Effects of temperature and salinity on osmoregulation and growth of Atlantic salmon (Salmo salar L.) smolts in seawater

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Abstract

One of the main developmental events of the smoltification process of Atlantic salmon (Salmo salar L.) is the pre-adaptation to an increase in salinity. Seawater acclimation involves a series of physiological changes which are critical for subsequent performance. The aim of this study was to monitor some important physiological mechanisms involved in seawater adaptation under different salinity (28 and 34‰) and temperature (4 and 8°C) regimes. An increase in plasma chloride levels and a decrease in muscle water was observed in all groups after 24 h of seawater exposure. Salinity did not affect plasma chloride levels nor tissue moisture, and no interactions between temperature and salinity were found. Temperature affected plasma chloride levels significantly after 12 h of seawater exposure, with the 4°C groups having lower levels than the 8°C groups. Between days 1 and 14, muscle water was observed to increase and then stabilise in the 8°C groups, while the low temperature groups required a further 14 days until tissue moisture was at levels similar to the freshwater group. After an initial reduction, both groups at 8°C showed elevated and stable gill Na⁺,K⁺-ATPase activities compared with the low temperature groups, which showed a long-term decrease. Salinity did not affect gill Na⁺,K⁺-ATPase activity and no interactions between temperature and salinity were found. During the first 2 months of seawater exposure, the growth pattern was affected by temperature only, while higher growth rate in brackish water at low temperature (4°C) in the period between days 64 and 90 indicates that a
reduction in salinity may improve long-term growth in the sea. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Smoltification in Atlantic salmon (Salmo salar L.) includes changes in morphology, behaviour and physiology which pre-adapt the fish to life in seawater (McCormick and Saunders, 1987; Hoar, 1988). The synchronous completion of smoltification is influenced by size (Boeuf et al., 1985) and several environmental factors including photoperiod (McCormick et al., 1987; Saunders and Harmon, 1990; Duston and Saunders, 1992) and temperature (Solbakken et al., 1994). Today, this knowledge is being used in the production of out-of-season smolts (Thrush et al., 1994). In 1994, the production of out-of-season salmon smolts (0 + ) in Norway was about 12 million smolts, or about 13% of the total smolt production (Anon, 1994) with numbers increasing in 1995–1996. The transfer of smolts to seawater in autumn is of great importance to the salmon industry as it reduces the rearing time in the hatchery, with growth in seawater starting several months prior to the natural smoltification in spring (see discussion in Thrush et al., 1994; Berge et al., 1995).

During seawater acclimation, salmon smolts experience hyper-osmotic stress, resulting in an initial and rapid increase in plasma ion concentrations (McCormick et al., 1989) and tissue dehydration (Blackburn and Clarke, 1987). This is followed by an adjustment period, the duration of which varies with fish size (Bjerknes et al., 1992), smolt status (McCormick et al., 1989; Sigholt and Finstad, 1990) and temperature (Sigholt and Finstad, 1990). The degree of disruption of the osmotic balance during seawater exposure is related to seawater tolerance which is in part related to the levels of gill Na⁺,K⁺-ATPase activity (Sigholt et al., 1995). In salmonids, a significant increase in gill Na⁺,K⁺-ATPase activity is normally observed after few days in seawater, followed by stabilisation at higher levels (Madsen and Naamansen, 1989; Berge et al., 1995).

One of the main problems related to out-of-season smolt transfers, e.g., during the period October–April, is the low and decreasing seawater temperature. In several experiments, Atlantic salmon pre-smolts and smolts acclimated to seawater at low temperatures have been reported to suffer increased osmotic disturbance (Virtanen and Oikari, 1984; Sigholt and Finstad, 1990), low growth rates and high mortality (Saunders et al., 1975). To reduce the osmotic disturbance, it has been suggested that smolts might be transferred to seawater at lower salinities, e.g., at fjord sites at 28–30‰, as earlier experiments indicate that survival of salmon parr is improved if the fish are gradually acclimated to full strength seawater (Bjerknes et al., 1992).

To elucidate the interrelationship between salinity and temperature on osmotic balance and growth in seawater, groups of smolts were transferred to two salinity regimes (28 and 34‰) at two temperatures (4 and 8°C). Hypo-osmoregulatory ability was assessed by measuring plasma chloride levels, gill Na⁺,K⁺-ATPase activity and muscle water content. Growth was monitored in individually tagged fish.
2. Materials and methods

