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Effects of daylength and winter fasting on growth and smolting of one-year-old Saimaa landlocked salmon (*Salmo salar m. sebago* Girard) under fish farm conditions

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The effects of extended autumn daylength and winter fasting on growth and smolting were studied in one-year-old Saimaa landlocked salmon. Underyearling juveniles were either exposed to extended light LD 20:4 regime (LD group) during August–October or reared under routine fish-farm illumination (ND group). After the photoperiod manipulation, LD fish were larger and a greater number of them belonged to upper size classes in comparison with ND fish. Upper size class fish from both photoperiod groups were selected for further experiments in which the fish were either fed or fasted during winter. Contrary to the LD fish, the ND fish increased their length and weight during January–March by which they achieved the size of the LD fish. There was a clear size difference between the ND fed and fasted groups during March–April, but the fasted fish compensated for their growth suppression soon after the feeding was started again. The activity of gill Na^+,K^+-ATPase was lower in the ND fasted group during January–March as compared with that of the ND fed group suggesting that smolting may be switched off by poor nutritional conditions. The different gill Na^+,K^+-ATPase profiles between the ND and the LD fish may indicate disturbed endogenous smolting cycle of the LD fish. However, despite these differences, all groups, independently of the treatment, completed their smolting at the same time in June as indicated by elevated gill Na^+,K^+-ATPase activity, enhanced salinity tolerance, and hypoosmoregulatory ability. Lower levels of plasma sodium, chloride and magnesium in freshwater observed in all groups during spring–early summer coincident with other changes typical for smolting indicate gradually increasing maladaptation of the fish to freshwater. These results as well as an observed increase in potassium concentration in freshwater during spring-summer suggest that downstream migration is more like a passive than active process.

Introduction

Saimaa salmon (*Salmo salar m. sebago* Girard) became isolated to freshwater after the last ice age (Berg 1985). Under natural conditions this salmon displays a life history similar to that of anadromous Atlantic salmon (*Salmo salar* L.), except that after spending 2 to 4 years in the river, juveniles migrate downstream into the lake instead of into the sea (Seppovaara 1962). The building of hydroelectric dams, however, has destroyed all the natural breeding areas of Saimaa landlocked salmon. Hence, the present stock of this species is maintained by annual stockings of hatchery-reared smolts near to their previous reproduction areas (Pursiainen *et al*. 1998). Even though juveniles of Saimaa salmon never encounter the sea they undergo transformation processes associated with smolting that are similar to those of their anadromous conspecifics (Kiiskinen *et al*. 2002). Traditionally, developmental changes at smolting have been interpreted as indicators that the fish are preparing for seawater. Accordingly, migration downstream is considered as an active process. Alternatively, the idea of smolting has been suggested as evidence of maladaptation of the fish to freshwater (Langdon & Thorpe 1985, Simpson 1985, Primmett *et al*. 1988, Thorpe & Moore 1997). This view considers migration as a passive process brought on by gradual deterioration of freshwater osmoregulatory capacity (Thorpe 1989, Thorpe & Moore 1997). In a previous study with Saimaa salmon, a temporary loss of freshwater osmoregulatory capacity was observed coincident with other changes associated with smolting (Kiiskinen *et al*. 2002). The question concerning the nature of smolting and subsequent migration is important, for example, considering the timing of stocking.

Under good growing conditions, development of a bimodal size-frequency distribution during the first growing season is commonly observed in Atlantic salmon. Separation into modal groups is associated with changes in feeding motivation and appetite, and therefore development strategy adopted by these fish (Metcalfe *et al*. 1988; Metcalfe 1998). The larger, faster growing upper modal group (UMG) fish represent potential one-year-old smolts, whereas the smaller, slower growing lower modal group (LMG) fish require at least one additional year before completing smolting (Thorpe 1977, Bailey *et al*. 1980, Kristinsson *et al*. 1985). Several papers have documented that artificially extended daylength has a growth promoting effect on juvenile Atlantic salmon (McCormick *et al*. 1987, Saunders *et al*. 1989, Thorpe *et al*. 1989, Saunders & Harmon 1990, Stewart *et al*. 1990). There is also evidence that the number of fish entering the UMG may be increased by affecting growth opportunities, i.e. photoperiod manipulation (Thorpe *et al*. 1989, Saunders *et al*. 1989, Saunders & Harmon 1990, Stewart *et al*. 1990). However, photoperiods other than the natural one may seriously affect parr-smolt transformation (Saunders *et al*. 1985, McCormick *et al*. 1987) or alter the timing of smolting (Saunders *et al*. 1989, Saunders & Harmon 1990). A number of studies agree that experience of a period of short-day regime/winter daylength following artificially extended daylength may be the way to combine the aspects of growth and smolting (Björnsson *et al*. 1989, Saunders *et al*. 1989, Stefansson *et al*. 1990, Stefansson *et al*. 1991, Thrush *et al*. 1994, Berge *et al*. 1995, Duston & Saunders 1995, Sigholt *et al*. 1995, Duncan & Bromage 1998).

The general aim of the present study was to look further into seasonal variation in smolt characteristics of Saimaa landlocked salmon in order to find out indicators that best describe smolt status of hatchery-reared juveniles. However, considering the fact that the present stock of endangered Saimaa landlocked salmon is totally dependent on stocking of cultivated juveniles there is also need to obtain more information on the effects of different hatchery practises on growth and smolting. Based on the findings of our previous study, it is known that Saimaa salmon can smolt as one-year-old if their early development was accelerated by using warm water (Kiiskinen *et al*. 2002). The present series of experiments were designed to examine the effects of extended daylength in autumn on growth of underyearling Saimaa salmon and their smolting the following spring in association with increasing daylength and water temperature. In order to combine the aspects of growth and smolting the fish were allowed to experience

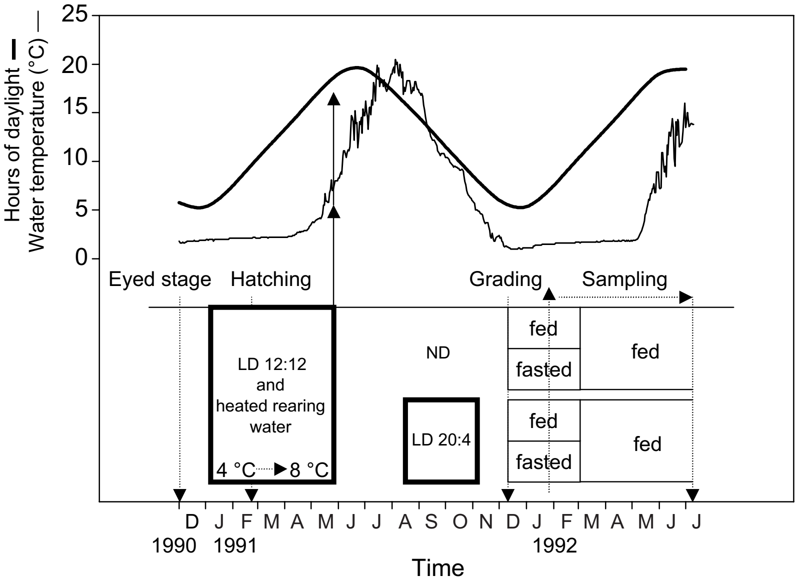

Fig. 1. Treatment history of one-year-old Saimaa landlocked salmon used in experiments from the eyed stage until the end of sampling period. Photoperiod of rearing hall (daylight hours) and rearing temperature (°C) are also shown.

a short-day regime/winter daylength following the period of artificially extended daylength. The experiments were also designed to study possible effects of nutritional status of the fish on smolting by feeding or fasting the fish during the winter. Attention was also paid to question why smolt phase is retained in salmon that spends its whole life cycle in freshwater.

