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Physiological and hormonal differences among Atlantic salmon parr and smolts reared in the wild, and hatchery smolts

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Abstract

Atlantic salmon which had been released as fry in tributaries of the Connecticut River (Northeastern USA) were captured during the migratory period (9 May to 6 June) 1 to 2 years later. Migrants (smolts) were captured above a dam on the main-stem; non-migrants (parr) were captured in two tributaries. Migrants were significantly larger than non-migrants (16.9 and 13.0 cm, respectively), but had a 20% lower condition factor. Relative to non-migrants, migrants had significantly higher gill Na^+,K^+-ATPase activity (5-fold), plasma glucose (1.8-fold), plasma thyroxine (8-fold), plasma cortisol (5-fold) and plasma growth hormone (100-fold). There were no significant differences in plasma $[Na^+]$, $[K^+]$ and $[Ca^{2+}]$ between migrants and non-migrants. Most of the differences seen between non-migrants and migrants were similar to those that occurred seasonally in a captive (hatchery) population of the same stock of Atlantic salmon. However, hatchery smolts had lower levels of plasma thyroxine and exhibited no significant change in plasma growth hormone from February to June. The large differences between migrants and non-migrants may be related to both the parr–smolt transformation and migration.

1. Introduction

Almost all of our knowledge of the physiology of the parr–smolt transformation is derived from laboratory or hatchery-reared populations. These studies have permitted sufficient access to fish and control over the rearing environment to

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unravel some of the most important endocrine and physiological changes that occur during this period (McCormick and Saunders, 1987; Hoar, 1988). However, the ability of environmental change to strongly influence the timing and intensity of changes during the parr–smolt transformation (Wedemeyer et al., 1980) indicates that significant differences may exist between wild and hatchery-reared populations of anadromous salmonids.

Only a few studies have examined the physiology of wild migratory smolts (Hoar, 1939; Power, 1959; Chernitsky, 1980, 1986; Youngson and Simpson, 1984; McCormick et al., 1985; Ackman and Takeuchi, 1986; Cunjak et al., 1990; Varnavsky et al., 1992), and only a few physiological parameters have been examined to date. Our purpose in this study was to determine whether physiological and endocrine changes that occur in captive populations also occur under natural (wild) conditions, and to search for potential differences between fish reared in captivity and in the wild. To accomplish this, Atlantic salmon juveniles that had been released as fry were captured as migrants (smolts) and non-migrants (parr), and compared to each other and to the same stock of fish reared in a hatchery.

2. Materials and methods

2.1. Hatchery sampling

Fish in this study are part of a restoration program for Atlantic salmon on the Connecticut River, where native Atlantic salmon were extinguished by dam construction over 150 years ago. Fish are of Penobscot River (Maine, USA) origin but for the last seven generations have been the progeny of adults (2–3-sea-winter fish) returning to the Connecticut River. Fish were reared from eggs to smolts in 1 year at the White River National Fish Hatchery in Bethel, VT (USA) using standard hatchery practices. Water used for rearing was a mixture of filtered water of the White River, and well water. Temperature fluctuated seasonally and ranged from 3 to 20°C.

Fish were sampled on 17 February, 10 and 24 March, 14 and 28 April, and 12 and 25 May between 09.00 and 11.00 h Eastern Standard Time. Fish were starved for 16 h prior to sampling. Temperatures on these dates were 3, 3, 3, 4, 9, 10 and 15°C, respectively. Fish were crowded into a small area of the pond with a blocking net to facilitate capture with a dip net. After anesthesia in 100 mg/l MS-222, length (snout to fork of tail) and weight were measured and blood was collected from the caudal vessels into heparinized syringes. Blood was centrifuged at 5000 *g* for 5 min, plasma separated and frozen at −80°C. Severed gill filaments were placed in 100 μl, ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at −80°C.

