Profiles in growth, smoltification, immune function and swimming performance of 1-year-old masu salmon
Oncorhynchus masou masou reared in water flow

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ABSTRACT: Effects of water flow on the growth, smoltification, immune function and swimming performance were studied in cultured 1-year-old masu salmon Oncorhynchus masou masou. The experimental fish were exposed to 2, 13 or 23 cm/s flow for 11 months. Fish exposed to 13 and 23 cm/s flows had larger body size after 11 months and higher condition factor after 2 months than those exposed to 2 cm/s flow. Blood levels of growth hormone and insulin-like growth factor-I were unaffected by flow conditions. The higher flow delayed progress of smoltification, whereas there was no clear difference in blood levels of thyroxine between the three flow groups. Superoxide anion produced by leukocytes was the highest in fish receiving 23 cm/s flow, whereas no difference was detected in the plasma immunoglobulin M concentration between the three flow groups after 11 months. Increased flow resulted in higher swimming performance during the rearing period. Correlations between the relative swimming speed and the specific growth rate (SGR) were observed. The swimming speed with the maximum SGR was estimated to be 1.04-fold the fork length per second. These results indicate that water-flow conditions can affect growth and fitness of juvenile masu salmon, which are desirable for both release programs and aquaculture of this species.

KEY WORDS: growth, growth hormone, immunocompetence, insulin-like growth factor-I, masu salmon, swimming performance, thyroxine, water flow.

INTRODUCTION

Water flow is a component of the natural habitat of salmonids, especially during their early life stage, and moderate exercise induced by the water flow has been reported to provide several advantages for salmon production in aquaculture.1–4 Masu salmon Oncorhynchus masou masou, a salmonid endemic to far east Asia, has a fluvial period lasting 1 or more years.5 The duration of the freshwater period is known to depend on the geographic distribution and the growth pattern of individuals of the same strain and in the same river.5–7 Masu salmon is very valuable for both fisheries and for aquaculture in Japan. However, the catch of the domestic stocks of masu salmon is diminishing from 3967 tons in 1984 to 2570 tons in 1998.8 Detailed data on the harvest of masu salmon cultured in Japan are not available, because the harvest data of salmonids, excluding rainbow trout Oncorhynchus mykiss and coho salmon Oncorhynchus kisutch, were combined,9 probably because the quantities harvested were too small to describe separately. Difficulties in stock enhancement programs and culture techniques for masu salmon hamper recovery or development of both the catch and culture production. Improvement of growth and fitness of fish is expected to encourage culture and/or release programs.

The present study was designed to ascertain whether water flow can be an effective tool promoting growth and fitness of masu salmon, as has been demonstrated in other salmonids: Arctic char Salvelinus alpinus,10,11 brown trout Salmo trutta12 and rainbow trout O. mykiss.13,14 We examined the effects of water flow introduced into
the rearing system of masu salmon on body size, condition factor, parr-smolt transformation, gonad development, immune functions and swimming performance, all of which are strongly connected with the effectiveness of culture and release programs. Endocrine responses of fish reared in the water flow were also examined to determine the relationships with growth properties including parr-smolt transformation through focusing on the blood levels of growth hormone (GH), insulin-like growth factor-I (IGF-I) and thyroxine (T4).

MATERIALS AND METHODS

Fish and experimental tanks

Nine hundred 1-year-old masu salmon, artificially fertilized on 18 September 1997, were used in the present study. The fish were successively bred and kept at Nikko Branch, National Research Institute of Aquaculture, Nikko, Tochigi, Japan. Fish were fed commercial pellets (Super 3; Oriental Yeast Co., Chiba, Japan) until satiation twice a day, 5 days a week, and maintained in a concrete, rectangular pond (4 m long×0.95 m width×0.35 m depth) set outdoors, which received continuously fresh water with negligible flow velocity (<1 cm/s), for 5 months prior to the present study. On 7 October 1998, 300 fish were selected randomly from the stock pond and introduced separately into each of three circular tanks (1640 mm in diameter, and 1250 mm in height) having different water-flow velocities, and reared there for 337 days until 9 September 1999. Initial body sizes were 122.6±0.9 mm (mean±SEM) in fork length and 19.0±0.4 g in body weight. Fish were fed commercial pellets (Super 4; Oriental Yeast Co.) at satiation levels from 9:00 h to 15:00 h every day using an automatic feeder operated by a spring coil (3K-12H, Clockwork feeder; FIAP, Nichimo Co., Tokyo, Japan). Water temperature was kept constant at 10°C throughout the experimental period.