Materials and methods

Fish and rearing conditions

Saimaa landlocked salmon from a cultivated (first hatchery generation) brood of Saimaa stock reared at Saimaa Fisheries Research and Aquaculture unit (62°05´N, 28°55´E) in south-eastern Finland were used in the experiments. The early development of the fish was accelerated by increasing the water temperature gradually from 4 to 8 °C approximately four weeks after the eyed embryo stage (12 December 1990) until 27 May 1991 when the ambient water temperature reached 8 °C (Fig. 1). Together with heated rearing water, constant LD 12:12 photoperiod was also used. From 28 June 1991, the fish (mean weight 0.144 g) were reared indoors in circular 3.3 m^2 plastic tanks (ca. 5000 individuals per tank) supplied with oxygenated water at a rate of 18–24 l min^{-1}. Concentration of oxygen was > 7 mg l^{-1} throughout the year. Illumination of the rearing hall was provided by artificial light followed by natural photoperiod with intensity of 2 lx at the surface of the rearing tanks (Fig. 1). Additional light came through windows. Working lights with intensity of 37 lx at the surface of rearing tanks ca. 07:00–16:00 were also used. Rearing temperature followed the natural temperature of lake Ylä-Enonvesi, the source of rearing water (Fig. 1). All the experimental fish were fed commercial dry feed (Ewos) by automatic dispenser (ITUMIC) which adjusted the amount of food by temperature and the size of the fish. Food was delivered throughout daylight hours.

Experimental and sampling procedures

On 22 August 1991, when the fish were one-summer-old, one rearing tank (ca. 5000 individuals, LD group) shielded by a black, light-proof plastic canopy, was exposed to nearly continuous light (a lamp with a 60 W tungsten bulb ca. 25 cm above the water level) with 4 hours twilight (decrease/increase) period at midnight. Water temperature followed the natural pattern. The rest of the fish were reared under routine farm illumination (ND group). On 30 October 1991, LD fish were restored to a normal photoperiod and on 14 November, the lengths and weights of 200 fish in both the LD and the ND groups were measured. On 2 December the fish in the LD and the ND groups were graded to upper and lower size groups using 12 mm and 10 mm bar graders, respectively. Two subgroups of 400 fish from the upper size group of LD and ND groups were chosen for further experiments conducted in 2.1-m^2 circular plastic tanks supplied with oxygenated water at a rate of 60–72 l min^{-1}. Two subgroups were fed continuously (LD,fed; mean weight 24.3 g and ND,fed; mean weight 17.0 g at the start), while the other subgroups were fasted (LD,fasted; mean weight 24.3 g and ND,fasted; mean weight 16.0 g) until 8 March, after which they were fed in the same way as the other groups. Rearing history and treatments are presented in Fig. 1.

Ten randomly selected fish from each experimental group were sampled at about one-month intervals during January–July 1992 (except February). The fish were starved for 48 h before sampling. Fish were killed by a sharp blow on the head to efficiently limit animal suffering, and then blood samples were collected from the caudal vessels into ammonium-heparinized syringes. Plasma was separated immediately by centrifugation. Plasma ion (Na^+, Cl^-, Mg^{2+} and K^+) concentrations from the fish of the freshwater groups were analysed during January–June. The fish were measured for total length to the nearest 0.1 cm and weighed to the nearest 0.1 g. The external smolt indices, i.e. distinctness of parr marks and percentage of silvered fish were estimated visually. Distinctness of parr marks was estimated on a scale of 0–4, where 0 indicates total absence of parr marks. The whole first gill arch and 1–4 g of lateral muscle close to the dorsal fin were removed. Gill and plasma samples were frozen in liquid nitrogen (–196 °C) immediately after sampling and then stored for less than a month at –80 °C before they were analysed. Water content of muscle was analysed soon after sampling.

Analytical procedures

The gill Na^+,K^+-ATPase activity was assayed with the method of Johnson *et al.* (1977) using the following conditions: ionic concentration in incubation was $MgCl_2$ 20 mM, KCl 600 mM, NaCl 240 mM and Na_2-ATP (Sigma) 10 mM, concentration of ouabain (Sigma) in determination of the Na/K-ATPase fraction was 5 mM, the incubation temperature was 25 °C and the time was 30 min. Protein levels were measured from the same homogenate as in the ATPase assay by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Muscle water content was determined as percent wet weight loss after drying the pieces at 105 °C for 24 h. Muscle lipid content was determined from the same dried samples by refluxing the sample for 24 h in 120 ml 94% ethanol with the Soxhlet method and calculated as percentage of wet weight. Plasma concentrations of Na^+ and K^+ were determined by flame photometry (FLM 3) (before analysis plasma was diluted 1:200), Cl^- with a Radiometer CMT 10 chloride titrator and Mg^{2+} spectrophotometrically using the reagent B-kit of Waco Co. (No. 999-83909).

Seawater challenge

Ten randomly selected fish from each experimental group were exposed at about one-month intervals during January–July (except February) to a seawater challenge test (SW test) for 48 hours at ca. 30‰ using ion-balanced salt (Instant Ocean). The volume of test water was 320 l per group and water was oxygenated by aquarium aerators. Tests were done at a constant temperature of 10 °C with a two- to five-day acclimation period (depending on the rearing temperature) before salt was added. The fish were not fed during acclimation or exposure. The mortality and muscle water content from these test fish were determined during January–July and concentrations of plasma ions (Cl^-, Na^+, Mg^{2+} and K^+) during April–June. Change in muscle water in seawater is expressed as a difference in water content of muscle between fish in freshwater and exposed to seawater.

Statistical procedures

Effects of time, autumn daylength and winter fasting on the different parameters were tested by three-way ANOVA. To normalise the variance, all percentage values were subjected to angular transformation (TP = $\sin^{-1} \sqrt{P}$, where P is a percentage value). The normality of length frequency distributions was tested by the Kolmogorov-Smirnov test.