2.2. Sampling of fish reared in the wild

The progeny of sea-run adults returning to the Connecticut River (stock described above) have been released into numerous tributaries of the Connecticut

River as non-feeding fry. These fish reside in the streams for 2–3 years prior to smolt migration (Ted Meyers, personal communication). Non-migrants (1- or 2-year-old parr) in this study were captured by electrofishing in Salmon Brook (a tributary of the Farmington River, CT) on 31 May (10.00 h, 16°C, $n=9$) and Westfield Brook (a tributary of the Westfield River, MA) on 6 June (11.00 h, 13°C, $n=8$). Upon capture, fish were immediately placed in anaesthetic and sampled as above. Migrants (2- or 3-year-old smolt) were captured by fly-rod catching at Cabot Station (a dam on the Connecticut River at Turners Falls, MA, 125 river miles from Long Island Sound) on 9 May (14.00 h, 13°C, $n=10$) and 14 May (09.00 h, 13.5°C, $n=10$). Migrants were observed at this site from 2 May through 8 June; migrant smolts have not been observed at this site at other times of the year. Fish that were angled were "landed" and placed in anesthetic within 30 seconds of hooking. In all cases care was taken to minimize the time between the initiation of capture (hooking or electroshocking) and blood removal, which did not exceed 3 min. Previous studies of unaesthetized salmonids indicate that changes in plasma cortisol are not detectable for the first 5 min following a severe handling stress (Sumpter et al., 1986). Gill and plasma samples were immediately placed on dry ice and subsequently stored at −80°C.

2.3. Analytical methods

Gill Na^+,K^+-ATPase activity was measured by the method outlined in McCormick (1993). Radioimmunoassay was used to measure growth hormone (Bolton et al., 1986 as modified by Björnsson et al., 1994), thyroxine (Dickhoff et al., 1978 as modified by Specker et al., 1989) and cortisol (Bisbal and Specker, 1991). Plasma glucose was measured by the hexokinase enzymatic method (Stein, 1963). Plasma sodium, potassium and calcium concentrations (free ions) were measured by ion-selective electrodes (AVL Scientific Corp., Roswell, GA, USA). Condition factor was calculated as $((\text{weight})/(\text{length})^3)\cdot 100$, with weight in g and length (fork length) in cm.

Morphological and physiological changes over time in hatchery fish were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test (initial sample, 17 February, as control; $P<0.05$). Comparisons of the three groups (migrant and non-migrants in the wild, and hatchery smolts) were carried out by the non-parametric Kruskal–Wallis test; if significant differences occurred ($P<0.05$) then pair-wise comparisons were made using the Mann–Whitney test. Statistical analyses were made using statistical software CRUNCH 4.0 (Oakland, CA).

3. Results

3.1. Hatchery fish

Length and weight remained relatively constant from February through mid-May (Table 1). The apparent decrease in length and weight (not statistically

Table 1
Changes in physiology of juvenile Atlantic salmon from White River National Fish Hatchery. Ten fish were sampled at each sampling period except the last when 8 fish were sampled. Asterisk indicates a significant difference from the first sampling period

	17 Feb	10 Mar	24 Mar	14 Apr	28 Apr	12 May	28 May
Length (cm)	19.8±0.4	20.1±0.4	19.9±0.4	20.4±0.3	20.5±0.3	21.2±0.3	18.6±0.9
Weight (g)	86.4±6.1	90.8±5.2	90.0±6.5	91.6±4.3	92.8±4.5	94.4±3.7	70.6±7.3
Condition Factor	1.10±0.02	1.11±0.01	1.11±0.02	1.08±0.02	1.12±0.02	0.99*±0.02	1.10±0.06
Gill Na^+,K^+-ATPase	3.7±0.5	8.7*±0.7	9.8*±0.7	12.8*±0.6	13.0*±1.3	15.2*±1.6	6.4±0.5
Plasma thyroxine (ng/ml)	14.6±1.0	18.4±1.5	13.5±1.8	21.6±4.2	22.5±1.7	30.2*±3.2	20.1±5.1
Plasma cortisol (ng/ml)	55.7±5.4	62.5±2.8	56.2±3.2	52.9±4.7	68.4±4.8	95.0*±8.7	99.4*±8.1
Plasma growth hormone (ng/ml)	0.4±0.08	0.6±0.22	0.5±0.07	0.4±0.04	0.7±0.08	0.7±0.05	1.1*±0.31
Plasma glucose (mM)	7.2±0.5	5.6±0.3	7.2±0.4	6.6±0.4	7.1±0.2	6.7±0.5	6.9±0.6
Plasma Na^+ (mM)	162.8±1.5	168.7±2.2	161.7±1.1	163.4±1.5	162.4±1.5	163.0±2.6	153.9*±3.4
Plasma K^+	3.0±0.8	1.9±0.3	2.0±0.2	1.7±0.2	2.7±0.2	2.5±0.3	5.4*±0.4
Plasma Ca^{2+}	1.52±0.06	1.75*±0.04	1.57±0.03	1.66±0.03	1.66±0.02	1.64±0.03	1.45±0.06