The three flow velocities were created by introducing oxygen-rich, fresh water into the tank from the outer edge and by draining the wastewater from the center bottom of the tank. The inlet water was introduced just below the water surface of the tanks using two 25 mm PVC pipes set 180° apart from each other along the tank wall at a rate of 60 L/min. The drain was connected to a standpipe set outside the tank by which the water levels were adjusted. The direction of the inlet pipes was set straight downward for the minimum velocity tank and horizontally, parallel to the outer edge, in order to create a clockwise flow for the medium and maximum velocity tanks. The maximum velocity tank was equipped with a pump (Magnet Pump, PMD-2531B2; Sanyo, Tokyo, Japan) set outside the tank.

The fish were distributed widely in tanks except for the center column having the drain at the bottom (300 mm in diameter) where vortex occurred especially in the two tanks with higher flow velocities. The flow velocities were determined using a flowmeter (UC-3; Tamaya Corp., Tokyo, Japan) for each tank at nine different horizontal and vertical points of the tank, except the center column, along a radius 90° from the two inlet pipes. The average velocities were 2.4±1.5 cm/s (mean±SD) in the minimum water-flow tank, 12.7±3.5 cm/s in the medium water-flow tank and 23.3±5.9 cm/s in the maximum water-flow tank. The latter two flow velocities were equivalent to relative swimming speeds ranging from 1.88 to 0.54 fork length (FL) per second throughout the 11-month experiment. This range includes the cruising speed (1.15 FL/s) of masu salmon in natural waters. In the present paper, the minimum flow was sometimes paraphrased to ‘no flow’ versus ‘flow’ for the medium and maximum flows, because it was as weak as negligible.

Sampling, measurement and assays

Thirty or more fish were randomly sampled from each tank monthly or bimonthly, anesthetized with 50 p.p.m. benzocaine and processed to record fork length, body weight and to identify the silvering stage. Stage of silvering was determined by categorizing fish into one of five categories based on the external appearance according to a modified criterion after Kubo:6 P, fish showing clear parr marks without silver on either side of the body and without fin pigmentation; S1, fish with slight silver coloration and fin pigmentation irrespective of the appearance of parr marks; S2, fish with silver color and fin pigmentation showing parr marks that are not clear but detectable from any angles; S3, fish where the parr marks can barely be detected depending on the light condition, showing clear silver color and fin pigmentation; and S4, fish showing markedly clear silver color without a parr mark. Condition factor (CF) was obtained using the following formula: CF = BW/FL^3 × 10^6. Specific growth rate was calculated according to the following formula: SGR(%) = [(Ln(BWt2) – Ln(BWt1))/ (t2 – t1)] × 100, where t1 and t2 indicate the time designated by day when body weight was recorded.

