

Role of the cortisol on the onset of downstream migration in hatchery reared chum salmon *Oncorhynchus keta* fry

Daisuke OJIMA^{1,2}, Tatsuki YOSHINAGA¹, Masafumi HARADA¹, Munehico IWATA^{1*}

¹Laboratory of Ecophysiology, School of Fisheries Sciences, Kitasato University, Sanriku, Ofunato, Iwate 022–0101, Japan

*E-mail: muiwata@kitasato-u.ac.jp

²Present address: Norwegian College of Fishery Science, University of Tromsø N-9037 Tromsø, Norway

► Received 11 January 2007; Accepted 26 February 2007

Abstract—The relationship between downstream migration and plasma cortisol (F) level in chum salmon fry was investigated. The releases of chum fry (age 0⁺, BW: 0.8–1.8 g) were carried out twice either in a day and a night. Approximately a million individuals of chum fry were released from the hatchery raceways to a hatchery pool, and the fry schools spontaneously moved down from the pool to the hatchery creek. The fish were sampled in the raceways (initial: before the migration), in the pool (staying: preparing period for the onset of migration), and in the creek (migrating: immediately after onset of the migration). The plasma concentrations of the migrating fry were significantly higher than those of the staying fry at 1 h after the releases in both the day- and the night-release. In addition, the F levels of the staying and migrating fry in the night-released were significantly higher than those of the day-released. Results can be interpreted that the F secretion ability may active with higher stress sensitivity in night. Chum fry show the downstream migration in nighttime in the early migratory season. Thus, the cortisol can relate to the onset of the downstream migration in the chum salmon fry.

Key words: chum salmon, cortisol, downstream migration, *Oncorhynchus keta*

Introduction

Many salmonid species, including genera *Oncorhynchus*, *Salmo*, and *Salvelinus*, are anadromous, and their juveniles migrate down to the ocean (downstream migration) after smoltification that involves morphological, physiological, and behavioral changes. Many studies have been conducted on the endocrine system of the smoltification in salmonids, and cortisol (F), gonadal steroids, growth hormone (GH), insulin, prolactin, and thyroid hormones have been reported to play a role in the smoltification (reviewed by Hoar 1988, Boeuf 1993).

The thyroxine (T₄) is probably the most well studied hormone in the smoltification. Many authors have demonstrated that the plasma thyroxine levels increased during the smoltification in fresh water (Dickhoff et al. 1982, Yamauchi et al. 1984, Boeuf et al. 1989). During a late stage of the smoltification, that is downstream migratory season, a surge of plasma T₄ (T₄ surge) occurs, suggested to be induced by environmental stimuli such as lunar phase and changes in freshwater quality (Grau et al. 1981, Nishioka et al. 1985, Hoffnagle and Fivizzani 1990, Specker et al. 2000, Iwata et al. 2003). Indeed, the plasma T₄ level in the migrating fish was higher than that of the non-migrating one (Youngson and Simpson 1984, Fujioka et al. 1990, Høgåsen and Prunet 1997, Iwata et al. 2003). These well-observed phenomena

have led a hypothesis that the T₄ surge is the key factor for the onset of downstream migration (Iwata 1995). In our recent study, however, it was found that a treatment of the exogenous T₄ increased the plasma T₄ level in the chum salmon *O. keta* fry, but it did not yield the onset of downstream migratory behavior (Ojima and Iwata 2007 in press). This fact implies that the T₄ surge is not a sole factor responsible for initiating the migration, and other unknown factors may trigger the downstream migration.

In the natural river, changes in water quality by rainfall stimulated the downstream migration in masu salmon *O. masou* during migration season (Yamauchi et al. 1985, Fujioka et al. 1990). Further, a turbid water treatment in an experimental tank can trigger the onset of downstream behavior in Atlantic salmon *S. salar* and chum salmon fry (Specker et al. 2000, Iwata et al. 2003, Ojima and Iwata 2007 in press). These results suggest that the turbidity is the key environmental factor for the onset of downstream migration in salmonids. On the other hand, the environmental stimulus such as turbidity is considered to be as a stress for salmonid juveniles (Newcombe and MacDonald 1991). Generally, the stress response involves behavioral and physiological changes in teleosts for avoidance and adaptation against the various stressors (Schreck et al. 1997). Thus, the environmental stressors can affect the onset of downstream migration through the stress response mechanism.