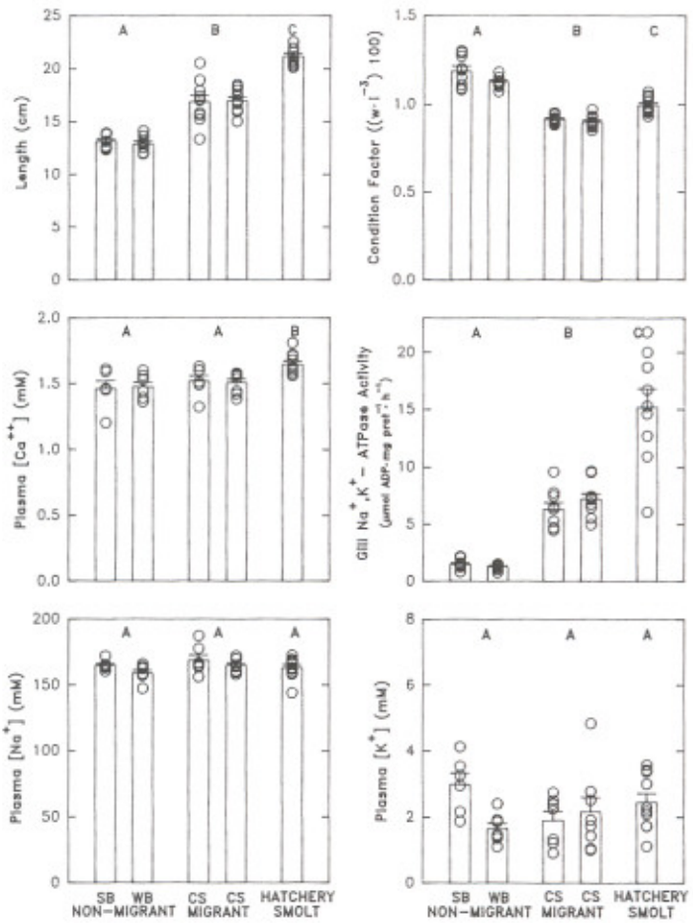

Fig. 1. Length, condition factor, gill Na^+,K^+-ATPase activity and plasma ions of juvenile Atlantic salmon. Non-migrants (parr) reared in the wild were sampled by electrofishing on 31 May and 6 June on Salmon Brook (SB, $n=9$) and Westfield Brook (WB, $n=8$), respectively. Migrants (smolts) reared in the wild were captured by angling on 9 May ($n=10$) and 14 May ($n=10$) at Cabot Station (Turners Falls, MA) on the Connecticut River. Hatchery smolts were sampled at White River National Fish Hatchery on 12 May ($n=10$; see Table 1). Bars are mean ± standard error; circles are values for individual fish. Different letters above histograms indicate significant differences between groups.

significant) in late May was probably an artefact of sub-sampling a large population. Condition factor of hatchery fish remained constant from February through April, then decreased significantly (10%) in mid-May.

Gill Na^+,K^+-ATPase activity increased throughout the sampling period from a low of 3.7 μmol ADP·mg prot^{-1}·h^{-1} in mid-February to a peak of 15.2 μmol ADP·mg prot^{-1}·h^{-1} in mid-May, a 4-fold increase over the initial levels (Table 1). Mean plasma thyroxine values ranged between 13 and 18 ng/ml in February and March. In April plasma thyroxine was more variable and rose slightly to a

