Effect of short-term decrease in water temperature on body temperature and involvement of testosterone in steelhead and rainbow trout, *Oncorhynchus mykiss*

Go Miura [a,*], Arimune Munakata [b], Takashi Yada [c], Carl B. Schreck [d], David L.G. Noakes [e], Hiroyuki Matsuda [a]

[a] Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama, Kanagawa 240–8501, Japan
[b] Department of Biology, Miyagi University of Education, Sendai, Miyagi 980–0845, Japan
[c] National Research Institute of Aquaculture, Fisheries Research Agency, Nikko, Tochigi 321–1661, Japan
[d] Oregon Cooperative Fisheries and Wildlife Research Unit, US Geological Survey, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331–3803, USA
[e] Department of Fisheries and Wildlife, Oregon Hatchery Research Center, Oregon State University, OR 97331–3803, USA

ARTICLE INFO

ABSTRACT

The Pacific salmonid species *Oncorhynchus mykiss* is separated into a migratory form (steelhead trout) and a non-migratory form (rainbow trout). A decrease in water temperature is likely a cue triggering downstream behavior in the migratory form, and testosterone inhibits onset of this behavior. To elucidate differences in sensitivity to water temperature decreases between the migratory and non-migratory forms and effect of testosterone on the sensitivity, we examined two experiments. In experiment 1, we compared changes in body temperature during a short-term decrease in water temperature between both live and dead steelhead and rainbow trout. In experiment 2, we investigated effects of testosterone on body temperature decrease in steelhead trout. Water temperature was decreased by 3 °C in 30 min. The body temperature of the steelhead decreased faster than that of the rainbow trout. In contrast, there was no significant difference in the decrease in body temperature between dead steelhead and rainbow trout specimens. The body temperature of the testosterone-treated steelhead trout decreased more slowly than that of control fish. Our results suggest that the migratory form is more sensitive to decreases in water temperature than the non-migratory form. Moreover, testosterone might play an inhibitory role in sensitivity to such decreases.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Pacific salmonids (*Oncorhynchus* spp.) such as *O. mykiss* are categorized into two different forms: the migratory form (steelhead trout) that migrates from rivers to the sea (downstream migration), and the non-migratory form (rainbow trout) that remains permanently in the natal river (Needham and Behnke, 1962; Ewing et al., 1994). Many anadromous Pacific salmonids such as the steelhead trout undertake downstream migration in the early spring after smoltification. Downstream migratory behavior, an initial step of downstream migration, frequently occurs in conjunction with factors such as rainfall, snow melt influx, and a diurnal decrease in water temperature overnight (Yamauchi et al., 1984, 1985; Iwata, 1996; Munakata, 2012). Because migratory and non-migratory juveniles grow together in the same stream environment before the occurrence of downstream migration, we hypothesized that the migratory form would be more sensitive to environmental changes than the non-migratory form. Although teleosts are ectothermic animals, body temperature is not always equivalent to that of the surrounding water. Even if the well-known examples of endothermic fishes are excluded (Block et al., 1993), there are examples of fishes in which changes in body temperature occur more slowly than changes in surrounding water temperature (Arctic charr (*Salvelinus alpinus L.*) and eight Lake Michigan fishes including *O. mykiss*; Spigarelli et al., 1977; Christiansen et al., 1991).

On the other hand, previous studies showed that yearling (1+) precociously mature fish in masu (*O. masou*) and Atlantic salmon (*Salmo salar*) showed neither the smoltification nor the downstream migration and lived continuously in their home rivers (Machidori and Kato, 1984; Bagliniere and Maisse, 1985; Thorpe, 1986). Recent studies also demonstrated that sex steroids such as testosterone (T) and 11-ketoandrostendione (11-KA) inhibited the occurrence of downstream migratory behavior in masu and Atlantic salmon smolts (Berglund et al., 1994; Munakata et al., 2000, 2001). Such phenomena suggest that some sex steroids physiologically influence the sensitivity to environmental changes in a negative fashion.

* Corresponding author at: Department of Fisheries and Fishing Port, East Regional Promotion Office, Miyagi Prefecture Government, 1-4-32, Higashi-Nakasato, Ishinomaki, Miyagi 986–0812, Japan. Tel.: +81 225 95 1473; fax: +81 225 96 2698.
E-mail addresses: yhfnx644@yahoo.co.jp (G. Miura),
munakata@staff.miyakyo-u.ac.jp (A. Munakata), yadat@fra.affrc.go.jp (T. Yada),
carl.schreck@oregonstate.edu (C.B. Schreck), david.noakes@oregonstate.edu
(D.L.G. Noakes), matsuda@ynu.ac.jp (H. Matsuda).

If the migratory form is more sensitive to a decrease in water temperature than the non-migratory form, we hypothesized that the body temperature of the former would show a more rapid decrease than the latter. Furthermore, sex steroids such as T, inhibitor of the downstream migratory behavior (Munakata et al., 2000, 2001), may have a negative effect on the body temperature decrease. However, it is still unclear whether the migratory form shows higher sensitivity to decrease in surrounding water temperature than the non-migratory form and whether the sex steroid inhibits this sensitivity. In the present study, we compared changes in body temperature in live steelhead and rainbow trout during a short-term decrease in water temperature. We also made the same comparison between dead steelhead and rainbow trout to assess if differences in body temperature decreases are physiologically controlled. Furthermore, we compared changes in body temperature in T-treated and control steelhead trout during same decrease to investigate the inhibitory effect of T on body temperature decreases.