The cortisol is a representative stress hormone that regu-

lates physiological stress response (Schreck et al. 1997). The cortisol also has an important role in the smoltification and downstream migration in salmonids. The plasma cortisol level increased as well as the T_4 during the migratory season (Boeuf 1993). The cortisol cooperates with the growth hormone (GH), and activates a seawater-adaptability by increasing a gill Na^+ , K^+ -ATPase activity (McCormick 1995). The migrating fish have higher gill Na^+ , K^+ -ATPase activity than that of the staying one (Hart et al. 1981, Zaugg 1981, Ewing and Rodgers 1998, McCormick and Björnsson 1994). Further, the cortisol-treated coho salmon *O. kisutch* stimulated seawater preference (Iwata et al. 1990). These findings show that the cortisol may play an important role in the downstream migration, as well as smoltification.

Based on the above-mentioned observations, we hypothesized that the cortisol can trigger the downstream migration in the chum salmon fry. We released the chum fry from hatchery raceways to a pool in either a day or a night, and compared the plasma cortisol levels between the staying and migrating chum fry.

Materials and methods

Fish and Study sites

Chum salmon fry, derived from artificial fertilization, were reared for 3 months after hatching at the Otsuchi Salmon Hatchery, Iwate Prefecture, Japan. The fry were maintained under a natural photoperiod and were fed a commercial diet after yolk absorption in the hatchery raceways. The study site (Fig. 1) comprised raceways, pools, and creek at the Otsuchi Salmon Hatchery. The hatchery was located at the upper end of a tributary of the Otsuchi River. Waters at

the Otsuchi Salmon Hatchery are supplied from wells and springs at the 3 ha hatchery site. All the waters flow into the creek, and springs were present at the sandy bottoms of the pool and the creek. The water temperature at the site was stable, in a range of 11–12°C, during the experiments.

Release and collecting samples

Releases of the fry were carried out twice either in a day or a night. Approximately a million individuals of the chum fry were released from the hatchery raceways by opening gates at 18:00 on 16 March, 2005 (night release; body weight, BW, 1.2 ± 0.05 g; mean \pm SD), and at 10:00 on 25 March, 2005 (day release; BW, 1.4 ± 0.05 g). The fry were flushed out from the raceways and subsequently discharged into the hatchery pool through a drain channel (Fig. 1). The fry schools spontaneously moved from the pool to the hatchery creek and reached the Otsuchi River. The distance from the hatchery to the estuary is approximately 1700 m. The released fry were experienced fall stimulus three times: at the raceway to drain channel (100 cm high), at the drain channel to the pool (50 cm high), and at the pool to the creek (60 cm high). Sunrise and sunset time were 05:43 and 17:40 on 16 March, and 05:30 and 17:49 on 25 March, respectively. The new moon might affect downstream migration in salmonids (Grau et al. 1981), but the moon phases were crescent on 16 March and full moon on 25 March.

Blood sampling

We defined the behavior moving over the dam down to the hatchery creek as the onset of the downstream migration behavior. A group of 40–50 individuals of the chum fry were caught by a net at the hatchery raceway (initial; 30 min before the gate opening), at the pool (St. 1, staying fry at 10