Results

Size and external smolt indices

The size (mean ± S.E) of LD fish was larger (length 12.1 ± 0.1 cm and weight 17.0 ± 0.5 g) on 14 November as compared with that of ND fish (length 9.6 ± 0.1 cm and weight 8.7 ± 0.4 g). The lengths of the LD fish were normally distributed (Kolmogorov-Smirnov: $P > 0.10$), whereas the distribution of the lengths of the ND fish was skewed to the right and was not normal (Kolmogorov-Smirnov: $P < 0.001$; Fig. 2). The mean weight of the LD and the ND fish after grading on 2 December was 25 g and 15 g, respectively. The length and weight of the fish were significantly affected by time as well as by both treatments and in length there was a significant interaction between winter fasting and autumn daylength (Table 1). The LD fish showed no change in size during January–April whereas the fish in the ND fed group increased in length and weight during January–March by which time they achieved the size of the LD fish (Fig. 3). The larger size difference between the ND fed and fasted groups during March–April as compared with that in the LD groups is explained by differences in growth rate of the fed groups. During June–July, the size differences between the groups were small and the fish showed increased growth rate as compared with that in the previous months.

Distinctness of parr marks weakened after May in all groups (Appendices 1–4). In May–June, although the coloration of the fish was brighter, in none of the sampling months were the fish fully silvered (Appendices 1–4). Sex ratios are also presented in Appendices 1–4.

Lipid and water content of muscle in freshwater

The effect of muscle water content on muscle lipid content was initially included as covariate in the analysis, but it was not significant, and thus it was rejected from the model. Time was the single factor significantly affecting the lipid content of muscle, but there were interactions between time and winter fasting and between time and autumn daylength (Table 1). A slight increase in lipid content of muscle followed by a decrease during spring-summer was observed in all groups, but the change took place earlier in both fed groups as compared with the fasted groups as well as earlier in the ND fasted than the LD fasted group (Fig. 4a). The lowest levels of lipid content of muscle in fed groups were observed during June–July.

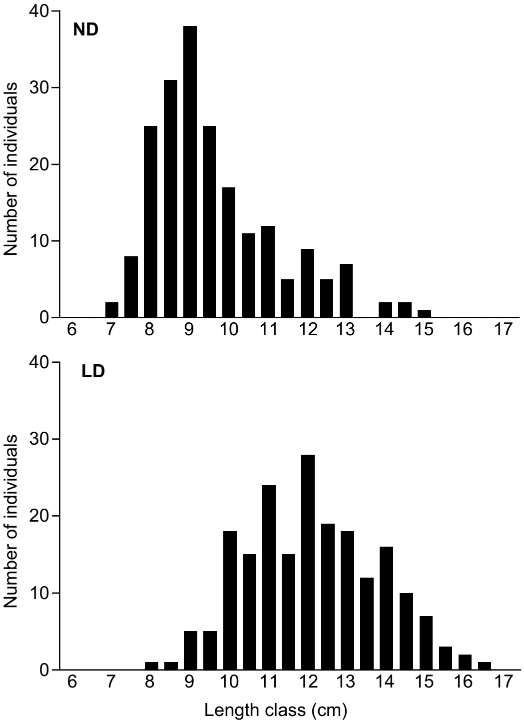

Fig. 2. Length-frequency distributions of underyearling Saimaa landlocked salmon on 14 November 1991 reared under routine farm illumination (ND) or exposed to LD 20:4 between 22 August and 30 October 1991 (LD).

The effects of all studied factors were significant on water content of muscle in freshwater (Table 1). In the ND and the LD fed groups, there was a clear decrease in muscle water content in freshwater as spring progressed (Fig. 4c). Contrary with fed groups, fasted groups showed an increase in water content of muscle during Janu-

Table 1. Effects of time (T), winter fasting (F) and autumn daylength (D) on physiological parameters of one-year-old Saimaa landlocked salmon tested by three-way analysis of variance.

Source	df	MS	*F*	*p*
Length (cm)				
T	5	43.453	52.15	0.000
F	1	28.084	33.70	0.000
D	1	32.476	38.97	0.000
T × F	5	0.714	0.86	0.511
T × D	5	0.992	1.19	0.315
F × D	1	5.468	6.56	0.011
T × F × D	5	1.165	1.40	0.226
Error	215	0.833		
Weight (g)				
T	5	1649.576	41.77	0.000
F	1	1479.586	37.47	0.000
D	1	1516.476	38.40	0.000
T × F	5	77.824	1.97	0.084
T × D	5	46.665	1.18	0.319
F × D	1	79.075	2.00	0.158
T × F × D	5	39.456	1.00	0.419
Error	215	39.488		
Muscle lipids (% ww)				
T	5	2.143E-03	6.47	0.000
F	1	1.034E-03	3.12	0.079
D	1	1.22E-04	0.37	0.544
T × F	5	1.270E-03	3.84	0.002
T × D	5	1.233E-03	3.72	0.003
F × D	1	6.548E-04	1.98	0.161
T × F × D	5	6.502E-05	0.20	0.964
Error	216	3.311E-04		
Muscle water (%) in FW				
T	5	1.773E-03	61.86	0.000
F	1	7.984E-03	278.59	0.000
D	1	4.366E-04	15.23	0.000
T × F	5	2.061E-03	71.93	0.000
T × D	5	7.036E-05	2.46	0.035
F × D	1	1.472E-06	0.05	0.821
T × F × D	5	3.625E-05	1.27	0.280
Error	216	2.866E-05		
Muscle water (%) in SW				
T	5	0.522	27.38	0.000
F	1	0.445	23.32	0.000
D	1	1.261E-03	0.07	0.797
T × F	5	0.526	27.56	0.000
T × D	5	1.500E-02	0.79	0.560
F × D	1	2.319E-02	1.22	0.272
T × F × D	4	1.111E-02	0.58	0.676
Error	190	1.907E-02		
Gill Na$^+$,K$^+$-ATPase				
T	5	168.766	68.79	0.000
F	1	10.250	4.18	0.042
D	1	207.225	84.47	0.000
T × F	5	6.670	2.72	0.021
T × D	5	28.648	11.68	0.000
F × D	1	0.101	0.04	0.840
T × F × D	5	3.503	1.43	0.215
Error	215	2.453		

ary–March (Fig. 4c). The level of muscle water content in both fasted groups was markedly higher during March–May as compared with that of the fed groups, which explains the interaction found between time and winter fasting. Due to a marked decrease of muscle water content in fasted groups from May onwards, there were no differences compared to fed groups in July. Although there was an interaction between time and autumn daylength, differences between the ND and the LD fish were small.

