Aquaculture 362–363 (2012) 127–136

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Growth, osmoregulation and endocrine changes in wild Atlantic salmon smolts and post-smolts during marine migration

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ARTICLE INFO

Article history:
Received 2 November 2009
Received in revised form 27 April 2011
Accepted 1 July 2011
Available online 13 July 2011

Keywords:
Energetics
Growth hormone
IGF-I
Thyroid hormones
Na^+,K^+-ATPase
IGF-I receptor

ABSTRACT

We have examined physiological parameters associated with seawater adaptability, growth and energetics, as well as major endocrine regulators of these processes in wild migrating Atlantic salmon smolts and post-smolts from the river through the fjord, coastal areas and the open ocean. Muscle RNA/DNA ratio suggests that growth rate increases soon after entry into seawater and continues to increase after the post-smolts reach the offshore banks and the feeding grounds in the Norwegian Sea. Post-smolts prioritize rapid growth and protein deposition in spring and summer, and their energy intake during this period is so high that deposition of energy is possible in addition to muscle growth. An increase in thyroxine (T_4) and triiodothyronine (T_3) levels was observed, suggesting a major activation of hepatic conversion of T_4 to T_3 in post-smolts in seawater, probably related to the high metabolic activity and rapid growth and development of the post-smolts. Decreased plasma growth hormone (GH) levels were observed from the river through the fjord, with levels around 2 ng ml^{-1} in rapidly growing post-smolts, concurrent with an increase in circulating insulin-like growth factor I (IGF-I). An increase in pituitary GH expression levels and hepatic GH receptor (GH-R) and local IGF-I mRNA levels suggest a physiological basis for the changes in circulating GH and IGF-I levels. Receptor expression in brain and pituitary suggests that both hormones are actively involved in the growth and differentiation of these tissues during the critical early marine phase. Gill Na^+,K^+-ATPase (NKA) activity increased to post-smolt levels above 20 μmol ADP mg prot.$^{-1}$ h^{-1}, probably representing long-term NKA activity levels of Atlantic salmon in seawater. Concurrent with the changes in NKA activity the expression of the NKA α1b isoform remained high in post-smolts, while the expression of the NKA α1a decreased from smolts to post-smolts. Both cystic fibrosis transmembrane conductance regulator (CFTR) I and II showed a reduction in mRNA levels from smolts to post-smolts, and remained stable at low expression levels in seawater.

1. Introduction

Following parr–smolt transformation and downstream migration, many stocks of Atlantic salmon (*Salmo salar* L.), including most Norwegian stocks, begin their oceanic migration in large fjord systems and archipelagos before reaching the open ocean. Although our knowledge about the ecology of wild Atlantic salmon post-smolts is limited, previous studies from Norwegian and British waters have suggested that these life stages spend less than a month in the fjords and coastal waters (Dutil and Coutu, 1988; Holm et al., 1982; Hvidsten and Lund, 1988; Thorpe, 1994) before continuing their migration towards the richer feeding grounds in the ocean. During their marine migration, the diet of Atlantic salmon post-smolts changes, and feeding conditions and early marine growth have been postulated to be critical to the overall marine survival and year-class strength of Atlantic salmon (Andreassen et al., 2001; Friedland et al., 2000, 2009; Haugland et al., 2006; McCarthy et al., 2008; Peyronnet et al., 2007; Rikardsen et al., 2004). In the northeast Atlantic, post-smolts are generally found in close relation with the North Atlantic Current (Holm et al., 2000, 2004; Holst et al., 2000; Shelton et al., 1997). In autumn and winter, salmon are present north of the Faroe Islands, feeding mainly on small mesopelagic fish and crustaceans, (Jacobsen and Hansen, 2001) in areas where Atlantic and Arctic water masses meet (Jákupsstovu, 1988).

The completion of parr–smolt transformation and downstream migration represents the culmination of a series of physiological and behavioral changes which are pre-adaptive for seawater entry (Hoar, 1988), with further adaptations taking place in response to seawater (see e.g. Björnsson, 1997; Björnsson et al., 1998; Handeland et al., 1996, 1998, 2000; McCormick, 1995, 2009; McCormick et al., 1989;

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doi:10.1016/j.aquaculture.2011.07.002

Nilsen et al., 2003, 2007, 2008; Stefansson et al., 2003, 2008). These physiological responses represent a critical part of the adaptive process to ocean conditions and studies have suggested that they confer substantial selective advantages during the critical early marine phase of anadromous salmonids (Andreassen et al., 2001; Levings et al., 1994; Stefansson et al., 2003). Despite the proposed critical role of rapid physiological adaptations for survival and growth, information on the physiological and endocrine changes in wild salmonids during their early marine phase is very limited.

We hypothesize that significant physiological adjustments are made during this period, concurrent with changes in behavior and feeding. Specifically, our hypothesis is that muscle growth (protein synthesis and deposition) is prioritized from the beginning of the oceanic migration in Atlantic salmon post-smolts, concurrent with significant osmoregulatory adjustments, in terms of adaptive changes in the Na^+,K^+-ATPase (NKA) system, the Na^+,K^+,$2Cl^-$ co-transporter (NKCC) and the cystic fibrosis transmembrane conductance regulator (CFTR) in the gills. Further, we propose that these changes are regulated by the key components of the endocrine system; hence we describe changes in the GH–IGF-I system and the thyroid hormones. The objective of this study was, therefore, to examine several important physiological parameters associated with seawater adaptability, growth and energetics, as well as major endocrine regulators of these processes in wild Atlantic salmon smolts and post-smolts during their migration from the river through the fjord, coastal areas and into the open ocean.

2. Materials and methods

2.1. Study area and fish material

The fish used in this study were sampled in 2002 at the following locations; the Vosso River in western Norway, the Trondheimsfjord in central Norway, two offshore banks (the Halten bank and the Sklinna bank off the Norwegian coast) and the major summer feeding area (the Norwegian Sea, Fig. 1, Jákupsstovu, 1988). Smolts from the Vosso River were captured in fresh water (FW) by use of a fish wheel (smolt screw, Meehan, 1961) located near the estuary at Bolstad. The fish wheel rotates with the river current, lifting the smolts gently into a flow-through cage where they are kept until sampling. Post-smolts were captured with a modified surface trawl (Haugland et al., 2006; Holst and McDonald, 2000; Valdemarsen and Misund, 1995) in the fjord and offshore banks during surveys carried out in May and early June 2002 (Table 1). The summer feeding area in the Norwegian Sea was sampled in late June 2002. Briefly, the trawl was fitted with extra flotation on the headline to sample the upper 14 m and was hauled at 3–5 knots. The cod end of the trawl was modified with a live fish capture device, the FISH-LIFT (Holst and McDonald, 2000), which is essentially a floating aquarium. Tow duration ranged from 30 min to 1 h. Our own video observations during trawling have demonstrated that post-smolts are able to sustain the trawling speed, maintaining position inside the trawl and FISH-LIFT for extended periods of time, suggesting that capture stress is not a major concern. Water temperature was 5.9 °C in the river on 11 May 2002. Average temperatures (0–5 m depth) in the marine zones ranged from 10.0 °C in the fjord, 11.5 °C offshore and 11.7 °C in the Norwegian Sea (Table 1). For further details on trawling and sampling see Haugland et al. (2006).