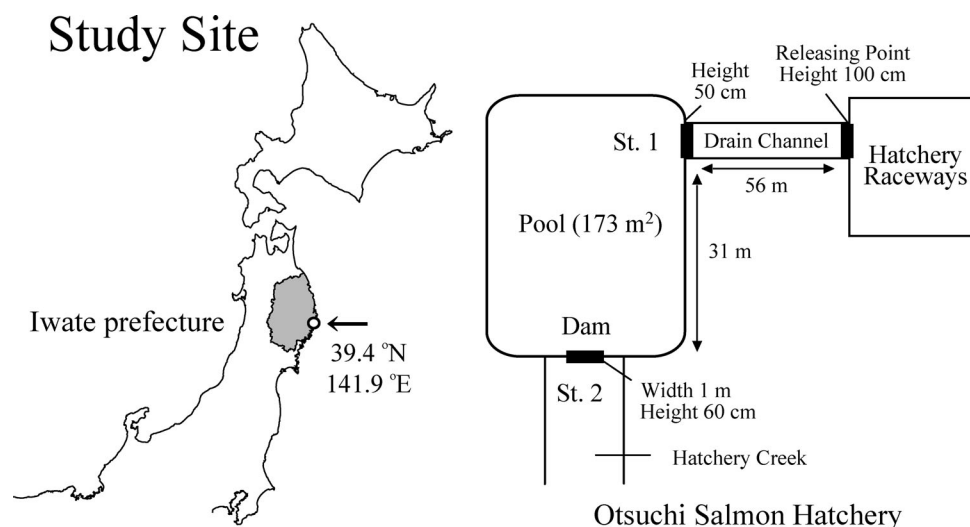


Fig. 1. This study was carried out at the Otsuchi Salmon Hatchery Iwate, Japan (left panel). Chum salmon fry was released from hatchery raceways through the drain channel to a hatchery pool (right panel). The fry formed schools in the pool and migrated across the concrete dam to a hatchery creek. Staying fry in the pool for school formation were collected at Station 1. Migrating fry were sampled at St. 2 in the creek.

min, 1 h, and 3 h after the release), and at the creek (St. 2, migrating fry at 1 h and 3 h after the release). The migrating fry were caught within a few seconds in the creek after falling down from the dam. The fry were immediately transferred into a 15-L bucket containing 0.04% v/v 2-phenoxyethanol as an anesthetic. The caudal peduncle was amputated, and blood sample was collected in a heparinized capillary tube. Each blood sampling was completed within 3 min to prevent the cortisol secretion by handling (Sumpter et al. 1986). The capillaries were centrifuged at 12000 *g* for 10 min after sealing. The 20- μ l blood plasma samples were pooled from two or three individuals, and transferred to a micro test tube and stored at -35°C until analysis. Plasma cortisol concentration was determined by a non-isotopic time-resolved fluoroimmunoassay (TR-FIA) according to Yamada et al. (2002).

Statistics

Statistical analyses were conducted for (1) time course changes in the plasma cortisol concentrations after the release, and (2) a comparison of the F concentrations between the staying and the migrating fry, and between the day and the night-released groups. Prior to the statistical tests, the Kolmogorov-Smirnov test of distributions was applied to exclude extremely high and low values of the F concentrations in each group. Statistically significant differences among the means of F concentration were analyzed by a one-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni test. The comparison of the F concentrations between the migrating and staying fry, and the day and night-released fry were carried out by the Mann-Whitney U test. All statistical analyses were computed by a SPSS 13.0J (SPSS).

Results

Cortisol levels in the staying and migrating fry

In the day-released group, the plasma cortisol concentrations increased significantly in both the staying fry in the pool and the migrating fry at the creek after the release (Fig. 2, $P < 0.05$). The F concentrations did not change from 1 to 3 h after the release. The F concentration of the migrating fry was significantly higher than that of the staying one at 1 h after the release ($P = 0.001$).

In the night-released group, the plasma F concentrations increased significantly in both the staying and migrating fry after the release (Fig. 2, $P < 0.05$). The F concentration of the migrating fry was significantly higher than that of the staying one at 1 h after the release ($P = 0.011$).