Half of the fish, of which the body size and silverying stage were recorded, were allowed to recover from the anesthetic and returned to the
same tank after the measurement mentioned above. The other half were used to obtain blood samples under anesthetic and killed to identify the sex and to weigh the gonad (GW). Gonad somatic index (GSI) was calculated as follows: GSI = GW/BW×100. Blood was sampled through caudal vessels using heparinized capillary tubes to determine the plasma levels of growth hormone (GH), insulin-like growth factor-I (IGF-I), thyroxine (T4) and immunoglobulin M (IgM), and the level of superoxide anion, an active oxygen radical, produced by the leukocytes in the circulating blood. Growth hormone, IGF-I and T4 were determined monthly or bimonthly during the experimental period, while the plasma IgM concentration and the level of superoxide anion were determined at the final sampling alone, 11 months after the beginning of the experiment. Blood for analyses of GH, IGF-I, T4 and IgM was centrifuged at 13 000 g for 5 min, and the plasma obtained was frozen at −80°C until assay, while the superoxide anion level was determined immediately after blood sampling. Plasma levels of GH and IGF-I were measured by radioimmunoassay following Bolton et al. and Moriyama et al., respectively. T4 was assayed with a time-resolved fluorometer by the method of Satoh et al. The IgM concentration was assayed by ELISA according to Nagae et al. The superoxide anion level was determined as a potential killing ability according to a modified method, originally described by Rook et al. and Secombes. Briefly, the plasma, including the leukocyte layer, obtained through 1000 g centrifugation of 200 µL blood using heparinized capillary tubes, was diluted with 100 µL Minimum Essential Medium (MEM) (Code: 05902; Nissui Pharmaceutical Co., Tokyo, Japan) solution (9.4 µg MEM/mL distilled water), which was previously autoclaved and then received 12.5 µL of 10% sodium bicarbonate and 2.92 mg L-glutamine (Code: 5908, Nissui Pharmaceutical Co.) per 10 mL MEM solution, and was divided into two fractions. One fraction received an equal volume of nitroblue tetrazolium (NBT; N-6876; Sigma Chemical Co., St Louis, MO, USA) solution, which was diluted with MEM solution (2 mg NBT/mL MEM solution) and another fraction received an equal volume of NBT solution including Zymosan A (Z-4250; Sigma Chemical Co.; 5 mg Zymosan A/mL NBT solution). After a 1 h incubation at 10°C, 13 times the volume of N,N-dimethylformamide (045-02916; Wako Pure Chemical Industries, Osaka, Japan) was added to each fraction and the fraction was centrifuged at 1500 g for 15 min at 4°C. The optical densities (OD) were determined using a microplate reader (MTP-120; Corona Electric Corp., Ibaragi, Japan) at 540 nm 1 h after the centrifugation at room temperature. The potential killing ability was calculated by subtracting the OD of the fraction receiving NBT from that of NBT including Zymosan A. Measurement of swimming performance

Swimming performance was measured monthly or bimonthly as the maximum time for which a fish was able to swim continuously in a stamina tunnel (PF-70B; Japan Aquatech Inc., Nagasaki, Japan) having a rectangular swim-chamber (300 mm width×700 mm length×200 mm depth) against a water velocity of 1 m/s. Water velocities were gradually elevated from 0 to 1 m/s in 60 s. The tolerable swim time was measured after attaining the final velocity of 1 m/s. Six masu salmon selected randomly from each tank were individually tested in the stamina tunnel. The swimming duration was determined after 15 min adaptation period in the tunnel.

Statistical analyses and calculation

Significant differences (P<0.05) of mean values were examined by F-test and t-test between flow groups and by Duncan's multiple range test following ANOVA for comparison over time within the same flow group. Regression lines showing the relationship between the maximum swim time and fork length were compared statistically according to Snedecor and Cochran. Significances of the regression curves between the SGR and the relative swimming speed were analyzed using the STATVIEW statistical package, SAS Institute Inc., Cary, NC, USA. Because of limited sample sizes, data were not separated by sex and by maturation stage but pooled unless otherwise stated.