2. Materials and methods

2.1. Fish

In experiment 1, two-year-old (2+) diploid steelhead and 1+ diploid rainbow trout (Oncorhynchus mykiss, Salmonidae) were obtained from the Oregon Hatchery Research Center (OHRC), Alsea, OR, USA on March 9, 2011. Before the experiment, the fish were reared in round outdoor fiber reinforced plastic (FRP) tanks (2 m in diameter with a water depth of 1 m) supplied with flow-through river water derived from Fall Creek at 7.0–11.0 °C. All fish were reared under a natural photoperiod and fed commercial trout pellets (Bio Oregon, Longview, WA, USA) twice per day (at 7:00 and 16:00 h) at a daily ration of 2% body weight (BW).

In experiment 2, 2+ diploid steelhead trout were obtained from the Nikko Branch, Fisheries Research Agency (FRA), Tochigi prefecture, Japan on May 27, 2011. Before the experiment, the fish were reared in outdoor concrete pond (2 × 8 m with a water depth of 0.5 m) supplied with flow-through spring water at 9.5–10.0 °C. All fish were reared under a natural photoperiod and fed commercial trout pellets (Scientific Feed Laboratory Co., Ltd., Tokyo, Japan) twice per day (at 9:00 and 15:00 h) at a daily ration of 2% BW.

2.2. Experiment 1; Effects of decreased water temperature on body temperature of steelhead and rainbow trout

On March 9, 2011, four specimens each type of trout were chosen from the stock FRP tanks based on their standard body length (BL) and BW, and were anesthetized in MS222 solution (150 mg L^{-1}, buffered to pH 7.0 with sodium bicarbonate in ambient river water). Then an acoustic tag (V9TP-1L, 9 mm in diameter × 40 mm, mass in water/air of 2.7/5.2 g, Vemco Inc., Halifax, NS, Canada) was implanted into the abdominal cavity through a 10-mm-long incision that was made by scalpel, and the incision was sutured at one point by surgical silk thread. The tag measures and transmits temperature in the cavity of fish at a frequency of 69 kHz. Tag-implanted fish were placed together in a round indoor FRP tank (84 cm in diameter with a water depth of 64 cm) supplied with flow-through river water derived from Fall Creek. All fish were reared at a 11:13 h light:dark cycle and fed commercial trout pellets twice per day (at 7:00 and 16:00 h) at a daily ration of 2% BW for 11 days until the closing of their wounds. Two temperature logging devices (MDS-MkV/T, JFE Advantech Co., Ltd., Hyogo, Japan) were strung at a depth of 10 cm and 60 cm in the tank. An acoustic receiver (VR2W-69 kHz, Vemco Inc., Halifax, NS, Canada) was also situated near the surface of the water to record the body temperature data from the tags implanted in the fish (Figs. 1 and 2). Flow rate and water temperature were maintained at 35–36 L min^{-1} and 7.0–11.0 °C, respectively. Changes in body temperature during artificial decreases in water temperature were
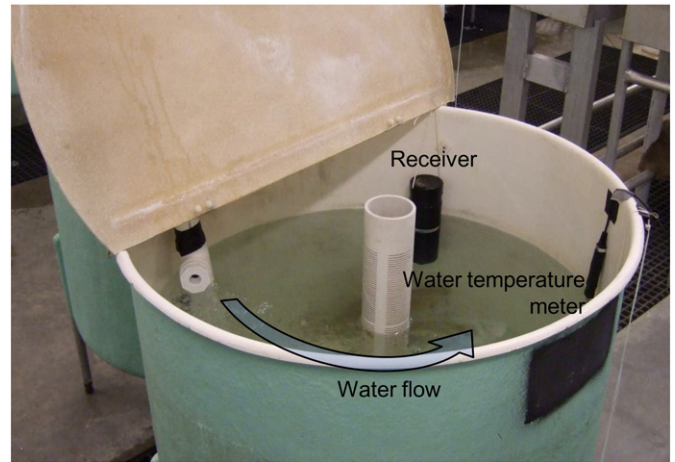

Fig. 1. Experimental tank in experiment 1. Tag-implanted fish were placed together in a round indoor fiber reinforced plastic (FRP) tank (84 cm in diameter with a water depth of 64 cm). An acoustic receiver (VR2W-69 kHz, Vemco Inc., Halifax, NS, Canada) was situated near the surface of the water to record body temperature of tag-implanted fish. Water temperature were maintained uniform anywhere from surface to bottom and the receiver could record data of tag-implanted fish from anywhere in the tank.

measured from March 20 to 22. On March 20 (at 14:30 h), 21 (at 9:30 and 14:00 h), and 22 (at 7:00 and 11:30 h), water temperature in the tank was increased from 7.3–9.1 °C to 10.3–12.1 °C (by 3.0 °C increments) by adding heated river water (12.0–13.0 °C) within 30 min (Fig. 3). An hour later, it was decreased to 7.3–9.1 °C by adding ambient-temperature river water (7.3–9.1 °C) within 30 min, and was kept at a constant level for 90 min. After the five trials on the afternoon of March 22, fish were killed by immersion in a lethal concentration of MS222 solution (350 mg L^{-1}, buffered to pH 7.0 with sodium bicarbonate in ambient river water). BL and BW were measured to the nearest 0.1 cm and 0.1 g, respectively, and the condition factor (CF; 100 × (BW × BL^{-3})) was calculated. Each of the specimens was then separately wrapped in a 5 mm mesh nylon net and suspended at mid-depth in the tank to avoid direct contact with other fish. Body temperature was measured twice (at 17:30 and 21:00 h) on the afternoon of March 22 during the decrease in water temperature as described above (Fig. 3).