Cortisol levels in the day- and night-released groups

The plasma F levels of the chum fry at the raceway did not differ between the fry groups sampled in the day and night (Fig. 2). In the staying fry in the pool, the plasma F lev-

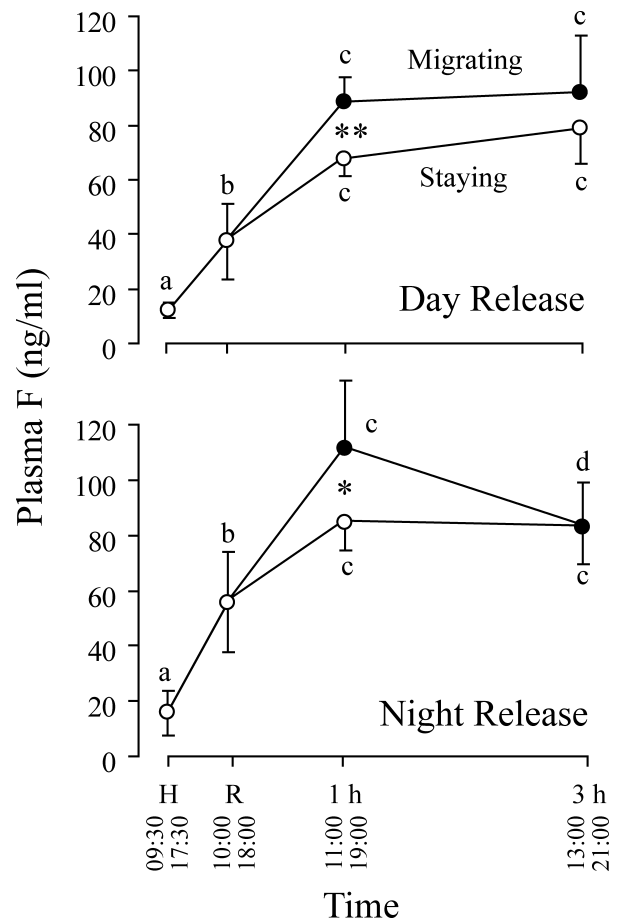


Fig. 2. The changes in plasma cortisol (F) concentrations in the staying and migrating chum salmon fry after the releases. The fry were released at 10:00 (day release) and 18:00 (night release). Plasma samples were collected at the raceway, the pool (St. 1), and the creek (St. 2). "H" and "R" show the fry before the release in the hatchery raceway, and just after the release (after 10 min) in the pool, respectively (Fig. 1). The plasma F concentrations are means \pm SD ($n = 9-10$). Asterisks indicate a significant difference between the staying and migrating fry at the coincident time after the release (* $P < 0.05$, ** $P < 0.01$). Different letters on the plots show the significant difference in the time-course change of the F concentration in each group ($P < 0.05$).

els of the night-released fry just after and 1 h after the release (56.0 and 84.8 ng/ml) were significantly higher than the corresponding samples of the day-released (37.1 and 67.6 ng/ml) ($P = 0.028$ and 0.001 , respectively). Similarly, in the migrating fry, the F of the night-released fry (111.6 ng/ml) was significantly higher than that of the day-released one (88.1 ng/ml) at 1 h after the release ($P = 0.014$). The F concentrations in the day-released migrating fry did not change from 1 to 3 h, while it decreased significantly in the night-released migrating one ($P < 0.05$).

Discussion

The most significant finding of this study is that the

plasma F concentration of the migrating chum salmon fry at the creek is significantly higher than that of the staying fry in the pool at 1 h after the release either in the day- and the night-release (Fig. 2). This result suggests that the chum fry with high plasma F level showed an active downstream migration. After the release, both the staying and migrating fry were exposed to various environmental changes such as fall-stimuli, increasing velocity, and turbidity of water. The migrating fry were exposed to the fall-stimulus at the dam in addition to the stimuli that the staying fry underwent. Therefore, it seems that the fall-stimulus at the dam might affect to increase the plasma F in the migrating fry. However, because the migrating fry were caught at the creek within a few seconds after the fall from the dam, the fall stimulus at the dam did not affect the plasma F levels in the migrating fry. Accordingly, it is suggested that the migrating fry at 1 h after the release had high secretion ability of F. On the other hand, at 3 h after the release, the plasma F concentrations between the migrating and staying fry were at the same levels (Fig. 2). In addition, the plasma F concentrations did not increase from 1 to 3 h in both the day and night release (Fig. 2). These results indicate that the F secretion was inactivated by a negative feedback system at 3 h after the release. In teleosts, it is known that the F secretion initiates in seconds to minutes after the stimuli, and recovers in minutes to hours by the negative feedback (Schreck et al. 1997). Thus, the negative feedback might inactivate the F secretion at 3 h after the release, resulting as the similar F levels between the migrating and the staying fry.