2.2. Sampling

Smolts were gently dip-netted from the fish wheel and kept in a container with flow through fresh water. On board the research vessel, post-smolts were sorted from the rest of the fish in the FISH-LIFT, and kept in a container with flow-through seawater (SW). Within minutes after capture and sorting, smolts and post-smolts

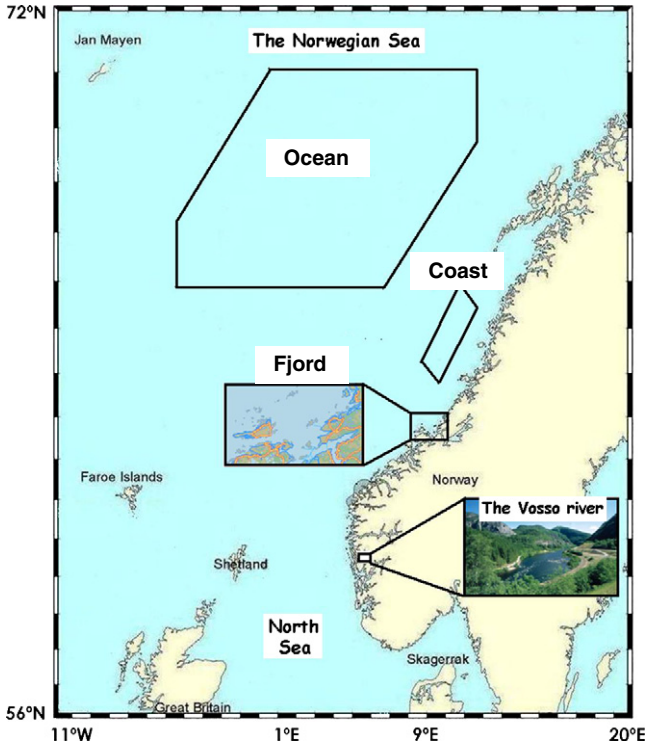

Fig. 1. Map of the study area with reference to sampling areas. Atlantic salmon smolts were sampled from the Vosso River, while post-smolts were sampled from the outer Trondheimsfjord/Frohavet (Fjord), the Halten bank and Sklinna bank (Coast) and the Norwegian Sea (Ocean).

were quickly dip-netted, anesthetized directly in 100 mg l^{-1} buffered tricaine methanesulphonate (MS222; Sigma, St. Louis, MO, USA) and blood was collected from the caudal vessels using 1 ml heparinised syringes. Plasma was separated by centrifugation, frozen on dry ice and stored at −80 °C for subsequent hormone analysis. All fish were weighed (wet weight) and measured (fork length) and the condition factor (CF) was calculated (CF = body weight × 100 × fork length^{-3}). Tissues for determination of mRNA levels and protein abundance were quickly dissected out and frozen directly on dry ice. For Na^+,K^+-ATPase (NKA) activity analysis, the second gill arch on the left side was dissected out, immersed in SEI buffer (250 mM sucrose, 10 mM Na_2-EDTA, 50 mM imidazole at pH 7.3), frozen on dry ice and stored at −80 °C until analysis. The fish were then opened by an incision along the mid-ventral line, their liver weight determined and their stomach contents removed. Post-smolts caught in the Norwegian Sea had a high forage ratio and high proportion of 0-group herring (for further details on analysis of stomach contents see Haugland et al., 2006). Hepatosomatic index was calculated as HSI = liver weight × 100 × body weight^{-1}.

2.3. Analysis

2.3.1. Energetics and muscle moisture

Moisture content was determined as the difference between wet and dry weight (after drying the carcass to a stable dry weight at 70 °C). The carcasses were then homogenized and lipid, protein and energy content of the homogenate were determined. Total protein was determined in a Leco FP-528, utilizing the principle of combustion of a sample and analysis of N_2 gas (Leco, St. Joseph, Michigan, USA). Energy content was analyzed in an IKA C 2000 combustion calorimeter (IKA GmbH, Staufen, Germany). Lipid contents were determined gravimetrically following ethyl acetate/isopropanol extraction, filtration and evaporation of solvent.

Table 1
Mean (standard error of mean, se) fish size (fork length), condition factor (K-factor), hepato-somatic index (HSI) and temperature during sampling periods of wild Atlantic salmon. Smolts were sampled from the Vosso River, while post-smolts were sampled from the outer Trondheimsfjord/Frohavet (Fjord), the Halten bank and Sklinna bank (Coast) and the Norwegian Sea (Ocean). HSI values not sharing a common subscript are significantly different ($p<0.05$).

Location	Fork length (se)	K-factor (se)	HSI (se)	Temperature, °C (se)	Sampling date
Vosso (River) (n = 5–11)	12.4 (0.3)	0.95 (0.02)	0.55a (0.09)	5.9	11 May 2002
Frohavet (Fjord) (n = 4–31)	11.7 (0.2)	0.95 (0.01)	1.24b (0.08)	10.0 (0.14)	25–29 May 2002
Offshore (Coast) (n = 4–12)	13.6 (0.5)	0.99 (0.02)		11.5 (0.07)	31 May–4 June 2002
Norwegian Sea (Ocean) (n = 6–38)	20.9 (0.8)	1.26 (0.02)	1.73c (0.09)	11.7 (0.08)	22–28 June 2002

2.3.2. RNA/DNA ratio

Nucleic acids of white muscle tissue of smolts and postsmolts were assayed according to the procedure of Le Pecq and Paoletti (1966) and Boer (1975). Briefly, frozen muscle tissue samples of 15–25 mg were cut in line with the anterior of the dorsal fin, just above the lateral line. The samples were immediately transferred to 1.5 ml Eppendorf tubes containing ice-cold Tris-EDTA buffer and homogenized by two separate 5-second ultrasound pulses (Hielscher UP50H). The samples were then shaken for 15 min and centrifuged at 6000 rpm and 4 °C for 8 min, before 80 µl of the supernatant was transferred to a new vial. The total nucleic acid concentration (RNA + DNA) was determined fluorometrically with a Perkin Elmer HTS plate reader (excitation: 360 nm, emission: 595 nm) by adding 200 µl ethidium bromide to 10 µl of the supernatant in triplicates on a micro plate. DNA concentration was determined by the same method after incubation of the remaining aliquot supernatant with 3 µl of 0.2 mg $mL^{\div 1}$ RNAse (Sigma, Ribonuclease A, R-5503) for 30 min at 37 °C.