2.3. Experiment 2; Effects of decreased water temperature on body temperature of testosterone-treated steelhead trout

On May 27, 2011, 14 steelhead trout were chosen from the stock pond based on their BL and BW, and 7 each were placed in two round indoor FRP tanks (1.2 m in diameter with a water depth of 0.4 m) supplied with flow-through spring water. Both groups were reared under a natural photoperiod for 11 days. One group was fed
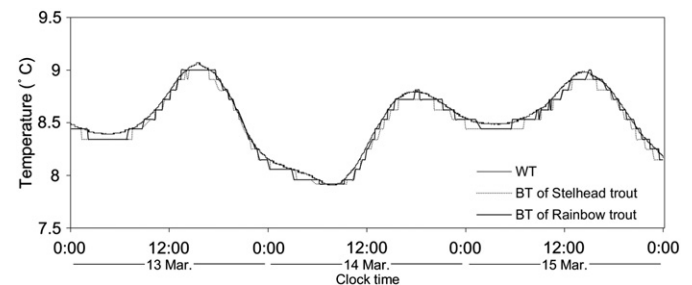

Fig. 2. Body temperature (BT) of one steelhead and one rainbow trout under ambient water temperature (WT) in experiment 1. Each line represents water or body temperature data obtained by the temperature logger or acoustic receiver. There was no difference between body temperature of steelhead and rainbow trout under the stable water temperature.
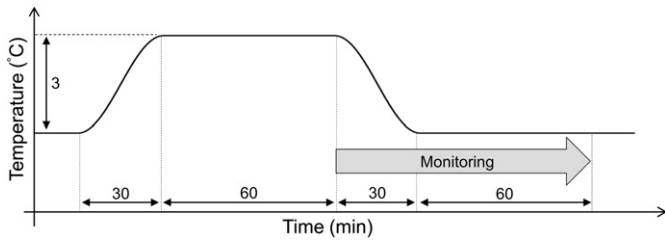
Fig. 3. Schematic illustration of changes in water temperature during the experiments. Water temperature was increased by 3 °C within 30 min and kept constant for the next 1 h. Thereafter, it was decreased by 3 °C within 30 min and kept constant for the next 1 h. Body temperature was monitored for 90 min after the start of the decrease in water temperature.

commercial trout pellets containing 25 μg g^{-1} of T twice per day (at 9:00 and 15:00 h) at a daily ration of 2% BW and defined T-treated group, while the other was fed T-free diets at the equal amount of T-treated group. Flow rate and water temperature were maintained at 21–22 L min^{-1} and 9.5–10.0 °C, respectively. On June 7, four steelhead trout were chosen randomly from each group and were anesthetized in 50 ppm ethyl-p-aminobenzoate solution. Then an acoustic tag (V9TP-1L) was implanted into the abdominal cavity through a 10-mm-long incision that was made by scalpel, and the incision was sutured at one point by surgical silk thread. Adipose fin in T-treated fish was cut off for identification. Tag-implanted fish were returned to each original tank and reared for 50 days until the closing of their wounds. Changes in body temperature during artificial decreases in water temperature were measured from July 27 to 29. On the afternoon of July 27, tag-implanted T-treated and control fish were placed together in another round indoor FRP tank (1.2 m in diameter with a water depth of 0.2 m) supplied with flow-through spring water. Both T-treated and control fish were reared under a natural photoperiod and fed T-free diets twice per day at a daily ration of 2% BW until the end of the experiment. Two temperature logging devices (MDS-MkV/T) were strung at a depth of 5 cm and 20 cm in the tank. An acoustic receiver (VR2W-69 kHz) was also situated near the surface of the water. Flow rate and water temperature were maintained at 21–22 L min^{-1} and 9.5–10.0 °C, respectively. Flow velocity was generated in 15–16 cm s^{-1} by using symmetrically-attached two submersible pumps (Rio+ 1700, Kamihata Fish Industries Ltd., Hyogo, Japan). On July 27 (at 15:00 and 19:30 h), 28 (at 10:00 and 14:30 h), and 29 (at 8:00 h), water temperature in the tank was increased from 9.5–10.0 °C to 12.5–13.0 °C (by 3.0 °C increments) by adding heated spring water (23.0–24.5 °C) within 30 min (Fig. 3). An hour later, it was decreased to 9.5–10.0 °C by adding ambient-temperature spring water (9.5–10.0 °C) within 30 min, and was kept at a constant level for 90 min. After the five trials on the afternoon of July 29, fish were anesthetized in 50 ppm ethyl-p-aminobenzoate solution, and blood was taken from caudal vasculature into heparinized syringe in order to measure plasma T level. The blood was centrifuged to obtain plasma. These samples were stored at −78 °C until the hormone measurement. After the plasma sampling, BL and BW were measured to the nearest 0.1 cm and 0.1 g, respectively, and the CF was calculated.

2.4. Plasma testosterone measurement

Commercially available enzyme immunoassay kits (RIDASCREEN Testosterone, R-Biopharm AG, Darmstadt, Germany) were used to measure plasma T levels.

2.5. Temperature analysis

In experiments 1 and 2, body temperature data of each fish for 90 min after the start of the decrease in water temperature were obtained at intervals of 1–43 min by the receiver (Fig. 4). Water temperature data were obtained concurrently at intervals of 1 min by the
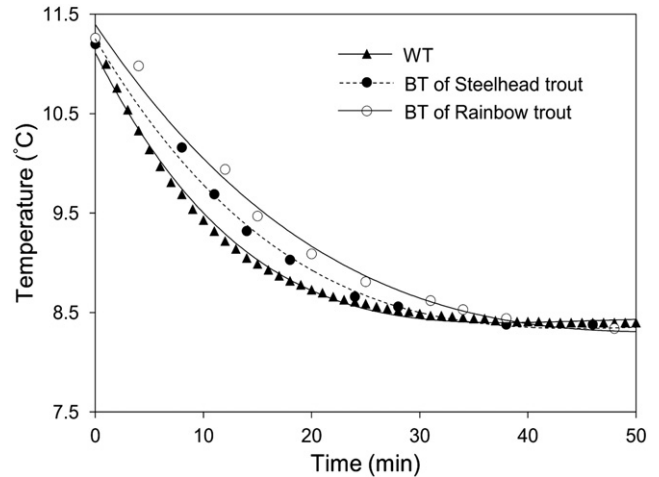