The role of cortisol may be maintenance of the high seawater adaptability and preference, and further activates the onset of downstream migration. The cortisol activates the seawater adaptability by increasing the gill Na^+ , K^+ -ATPase activity (McCormick 1995), and seawater preference (Iwata et al. 1990). In salmonid juveniles, the gill Na^+ , K^+ -ATPase activity of the migrating fish is higher than that of the non-migrating one (Ewing and Rodgers 1998, McCormick and Björnsson 1994). Therefore, in the present study, the fry with high secretion of F is likely to initiate downstream migration after acquiring the high seawater adaptability and preference. On the other hand, a neurohormone that initiates the F secretion may be related to the downstream migration in the fry. Corticotropin-releasing hormone (CRH) is the initiating hormone in response to the stress by the hypothalamic-pituitary-interrenal (HPI) axis. It stimulates secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, thereby regulating the peripheral responses to stress. The CRH is also involved in the control system of many kinds of behavior, particularly those that are expressed as the stress response (Butler et al. 1990, Takahashi et al. 1989, To et al. 1999). The CRH can alter the downstream movement of juvenile Chinook salmon *O. tshawytscha* in a simulated stream environment (Clements and Schreck 2004). Moreover, the CRH

caused locomotor activity in a dose-dependent manner in Chinook salmon (Clements et al. 2002). In the present study, the plasma F level of the migrating fry was higher than that of the staying one. Therefore, in the migrating fry the CRH might be secreted actively and achieved downstream movement as a result.

At just after and 1 h after the release, the plasma F concentrations of the night-released fry were significantly higher than that of the day-released one in both the staying and migrating fry (Fig. 2). The circadian rhythms of the plasma F level in some salmonids have been observed with the peak in nighttimes (Thorpe et al. 1987, Laidley and Leatherland 1988, Yamada et al. 2002). Thus, in the nighttime, the secretion ability of F in the fry can be more active than the daytime. The rapid decrease of plasma F level of the night-released migrating fry after 1 h may also reflect higher metabolism in cortisol in nighttime. In the early migratory season, chum salmon and other salmonids show downstream migration in nighttime (Hoar 1951, Neave 1955, Aarestrup et al. 2002, Riley et al. 2002, Carlsen et al. 2004), while their migrations in the late season occurs both in day and night (Moore et al. 1995, Iwata et al. 2003). The circadian rhythm of the secretion ability of F may relate to the downstream migration in nighttime.

In conclusion, the present study demonstrates that the migrating chum fry with high secretion ability of F showed active downstream migration. The active F secretion enables the migrating fry to maintain high level of seawater adaptability and preference, and have active CRH secretion in their brain. Consequently, the migrating fry might show active downstream migration. Moreover, because the F secretion has the circadian rhythm with the peak at night, the cortisol may relate to downstream migration in the nighttime.

Acknowledgements

The authors thank Mr. T. Sasaki, and other staffs of the Otsuchi Salmon Hatchery for their helpful assistance of this study. Thanks for technical assistance are also due to Mr. S. Abe. The authors also thank Drs. H. Chiba, H. Yamada and Y. Fujimoto for valuable comments and suggestions.

References

- Aarestrup, K., Nielsen, C. and Koed, A. 2002. Net ground speed of downstream migrating radio-tagged Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) smolts in relation to environmental factors. *Hydrobiologia* 483: 95–102.
- Boeuf, G. 1993. Salmonid smolting: a pre-adaptation to the oceanic environment. *In* Fish Ecophysiology. Rankin, J.C. and Jensen, F. B. (Eds.), pp. 105–135, Chapman & Hall, Tokyo.
- Boeuf, G., Le Bail, P.Y. and Prunet, P. 1989. Growth hormone and thyroid hormones during Atlantic salmon *Salmo salar* L., smolting and after transfer to seawater. *Aquaculture* 82: 257–68.
- Butler, P. D., Weiss, J. M., Stout, J. C. and Nemeroff, C. B. 1990.

- Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J. Neurosci.* 10: 176–183.
- Carlsen, K. T., Berg, O. K., Finstad, B. and Heggberget, T. G. 2004. Diel periodicity and environmental influence on the smolt migration of Arctic charr, *Salvelinus alpinus*, Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, in northern Norway. *Environ. Biol. Fish.* 70: 403–413.
- Clements, S. and Schreck, C. B. 2004. Central administration of corticotropin-releasing hormone alters downstream movement in an artificial stream in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Gen. Comp. Endocrinol.* 137: 1–8.
- Clements, S., Schreck, C. B., Larsen, D. A. and Dickhoff, W. W. 2002. Central administration of corticotropin-releasing hormone stimulates locomotor activity in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Gen. Comp. Endocrinol.* 125: 319–327.
- Dickhoff, W. W., Folmar, L. C., Mighell, J. L. and Mahnken, C. V. W. 1982. Plasma thyroid hormones during smoltification of yearling and underyearling coho salmon and yearling chinook salmon and steelhead trout. *Aquaculture* 28: 39–48.
- Ewing, R. D. and Rodgers, J. D. 1998. Changes in physiological indices of smolting during seaward migration of wild coho salmon, *Oncorhynchus kisutch*. *Aquaculture* 168: 69–83.
- Fujioka, Y., Fushiki, S., Tagawa, M., Ogasawara, T. and Hirano, T. 1990. Downstream migratory behavior and plasma thyroxine levels of Biwa salmon *Oncorhynchus rhodurus*. *Nippon Suisan Gakkaishi* 56: 1773–1779.
- Grau, E. G., Dickhoff, W. W., Nishioka, R. S., Bern, H. A. and Folmar, L. C. 1981. Lunar phasing of the thyroxine surge preparatory to seaward migration of salmonid fish. *Science* 211: 607–609.
- Hart, C. E., Concannon, G., Fustish, C. A. and Ewing, R. D. 1981. Seaward migration and gill Na⁺, K⁺-ATPase activity of spring chinook salmon in an artificial stream. *Trans. Am. Fish. Soc.* 110: 44–50.
- Hoar, W. S. 1951. The behaviour of chum, pink and coho salmon in relation to their seaward migration. *J. Fish. Res. Bd. Can.* 8: 241–263.
- Hoar, W. S. 1988. The Physiology of smolting salmonids. *In* *Fish Physiology*, vol. XIB. Hoar, W.S. and Randall, D.J. (Eds.), pp. 275–343, Academic Press, Tokyo.
- Hoffnagle, T. L. and Fivizzani, A. J. 1990. Stimulation of plasma thyroxine levels by novel water chemistry during smoltification in Chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 47: 1513–1517.
- Høgåsen, H. R. and Prunet, P. 1997. Plasma levels of thyroxine, prolactin, and cortisol in migrating and resident wild Arctic char, *Salvelinus alpinus*. *Can. J. Fish. Aquat. Sci.* 54: 2947–2954.
- Iwata M. 1995. Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: A review. *Aquaculture* 135: 131–139.
- Iwata, M., Yamauchi, K., Nishioka, R. S., Lin, R. and Bern, H. A. 1990. Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). *Mar. Behav. Physiol.* 17: 191–201.
- Iwata, M., Tsuboi, H., Yamashita, T., Amemiya, A., Yamada, H. and Chiba, H. 2003. Function and trigger of thyroxine surge in migrating chum salmon *Oncorhynchus keta* fry. *Aquaculture* 222: 315–329.
- Laidley, C. W. and Leatherland, J. F. 1988. Circadian studies of plasma cortisol, thyroid hormone, protein, glucose and ion concentration, liver glycogen concentration and liver and spleen weight in rainbow trout, *Salmo gairdneri* Richardson. *Comp. Biochem. Physiol.* 89A: 495–503.