2.3.3. RNA isolation and cDNA synthesis

Total RNA was extracted from approximately 50 mg tissue using TRI reagent (Sigma) as outlined by Chomczynski (1993). The RNA quantity and integrity was determined by using a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the Agilent 2100 expert bio analyzer (Agilent technologies, Santa Clara, CA, USA), respectively. Total RNA were treated with RQ1 RNase-free DNase (Promega) and cDNA reversely transcribed using 0.5 µg total RNA and random nonamers in conjunction with the Reverse Transcription Core kit (Eurogentec, RT-RTCK-05) following the manufactures instructions.

2.3.4. Real-time quantitative PCR assays

Real-time quantitative PCR (Q-PCR) TaqMan assays were used to quantify in vitro expression of NKA-α1a, NKA-α1b and NKCC1 in the gill as described by Nilsen et al. (2007) and Stefansson et al. (2007). GH receptor, IGF-I receptor and IGF-I mRNA expression were measured using FAM labeled TAMRA probes (Nilsen et al., 2008; Wargelius et al., 2005). Briefly, for each assay, triplicate five fold cDNA dilution series made from total RNA from different exposure groups were used to determine amplification efficiencies (E) calculated as the slope from the plot of log RNA concentration versus threshold cycle (Ct) values using the following formula: $E = 10^{(-1/slope)}$. This efficiency was used to correct for difference in amplification efficiency when calculating gene expression according to Pfaffl (2004). Expression in most tissues is presented as relative to the endogenous normalization gene, EF1α, according to Olsvik et al. (2005). In the pituitary samples the EF1α did not meet the requirements of a stable reference gene, hence the ribosomal protein L 23, RPL 23, was used as reference gene for pituitary. SYBR-based quantitative PCR (Applied Biosystems, Carlsbad, CA, USA) assays were used to measure expression in the pituitary. The RPL 23 forward and reverse primers were ATGCTGCCAGCATTTGAAGCAATCCT and CTTTACATCATCTCTGTCAAGGGCATCAA, respectively. The PCR consisted of 10 µl cDNA, 400 nM of each primer and SYBR Green Universal Master mix in a total reaction volume of 25 µl. The thermal cycling protocol consisted of 2 min at 50 °C, 10 min at 95 °C followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. The primers for target genes have been published earlier (Nilsen et al., 2007).

2.3.5. Western blots

NKA and NKCC protein abundance was determined by Western blots following the procedure described by Pelis et al. (2001) and Stefansson et al. (2007). Briefly, NKA and NKCC abundance was detected using a mouse monoclonal antibody specific for chicken α-subunit (α5, Takeyasu et al., 1990) and a mouse monoclonal antibody directed against 310 amino acids at the carboxyl terminus of human colonic NKCC1, respectively. The NKA (α5; developed by D. M. Fambrough, Johns Hopkins University, MD, USA) and NKCC (T4; developed by Christian Lytle and Bliss Forbush III) antibodies were obtained from the Developmental Studies Hybridoma Bank, University of Iowa, Department of Biological Sciences, Iowa City, IA, USA. Thawed gill tissue was homogenized using an ice-cold glass homogenizer in SEI buffer containing protease inhibitors (1 complete-mini tab per 10 ml SEI, Roche Diagnostics Corporation, Indianapolis, IN, USA) and centrifuged at 3000 rpm for 7 min. The resulting pellet of subcellular material was resuspended in 5 volumes of SEI buffer containing 0.1% sodium deoxycholate. After centrifugation at 2060 × *g* for 6 min, supernatant was diluted with Laemmli's buffer and heated at 60 °C for 15 min. This crude membrane preparation is similar to that used by Zaugg (1982) and results in 4-fold enrichment of membrane bound proteins. Sample volume of 10 µg total protein was separated by 7.5% and 6% SDS-PAGE for NKA and NKCC, respectively. After two hours, the gels were blotted onto Immobilion P (PVDF) membranes (Millipore, Bedford, MA, USA) overnight on ice and incubated in blocking buffer (PBS containing 0.05% Triton X-100 and 2% skimmed milk) for 1 h at room temperature. After rinsing of membranes in PBS-Tx, membranes were incubated with anti-NKA (α5; 1:2000) or anti-NKCC (T4; 1:1000) antibodies. Membranes were rinsed and incubated with secondary peroxidase-conjugated antibodies (1:1000) for 1 h and reacted with diaminobenzidine solution until bands were visible. Color development was stopped with deionised water, membranes dried and digital photographs taken. Band staining intensity quantified using ImageJ processing and analysis software (see Pelis et al., 2001).

2.3.6. Na^+,K^+-ATPase activity

Gill filaments were thawed on the day of assay, the storage buffer discarded, and gill Na^+,K^+-ATPase activity analyzed according to McCormick (1993). Briefly, this kinetic assay utilizes the hydrolysis of ATP, which is enzymatically coupled to the conversion of NADH to NAD^+ by pyruvate kinase and lactic dehydrogenase with or without the addition of ouabain, a specific inhibitor of NKA. Readings were done at 340 nm for 10 min at 25 °C. Protein in homogenate was determined by a bicinchoninic acid method (Smith et al., 1985) and NKA activity is expressed as µmol ADP mg $prot.^{\div 1}$ $h^{\div 1}$.

2.3.7. Hormone levels

Plasma growth hormone (GH) levels were quantified using a specific salmon GH radioimmunoassay according to Björnsson et al. (1994). Plasma insulin-like growth factor I (IGF-I) levels were measured by a radioimmunoassay validated for salmonids (Moriyama

et al., 1994). Plasma L-thyroxine (T_4) and triiodothyronine (T_3) levels were assessed by competitive immunoassays (EIA) according to Kulczykowska et al. (2004) and Stefansson et al. (2007).