RESULTS

Gonad observations revealed that there was no precociously maturing female in any flow groups throughout the experiment (Fig. 1), while many precociously maturing males appeared irrespective of flow conditions (Fig. 2). No clear differences in gonad development were observed in females among the three flow conditions. The proportion of precociously maturing males in the maximum flow group was low, while the mode of the GSI was contrarily higher than in fish receiving no flow (P<0.05) and medium flow (P<0.01) in the spawning season, at the end of the experiment. The fish reared in the medium flow velocity exhibited larger fork length than fish in the
medium and maximum flows were significantly larger than the fish reared in the minimum flow velocities (Fig. 3). The change in body weight showed a similar pattern to that in fork length and,

minimum and maximum flow velocities after 10 months. At 11 months, the fork length of fish receiving the maximum flow caught up with that of the medium flow group. Both the fish reared in the

**Fig. 1** Proportion frequency in percentage of gonad somatic index (=gonad weight/body weight×100) of female by flow velocity over time. Mini. flow velocity, 2 cm/s; Medi. flow velocity, 13 cm/s; Maxi. flow velocity, 23 cm/s.
yet, the differences in body weight between the three groups were clearer than those observed in fork length (Fig. 3). The condition factor of fish exposed to flow was often significantly higher than that of the no-flow group (Fig. 3). A significant difference in the condition factor appeared as early as 60 days. Differences in both body size and condition factor between females, immature males and
precociously maturing males became clear in and after June, after 240 days, although sample size on June was much smaller (Table 1). Females and precociously maturing males all grew larger in the three groups. Females receiving the medium flow were significantly larger than those receiving the minimum and maximum flows in August and September. Precociously maturing males had higher condition factor than females and immature males in all flow groups in and after June. Fish reared in the maximum flow exhibited higher condition factor than those in the minimum and medium flows, irrespective of the differences in sex and maturation stage.

Smoltification proceeded at different rates among the three groups (Figs 4,5). Figure 5 depicts the proportion of the silvering stage of immature masu salmon reared at different water velocities over time (excluding the precociously maturing males). The proportion of parr declined later in the higher velocity groups, as indicated by large differences in the incidence of parr between groups at 90–120 days (Figs 4,5) and, thereafter, parr reappeared earlier in the minimum velocity group than in the medium and maximum velocity groups (Fig. 4).

Significant changes over time were detected in all flow groups for the plasma GH level ($P<0.01$ for all groups), in the maximum flow group ($P<0.01$) and in the minimum flow group ($P<0.05$) for the IGF-I level (Fig. 6). However, both GH and IGF-I levels did not exhibit any remarkable differences between the three flow groups except for a few cases. They also did not show any significant difference between immature and mature fish.

There were significant differences in T4 levels between immature and mature fish. Remarkable differences in the T4 level were not observed precociously maturing males became clear in and after June, after 240 days, although sample size on June was much smaller (Table 1). Females and precociously maturing males all grew larger in the three groups. Females receiving the medium flow were significantly larger than those receiving the minimum and maximum flows in August and September. Precociously maturing males had higher condition factor than females and immature males in all flow groups in and after June. Fish reared in the maximum flow exhibited higher condition factor than those in the minimum and medium flows, irrespective of the differences in sex and maturation stage.

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between the flow groups, although significant changes over time were detected in the maximum (P < 0.01) and minimum (P < 0.05) flow groups (Fig. 6).

Plasma IgM concentration did not show any significant difference between groups and between immature and mature fish. The superoxide anion level was significantly higher in fish receiving the maximum flow velocity than in those receiving the minimum and medium flows after 11 months (Fig. 7). Precociously maturing males exhibited significantly higher potential killing ability than immature fish in the medium flow group (P < 0.05) (Table 2).

Fish reared in higher water velocity clearly exhibited much greater swim time in the stamina tunnel (Fig. 8). There was no difference in swimming performance between parr and smolt in any flow condition (data not shown). The regression lines showing relationships between the maximum swim time and fork length had a steeper slope in fish receiving higher flow velocities (Fig. 9). Significant differences of regression lines were detected both between the minimum and maximum groups (P < 0.01) and between the medium and maximum groups (P < 0.05).

The swimming speeds that corresponded to the maximum specific growth rate were calculated to be 0.76 FL/s ($R^2 = 0.591$, $P = 0.0280$) for the medium water-velocity group and 1.31 FL/s ($R^2 = 0.668$, $P = 0.0121$) for the maximum water-velocity group. When both groups were combined, the swimming speed maximizing the SGR was estimated as 1.04 FL/s ($R^2 = 0.256$, $P = 0.0696$) (Fig. 10).