- McCormick, S. D. 1995. Hormonal control of gill Na⁺, K⁺-ATPase and chloride cell function. *In* *Cellular and molecular approaches to fish ionic regulation*. Wood, C.M. and Shuttleworth, T. J. (Eds.), pp. 285–315, Academic Press, San Diego.
- McCormick, S. D. and Björnsson, B. Th. 1994. Physiological and hormonal differences among Atlantic salmon parr and smolts reared in the wild, and hatchery smolts. *Aquaculture* 121: 235–244.
- Moore, A., Potter, E. C. E., Milner, N. J. and Bamber, S. 1995. The migratory behaviour of wild Atlantic salmon (*Salmo salar*) smolts in the estuary of the River Conwy, North Wales. *Can. J. Fish. Aquat. Sci.* 52: 1923–1935.
- Neave, F. 1955. Notes on the seaward migration of pink and chum salmon fry. *J. Fish. Res. Bd. Can.* 12: 369–374.
- Newcombe, C. P. and MacDonald, D. D. 1991. Effects of suspended sediments on aquatic ecosystems. *N. Am. J. Fish. Manage.* 11: 72–82.
- Nishioka, R. S., Young, G., Bern, H. A., Jochimsen, W. and Hiser, C. 1985. Attempts to intensify the thyroxine surge in coho and king salmon by chemical stimulation. *Aquaculture* 45: 215–225.
- Ojima, D. and Iwata, M. The relationship between thyroxine surge and onset of downstream migration in chum salmon *Oncorhynchus keta* fry. *Aquaculture* (2007 in press).
- Riley, W. D., Eagle, M. O. and Ives, S. J. 2002. The onset of downstream movement of juvenile Atlantic salmon, *Salmo salar* L., in a chalk stream. *Fish. Manage. Ecol.* 9: 87–94.
- Schreck, C. B., Olla, B. L. and Davis, M. W. 1997. Behavioral responses to stress. *In* *Fish stress and health in aquaculture*. Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schreck, C.B. (Eds.), pp. 145–170, Cambridge University Press, Cambridge.
- Specker, J. L., Eales, J. G., Tagawa, M. and Tyler III, W. A. 2000. Parr-smolt transformation in Atlantic salmon: thyroid hormone deiodination in liver and brain and endocrine correlates of change in rheotactic behavior. *Can. J. Zool.* 78: 696–705.
- Sumpter, J. P., Dye, H. M. and Benfey, T. J. 1986. The effects of stress on plasma ACTH, α -MSH, and cortisol levels in salmonid fishes. *Gen. Comp. Endocrinol.* 62: 377–385.
- Takahashi, L. K., Kalin, N. H., Vanden Burgt, J. A. and Sherman, J. E. 1989. Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. *Behav. Neurosci.* 103: 648–654.
- Thorpe, J. E., McConway, M. G., Miles, M. S. and Muir, J. S. 1987. Diel and seasonal changes in resting plasma cortisol levels in juvenile Atlantic salmon, *Salmo salar* L. *Gen. Comp. Endocrinol.* 65: 19–22.
- To, C. T., Anheuer, Z. E. and Bagdy, G. 1999. Effects of acute and chronic treatment of CRH-induced anxiety. *Neuroreport* 10: 553–555.
- Yamada, H., Satoh R., Ogoh, M., Takaji, K., Fujimoto, Y., Hakuba, T., Chiba, H., Kambegawa, A. and Iwata, M. 2002. Circadian

- changes in serum concentrations of steroids in Japanese char *Salvelinus leucomaenis* at the stage of final maturation. Zool. Sci. 19: 891–898.
- Yamauchi, K., Koide, N., Adachi, S. and 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. and Harako, T. 1985. Physiological and behavioral changes occur-
ring during smoltification in the masu salmon, *Oncorhynchus masou*. Aquaculture 45: 227–235.
- Youngson, A. F. and Simpson, T. H. 1984. Changes in serum thyroxine levels during smolting in captive and wild Atlantic salmon, *Salmo salar* L. J. Fish Biol. 24: 29–39.
- Zaugg, W. S. 1981. Advanced photoperiod and water temperature effects on gill $\text{Na}^+\text{-K}^+$ -adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 38: 758–764.