2.4. Statistics

All statistical analyses were performed with Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA, http://www.statsoft.com). In the case of deviations from normality of distributions (Shapiro–Wilk test; Zar, 1996) and homogeneity of variances (Levene's F-test) data were log-transformed (Zar, 1996) to meet the requirements of parametric models. All parameters were analyzed using a one-way ANOVA, followed by a Newman–Keuls test in case of significant ANOVAs. A significance level of 0.05 was used. Data are presented as mean ± standard error of the mean (se).

3. Results

3.1. Growth, energetics and size relations

The fork length of the fish in the present study ranged from 11.7 to 12.4 cm for smolts caught in the river and fjord, 13.6 cm offshore and 20.9 cm for fish caught in the Norwegian Sea (Table 1). Condition factor ranged from 0.95 for fish caught in the river and fjord to 1.26 for post-smolts caught in the Norwegian Sea. The hepatosomatic index increased significantly from 0.55% in the Vosso River, through the fjords and offshore waters to a maximum of 1.73% in the Norwegian Sea (Table 1).

Muscle RNA/DNA ratio, an index of protein synthesis capacity, was 1.42 ± 0.11 in the river, increased to 2.37 ± 0.11 in the fjord, 2.74 ± 0.22 offshore, peaking at 3.04 ± 0.10 in the Norwegian Sea (Fig. 2a). Energy content and lipid levels (on a dry weight basis) were low in smolts in the river and fjord, increasing significantly in post-smolts caught in the Norwegian Sea (Fig. 2c, d). Protein content (on a dry weight basis) was 78.5 ± 0.46% in smolts from the river, decreasing slightly in samples from the offshore banks and the Norwegian Sea (Fig. 2b). Muscle moisture was highest at 77.5% for smolts in fresh water and decreased transiently in fish caught in the fjord, with a significant increase in post-smolts in the Norwegian Sea to a level of 76.0% (Fig. 3).

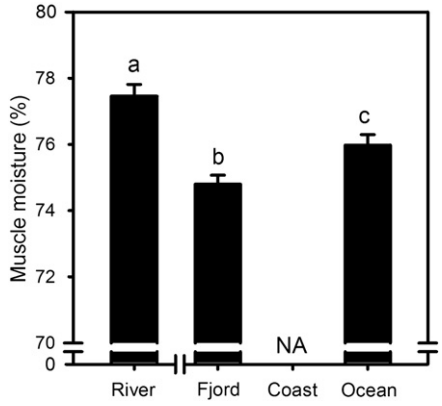

Fig. 3. Mean (se) muscle moisture of wild Atlantic salmon smolts and post-smolts during marine migration. Refer to Fig. 1 for description of zones.

3.2. Endocrinology and osmoregulatory physiology

Thyroxin (T_4) levels were relatively low in smolts in the river, increasing approximately 4-fold in fish caught in the fjord, remaining high in post-smolts offshore and on the feeding grounds in the Norwegian Sea (Fig. 4). Triiodothyronine (T_3) levels were relatively low in smolts in the river, increasing throughout the post-smolt

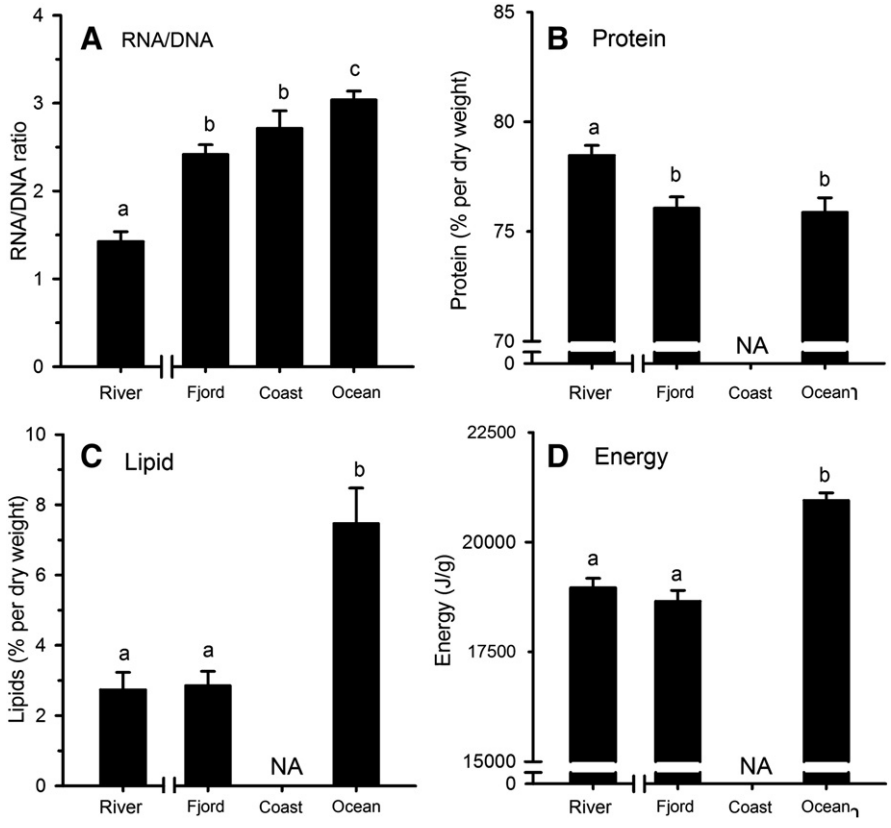

Fig. 2. Mean (se) RNA/DNA ratio (a), muscle protein levels (b), lipid levels (c) and energy contents (d) of wild Atlantic salmon smolts and post-smolts during marine migration. Refer to Fig. 1 for description of zones.

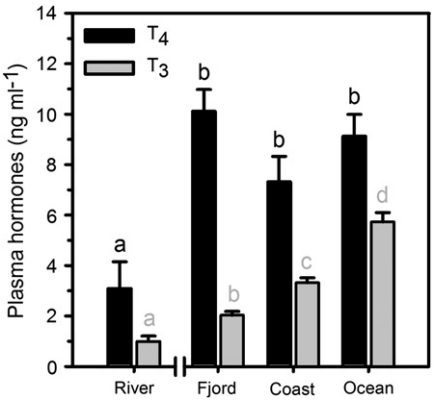

Fig. 4. Mean (se) circulating thyroxin (T_4) and triiodothyronine (T_3) levels of wild Atlantic salmon smolts and post-smolts during marine migration. Refer to Fig. 1 for description of zones.