Fig. 4. Best-fit polynomial models minimizing the Akaike information criterion (AIC) of water temperature (WT) and body temperature (BT) of one steelhead and one rainbow trout in one of the trials in experiment 1. Each dot represents water or body temperature data obtained by the temperature logger or acoustic receiver, and each line represents the selected best-fit model.

data loggers. We estimated parameters that explained the decreases in body and water temperature by polynomial models (from second- to fourth-order) by an ordinary least squares method for individuals. Then we calculated the Akaike information criterion (AIC) for each model and chose the best model based on the minimum AIC (Fig. 4). On the basis of the selected models, temperature and the gradient of the decrease were estimated every 5 min for a total of 30 min soon after the start of the decrease in water temperature.

2.6. Statistical analysis

The differences in mean BL, BW, and CF between the steelhead and rainbow trout and the T-treated and control steelhead trout, and the differences in the average and the gradient of the decrease in estimated water temperature and body temperature of the two types of trout were analyzed by Student's t-test (Ekuseru-toukei 2010, Social Survey Research Information Co., Ltd., Tokyo, Japan). On the other hand, the difference in mean plasma T level between the T-treated and control steelhead trout and the differences in mean temperature between the estimated water and body temperature of T-treated steelhead trout from 20 to 30 min after the start of the water temperature decrease in experiment 2 were analyzed by Welch's t-test because the within-group variances differed significantly ($p < 0.05$) between the two groups as the results of f-test. In the illustrations, values are presented as mean ± standard error of the mean (SEM) in the original scale, if not stated otherwise.

3. Results

3.1. Experiment 1; Effects of decreased water temperature on body temperature of steelhead and rainbow trout

The initial and final mean CF of the steelhead trout were significantly ($p < 0.05$) lower than those of the rainbow trout (Table 1). There was no significant difference between the mean body temperature of the steelhead trout and water temperature, whereas the mean body temperature of the rainbow trout was significantly ($p < 0.05$) higher than the water temperature at 10 and 15 min after the start of the decrease in water temperature (Fig. 5a). The gradient of the decrease in body temperature of the steelhead trout was significantly ($p < 0.05$) higher than that of the rainbow trout at 10 and 20 min after the start of the decrease (Fig. 5b).

Table 1
Initial and final standard body length (BL), body weight (BW), and condition factor (CF) (mean ± SEM) of tag-implanted steelhead and rainbow trout. Significant differences in CF between trout at initial and final time points are indicated by * ($p < 0.05$) and ** ($p < 0.01$), respectively.

Group	N	BL (cm)	BW (g)	CF	Sampling date
Initial data					
Steelhead trout	4	25.9 ± 0.4	229.3 ± 20.1	1.31 ± 0.06	March 9, 2011
Rainbow trout	4	24.9 ± 0.3	234.5 ± 11.3	1.52 ± 0.03*	March 9, 2011
Final data					
Steelhead trout	4	26.0 ± 0.5	218.6 ± 16.0	1.24 ± 0.04	March 22, 2011
Rainbow trout	4	25.6 ± 0.4	254.5 ± 14.8	1.52 ± 0.04**	March 22, 2011

The mean body temperature of the dead steelhead and rainbow trout was significantly ($p < 0.05$) higher than water temperature from 5 to 25 min and 10 to 20 min after the start of the decrease, respectively (Fig. 6a). However, there was no difference in mean body temperature and the gradient of the decrease in body temperature between the dead steelhead and rainbow trout (Figs. 6a and 6b).
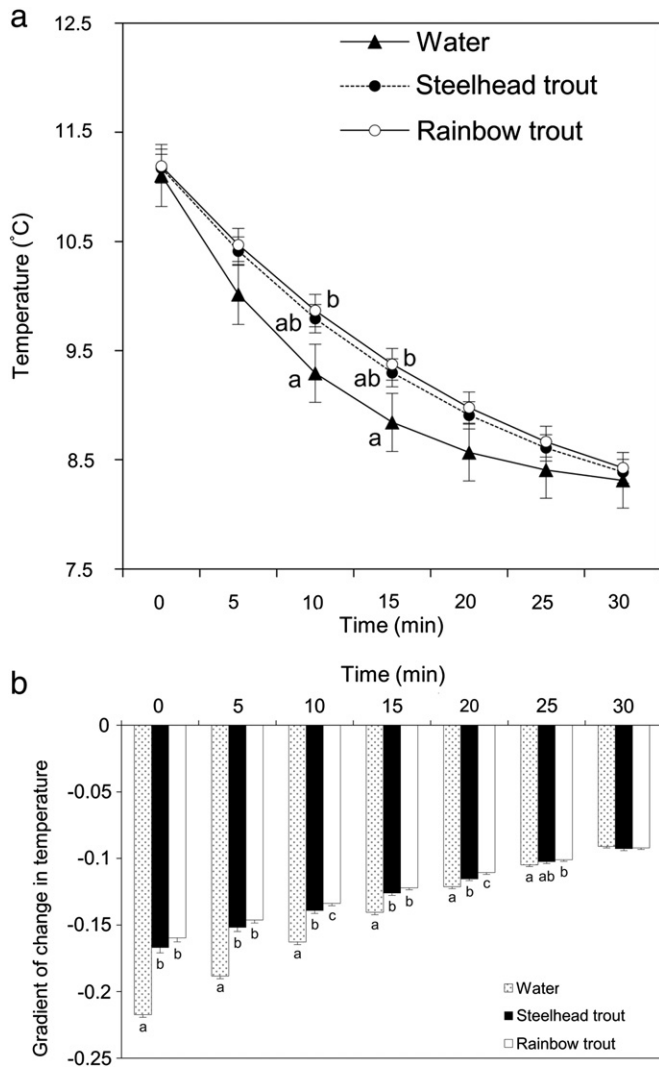

