are not able to find optimal routes during migration. Makiguchi et al. (2007) compared migration behaviours of chum and pink salmon in the Shibetsu River (at the present study site) and showed that male pink salmon tended to swim in shallow depths in spite of relatively high currents. The authors suggested that pink salmon

were unable to find energy efficient routes for their migration because of the relatively shallow depth of the river compared to the body depth of the fish. When salmon migrate upstream to spawn, they inevitably encounter shallow streams in which surface waves generate a drag. This affects the migration route and energy utilisation in salmon (Hughes 2004), and may also contribute to inefficient swimming in pink salmon. Second, in Shibetsu River pink salmon may not face a selection pressure from the cost of migration required to successfully reach the spawning ground since the distance they migrate up the is not great (approximately 20 km; Makiguchi Y, Nii H, Nakao K, unpublished data). Previous studies have reported that during upstream migration, male sockeye and pink salmon use more energy than

females when the fish migrate through difficult routes (Hinch & Rand 1998; Standen et al. 2002). Here, we estimated the energy used by pink salmon using an equation derived from the swimming respiratory experiment. This analysis showed that for a migration distance of 20 km at an average swimming velocity (0.65 m·s^{-1} for males and 0.56 m·s^{-1} for females) and with a constant water current, a COT of 11263.6 mg O_2 kg^{-1} is expected for males and of 9008.3 mg O_2 kg^{-1} for females, based on our energy model. We converted the data on oxygen uptake from mg O_2 to MJ·kg^{-1} using standard oxy-caloric values (14.1 J·mg O_2^{-1}; Videler 1993). Energy use (MJ·kg^{-1}) during spawning migration in the present study was estimated by multiplying with 14.1 J·mg O_2^{-1} and resulted in 0.159 MJ·kg^{-1} for males and 0.127 MJ·kg^{-1} for females, respectively. The somatic energy density of pink salmon estimated by Crossin et al. (2003) is similar to that used in the present study, namely, 2.93% of body weight (kg) for males and 2.00% for females. These estimates appear consistent with the energy requirements of the fish: pink salmon in Weaver Creek, British Columbia migrate 161 km from the river mouth to the spawning ground and proximate constituent analyses estimate they spend 11–16% of gross somatic energy reserves (0.60 MJ·kg^{-1} for males and 1.04 MJ·kg^{-1} for females) (Crossin et al. 2003). These estimates would suggest that pink salmon in the Shibetsu River might have a somatic energy reserve for mating and reproductive success after arrival at their spawning ground, because they have only a short migration distance (20 km). There are no reports on whether factors such as a migration distance or genetic differences can affect secondary sexual characteristics in pink salmon populations. However, Kinnison et al. (2003) reported a trade-off between the energy costs of migratory distance and that of developing a dorsal hump in male chinook salmon (*O. tshawytscha*) in a New Zealand population. Male chinook salmon with
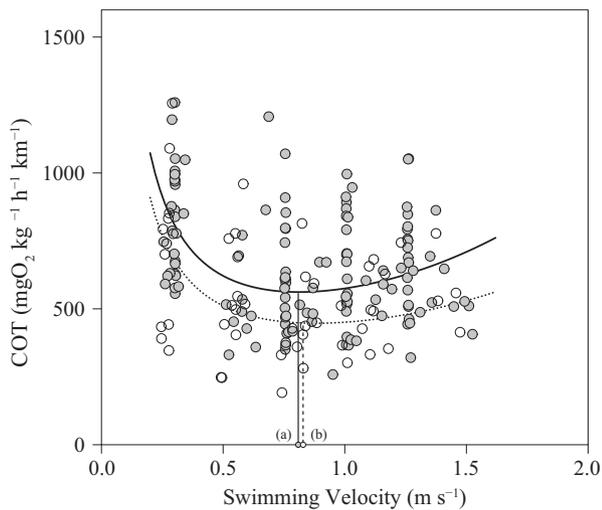

Fig. 4. Costs of transport (COT; mgO$_2$·kg^{-1}·h^{-1}·km^{-1}), i.e., the amount of oxygen used per unit mass per unit distance by male (closed circle) and female (open circle) pink salmon, as a function of swimming velocity U (m·s^{-1}). The relationship can be described by either COT $(U) = (167.9e^{1.23U})U^{-1}$ for males (solid line) or COT $(U) = (144.9e^{1.14U})U^{-1}$ for females (dotted line). The swimming velocities in the minimum COT values are 0.81 m·s^{-1} for males (a) and 0.88 m·s^{-1} (b) for females.

Table 3. Linear mixed model test the sex effects on dependent variables; swimming velocity (m·s^{-1}), swimming velocity (BL·s^{-1}), ground speed (m·s^{-1}), ground speed (BL·s^{-1}), swimming efficiency index (SEI), oxygen uptake (MO$_2$) and cost of transport (COT) in pink salmon. Values are shown in mean ± SD. A likelihood ratio test was used to the significance of the fixed effects compared with null model (compared to model without sex parameter).

Dependent variables	Estimate		χ^2	ΔAIC	P-value
	Male	Female			
Swimming velocity (m·s^{-1})	0.65 ± 0.31	0.56 ± 0.21	3.35	1.35	0.07
Swimming velocity (BL·s^{-1})	1.14 ± 0.49	0.99 ± 0.41	2.49	0.49	0.12
Ground speed (m·s^{-1})	0.08 ± 0.06	0.08 ± 0.04	0.00	2.05	0.96
Ground speed (BL·s^{-1})	0.14 ± 0.10	0.14 ± 0.08	0.00	2.00	0.99
SEI	0.14 ± 0.11	0.14 ± 0.06	0.00	2.00	0.99
MO$_2$ (mg O$_2$·kg^{-1}·h^{-1})	428.39 ± 185.49	290.82 ± 78.92	4.25	2.25	0.04
COT (mg O$_2$·kg^{-1}·km^{-1})	1324.55 ± 1336.82	760.53 ± 163.52	0.04	1.96	0.85

χ^2, difference in deviance for model relative to deviance of the null model; AIC, Akaike's information criteria; ΔAIC, difference for model relative to AIC of the null model; CI, 95 % confidence interval for a coefficient.

long migrations, such as 100 km up the Glenafiffe Stream, tend to be less deep-bodied than those with a shorter migration, such as 17 km up Silverstream. Therefore, we speculate that the secondary sexual traits of pink salmon in the Shibetsu River may be more limited by mating and reproductive success under sexual selection than migrating success to spawning ground under natural selection.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The swimming respirometer showing (a) the swimming chamber, (b) the motor and propeller controller, (c) oxygen sensor probe, (d) oxygen sensor controller, and (e) the propeller that generates the water current.

Figure S2. Oxygen uptake (MO_2; mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) in male pink salmon as a function of log_{10}-transformed hump height (a) and log_{10}-transformed upper jaw length (b).