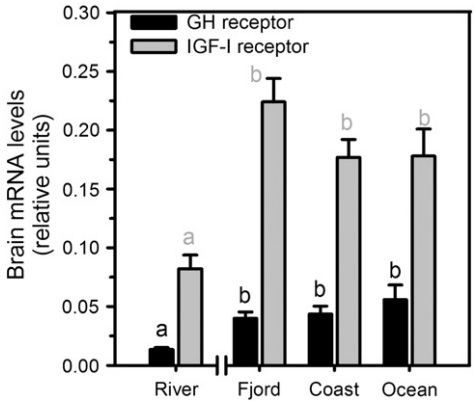

Fig. 6. Mean (se) brain GH and IGF-I receptor mRNA levels of wild Atlantic salmon smolts and post-smolts during marine migration. Refer to Fig. 1 for description of zones.

period, reaching peak levels of 5.7 ± 0.36 ng ml^{-1} in rapidly growing oceanic post-smolts.

Mean plasma growth hormone levels in smolts caught in river Vosso were 20.5 ± 6.8 ng ml$^{\div 1}$ (Fig. 5a). Plasma GH levels gradually decreased in fish caught in seawater, reaching levels between 2.1 and 2.3 ng ml$^{\div 1}$ in fish caught offshore and in the Norwegian Sea, representing circulating levels of fully adapted, fast growing marine post-smolts. A contrasting development was observed for circulating IGF-I levels, which increased from 27.7 ± 2.3 ng ml$^{\div 1}$ (Fig. 5a) in the river to 64.0 ± 2.0 ng ml$^{\div 1}$ in fish caught on the high seas feeding grounds. Despite the low circulating GH levels observed in the offshore and high seas feeding grounds, GH production, represented by pituitary mRNA levels increased 3-fold from the fjord through the Norwegian Sea (Fig. 5c). Concurrent with these changes, an increase in liver IGF-I expression levels was observed, along with a stable expression of the GH receptor in the liver (Fig. 5b). GH receptor expression in the pituitary was variable, and no significant changes were observed (Fig. 5d), while IGF-I receptor expression increased several fold, peaking in rapidly growing oceanic post-smolts. Further, in wild Atlantic salmon smolts and post-smolts, IGF-I receptor and GH receptor mRNA levels increased in the brain from fish caught in the river through the fjords and in the Norwegian Sea (Fig. 6).

In the gill, GH receptor expression increased transiently in post-smolts caught in the fjord, with a subsequent decline offshore and in the open ocean, suggesting an important role of GH in adaptation to

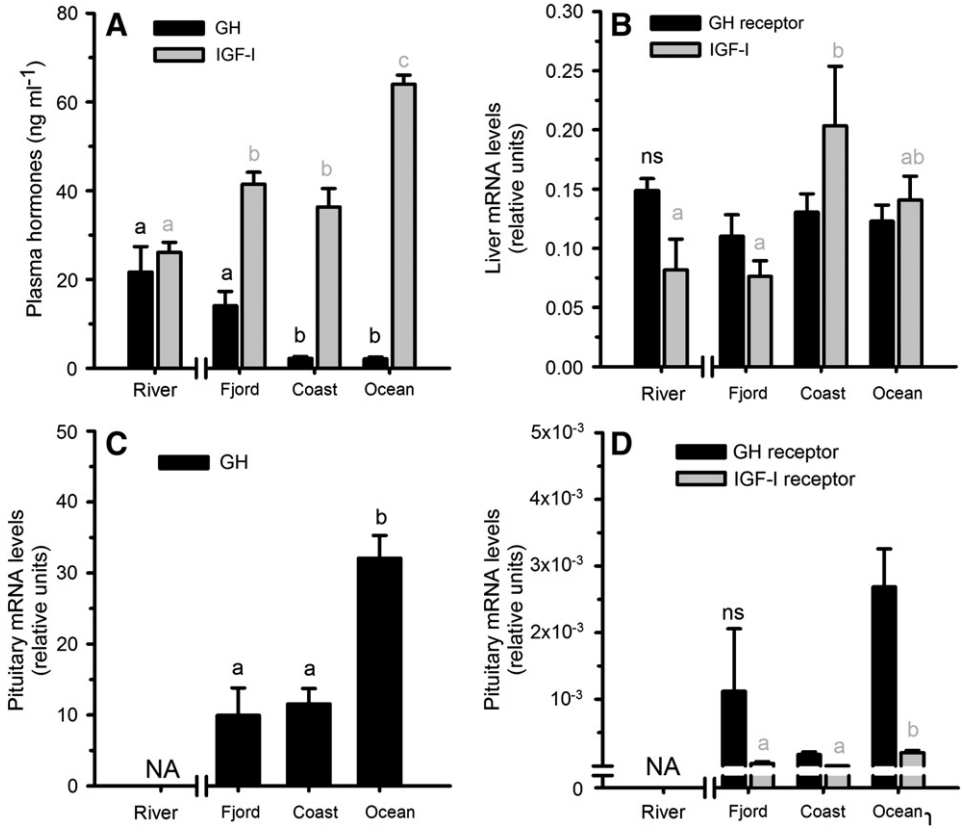

Fig. 5. Mean (se) circulating levels of growth hormone (GH) and insulin-like growth factor I (IGF-I) (a), liver GH and IGF-I receptor mRNA levels (b), pituitary GH mRNA levels (c) and pituitary GH and IGF-I receptor mRNA levels (d) of wild Atlantic salmon smolts and post-smolts during marine migration. Refer to Fig. 1 for description of zones.

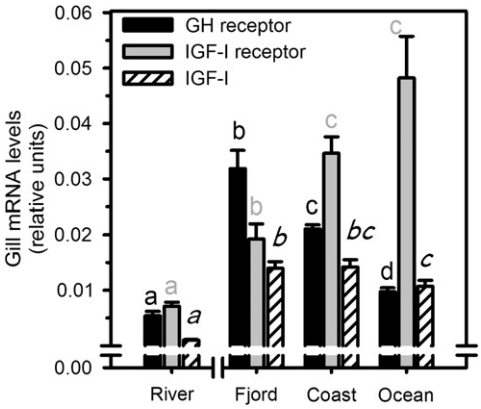

Fig. 7. Mean (se) gill GH and IGF-I receptor mRNA levels, and local IGF-I expression of wild Atlantic salmon smolts and post-smolts during marine migration. Refer to Fig. 1 for description of zones.

seawater (Fig. 7). A similar expression pattern was observed in gill IGF-I production, showing an increase between fish caught in the river and the fjord, with a gradual reduction offshore and in the Norwegian Sea. In contrast, IGF-I receptor mRNA levels increased significantly through the fjord, offshore and in the ocean, suggesting important roles of IGF-I in adaptation to the marine environment.