Fig. 5. Mean water and body temperatures (a) and mean gradients of decrease in water and body temperatures (b) of live tag-implanted steelhead and rainbow trout. Error bars represent SEM. Different letters indicate significant ($p < 0.05$) differences among temperatures or gradients of decrease in temperature at the same time point.
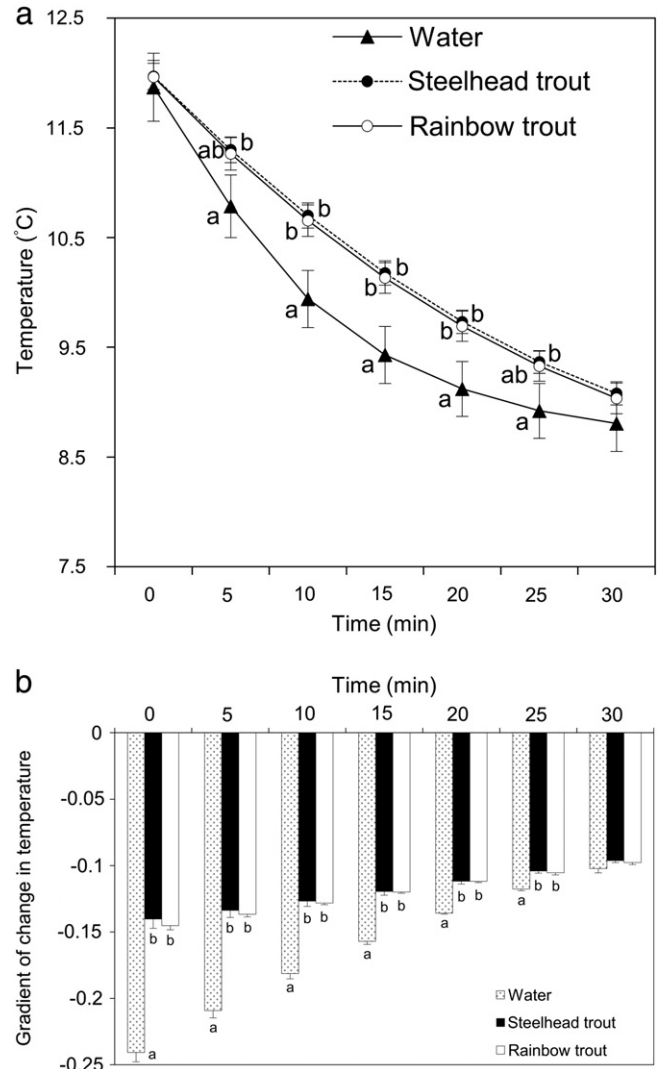

Fig. 6. Mean water and body temperatures (a) and mean gradients of decrease in water and body temperatures (b) of dead tag-implanted steelhead and rainbow trout. Error bars represent SEM. Different letters indicate significant ($p < 0.05$) differences among temperatures or gradients of decrease in temperature at the same time point.

3.2. Experiment 2; Effects of decreased water temperature on body temperature of testosterone-treated steelhead trout

There was no significant difference in initial and final mean BL, BW, and CF between the T-treated and control steelhead trout. The mean plasma T level of the T-treated group was significantly ($p < 0.05$) higher than that of the control group (Table 2). The mean body temperature of the two groups was significantly ($p < 0.05$) higher than water temperature from 5 to 30 min after the start of the decrease in water temperature (Fig. 7a). The mean body temperature of the T-treated group was significantly ($p < 0.05$) higher than that of the control group from 15 to 30 min after the start of the decrease. The gradient of the decrease in body temperature of the T-treated group was significantly ($p < 0.05$) lower than that of the control group at 25 min after the start of the decrease (Fig. 7b).

4. Discussion

In experiment 1, live steelhead trout showed a higher gradient for a decrease in body temperature compared to rainbow trout during a short-term decrease in water temperature. In contrast, there was no

difference in the gradient between dead steelhead and rainbow trout. These results suggest that the steelhead trout is more sensitive to decreases in water temperature than the rainbow trout due to some physiological factors. In experiment 2, T-treated steelhead trout showed a higher body temperature and a lower gradient for a decrease in body temperature compared to control steelhead trout during the decrease. This result indicates that T is a physiological factor that influences the inhibition of decreasing body temperature in the steelhead trout.

Downstream migration of Atlantic, masu, sockeye (*O. nerka*), pink (*O. gorbuscha*), and chum salmon (*O. keta*) as well as the migratory form of *O. mykiss*, steelhead trout, occurs in the spring (Bjorn, 1971; Kato, 1991; Giorig et al., 1997; McCormick et al., 1998; McGinnity et al., 2007; Pavlov et al., 2008). In this season, short-term decreases in water temperature in the stream are frequently caused by rainfall, melting snow influx, and increased daily fluctuations of air temperature. Salmonid smolts exhibited the downstream migratory behavior mainly at dusk and night (Hoar, 1951; Munakata et al., 2000; Riley et al., 2002). In this period, water temperature in the stream shows clear nocturnal decrease following the peak temperature in midafternoon associated with the diurnal variation in air temperature. Moreover, it was also reported that the downstream behavior occurred during and after the rainfall and snow runoff (Solomon, 1978a,b; Yamauchi et al., 1984, 1985; Iwata, 1996; Munakata, 2012). Changes of body temperature associated with these water temperature changes mean sensitivity rather than adaptation, because variability of body temperature leads to instability of internal physiological mechanisms. Therefore, it was thought that decrease in water temperature was negative stimulus for steelhead and rainbow trout. It was reported that cortisol, one of physiological stress response hormons (Barton, 2002), triggered onset of downstream migratory behavior in masu salmon (Munakata et al., 2007). Our previous study also showed that minutes water temperature decrease triggered increase in plasma cortisol levels in several salmonid smolts. These findings indirectly suggested that the onset of downstream migratory behavior was triggered partly by such short-term decreases in water temperature during spring.