2.1. Fish stock and rearing conditions

This experiment was carried out indoors between January and September 1995, at the Industrial Laboratory in the Bergen High Technology Centre. In December 1994, the fish (MOWI strain, \( n = 750 \), initial mean weight = 54.9 SD ± 21.7 g, mean length = 14.5 SD ± 1.8 cm, age: 0+) were brought in from Søvereid Fish Farming Industry (Søvereid, Norway) and placed in four 1 m\(^2\) grey rearing tanks (500 l), covered with lids to prevent light leakage. Light was provided by a fluorescent daylight tube mounted under the tank cover (200 lx at the bottom of the tanks), and natural photoperiod including dusk and dawn was simulated by a computer program. A commercial dry diet (Nutra svev, T. Skretting, Stavanger, Norway) was fed in excess (110%) according to fish size and temperature (Austereng et al., 1987) during light hours. The temperature regime in freshwater followed the natural ambient temperature increasing from 2 to 3°C during January–March to approximately 10°C in late May. Flow rate was maintained at about 20 l min\(^{-1}\) (\( \text{O}_2 \) saturation > 80%). On 28 April, a randomly collected sub-population of 40 individuals from each tank was individually tagged (Carlin tags, McAllister et al., 1992) for growth measurements.

2.2. Experimental design

Monthly between January and May, 12 fish were collected and their hypo-osmoregulatory ability assessed by measuring plasma chloride levels after a 24-h seawater challenge test (34.5%, Blackburn and Clarke, 1987). At the same time, gill Na\(^+\),K\(^+\)-ATPase activity was analysed from gill filaments collected from 12 fish in freshwater. The fish were starved for 24 h prior to sampling and seawater exposure. In mid-May, all smolts showed normal morphological signs of smolting, i.e., dark fin margins, absence of parr marks and loose silvery scales.

The smolts were transferred to seawater on 27 May and distributed randomly into four separate rearing tanks in a 2 \( \times \) 2 factorial design; high and low salinity (28 and 34\%, range ± 0.1%) and high and low temperature (4 and 8°C, range ± 0.1°C). Five days prior to seawater transfer, the fish were acclimated to the new temperatures while still in freshwater. The temperature acclimation procedure in all groups included a 2°C temperature reduction between days 0 and 1, followed by daily 1°C temperature reductions between days 1 and 5. The transfer from freshwater to the different qualities of seawater was obtained by changing the water supply into the tanks, which produced stable experimental conditions within 1 h.

2.3. Sampling procedures

Blood and muscle samples were collected randomly from eight fish in each group at the following times: in freshwater 1 day before seawater transfer, after 12 h, 1, 2, 4, 8,
14, 30 and 60 days in seawater. All fish were starved 24 h prior to sampling and were killed by a blow to the head. Blood was collected with heparinized syringes from the caudal peduncle, plasma obtained by centrifugation at 4°C and 4000 rpm (rotor diameter 20 cm), and analysed for chloride levels (mM) in duplicate 20 μl samples in a Radiometer CMT 10 titrator. White muscle was collected with a cross-section in the tail (approximately 1 cm³) and water content calculated as weight loss after 96 h at 80°C. Gill filaments were frozen in SEI buffer at −80°C and subsequently analysed for gill Na⁺,K⁺-ATPase activity using the method of McCormick (1993). Fork length (to nearest 0.1 cm) and weight (to nearest 0.1 g) of the individually tagged smolts in each group were determined at monthly intervals in seawater to study individual growth in seawater adapted smolts. Specific growth rate was calculated as \( \frac{\ln W_2 - \ln W_1}{\Delta T} \) where \( \Delta T \) is the number of days between times \( T_1 \) and \( T_2 \).

2.4. Statistical analysis

Values in figures and table are means (± SE). Prior to the statistical analysis, all data were log-transformed and tested for normality and homogeneity of variance among the different groups using a Kolmogornov–Smirnov test and a Hartley \( F \)-max test, respectively (Sokal and Rolf, 1995). Mean values within and between groups were compared by one-way and two-way ANOVA, respectively, followed by Student–Newman–Keul post-hoc tests if the treatment had a significant effect. All data from individually tagged fish were analysed statistically by MANOVA.

3. Results

3.1. Development of hypo-osmoregulatory ability in freshwater between January and May

Between January and March, no significant changes were observed in gill Na⁺,K⁺-ATPase activity (Fig. 1). However, from April to May, gill Na⁺,K⁺-ATPase activity increased threefold (\( p < 0.01 \)), concurrent with increases in temperature and day length. Correspondingly, plasma chloride levels from the seawater challenge tests decreased from January (≈ 186 mmol l⁻¹, SE ± 6.6) to March (≈ 146 mmol l⁻¹, SE ± 2.1, \( p < 0.01 \)). Between March and May, no further changes were observed in plasma chloride levels, with final levels in May (≈ 144 mmol l⁻¹, SE ± 1.1).