Gill Na^+,K^+-ATPase activity of fish caught in the river averaged 8.6 ± 0.9 μmol ADP mg prot.$^{-1}$ h^{-1} (Fig. 8a), increasing to 21–25 μmol ADP mg prot.$^{-1}$ h^{-1} in the fjords and offshore, representing activity levels of fully acclimated marine post-smolts. There was a significant reduction in NKA α1a mRNA levels from the river through the fjord and into the fully marine zones, reaching non-quantifiable mRNA levels offshore and in the Norwegian Sea (Fig. 8b). In contrast, NKA α1b mRNA levels were high in smolts from the river and did not change significantly in the fjord or offshore. NKA α-subunit protein levels decreased between smolts and post-smolts in the fjord, and remained stable in fish caught offshore (Fig. 8c). NKCC1 transcript levels decreased significantly from the river through the fjord, with a further reduction in the Norwegian Sea (Fig. 8b). NKCC protein abundance did not change significantly from the river through the fjords and offshore (Fig. 8c). Expression patterns for CFTR-I and II were similar, showing a reduction between smolts caught in the river and post-smolts caught in the marine zones (Fig. 8d).

4. Discussion

4.1. Growth and size relations

The post-smolts caught in the Norwegian Sea had approximately doubled their length since they left the river as smolts (Table 1). Based on smolt age and recaptures of tagged fish we know that these fish are a mixture of European fish (Holm et al., 2003) and when caught in June, the majority has spent two–three months in the sea. While it is possible to view the present data as a simple developmental series of changes during smolt migration from the river, through the fjord, out to the open sea and full oceanic conditions, this interpretation has to be made with caution, as fish which are caught off the Norwegian coast and in the Norwegian Sea originate from different regions, representing different segments of the smolt run, and having spent different periods of time in seawater. However, the present material represents the physiological and endocrine status of Atlantic salmon post-smolts at different stages of marine migration through the first spring and early summer. Assuming that the post-smolts caught in the Norwegian Sea have been in the ocean for 90 days, and that their smolt size was approximately 12 cm, the average growth in length per day over this period has been approximately 1 mm. Although this is a high estimate of growth rate, these calculations are in agreement with

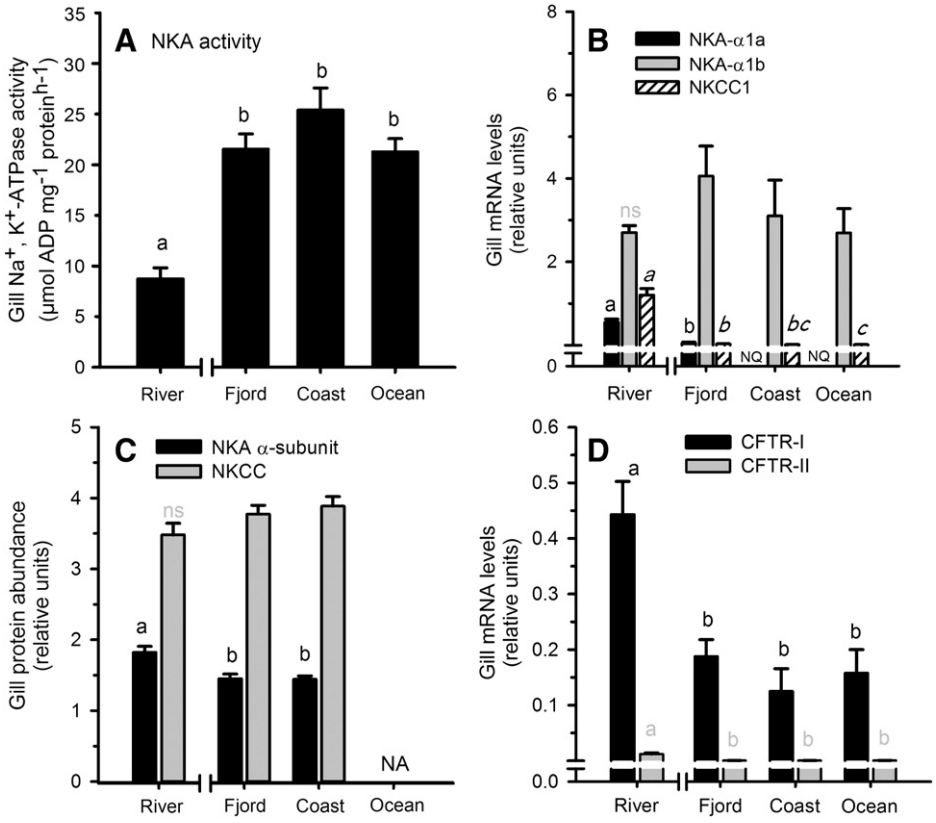

Fig. 8. Mean (se) gill Na^+,K^+-ATPase, NKA, activity (a), NKA α1a, α1b and NKCC1 mRNA levels (b), gill NKA α-subunit and NKCC protein levels (c) and gill CFTR-I and II mRNA levels (d) of wild Atlantic salmon smolts and postsmolts during marine migration. Refer to Fig. 1 for description of zones.

previous reports of marine growth of larger Atlantic salmon. Jacobsen (2000) found an average daily growth in length of 0.45 mm of older post-smolt salmon tagged north of the Faroe Islands and recovered in home waters and rivers throughout the distribution range of Atlantic salmon. Our results further corresponded with those of Jensen (1980) who found a growth rate of 0.3 mm per day of adult salmon tagged in West Greenland and recaptured in North America and 0.5 mm per day of those recaptured in British, Scandinavian and Russian rivers.