In our present results, there is an elevated regulation of body temperature in the non-migratory rainbow trout and T-treated steelhead trout. Since the steelhead trout had lower CF than the rainbow trout, CF appeared to be responsible for changes in body temperature during the decrease in water temperature. In several Atlantic and Pacific salmonids including *O. mykiss*, the migratory form (smolt) also has a lower CF than the non-migratory form (parr) due to smoltification progresses (Ewing et al., 1994; Sigholt et al., 1998; Beckman et al., 2003; McCormick et al., 2003; Munakata et al., 2012). In contrast, a previous study on eight Lake Michigan fishes including *O. mykiss* whose body sizes and shapes varied widely found that the time it took for body temperature to decrease during decreases in water temperature (in 8–10 °C decrements) was positively correlated with

BW (Spigarelli et al., 1977). In general, a decrease in CF increases the ratio of surface area to volume, which can increase the efficiency of body surface heat exchange between the body and the surrounding water, according to classical heat exchange laws such as Newton's law of excess temperature (Stevens and Fry, 1970; Neil and Stevens, 1974; Spigarelli et al., 1977). Thus it is suggested that a rapid decrease in the body temperature of steelhead trout depends on body surface heat exchange proportional to the ratio of surface area to volume.

After lethal anesthesia, however, the differences in the decrease in body temperature between steelhead and rainbow trout disappeared. This indicates that some physiological factors play a role in changes in body temperature in addition to physical factors such as body surface heat exchange. Indeed, Japanese eel (*Anguilla japonica*) with a low CF (mean ± SEM; 0.17 ± 0.01) and common carp (*Cyprinus carpio*) with a high CF (mean ± SEM; 2.39 ± 0.12) show an almost equivalent decrease in body temperature with decrease in water temperature (unpublished data). Hence, we speculate that there are some physiological factors regulating heat exchange.

Result of experiment 2 showed that T had inhibitory effect on the body temperature decrease in the steelhead trout under the same CF. Except for scombrid fish (Scombridae) that maintain their body temperature at a higher level than water temperature (Carey and Teal, 1966;
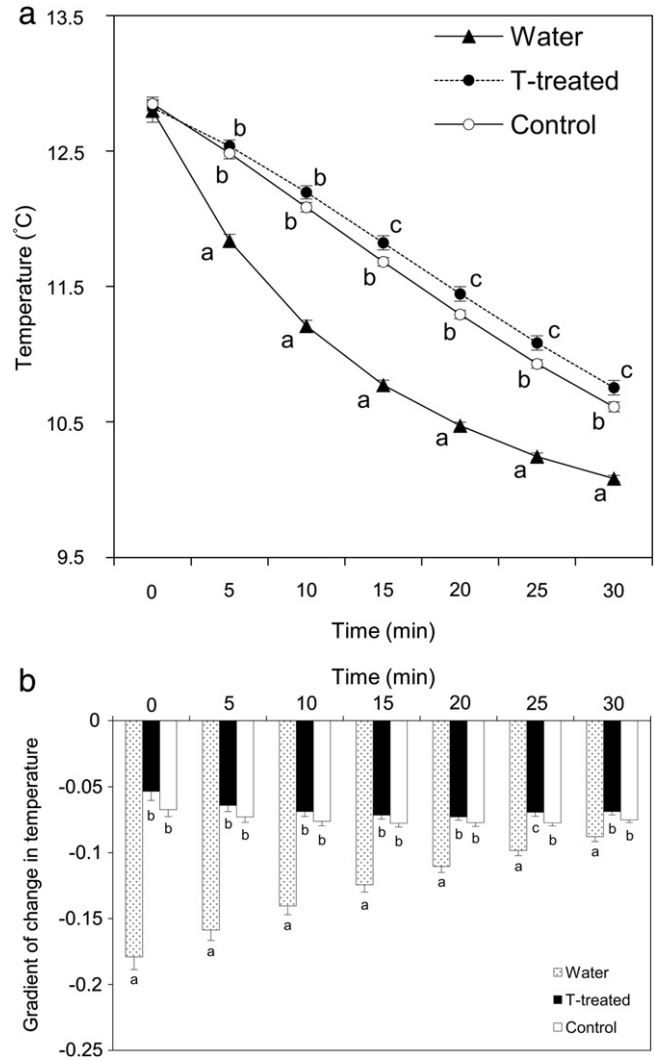

Fig. 7. Mean water and body temperatures (a) and mean gradients of decrease in water and body temperatures (b) of tag-implanted testosterone treated (T-treated) and control steelhead trout. Error bars represent SEM. Different letters indicate significant ($p < 0.05$) differences among temperatures or gradients of decrease in temperature at the same time point.

Table 2
Initial and final standard body length (BL), body weight (BW), condition factor (CF), and plasma testosterone (T) level (mean ± SEM) of testosterone treated (T-treated) and control steelhead trout. Significant difference in plasma T level between trout at final time point is indicated by * ($p < 0.05$).