**DISCUSSION**

The present study demonstrated that water flow can significantly affect the body size, condition...
factor, smoltification, potential killing ability of leukocytes, as well as swimming performance of juvenile masu salmon. Gonad development and blood levels of GH, IGF-I, T4 and IgM appeared unaffected by water flow conditions. Most of the significant effects are desirable for enhancing actual hatchery and culture production of this species.

The velocity of the ambient water is considered equal to the swimming speed of fish, as fish usually

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**Table 2** Optical density of superoxide anion produced by leukocytes in the spawning season of masu salmon reared for 11 months at different water velocities

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IM, immature fish; M, precociously maturing male. Means sharing a common alphabetical symbol or without symbols at each line or between IM and M do not differ significantly. Capital letters are true for comparison between flow velocities and lowercase letters are for between IM and M. Mini. flow velocity, 2 cm/s; Medi. flow velocity, 13 cm/s; Maxi. flow velocity, 23 cm/s.

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**Fig. 5** Proportion of the silvery stage of immature masu salmon (excluding mature males) reared at different water velocities over time. Because sex identification and gonad weight measurement were not performed on 150, 210 and 270 days after the experiment began, no data were available on those days. \( n = 10–35 \). Refer to the text for the definition of each stage. Mini. flow velocity, 2 cm/s; Medi. flow velocity, 13 cm/s; Maxi. flow velocity, 23 cm/s.

**Fig. 6** Changes over time in plasma growth hormone (GH; top), insulin-like growth factor-I (IGF-I; middle) and thyroxine (T4; bottom) of masu salmon reared at different water velocities. Mean and the SEM are shown (\( n = 15 \) for GH, 8–15 for IGF-I and T4). Small letters a, b and c denote significant difference \( (p<0.05) \) between the three groups. The bottom figure shows immature fish alone because of significant differences between immature and mature fish. Mini. flow velocity, 2 cm/s; Medi. flow velocity, 13 cm/s; Maxi. flow velocity, 23 cm/s.
by Davison et al. 23). The growth of Arctic charr *Salvelinus alpinus*, 10,11 brown trout *Salmo trutta* 12 and rainbow trout *O. mykiss* 13,14 improves with exercise, while chinook salmon *Onchorhynchus tshawytscha* 24,25 and sockeye salmon *Oncorhynchus nerka* 24 show no effect or retardation of growth. The relative swimming speeds of masu salmon that optimized the specific growth rate (RSSGR) were calculated to be 0.76 and 1.31 FL/s for fish receiving medium and maximum flows, respectively. Both speeds are considered in the range allowing masu salmon to swim aerobically, compared with the values in the other salmonids. 23

**Fig. 7** Plasma IgM concentration (upper) and optical density (OD) of superoxide anion produced by leukocytes (lower) in the spawning season of masu salmon reared for 11 months at different water velocities. Mean and the SEM are shown (*n*=6). Mini., 2 cm/s; Medi., 13 cm/s; Maxi., 23 cm/s.

**Fig. 8** Change over time in the maximum swim time of masu salmon against 1 m/s flow. Mean and the SEM are shown (*n*=6). Small letters a, b and c denote significant difference (*P*<0.05) between the three groups. Mini. flow velocity, 2 cm/s; Medi. flow velocity, 13 cm/s; Maxi. flow velocity, 23 cm/s.

swim against the ambient flow and try to maintain their position. In other words, introduction of water flow into the rearing condition has the same effect as exercise training on fish. Sustained aerobic exercise 23 is known to increase body growth and feed efficiency etc. in salmonids, although some studies have reported negative, rather than positive, effects of exercise (reviewed

**Fig. 9** Relationships between the maximum swim time and fork length. Mini. flow velocity, 2 cm/s; Medi. flow velocity, 13 cm/s; Maxi. flow velocity, 23 cm/s.