Our data on muscle RNA/DNA ratio (Fig. 2a) suggest that growth rate increases soon after entry into seawater and continues to increase after the post-smolts reach the offshore banks and the feeding grounds in the Norwegian Sea. The RNA/DNA ratio is used as an index of instantaneous growth rate because it is positively correlated with direct measures of growth (Bulow, 1987). Thorpe et al. (1982) and Varnavskiy et al. (1991) found significant positive correlations between growth rate and RNA/DNA in parr of Atlantic salmon and presmolt/smolt of coho salmon (*Oncorhynchus kisutch*), respectively. Recently, MacLean and Caldarone (2008) have provided evidence of a strong correlation between muscle RNA/DNA ratio and recent growth rate of Atlantic salmon smolts; however, they raise concerns about the use of RNA/DNA ratio as a measure of growth in fish from different temperature environments (see also Ferguson and Danzmann, 1990). With the exception of temperature in the river, which was approximately 6 °C at the time of sampling, temperatures in the present study were within a narrow range of 10–11.7 °C, characteristic of well-mixed marine water masses, suggesting that temperature was not a confounding factor for the interpretation of RNA/DNA ratios. Indeed, recalculating our data on RNA/DNA ratio and temperature according to the growth model of Buckley (1984) confirms our conclusions. Our results suggest that the somatic growth of post-smolts is high during their migration through the fjord and along the coast. Results from Andreassen et al. (2001) and Rikardsen et al. (2004) support this as they found high forage ratios among post-smolts in several fjords including the Trondheimsfjord and Frohavet. Our results further suggest that post-smolts prioritize rapid growth and protein deposition during the feeding migration in spring and summer (Fig. 2b), and that their energy intake during this period is high enough to allow deposition of energy, in addition to muscle growth. Condition factor was low in smolts and early post-smolts, but increased to high levels in oceanic fish, concurrent with an increase in lipid levels, hepato-somatic index and total energy content (Table 1, Fig. 2c, d). These findings are in line with the findings of Stefansson et al. (2003) that smolts continue to have low energy reserves during the early marine phase (fjord/coast). The increasing RNA/DNA ratio combined with the low energy reserves observed indicate that the post-smolts prioritize somatic growth throughout their early marine migration, and that surplus energy is stored as lipid (Fig. 2c, d) and glycogen, indicated by the increase in hepato-somatic index (Table 1) by the time the fish leave the Norwegian coast. Stomach content analyses show that in 2002 post-smolts caught in the Norwegian Sea had a high forage ratio and high proportion of 0-group herring by mass (Haugland et al., 2006). Norwegian spring spawning herring spawn along the Norwegian west coast in February–March. The 2002 year class was large and a significant amount of the larvae drifted westwards and into the Norwegian Sea where they overlapped with catches of post-smolts (J. C. Holst, unpublished observations).

On entry into seawater, smolts experience hyper-osmotic stress, often followed by a transient drop in tissue (muscle) moisture (Handeland et al., 1998, 2000; Sigholt and Finstad, 1990; Stagg et al., 1989). In contrast, Stefansson et al. (2003) observed stable muscle moisture levels when investigating wild post-smolts migrating through the Trondheimsfjord and Frohavet, and concluded that muscle moisture was regulated within 77–79%. The levels of muscle moisture observed for smolts in the Vosso River in the present study (Fig. 3) were consistent with the freshwater levels observed by Stefansson et al. (2003), while the levels observed for post-smolts in full salinity seawater in Frohavet were slightly lower (74.8%). Muscle moisture levels of rapidly growing fish taken in the Norwegian Sea (76.0%) were in line with levels observed by Handeland et al. (1998, 2000) and Stefansson et al. (2003), and probably represent long-term moisture levels of wild post-smolt Atlantic salmon.

4.2. Endocrine status

Thyroid hormones are involved in a wide range of physiological, developmental and behavioral processes in vertebrates, including teleosts (see Dufour and Rousseau, 2007 and McCormick, 2009 for reviews). Salmonid smoltification is typically associated with an increase in circulating T_4 levels, while T_3 levels are generally regulated within a more narrow range (Dickhoff et al., 1985, 1997; Ebbesson et al., 2000, 2008; McCormick et al., 2007; Stefansson et al., 2007). Exposure of fully smoltified salmon to seawater causes a further increase in plasma T_4 levels (McCormick and Saunders, 1990; Young et al., 1995). The thyroid hormone levels in smolts in the river (Fig. 4) are in line with previous observations of circulating T_4 and T_3 levels in smolts in our laboratories (Ebbesson et al., 2008; Stefansson et al., 2007). In the present study, however, a further increase in both T_4 and T_3 levels was observed between smolts in the river and post-smolts in seawater. The high T_4 levels and the continued increase in circulating levels of the biologically more active form, T_3, suggests a major activation of hepatic conversion of T_4 to T_3 in post-smolts in seawater, probably related to the high metabolic activity and rapid growth and development of the post-smolts on the summer feeding grounds in the Norwegian Sea.

The GH–IGF-I system (often referred to as the GH–IGF-I axis, but see Reinecke et al., 2005) is a major regulator of growth and development in fish, including the Atlantic salmon during parr–smolt transformation (for review see Reinecke et al., 2005; Stefansson et al., 2008). Overall, a reduction in circulating GH levels was observed from the river (20.5 ng $ml^{\div 1}$) through the fjord, with levels around 2 ng $ml^{\div 1}$ in rapidly growing post-smolts caught offshore and in the Norwegian Sea (Fig. 5a). Concurrent with this reduction in circulating GH, an increase in circulating IGF-I levels was observed, reaching levels of approximately 65 ng $ml^{\div 1}$ in the Norwegian Sea. Low circulating GH levels concomitant with relatively high IGF-I in rapidly growing fish are in agreement with observations by Stefansson et al. (1991) and Nordgarden et al. (2006) in Atlantic salmon post-smolts and by Larsen et al. (2001) and Beckman et al. (2004a) in coho salmon. Our observations on changes in pituitary GH expression levels (Fig. 5c) along with hepatic GH-R and local IGF-I mRNA levels (Fig. 5b) suggest a physiological basis for the present changes in circulating GH and IGF-I levels. From relatively low levels in the fjord and offshore, there was a significant increase in pituitary GH mRNA levels in fish caught in the Norwegian Sea, pointing to an increase in GH production in rapidly growing oceanic post-smolts and suggesting a rapid turnover of GH to sustain a high production of circulating IGF-I from the liver. Concurrent with this increase in GH production, GH receptor expression in the liver was maintained at stable levels, and IGF-I mRNA levels were high offshore and in the ocean. Taken together, our results suggest that GH is quickly removed from circulation by high GH-R activity in the liver (and other tissues, see below), stimulating hepatic IGF-I production, with IGF-I released into circulation from the liver, thus providing an explanation for the low GH and high IGF-I levels observed. Comparing RNA/DNA ratio (Fig. 2a) with circulating IGF-I levels (Fig. 5a), our results further support IGF-I as a candidate for measuring instantaneous growth in fish (Beckman et al., 2004b; Imsland et al., 2007; Picha et al., 2008).