Group	N	BL (cm)	BW (g)	CF	Plasma T level	Sampling date
Initial data						
T-treated	4	34.9 ± 0.4	539.0 ± 23.7	1.27 ± 0.06	–	June 7, 2011
Control	4	35.1 ± 0.5	592.5 ± 20.1	1.23 ± 0.03	–	June 7, 2011
Final data						
T-treated	4	35.7 ± 0.4	643.0 ± 24.3	1.42 ± 0.08	8.78 ± 3.41*	July 29, 2011
Control	4	35.7 ± 0.5	602.7 ± 29.7	1.36 ± 0.04	0.26 ± 0.05	July 29, 2011

Carey and Lawson, 1973), physiological maintenance of the body temperature of teleosts requires an elevation of aerobic capacity and a reduction of the rate of heat loss (Block et al., 1993). It was reported that sex steroids such as T and thyroid hormones were among the factors modulating energy metabolism through improvement of mitochondrial oxidative capacity in teleosts (Blake et al., 1984; Sangiao-Alvarellos et al., 2006). It was thought that these actions had a critical role in thermo regulation (Hazel, 1989). Furthermore, T promotes assimilation of muscle proteins (Yu et al., 1979; Ng et al., 1984; Connaughton and Taylor, 1995). As a result, rate of the basal metabolism is enhanced and body temperature of fish has stability against decrease in surrounding water temperature (Loughna and Goldspink, 1985). On the other hand, opercula movement associated with respiration stimulates thermal exchange between surrounding water and venous blood and accelerates decrease in body temperature of teleosts (Block et al., 1993). An inability to limit heat loss while respiring through gills accounts for ectothermy in teleosts. However, details of the biological difference between steelhead and rainbow trout and effect of T on such ectothermy especially in respiration are still unclear and require further study. Precociously mature 1+ masu and Atlantic salmon that grow faster than 1+ smolts do not show downstream migration and remain in the upper reaches of streams (Machidori and Kato, 1984; Bagliniere and Maisse, 1985; Thorpe, 1986). Recent studies have reported that sex steroids such as 11-KA and T inhibit the occurrence of downstream migratory behavior in smolts of Atlantic and masu salmon, respectively (Berglund et al., 1994; Munakata et al., 2000, 2001). Therefore, it is suggested that T regulates the onset of downstream migratory behavior through the physiological inhibition of decreasing body temperature such as the increase of the basal metabolic rate and/or the reduction of opercula movement.

In summary, the present study demonstrates that, in response to a short-term decrease in water temperature, the body temperature of steelhead trout decreases more rapidly than that of rainbow trout. As there was no significant difference in the decrease in body temperature between dead steelhead and rainbow trout, and the body temperature of T-treated steelhead trout decreased more slowly than that of control fish, we speculate that the decrease in body temperature of migratory form of *O. mykiss*, steelhead trout, is caused by lack of physiological inhibitors such as T in addition to reductions in CF resulting from smolt development. Further investigations of the relationship between this sensitivity and physiological changes are required.

Acknowledgments

References

Bagliniere, J.L., Maisse, G., 1985. Precocious maturation and smoltification in wild Atlantic salmon in the American massif, France. Aquaculture 45, 249–263.

Barton, B.A., 2002. Stress in fish: A diversity of response with particular reference to changes in circulating corticosteroid. Integr. Comp. Biol. 42, 517–525.

Beckman, B.R., Larsen, D.A., Dickhoff, W.W., 2003. Life history plasticity in chinook salmon: relation of size and growth rate to annual smolting. Aquaculture 222, 149–165.

Berglund, I., Lundqvist, H., Fångstam, H., 1994. Downstream migration of immature salmon (*Salmo salar*) smolts blocked by implantation of the androgen 11-ketoandrostenedione. Aquaculture 121, 269–276.

Bjorn, T.C., 1971. Trout and salmon movements in two Idaho streams as related to temperature, food, stream flow, cover, and population density. Trans. Am. Fish. Soc. 100, 423–438.

Blake, R.L., Roberts, F.L., Saunders, R.L., 1984. Parr-smolt transformation of Atlantic salmon (*Salmo salar*): activities of two respiratory enzymes and concentrations of mitochondria in the liver. Can. J. Fish. Aquat. Sci. 41, 199–203.

Block, B.A., Finnerty, J.R., Stewart, A.F.R., Kidd, J., 1993. Evolution of endothermy in fish: mapping physiological traits on molecular phylogeny. Science 26, 210–213.

Carey, F.G., Lawson, K.D., 1973. Temperature regulation in free-swimming Bluefin tuna. Comp. Biochem. Physiol. A 44, 375–392.

Carey, F.G., Teal, J.M., 1966. Heat conservation in tuna fish muscle. Proc. Natl. Acad. Sci. U.S.A. 56, 1464–1469.

Christiansen, J.S., Johnsen, H.K., Jobling, M., 1991. The combined effects of ambient temperature and enforced sustained swimming activity on body temperature of Arctic charr (*Salvelinus alpines L.*). J. Therm. Biol. 16, 351–355.

Connaughton, M.A., Taylor, M.H., 1995. Effects of exogenous testosterone on sonic muscle mass in the weakfish, *Cynoscion regalis*. Gen. Comp. Endocrinol. 100, 238–245.

Ewing, R.D., Barratt, D., Garlock, D., 1994. Physiological changes related to migration tendency in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 121, 277–287.

Giorig, A.E., Hillman, T., Stevenson, J.S., Heys, S.G., Peven, C.M., 1997. Factors that influence the downstream migration rates of juvenile salmon and steelhead through the hydroelectric system in the mid-Columbia River basin. N. Am. J. Fish. Manag. 17, 268–282.

Hazel, J.R., 1989. Cold adaptation in ectotherms: regulation of membrane function and cellular metabolism. Comp. Environ. Physiol. 4, 1–50.

Hoar, W.S., 1951. The behaviour of chum, pink and coho salmon in relation to their seaward migration. J. Fish. Res. Board Can. 8, 241–263.

Iwata, M., 1996. Downstream migratory behaviors and endocrine control of salmonid fishes. Bull. Natl Res. Inst. Aquac. Suppl. 2, 17–21.