Gill Na$^+$,K$^+$-ATPase activity and water content of muscle after 48 h exposure to seawater

The effects of all studied factors were statistically significant on activity of gill Na$^+$,K$^+$-ATPase (Table 1). Interactions were found between time and winter fasting as well as between time and autumn daylength. The fish in the LD fed and fasted groups showed no increase in gill Na$^+$,K$^+$-ATPase activity until a sharp rise in June (Fig. 4b). Although the activity of gill Na$^+$,K$^+$-ATPase was lower in the ND fasted group compared to the ND fed group during March–April, the increase in both ND groups occurred earlier than in the LD groups (Fig. 4b). However, the peak was reached simultaneously with the LD in June, but the level was higher than that of the LD fish. In July, the enzymatic activity declined sharply in all groups.

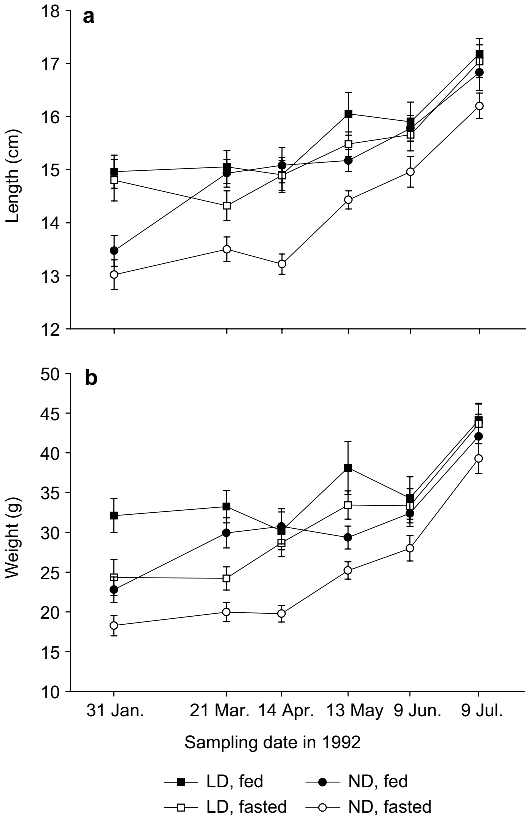

Fig. 3. Length (**a**) and weight (**b**) of one-year-old upper modal group of Saimaa landlocked salmon reared under routine farm illumination (ND) or exposed to LD 20:4 between 22 August and 30 October 1991 (LD) and either fed or fasted from 2 December 1991 to 8 March 1992. Symbols represent the mean values and vertical bars indicate standard error of the mean (S.E.).

Mortality in 48 h seawater challenge test was high in January in the fish of both fasted groups (80% and 100% in the LD and the ND fish, respectively), while mortality in the LD fed and ND fed groups was 10% and 20%, respectively. During the following months, the rate of mortality in the fasted groups decreased markedly (Appendices 2 and 4). Mortalities of the fish of the ND and LD fed groups are shown in Appendices 1 and 3, respectively. Time and winter fasting were the factors that affected the muscle water content in seawater (Table 1). In all groups, the ability to regulate the water content of muscle improved clearly as spring progressed (Fig. 4d). However, there were some differences in ability to regulate muscle water content in seawater between the fed and the fasted groups, especially concerning ND fish, as indicated by a significant interaction found between time and winter fasting.

Plasma ion concentrations in freshwater

The concentrations of all plasma ions in freshwater were significantly affected by time (Table 2). As the spring progressed, the fish in all groups showed a decreasing trend in plasma sodium, chloride and magnesium concentrations whereas the trend in potassium concentration was increasing (Figs. 5 and 6). Plasma sodium and chloride concentrations were also affected by winter fasting and in plasma magnesium

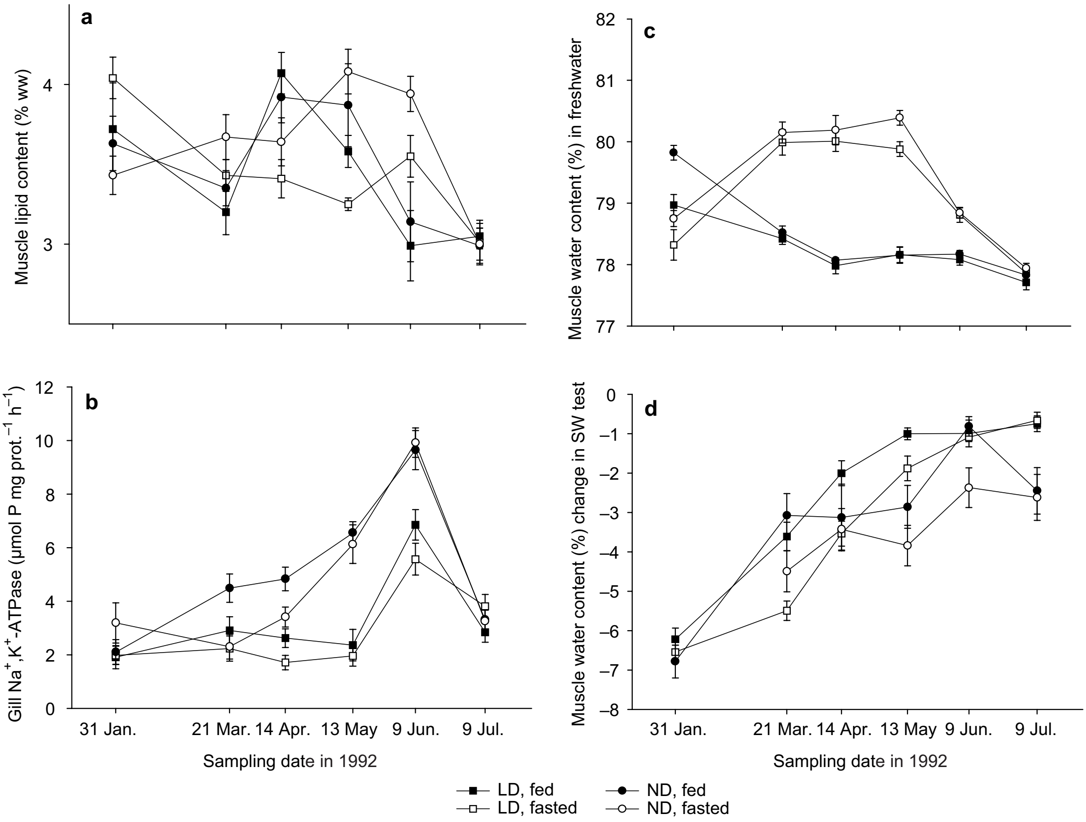

Fig. 4. Lipid content in muscle (**a**), gill Na$^+$,K$^+$-ATPase activity (**b**), water content in muscle in freshwater (**c**) and the change in muscle water content (**d**) after 48 h seawater exposure in one-year-old upper modal group of Saimaa landlocked salmon representing reared under routine farm illumination (ND) or exposed to LD 20:4 from 22 August to 30 October 1991(LD) and either fed or fasted from 2 December 1991 to 8 March 1992. Symbols as in Fig. 3, represent the mean values and vertical bars indicate standard error of the mean (S.E.).

concentration there was an interaction between time and winter fasting. Plasma potassium concentration was affected by autumn daylength and interaction was found between time and autumn daylength.