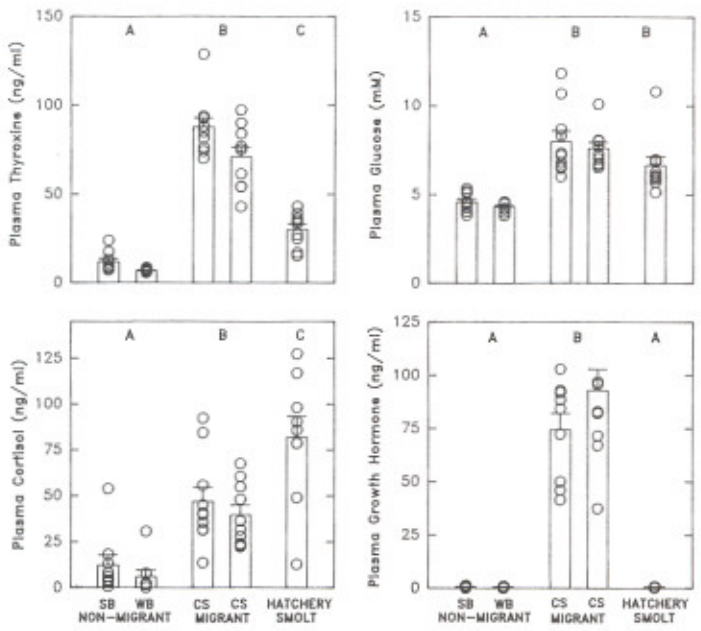

Fig. 2. Plasma thyroxine, cortisol, growth hormone and glucose of juvenile Atlantic salmon. See Fig. 1 legend and text for details.

peak value of 30 ng/ml in mid-May (a significant increase over February levels). Plasma growth hormone was low in February (mean: 0.4 mg/ml) and remained low throughout the study, although a small increase occurred at the end of May (mean: 1.1 ng/ml, $P<0.05$).

Because the fish were slightly disturbed by the process of crowding used to capture the fish 10–15 min prior to sampling, the reported levels of plasma cortisol probably do not reflect basal levels but rather a response to a mild stressor. The levels remained constant from February to mid-April, and then increased significantly in May.

Mean plasma glucose ranged between 5.6 and 7.2 mM throughout the study and did not vary significantly. Plasma sodium and potassium concentrations remained constant from February through mid-May, but decreased and increased, respectively, at the last sampling in late May. Plasma calcium (free ion concentration) increased slightly but significantly in mid-March, but otherwise remained constant.

Hatchery smolts sampled on 12 May were chosen for statistical comparison to fish reared in the wild. This date reflects the closest time and temperature to the field sampling of migrants reared in the wild, and the date at which gill Na^+,K^+-ATPase activity and plasma thyroxine peaked.

3.2. Fish reared in the wild

There was no significant difference in any of the measured parameters between non-migrants collected at two different sites, or between migrants on two different dates. Non-migrants were easily distinguished from migrants by their smaller size, clearly visible parr marks, low silvering and absence of darkened fin margins. Migrants were slimmer than non-migrants (as evidenced by their lower condition factor, Fig. 1), brightly silvered and had dark fin margins; parr marks were visible in only one individual.

Gill Na^+,K^+-ATPase activity ranged from 0.8 to 2.2 μmol ADP·mg prot^{-1}·h^{-1} in non-migrants, and from 4.5 to 9.6 μmol ADP·mg prot^{-1}·h^{-1} in migrants (Fig. 1). Mean gill Na^+,K^+-ATPase activity activity was 5-fold higher in migrants relative to non-migrants. Plasma sodium, potassium and calcium were not significantly different between migrants and non-migrants.

Plasma thyroxine, cortisol and growth hormone were substantially higher in migrants than in non-migrants, being 8-, 5-, and 100-fold higher, respectively (Fig. 2). There was no overlap in the values for plasma thyroxine and growth hormone between the two groups. Plasma glucose was 75% higher in migrants than non-migrants (Fig. 2).

4. Discussion

Fish released as fry into the wild were the progeny of sea-run fish, and have spent 1 to 3 years in streams prior to recapture in the present study. Because these fish have spent most of their lives in the wild, it is assumed that most of their behavior and physiology will be the same as wild fish (which are not currently present in the Connecticut River and its tributaries). Our initial assumption in this study was that fish captured in the main stem would be seaward-migrating smolts, and that those captured in the tributaries immediately after the migratory period would be non-migratory parr. This assumption was verified by the clear differences in size and appearance of fish captured at these different sites. The observed differences between hatchery smolts and smolts in the wild may be a combination of the different rearing environments (artificial versus natural) and the absence of an active migration in the captive, hatchery smolts. The differences are unlikely to be due solely to the larger size of the hatchery fish, as we have observed no significant difference in physiology of large (20–25 cm) and small (15–20 cm) hatchery fish (McCormick, personal observation).