Kato, F., 1991. Life histories of masu and amago salmon. In: Groot, C., Marbolis, L. (Eds.), Pacific salmon life histories. British Colombia Press, Vancouver, pp. 447–520.

Loughna, P.T., Goldspink, G., 1985. Muscle protein synthesis rates during temperature acclimation in a eurythermal (*Cyprinus carpio*) and a stenothermal (*Salmo gairdneri*) species of teleost. J. Exp. Biol. 118, 267–276.

Machidori, S., Kato, F., 1984. Spawning populations and marine life of masu salmon *Oncorhynchus masou*. Int. North Pac. Fish. Comm. 43, 1–138.

McCormick, S.D., Hansen, L.P., Quin, T.P., Saunders, R.L., 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 55, 77–92.

McCormick, S.D., O'Dea, M.F., Moeckel, A.M., Björnsson, B.T., 2003. Endocrine and physiological changes in Atlantic salmon smolts following hatchery release. Aquaculture 222, 45–57.

McGinnity, P., de Eyto, E., Cross, T.F., Coughlan, J., Whelan, K., Ferguson, A., 2007. Population specific smolt development, migration and maturity schedules in Atlantic salmon in a natural river environment. Aquaculture 273, 257–268.

Munakata, A., 2012. Migratory behaviors in masu salmon (*Oncorhynchus masou*) and the influence of endocrinological factors. Aqua-BioSci. Monogr. 5, 29–65.

Munakata, A., Amano, M., Ikuta, K., Kitamura, S., Aida, K., 2000. Inhibitory effects of testosterone on downstream migratory behavior in masu salmon, *Oncorhynchus masou*. Zool. Sci. 17, 863–870.

Munakata, A., Amano, M., Ikuta, K., Kitamura, S., Aida, K., 2001. The involvement of sex steroid hormones in downstream and upstream migratory behavior of masu salmon. Comp. Biochem. Physiol. B 129, 661–669.

Munakata, A., Amano, M., Ikuta, K., Kitamura, S., Aida, K., 2007. Effects of growth hormone and cortisol on the downstream migratory behavior in masu salmon, *Oncorhynchus masou*. Gen. Comp. Endocrinol. 150, 12–17.

Munakata, A., Amano, M., Ikuta, K., Kitamura, S., Aida, K., 2012. Involvement of sex steroids and thyroid hormones in upstream and downstream behaviors in masu salmon, *Oncorhynchus masou*. Aquaculture 362–363, 158–166.

Needham, P., Behnke, R., 1962. The origin of hatchery rainbow trout. Prog. Fish Cult. 24, 156–158.

Neil, W.H., Stevens, E.D., 1974. Thermal inertia versus thermoregulation in "warm" turtles and tunas. Science 184, 1008–1010.

Ng, T.B., Woo, N.Y., Tam, P.P., Au, C.Y., 1984. Changes in metabolism and hepatic ultra-structure induced by estradiol and testosterone in immature female *Epinephelus akaara* (Teleostei, Serranidae). Cell Tissue Res. 236, 651–659.

Pavlov, D.S., Kirillova, E.A., Kirillov, P.I., 2008. Patterns and some mechanisms of downstream migration of juvenile salmonids (with reference to the Utkholok and Kalkaveyem Rivers in northwestern Kamchatka). J. Ichthyol. 48, 937–980.

Riley, W.D., Eagle, M.O., Ives, S.J., 2002. The onset of downstream movement of juvenile Atlantic salmon, *Salmo salar* L., in a chalk stream. Fish. Manag. Ecol. 9, 87–94.

Sangiao-Alvarellos, S., Polakof, S., Arjona, F.J., García-López, A., Martín del Río, M.P., Martínez-Rodríguez, G., Míguez, J.M., Mancera, J.M., Soengas, J.L., 2006. Influence of testosterone administration on osmoregulation and energy metabolism of gilthead sea bream *Sparus auratus*. Gen. Comp. Endocrinol. 149, 30–41.

Sigholt, T., Åsgård, T., Staurnes, M., 1998. Timing of parr-smolt transformation in Atlantic salmon (*Salmo salar*): effects of changes in temperature and photoperiod. Aquaculture 160, 129–144.

Solomon, D.J., 1978. Migration of smolts of Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) in a chalkstream. Environ. Biol. Fish. 3, 223–229.

Solomon, D.J., 1978. Some observations on salmon smolt migration in a chalkstream. J. Fish Biol. 12, 571–574.

Spigarelli, S.A., Thommes, M.M., Beitinger, L.T., 1977. The influence of body weight on heating and cooling of selected Lake Michigan fishes. Comp. Biochem. Physiol. A 56, 51–57.

Stevens, E.D., Fry, F.E.J., 1970. The rate of thermal exchange in a teleost, *Tilapia mossambica*. Can. J. Zool. 48, 221–226.

Thorpe, J.E., 1986. Age at first maturity in Atlantic salmon, *Salmo salar L.*: fresh water period influences and conflicts with smolting. Can. Spec. Pub. Fish. Aquat. Sci. 89, 7–14.

Yamauchi, K., Koide, N., Adachi, S., Nagahama, Y., 1984. Changes in seawater adaptability and blood thyroxine concentrations during smoltification of the masu salmon, *Oncorhynchus masou*, and the amago salmon, *Oncorhynchus rhodurus*. Aquaculture 42, 247–256.

Yamauchi, K., Ban, M., Kasahara, N., Izumi, T., Kojima, H., Harako, T., 1985. Physiological and behavioral changes occurring during smoltification in the masu salmon, *Oncorhynchus masou*. Aquaculture 45, 227–235.

Yu, T.C., Sinnhuber, R.O., Hendricks, J.D., 1979. Effect of steroid hormones on the growth of juvenile coho salmon (*Oncorhynchus kisutch*). Aquaculture 16, 351–359.