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The difference of the two RSS_{SGR} would result from the adaptive response of fish to their ambient flow. Therefore, the value 1.04 FL/s obtained from the whole, pooled data could be a criterion available for masu salmon production where higher growth rates of fish are desired.

Duration of swimming at high velocity was greatly improved by the flow conditioning set in the present study. The maximum swim time against the swift current increased in proportion to the water velocities to which the fish had been acclimated. The improved swimming performance, especially an enhanced ability of swift movement acquired in the flow stimuli during the rearing period, would increase the chance for fish to get more food and to avoid dangers such as predation when released into natural waters. Therefore, higher survival rates would be expected if individuals possessing such improved ability are released.

The greater the flow in which the fish had been reared, the longer it took the fish to smolt, as evidenced by the duration of the color change. In some cases, the proportion of parr was higher in data excluding the precociously maturing males (Fig. 5) than in those including them (Fig. 4), contrary to expectation. This is probably due to the reduced number of specimens (Fig. 5), where the specimens were limited to those accompanied by sex identification and gonad weight measurement. The delay in the parr-smolt transformation induced by the flow treatments might be because the parr is much more efficient than the smolt for swimming against the water current, as shown by Glova and McInerney, and Thorpe and Morgan, and Virtanen and Forsman, although there was no difference in the swimming performance between individuals with different silvering stages regardless of flow condition in masu salmon (data not shown). The delay in smoltification observed in fish exposed to flow could also be related to the smaller body size compared with that in no-flow groups prior to the downstream period as shown in Fig. 3, because successful smoltification has been observed in juvenile salmon with large body size. Therefore, the delay in smoltification observed in groups reared under velocities forcing swimming is attributable to two possibilities: a direct effect shown by the physiologically higher adaptability to current in parr rather than in smolt, and an indirect effect via the size-dependent progress of smoltification.

The increase in fork length in response to current was not as remarkable as that observed for body weight and condition factor. This suggests that the growth along the body axis was not so affected by flow as the cross-section of muscle fibers. This might be explained by the fact that the fish receiving flow did not have constantly higher concentrations of plasma IGF-I, a hormone that assists cartilage growth and elongation of the body. The GH levels also did not exhibit any clear difference among the three flow groups, but gradual increases were observed commonly in the course of the experiment. Thus, the change in GH levels was opposite to the gradual decrease in IGF-I as time elapsed. Growth hormone stimulates synthesis of somatomedins in the liver, as well as protein in the body, and is under negative feedback control of IGF-I in the pituitary. Changes in GH levels, therefore, are likely to be influenced by the IGF-I level in each flow group, although the reason for the gradual decrease in the IGF-I level is unknown. Dickhoff et al. found a highly significant correlation between plasma IGF-I and instantaneous growth rate of chinook salmon in spring. However, no significant correlation was observed between the plasma IGF-I level and the SGR in any flow condition in the present study. A several-fold increase in plasma GH concentration has been demonstrated in steelhead trout *O. mykiss* and coho salmon *O. kisutch* under sustained exercise for 24 h. Differences in GH levels in fish receiving flow stimuli might be revealed as an effect of chronic training if compared using individuals previously trained in different flow conditions after exercise in much higher velocities.

The plasma level of T4 changed differently between immature and mature fish regardless of the flow condition. A prediction that T4 change of immature fish is associated with the progress of smoltification among the flow groups was not proved. It could be ascribed to the great variation.
in progress of parr-smolt transformation even within the same group. Higgs and Eales observed a higher thyroid hormone secretion rate as the result of exercise in brook trout Salvelinus fontinalis. Kiesling et al. reported that sustained exercise did not alter T4 level, whereas T3 level was increased in chinook salmon. Although there might be changes in T3 levels in masu salmon as well, it remains unknown.