Plasma ion concentrations after 48 h exposure to seawater

Except plasma potassium concentration, the ion concentrations in seawater were significantly affected by time (Table 2). Decreasing trends in plasma ion concentrations indicating elevated ability to osmoregulate in seawater were observed in all groups (Figs. 5 and 6). Also the effect of photoperiod was significant on all ion concentrations; the ability to osmoregulate in seawater was observed a month earlier in the LD than in the ND fish. Only in plasma potassium concentration was an interaction between time and autumn daylength found.

Discussion

After the photoperiod manipulation, the fish reared under LD 20:4 during August–November were larger than those of the control group (ND) showing that artificially extended daylength stimulates the growth of juvenile Saimaa salmon as it does in other Atlantic salmon (McCormick

Table 2. Effects of time (T), winter fasting (F) and autumn daylength (D) on plasma ion concentrarions of one-year-old Saimaa landlocked salmon in freshwater and after 48 h exposure to sea water tested by three-way analysis of variance.

Source	df	MS	*F*	*p*
Plasma Na^+ in FW				
T	4	1158.270	19.79	0.000
F	1	607.176	10.37	0.002
D	1	0.523	0.01	0.925
T × F	4	35.674	0.61	0.656
T × D	4	33.248	0.57	0.686
F × D	1	146.559	2.50	0.115
T × F × D	4	23.225	0.40	0.811
Error	166	58.528		
Plasma Cl^- in FW				
T	4	373.070	13.38	0.000
F	1	334.126	11.98	0.001
D	1	11.425	0.41	0.523
T × F	4	139.191	4.99	0.001
T × D	4	28.226	1.01	0.403
F × D	1	15.966	0.57	0.450
T × F × D	4	43.200	1.55	0.190
Error	166	27.884		
Plasma Mg^{2+} in FW				
T	4	0.176	8.24	0.000
F	1	4.018E-02	1.88	0.172
D	1	1.197E-02	0.56	0.455
T × F	4	0.108	5.07	0.001
T × D	4	9.446E-03	0.44	0.778
F × D	1	0.107	4.99	0.027
T × F × D	4	2.664E-02	1.25	0.294
Error	166	2.138E-02		
Plasma K^+ in FW				
T	4	39.471	20.46	0.000
F	1	5.382	2.79	0.097
D	1	84.466	43.79	0.000
T × F	4	4.491	2.33	0.058
T × D	4	14.408	7.47	0.000
F × D	1	7.097	3.68	0.057
T × F × D	4	2.750	1.43	0.228
Error	166	1.929		
Plasma Na^+ in SW				
T	3	5545.579	21.77	0.000
F	1	1297.176	4.74	0.032
D	1	112.915	4.37	0.039
T × F	3	588.512	2.31	0.081
T × D	2	354.663	1.39	0.254
F × D	1	229.696	0.902	0.345
T × F × D	2	196.036	0.77	0.466
Error	92	254.747		

Continues

Table 2. Continued.

Source	df	MS	*F*	*p*
Plasma Cl^- in SW				
T	3	4366.459	17.22	0.000
F	1	594.111	2.34	0.129
D	1	1235.497	4.87	0.030
T × F	3	467.317	1.84	0.145
T × D	2	229.115	0.90	0.409
F × D	1	226.006	0.89	0.348
T × F × D	2	430.250	1.70	0.189
Error	92	253.507		
Plasma Mg^{2+} in SW				
T	3	6.082	6.82	0.000
F	1	1.452	1.628	0.205
D	1	11.619	13.03	0.000
T × F	3	1.278	1.43	0.238
T × D	2	0.512	0.58	0.565
F × D	1	0.251	0.28	0.597
T × F × D	2	4.273	4.79	0.010
Error	92	0.892		
Plasma K^+ in SW				
T	3	0.950	0.21	0.887
F	1	1.172	0.26	0.610
D	1	57.862	12.97	0.001
T × F	3	1.930	0.43	0.730
T × D	2	18.243	4.09	0.020
F × D	1	9.832	2.20	0.141
T × F × D	2	1.005	0.23	0.799
Error	92	4.463		

et al. 1987, Björnsson *et al.* 1989, Saunders *et al.* 1989, Thorpe *et al.* 1989, Saunders & Harmon 1990, Stefansson *et al.* 1991, Berge *et al.* 1995, Sigholt *et al.* 1995). Besides the LD fish being generally larger, the proportion of the fish in the upper size classes was greater suggesting that the number of potential one-year-old smolts may be increased. This, however, needs further research.

Since neither the ND nor the LD fish showed bimodality in their length-frequency distribution on 14 November the fish from upper size classes were selected for further experiments by grading. During January–April, there was no change in size of the LD fish whereas the ND fish increased in length and weight during January–March, by which time they achieved the size of the LD fish. Between the ND fed and fasted groups there was a clear size difference during March–April, but soon after the feeding was started again, they compensated for their growth suppression and achieved the size of the fed group in May. From May onwards there were no size differences between any of the groups. Compensatory responses are more persistent and stronger among premigratory than among nonmigratory fish as well as among upper than lower modal group fish in association with different life-history strategies adopted by these fish (Nicieza & Metcalfe 1997). The hyperphagic response may be a mechanism underlying compensatory growth (Bull & Metcalfe 1997, Nicieza & Metcalfe 1997). In some cases, however, restricted-re-fed animals may show rapid recovery growth without becoming hyperphagic (Jobling 1995). This suggests the possibility of improved food conversion efficiency that is possibly linked to differences in metabolic expenditures between restricted-re-fed and continuously fed animals (Jobling 1995).