The clear and in some cases quite large differences between migratory smolts and non-migratory parr indicate that the physiological and endocrine changes found in many captive populations of smolting Atlantic salmon also occur in fish reared in the wild. Several of these parameters, such as growth hormone and cortisol, have not previously been measured in wild smolts in fresh water. The higher gill Na^+,K^+-ATPase activity in migrants relative to non-migrants in this study confirms the results of Cunjak et al. (1990) in which parr captured by electro-

fishing had low levels from late-May to September, and migrants had elevated levels (10-fold higher) in late May and early June.

Hoar's seminal work on changes in thyroid activity during smolting involved the collection of wild Atlantic salmon parr and smolts (Hoar, 1939). The thyroid has subsequently been implicated in the regulation of several aspects of smolting, and while plasma thyroxine increases in spring in smolts, its physiological role is still uncertain (Hoar, 1988). Our findings of higher plasma thyroxine in migrants confirms the study of Youngson and Simpson (1984) in which the high levels of plasma thyroxine in wild Atlantic salmon migrants were correlated with increased stream flows.

There was a remarkable 100-fold difference in plasma growth hormone between parr and smolt reared in the wild. This is particularly interesting in light of the absence of significant changes in plasma growth hormone in hatchery fish. Growth hormone can stimulate gill Na^+,K^+-ATPase activity in salmonids, increases in spring in plasma of many salmonids, and is thought to be involved in the increased seawater adaptability of smolts (Sakamoto et al., 1993). The absence of changes in plasma growth hormone in hatchery fish in the present study (even though gill Na^+,K^+-ATPase activity increased 4-fold) is not readily explicable, though it may relate to changes in clearance rate and tissue responsiveness (e.g. receptors) in this group of fish. It should be noted that the hatchery fish in this study are capable of rapid and substantial increases in plasma growth hormone; fish that were placed in a floating net pen in the Connecticut River exhibited increases in plasma growth hormone when the net was towed downstream (S.D. McCormick and B.Th. Björnsson, unpublished observation). The higher plasma growth hormone levels in migrants reared in the wild relative to both non-migrants and hatchery smolts may be due in part to active migration. Sustained exercise (due to a challenge with increased flow) has been shown to result in large and rapid increases in plasma growth hormone (Barrett and McKeown, 1989) in salmonids. The increases in plasma growth hormone may in part be related to increased "exercise" during migration, although the difference in activity of wild parr and smolts has yet to be examined. Clearly the regulation of growth hormone levels in smolts requires further investigation.

Plasma ions did not differ between migrants and non-migrants reared in the wild, providing no evidence that osmoregulatory perturbations are important in initiating or maintaining migration. However, other ionoregulatory parameters such as extracellular volume and intracellular ions have yet to be investigated. The hyperglycemia of smolts reared in the wild may be related to the energetics of migration, or to the general catabolic state that appears to be part of the parr–smolt transformation (McCormick and Saunders, 1987).

Based on our knowledge of the endocrine control of smolting (Hoar, 1988), it is proposed that the observed differences in circulating levels of hormones in migrants and non-migrants are causally related to the parr–smolt transformation (including the process of active migration and imprinting) in the wild. However, we cannot at present eliminate the possibility that some of the observed differences were due to changes over time (the non-migrants being sampled 2 weeks

later than migrants), though it is unlikely that this would explain the large differences in plasma growth hormone and thyroxine, or gill Na^+,K^+-ATPase activity which is not rapidly altered in salmonids (McCormick and Saunders, 1987). Some of the observed changes may have been due to the somewhat unnatural experience of migrants captured at impoundments of a power-generating dam. Whether some of the documented changes are causal to or the result of migratory behavior has yet to be demonstrated. More intensive sampling over time, particularly of smolts in the wild prior to migration, may elucidate the causal relationships between hormones and the physiological and behavioral changes in smolts.

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6. References

Ackman, R.G. and Takeuchi, T., 1986. Comparison of fatty acids and lipids of smolting and hatchery-fed and wild Atlantic salmon (*Salmo salar*). Lipids, 21: 117–120.