Plasma IgM concentrations were similar to those reported by Fuda et al. in masu salmon of a comparable life stage. They did not differ among the three flow groups. The reason remains unknown, although moderate training is also reported to exert little, if any, effect on serum immunoglobulin concentration in humans. In contrast, it is interesting that no decrease in IgM concentration was observed in precociously maturing males in comparison with immature fish, because sexually maturing salmonids have been known to exhibit immune suppression. For example, adult chinook salmon during freshwater migration generated relatively few antibody-producing cells from peripheral blood leukocytes. Slater and Schreck reported that testosterone was found to significantly reduce the plaque-forming response in vitro in chinook salmon. Suzuki et al. observed that plasma IgM levels decreased during the spawning season in rainbow trout.

The potential killing ability of leukocytes was the highest in the fish held at the maximum water velocity as evidenced by increases in superoxide anion level. No data are available on the potential killing ability of leukocytes at the beginning of the experiment; therefore, we cannot judge correctly whether exercise induced by the maximum flow did increase the level of leukocytes or if the non-exercise resting condition in the minimum flow decreased the level of leukocytes. However, since we ascertained in our recent study that the 0-year-old masu salmon reared in continuous flow condition exhibited significantly elevated superoxide anion level in their leukocytes (data not published), exercise induced by the maximum flow is considered to have increased the levels in the present study. To date, no information on the effects of aerobic exercise on the blood level of superoxide anion produced by leukocytes, especially neutrophils, has been reported in fish. It is known in humans that neutrophils possess β-adrenergic receptors and that epinephrine and other adrenergic agonists inhibit production of superoxide anion in neutrophils. Hack et al. found a strong inverse correlation between the blood level of epinephrine and superoxide anion production. As exercise generally increases plasma catecholamines through the activation of the sympathetic nervous system, accompanied by an elevated metabolic rate, the increased production of superoxide anion observed in fish receiving water flow appears to be inconsistent with the findings in humans. However, it should be noted that the epinephrine level is increased not only by exercise, but also by stress. Masu salmon frequently exhibit agonistic behavior in captivity, which supposes to be accompanied with social stress. Reduction of such agonistic behavior by the flow treatment has been found in Arctic charr S. alpinus. The same effect of flow mitigating aggressive behavior could be expected in masu salmon. This could explain why plasma epinephrine was decreased in fish receiving flow and, thus, superoxide anion production was increased, provided that the same function of epinephrine on neutrophils exists in masu salmon as in humans.

Precociously maturing males exhibited significantly higher potential killing ability of leukocytes than immature fish. This was an entirely unexpected immunoreponse, being contrary to that commonly induced by the immune-suppressive effects in normal maturing salmonids. No precociously maturing masu salmon in our study contracted fungal infections, whereas the normally maturing fish generally die because they become covered with severe fungi. This suggests that precociously maturing males possess higher immunocompetence, which is entirely different from that of the normal maturing, senescent fish. In humans, neutrophils comprise the majority of circulating leukocytes and are important to the body’s early response to bacterial and fungal infection. It is worth noting that water flow significantly enhances the level of the superoxide anion produced by leukocytes, although the significance of the superoxide anion countering lethal diseases still remains unknown in masu salmon. The ability of relative swimming speed in enhancing the potential killing ability was not determined because no data were available, except for the final sampling time. Although remarkably higher levels of superoxide anion were observed in fish receiving the 23 cm/s flow, they were obtained when the relative swimming speed was close to 1 FL/s and thus not very different from RSS$S_{GR}$ estimated above. This suggests that immunologically improved characteristics can be induced by water flow that permits fish to achieve an aerobic, sustainable swimming speed close to the cruising speed observed in natural waters.

Because the experimental tanks used in the present study were designed to generate constant water velocities throughout the experimental period, relative swimming speeds of fish could not be kept constant but decreased concomitantly.
with fish size. Moreover, as evidenced by the variability of silverying stages within a group, parr-smolt transformation was not always synchronized between individuals receiving the flow. Such experimental designs might prevent clearer responses of fish to the respective flow. Use of an improved experimental system allowing fish to swim at a constant relative swimming speed and/or adoption of siblings as the experimental fish to clarify the genetic relevance to flow stimuli would be preferable in future studies.

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