The determination of whether an individual

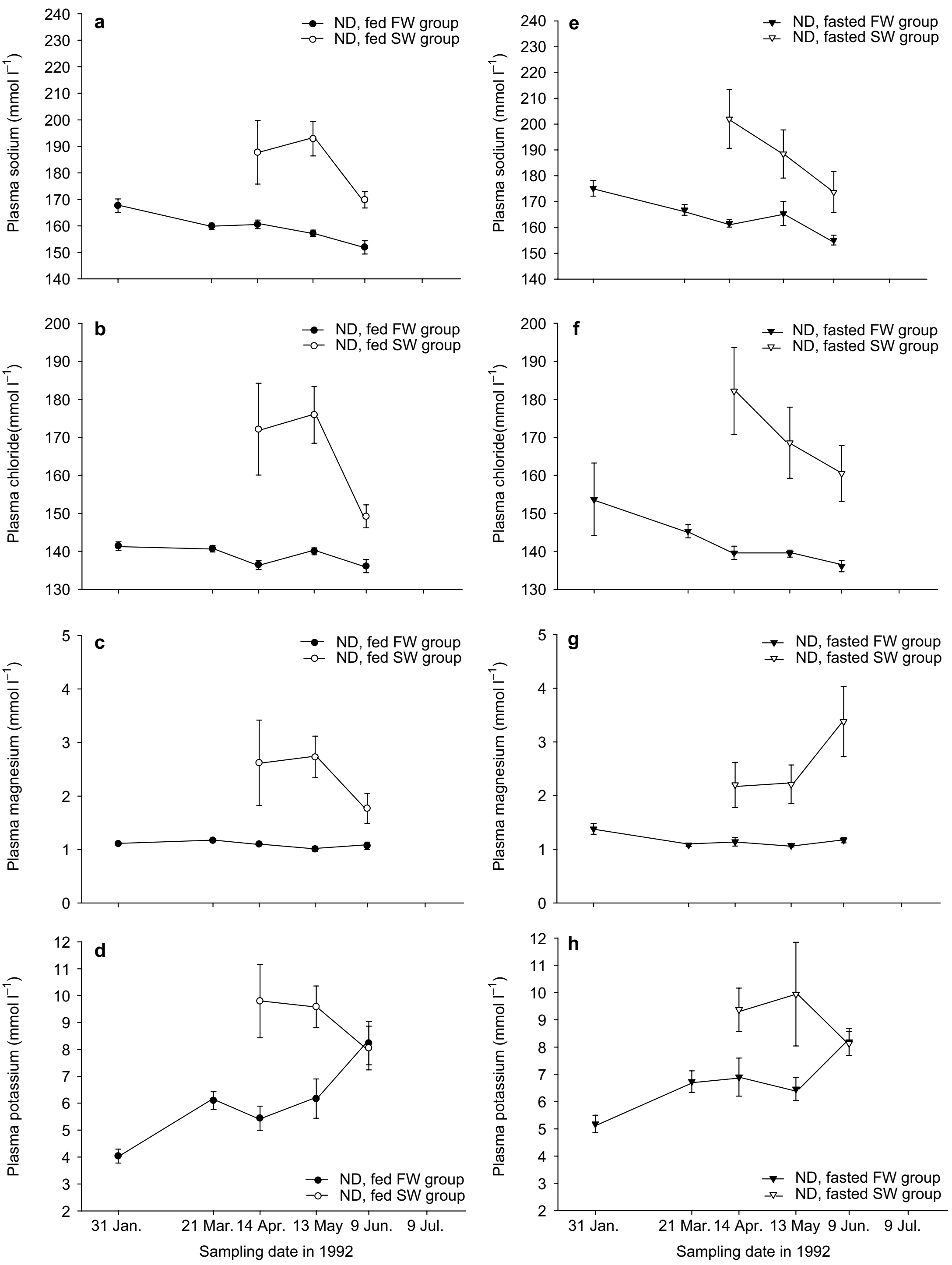

Fig. 5. Plasma sodium, chloride, magnesium and potassium concentrations in freshwater and following 48 h exposure to seawater in one-year-old upper modal group of Saimaa landlocked salmon reared under routine farm illumination and either fed (ND fed) or fasted (ND fasted) from 2 December 1991 to 8 March 1992 (**a**, **b**, **c** and **d**) and (**e**, **f**, **g** and **h**), respectively. Symbols represent the mean values and vertical bars indicate standard error of the mean (S.E.).

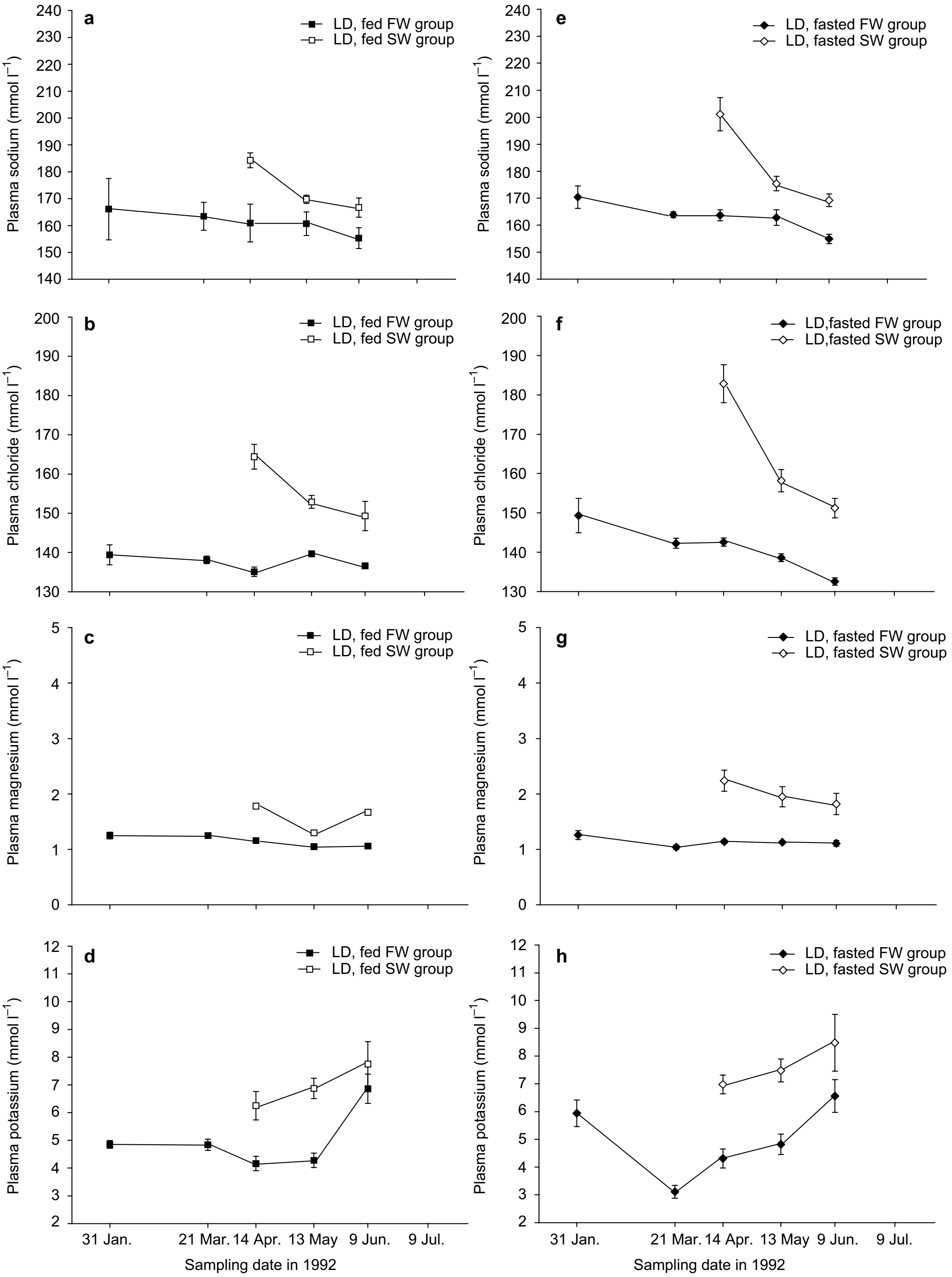

Fig. 6. Plasma sodium, chloride, magnesium and potassium concentrations in freshwater and following 48 h exposure to seawater in one-year-old upper modal group of Saimaa landlocked salmon exposed to LD 20:4 from 22 August to 30 October 1991 and either fed (LD fed) or fasted (LD fasted) from 2 December 1991 to 8 March 1992 (**a**, **b**, **c** and **d**) and (**e**, **f**, **g** and **h**), respectively. Symbols represent the mean values and vertical bars indicate standard error of the mean (S.E.).