Our findings further suggest important roles of GH and IGF-I at the level of the brain and pituitary, both as regulators of cellular proliferation and differentiation and as key elements in the feedback loops regulating production and release of GH and IGF-I (Figs. 5d, 6). GH-R mRNA levels in the pituitary showed high variability (Fig. 5d),

making it difficult to draw firm conclusions from the present material. At the level of the brain, however, GH receptor expression was higher in post-smolts from all marine zones compared to smolts from the river (Fig. 6), suggesting important roles of GH in the growth and differentiation of the brain in post-smolt salmon. In line with the changes in brain GH-R expression, brain IGF-I receptor mRNA levels were significantly higher in marine salmon than in freshwater smolts. Significant changes were also observed in IGF-I receptor mRNA levels at the level of the pituitary, with higher expression in post-smolts in the Norwegian Sea. Taken together, our results on circulating levels of GH and IGF-I together with mRNA expression data at the level of the brain and pituitary suggest that both hormones are actively involved in the growth and differentiation of these tissues during the critical early marine phase in the life cycle of the Atlantic salmon, as well as suggesting a possible mechanism for negative feedback on GH secretion (Björnsson et al., 2002).

4.3. Osmoregulatory changes

There are few studies of osmoregulatory changes during natural migration of Atlantic salmon from fresh water to sea water, the majority of published data are based on artificial transfer of smolts to sea water under controlled conditions, making direct comparisons of temporal changes difficult.

In addition to their effects on growth and overall metabolism, GH and IGF-I are major regulators of osmoregulatory ability in teleosts (Dickhoff et al., 1997; Mancera and McCormick, 1998; McCormick, 2009; McCormick and Bradshaw, 2006; Stefansson et al., 2008). In the present study, a transient 6–7 fold increase in GH-R and local IGF-I production at the level of the gill was observed, concurrent with a gradual increase in IGF-I receptor mRNA levels from the river throughout the marine zones (Fig. 7). Our findings support the key role of the GH–IGF-I system in the adaptation of Atlantic salmon smolts to seawater (Björnsson et al., 2002; McCormick, 2009; Nilsen et al., 2008).

Gill NKA activity increased from smolt levels of 8.6 μmol ADP mg prot.$^{\div 1}$ h$^{\div 1}$ to post-smolt levels in seawater above 20 μmol ADP mg prot.$^{\div 1}$ h$^{\div 1}$ in all marine zones (Fig. 8a). These values are higher than those reported by Stefansson et al. (2003), but are in line with Nilsen et al. (2007) and Stefansson et al. (2007), and likely represent long-term NKA activity levels of Atlantic salmon in seawater. Concurrent with the changes in NKA activity there was a change in the relative expression of α subunit isoforms (Fig. 8b). The expression of NKA α1b was high in smolts in the river, and remained high in post-smolts in seawater. In contrast, the expression of NKA α1a decreased significantly from smolts in freshwater to fish caught in the fjord, and reached non-quantifiable levels in post-smolts caught off shore and in the Norwegian Sea. These changes agree with the results reported by Nilsen et al. (2007) during smoltification and seawater transfer of Atlantic salmon, and further illustrate the concept of α1a and α1b as freshwater and seawater isoforms, respectively (Madsen et al., 2009; Nilsen et al., 2007; Richards et al., 2003). Concurrent with the changes in NKA activity and isoform expression, a reduction in NKCC1 mRNA levels was observed between smolts in freshwater and seawater post-smolts, with stable, low expression levels in the marine environment (Fig. 8b). NKCC protein abundance did not differ significantly among zones (Fig. 8c). These results are in apparent contrast to previous reports on an increase in NKCC in smolts in freshwater and after transfer to seawater (Pelis et al., 2001; Tipsmark et al., 2002), however, the present results agree with our previously published findings of a decrease in NKCC mRNA levels and protein abundance of post-smolts in seawater in June compared with smolts in freshwater in May (Nilsen et al., 2007).

There was a reduction in mRNA levels for both forms of CFTR (I and II) from smolts in freshwater to post-smolts in the fjord, and these levels then remained stable at relatively low expression levels in seawater (Fig. 8d). These findings are in contrast to those of Singer et al. (2002) who described elevated CFTR I mRNA levels during a two-week period after seawater transfer, whereas CFTR II was only transiently elevated after 24 h. Further, in the euryhaline spotted green pufferfish (*Tetraodon nigroviridis*) CFTR protein was only detected in seawater-acclimated fish (Tang and Lee, 2007), pointing to a key role of CFTR in seawater adaptation. According to current models for ion transport in seawater (Evans et al., 2005) an apical localization is described for the CFTR in the seawater chloride cell, whereas as basolateral localization is suggested for the freshwater model. Salmonids are unique in having two forms of CFTR (Marshall and Singer, 2002). However, the immunohistochemical localization of CFTR in salmonids has proven difficult. The question therefore remains open as to the different functions of the two forms of CFTR in Atlantic salmon, their sub-cellular localization and changes related to adaptation to seawater.

Atlantic salmon go through significant physiological changes during the post-smolt period (1st spring and summer in sea water), concurrent with changes in behavior and feeding. Muscle growth is prioritized from the beginning of the oceanic migration, with energy deposition occurring concurrent with an increase in growth rate. Adaptive changes are also observed at the level of the gill, with osmoregulatory adjustments which are required for life in sea water observed in the Na^+,K^+-ATPase system, the NKCC and CFTR. Further, we present evidence that these changes are regulated by the GH–IGF-I axis and the thyroid hormones. An increase in T_4 and T_3 levels suggest high metabolic activity and rapid growth and development of the post-smolts. Low plasma GH levels were observed in rapidly growing post-smolts, concurrent with an increase in circulating IGF-I. An increase in pituitary GH expression levels and high hepatic GH-R and local IGF-I mRNA levels suggest a physiological basis for the changes in circulating GH and IGF-I levels. Receptor expression in brain and pituitary suggest that both hormones are actively involved in the growth and differentiation of these tissues during the early marine phase in the life cycle of the Atlantic salmon.

Acknowledgments

We thank Bjørn Sveinsbø and Valentina Tronci at the University of Bergen, and Barbro Egnér at the University of Gothenburg, for excellent help in the lab. This study was funded in part by the Research Council of Norway and partly by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

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