Barrett, B.A. and McKeown, B.A., 1989. Plasma growth hormone levels in *Salmo gairdneri*: studies on temperature and the exercise intensity/duration relationship. Comp. Biochem. Physiol., 94A: 791–794.

Bisbal, G.A. and Specker, J.L., 1991. Cortisol stimulates hypo-osmoregulatory ability in Atlantic salmon, *Salmo salar* L. J. Fish Biol., 39: 421–432.

Björnsson, B.Th., Taranger, G.L., Hansen, T., Stefansson, S.O. and Haux, C., 1994. The interrelation between photopiod, growth hormone, and sexual maturation of adult Atlantic salmon (*Salmo salar*). Gen. Comp. Endocrinol., 93: 70–81.

Bolton, J.P., Takahashi, H., Kawauchi, H., Kubota, J. and Hirano, T., 1986. Development and validation of a salmon growth hormone radioimmunoassay. Gen. Comp. Endocrinol., 62: 230–238.

Chernitsky, A.G., 1980. Functional state of chloride cells of Baltic salmon (*Salmo salar*). Comp. Biochem. Physiol., 67A: 519–522.

Chernitsky, A.G., 1986. Quantitative evaluation of the degree of parr–smolt transformation in wild smolts and hatchery juveniles of Atlantic salmon (*Salmo salar* L.) by SDH activity of chloride cells. Aquaculture, 59: 287–297.

Cunjak, R.A., Saunders, R.L. and Chadwick, E.M.P., 1990. Seasonal variations in the smolt characteristics of juvenile Atlantic salmon (*Salmo salar*) from estuarine and riverine environments. Can. J. Fish. Aquat. Sci., 47: 813–820.

Dickhoff, W.W., Folmar, L.C. and Gorbman, A., 1978. Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 36: 229–232.

Hoar, W.S., 1939. The thyroid gland of the Atlantic salmon. J. Morphol., 65: 257–295.

Hoar, W.S., 1988. The physiology of smolting salmonids. In: W.S. Hoar and D. Randall (Editors), Fish Physiology, Vol. XIB. Academic Press, New York, pp. 275–343.

McCormick, S.D., 1993. Methods for non-lethal gill biopsy and measurement of Na^+,K^+-ATPase activity. Can. J. Fish. Aquat. Sci., 50: 656–658.

McCormick, S.D. and Saunders, R.L., 1987. Preparatory physiological adaptations for marine life in salmonids: osmoregulation, growth and metabolism. Am. Fish. Soc. Symp., 1: 211–229.

McCormick, S.D., Naiman, R.J. and Montgomery, E.T., 1985. Physiological smolt characteristics of anadromous and non-anadromous brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 42: 529–538.

Power, G., 1959. Field measurement of the basal oxygen consumption of Atlantic salmon parr and smolts. Arctic, 12: 195–202.

Sakamoto, T., McCormick, S.D. and Hirano, T., 1993. Osmoregulatory actions of growth hormone and its mode of action in salmonids: a review. Fish Physiol. Biochem., 11: 155–164.

Specker, J.L., Whitesel, T.A., Parker, S.J. and Saunders, R.L., 1989. Thyroidal response of Atlantic salmon to seawater challenge — predictor of growth in seawater. Aquaculture, 82: 307–318.

Stein, M.W., 1963. D-glucose, determination with hexokinase and glucose-6-phosphate dehydrogenase. In: H.U. Bergmeyer (Editor), Methods in Enzymatic Analysis. Academic Press, New York, pp. 117–122.

Sumpter, J.P., Dye, H.M. and Benfey, T.J., 1986. The effects of stress on plasma ACTH, alpha-MSH, and cortisol levels in salmonid fishes. Gen. Comp. Endocrinol., 62: 377–385.

Varnavsky, V.S., Sakamoto, T. and Hirano, T., 1992. Stunting of wild coho salmon (*Oncorhynchus kisutch*) in seawater: patterns of plasma thyroid hormones, cortisol, and growth hormone. Can. J. Fish. Aquat. Sci., 49: 458–461.

Wedemeyer, G.A., Saunders, R.L. and Clarke, W.C., 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev., 42: 1–14.

Youngson, A.F. and Simpson, T.H., 1984. Changes in serum thyroxine levels during smolting in captive and wild Atlantic salmon, *Salmo salar*. J. Fish Biol., 24: 29–39.