will undergo smolting the following spring occurs in late summer; if the size and growth rate of the fish exceed certain genetically determined threshold levels it will complete smolting in the following spring (Metcalfe 1998, Thorpe *et al.* 1998). Gill Na^+,K^+-ATPase activity is a commonly used indicator of smolt status. The fish in the ND fed group showed an increase in gill Na^+,K^+-ATPase activity during January–March reaching the peak value in June, whereas no such increase was observed in the ND fasted group until April–May. This suggests that smolting may be switched off if the fish's performance drops below expected levels (Metcalfe 1998). The fish in the ND fasted group, however, achieved smolt status similar to the ND fed group soon after the feeding was started as indicated by elevated gill Na^+,K^+-ATPase activity. Contrary to the ND fish, the fish in the LD groups showed no increase in gill Na^+,K^+-ATPase activity until a sharp rise in July. This suggests that autumn photoperiod manipulation disturbed the endogenous smolting cycle of these fish. This may also explain growth suppression observed in the LD fish during January–April. However, despite these differences both the ND and the LD fish appeared to complete their smolting at the same time in June as indicated by increased gill Na^+,K^+-ATPase activity, decreased lipid content of muscle as well as good salinity tolerance and hypoosmoregulatory ability. The pronounced depletion of lipids accompanying smolting may indicate that metabolic demands are severe — more severe than can be met by dietary means (Sheridan 1989). He also suggested that mobilized energy from lipid depletion might be channelled into hypoosmoregulatory adjustments. Although there was a decreasing trend in lipid content of muscle, the present results allow no conclusions about the energetic costs of smolting since there are no food intake data for the fish.

Along with gill Na^+,K^+-ATPase activity, smolt status is commonly evaluated by silvering of the skin and disappearance of parr marks. Despite other changes typical for smolting, in none of the sampling months were the ND or the LD fish fully silvered with totally disappeared parr marks. According to Kazakov and Kozlov (1985) the rate of silvering of Atlantic salmon juveniles reared in fish farms is greatly affected by the intensity of illumination, thus suggesting a strong dependence of the silvering dynamics on the background coloration or light conditions in which the Atlantic salmon juveniles live. Also Kato (1972) reported that juvenile rainbow trout (*Oncorhynchus mykiss*) reared in a blue tank attained smolt-like silvery coloration independent of season and considered silvering as camouflaging adaptation. Coloration of the fish in the present study may be explained by low illumination intensity of the rearing hall.

Gill Na^+,K^+-ATPase is known to play a central role in the salt-secretory function of the chloride cell (McCormick 1995). The rise of this enzyme activity, however, occurs long before it would be needed for seawater adaptation (Thorpe & Moore 1997) and without exposure to increasing salinity as showed by the present results. Several papers have suggested that the increase of gill Na^+,K^+-ATPase activity in freshwater would primarily reflect an attempt to compensate for ion losses associated with a gradual deteriorating of freshwater osmoregulatory capacity (Langdon & Thorpe 1985, Simpson 1985, Primmett *et al.* 1988, Reis-Henriques *et al.* 1996, Thorpe & Moore 1997). The present results support this since lower levels of plasma sodium and chloride were observed in all groups in freshwater during the period of elevated Na^+,K^+-ATPase activity. In freshwater, gill Na^+,K^+-ATPase may operate in series with the apical proton pump to energize the uptake of sodium from the water (Lin & Randall 1995). However, overall increase of gill Na^+,K^+-ATPase activity is also linked up with increased sodium efflux potential capable of activation in seawater (Primmett *et al.* 1988). In the ND fish of the present study, there was a relationship between increase of gill Na^+,K^+-ATPase activity and elevated capacity to hypoosmoregulate whereas the LD fish showed good capacity to hypoosmoregulate before any elevation of gill Na^+,K^+-ATPase activity in freshwater. This suggests that gill Na^+,K^+-ATPase may have been induced by exposure to seawater as reported by Berge *et al.* (1995) in yearling Atlantic salmon smolts. The results from Saimaa landlocked salmon (Kiiskinen *et al.* unpubl. data), however, suggest that induction of Na^+,K^+-ATPase is rather a consequence of current developmental state (smolting) of the fish than a single response to salinity.

A high water content of muscle during smolting found in the previous study with Saimaa salmon was also interpreted as an indication of diminished freshwater osmoregulatory capacity (Kiiskinen *et al.* 2002). Increased urine output reported in smolting Atlantic salmon by Eddy and Talbot (1985) may be seen as an attempt to restore water balance in freshwater by the fish that were becoming more permeable to water (Thorpe & Moore 1997). Since magnesium is mainly or entirely excreted through the kidney (Hickmann & Trump 1969) a decrease of plasma magnesium observed in the present study may indicate increased excretion rate of the kidney and thus suggests osmoregulatory difficulties in freshwater as well. Loss of smolt characteristics, as indicated by a decrease in gill Na^+,K^+-ATPase activity, was observed in both the ND fish and the LD fish in July. Although no clear reduction in ability to maintain muscle water content in seawater was observed in the LD or ND fish in July, it is not known whether this is an indication of high hypoosmoregulatory ability since plasma ion concentrations were not determined. An earlier study with hatchery-reared Saimaa salmon showed that both parameters are needed to estimate hypoosmoregulatory ability (Kiiskinen *et al.* 2002). Both the ND and the LD fish showed enhanced growth rate at the time when smolt characteristics were lost. Thus, a slow growth observed in the present as well as in the previous study (Kiiskinen *et al.* 2002) during smolting may be a consequence of loss of osmoregulatory capacity in freshwater.

As reported above, a temporary loss of freshwater osmoregulatory capacity appears to be characteristic for smolt development of Saimaa salmon. This is consistent with the idea that downstream movement would be more like a passive than an active process (Thorpe 1989, Thorpe & Moore 1997). In other words, arrival in the sea, or in the lake, may be seen as a consequence of what has been happening in the fish in freshwater. Indeed, reduced swimming performance has been reported in smolts of Atlantic salmon (Thorpe & Morgan 1978), Baltic salmon (*Salmo salar*; Virtanen & Forsman 1987), Coho salmon (*Oncorhynchus kisutch*; Smith 1982), and sea-run and lake-run stocks of brown trout (*Salmo trutta*; Pirhonen *et al.* 1998). Unlike other ion concentrations determined in the present study, plasma potassium concentration in all groups increased with increasing gill Na^+,K^+-ATPase activity. Increased level of potassium in plasma suggests that potassium is leaking out of the cells. In human physiology, a high level of potassium in the extracellular fluids has a depressor effect on conduction of nerve and muscle impulses along membranes (Guyton 1961) i.e. leaking of potassium out of the cells leads to weakening of the muscle function. This might explain the decrease in willingness of smolts to swim against a current. In a wider context, smolting and its component processes are suggested as a representation of failure to meet the necessary and sufficient conditions for maturation in the freshwater (stream) environment (Thorpe 1994). In other words, smolting is regarded as a set of subsidiary processes relative to maturation. Viewed this way, smolting represents a negative developmental decision chosen by a fish that has failed to mature as parr (Thorpe & Metcalfe 1998). This seems a reasonable explanation considering the question why smolt phase, involving a temporary maladaptation to freshwater, is retained in salmon that spends its whole life cycle in freshwater.

In conclusion, gill Na^+,K^+-ATPase was found to be the most reliable indicator of smolt status, no matter what role it plays. Silvering, instead, appears to be more or less unreliable. Artificially extended daylength promoted the growth of the LD fish during autumn and increased the proportion of the fish in the upper size classes, but it appeared to disturb the endogenous smolt cycle of these fish. Thus, if the aim is to produce one-year-old smolts the best result may be achieved simply by using warm water to accelerate the early development of the fish as was done in the case of the ND fish of the present study. If migration downstream is more like a passive than active process brought on by gradual deterioration of freshwater osmoregulatory capacity as suggested by the present as well as earlier results (Kiiskinen *et al.* 2002), the stockings of smolts should be made prior to completion of smolting. Additional benefits of early stocking time include lower stress level of handling and transport due to cold water temperature. Earlier stockings may also benefit the imprinting of the fish in the river that is connected to smolting process (Quinn 1993). Imprinting of the juveniles in the river is essential concerning the subsequent homing of spawners back in the river, where they are trapped for collection of milt and eggs for cultivation. As shown by the results from the ND fasted group, however, stockings should not be made too early either since smolting may be switched off if nutritional conditions are inadequate. It is poorly known how quickly cultivated juveniles learn to forage in natural environment.

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Appendix 1. External smolt indices (mean ± S.E.), mortality following 48 h exposure to seawater at ca. 30‰ salinity and sex ratio for one-year-old Saimaa landlocked salmon (*Salmo salar m. sebago* Girard) reared under normal hatchery conditions (ND fed group).

Parameter	Date in 1992					
	31 January	21 March	14 April	13 May	9 June	9 July
Temperature (°C)	1.6	1.8	1.9	3.4	8.5	14.0
Parr marks[1] (0–4)	4.0 ± 0.00	4.0 ± 0.00	3.9 ± 0.10	3.8 ± 0.13	3.3 ± 0.15	3.1 ± 0.10
Parr marks[2] (0–4)	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.8 ± 0.13
Silvered (%)	0	0	0	0	0	0
Sex (male/female)	5/5	3/7	4/6	5/5	5/5	6/4
Mortality (%) in SW test	20	30	10	20	0	0

[1] Visibility of parr marks on the surface of scales.
[2] Visibility of parr marks on the surface of skin.

Appendix 2. External smolt indices (mean ± S.E.), mortality following 48 h exposure to seawater at ca. 30‰ salinity and sex ratio for one-year-old Saimaa landlocked salmon (*Salmo salar m. sebago* Girard) reared under normal hatchery conditions, but fasted from 2 December 1991 to 8 March 1992 (ND fasted group).

Parameter	Date in 1992					
	31 January	21 March	14 April	13 May	9 June	9 July
Temperature (°C)	1.6	1.8	1.9	3.4	8.5	14.0
Parr marks[1] (0–4)	3.8 ± 0.13	3.9 ± 0.10	4.0 ± 0.00	4.0 ± 0.00	3.4 ± 0.16	3.2 ± 0.13
Parr marks[2] (0–4)	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.8 ± 0.13
Silvered (%)	0	0	0	0	0	0
Sex (male/female)	7/3	7/3	5/5	6/4	7/3	7/3
Mortality (%) in SW test	100	20	30	0	0	0

[1] Visibility of parr marks on the surface of scales.
[2] Visibility of parr marks on the surface of skin.

Appendix 3. External smolt indices (mean ± S.E.), mortality following 48 h exposure to seawater at ca. 30‰ salinity and sex ratio for one-year-old Saimaa landlocked salmon (*Salmo salar m. sebago* Girard) reared under LD 20:4 from 22 August to 30 October 1991 (LD fed group).

Parameter	Date in 1992					
	31 January	21 March	14 April	13 May	9 June	9 July
Temperature (°C)	1.6	1.8	1.9	3.4	8.5	14.0
Parr marks[1] (0–4)	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.7 ± 0.15	3.4 ± 0.16	2.8 ± 0.13
Parr marks[2] (0–4)	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.8 ± 0.13
Silvered (%)	0	0	0	0	0	0
Sex (male/female)	6/4	4/6	4/6	9/1	7/3	4/6
Mortality (%)	10	20	0	0	0	0

[1] Visibility of parr marks on the surface of scales.
[2] Visibility of parr marks on the surface of skin.

Appendix 4. External smolt indices (mean ± S.E.), mortality following 48 h exposure to seawater at ca. 30‰ salinity and sex ratio for one-year-old Saimaa landlocked salmon (*Salmo salar m. sebago* Girard) reared under LD 20:4 from 22 August–30 October 1991 and fasted for three months from December 1991–March 1992 (LD fasted group).

Parameter	Date in 1992					
	31 January	21 March	14 April	13 May	9 June	9 July
Temperature (°C)	1.61.8	1.93.4	8.514.0			
Parr marks[1] (0–4)	4.0 ± 0.00	4.0 ± 0.00	3.9 ± 0.10	3.9 ± 0.10	3.3 ± 0.21	3.0 ± 0.15
Parr marks[2] (0–4)	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.8 ± 0.10
Silvered (%)	0	0	0	0	0	0
Sex (male/female)	7/3	7/3	6/4	3/7	3/7	5/5
Mortality (%)	80	0	0	0	1 0	0

[1] Visibility of parr marks on the surface of scales.

[2] Visibility of parr marks on the surface of skin.