3.2. Plasma chloride levels and water balance in muscle tissue in seawater

A significant increase in plasma chloride levels was observed in all groups (\( p < 0.01 \), Fig. 2a and b) and a decrease in muscle water was seen in two groups (\( p < 0.01 \); in group 4°C, 28‰ and 4°C, 34‰, Fig. 3a and b) after 24 h of seawater exposure. Plasma chloride appeared to reach stable levels between 138–144 mmol l⁻¹ after 12 h in seawater, with no further changes in any groups during the experiment. Salinity did not affect either plasma chloride levels nor tissue moisture, and no interactions between
temperature and salinity were found. Temperature was, however, found to affect plasma chloride levels significantly after 12 h of seawater exposure, with the 4°C groups having significantly lower levels than the 8°C groups (p < 0.05). After 12 and 24 h of seawater exposure, muscle water content was significantly higher in the 8°C groups compared with the 4°C groups (p < 0.05). Between days 1 and 14, muscle water was observed to increase and then stabilise in the 8°C groups, while the low temperature groups required a further 14 days until tissue moisture was at levels similar to the freshwater group (p < 0.01, between days 1 and 30 in group 4°C, 34½). During this period, temperature was found to affect water content in muscle tissue significantly at days 8 and 14, with the 8°C groups having significantly higher water content than 4°C groups.

3.3. Gill Na⁺,K⁺-ATPase activity in seawater

A small increase in gill Na⁺,K⁺-ATPase activity was observed in groups 4°C, 28%e and 4°C, 34% during the first 24–48 h of seawater exposure, while a temporary decrease in gill Na⁺,K⁺-ATPase activity was observed at 12 and 24 h in groups 8°C, 34%e (p < 0.05) and 8°C, 28%, respectively (Fig. 4a and b). Between days 2 and 8, both groups at 8°C showed elevated and stable gill Na⁺,K⁺-ATPase activities compared with the low temperature groups, which showed a long-term decrease in enzyme activity (p < 0.05, in group 4°C, 34%). Gill Na⁺,K⁺-ATPase activity was significantly higher in the 8°C groups than the 4°C groups after 8 days of seawater exposure (p < 0.05). Salinity did not affect gill Na⁺,K⁺-ATPase activity and no interactions between temperature and salinity were found.
3.4. Mortality in seawater

There was no mortality during the first 8 days of seawater exposure (27 May–2 June) whereas on 3 and 5 June, one fish died in groups 8°C, 34%e and 4°C, 28%e. For the rest
Fig. 3. Muscle water content during exposure to 28°C (□) and 34°C (△) seawater at 4°C (A) and 8°C (B). Values are given as means ± SE (n = 8).

of this experiment, no mortalities occurred in the high temperature groups. At low temperature, four fish died in 34°C and three died in 28°C between 3 July and 23 August.
Fig. 4. Gill Na⁺, K⁺-ATPase activity during exposure to 28‰ (□) and 34‰ (△) seawater at 4°C (A) and 8°C (B). Values are given as means ± SE (n = 8).

3.5. Growth in seawater

The growth pattern during the first 2 months in seawater was significantly affected by temperature, but not by salinity (Fig. 5, Table 1). After 23 days in seawater, the mean
weight in group 8°C, 34‰ was significantly higher than in the low temperature groups (p < 0.05). For the rest of the experiment, the mean weights in the 8°C groups were significantly higher than that of the low temperature groups (p < 0.01) with no differences between high and low salinity at either temperature. Growth rates were not affected by salinity during the first 2 months of seawater exposure (Table 1). However, between days 64 and 90, significant differences in growth rates were observed among all treatments (p < 0.01). The highest growth rates were observed in the high temperature groups, with the high salinity groups (8°C, 34‰) showing greater growth than the low

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
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<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Salinity (%)</td>
<td>Percentage of growth per day</td>
<td>Percentage of growth per day</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>0.39 ± 0.10 a</td>
<td>0.44 ± 0.04 a</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>0.36 ± 0.05 a</td>
<td>0.36 ± 0.05 a</td>
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<tr>
<td>8</td>
<td>28</td>
<td>0.40 ± 0.04 a</td>
<td>0.97 ± 0.03 b</td>
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<tr>
<td>8</td>
<td>34</td>
<td>0.42 ± 0.05 a</td>
<td>1.08 ± 0.03 b</td>
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Significant differences (p < 0.01) within a period are indicated by different letters (Period 1: from tagging to day 23 in seawater; Period 2: days 23–64; Period 3: days 64–90).
salinity group (8°C, 28%), while the low temperature and high salinity group (4°C, 34%) showed the lowest growth rate, significantly below the 4°C, 28% group (p < 0.01).

4. Discussion

The smoltification process and the development of hypo-osmoregulatory ability are critical factors in ensuring survival and growth in full strength seawater. Hence, juvenile Atlantic salmon transferred outside the ‘smolt window’ suffer osmotic disturbances (Stagg et al., 1989), low growth (Björnsson et al., 1988) and high mortality (Berge et al., 1995). The development of seawater tolerance in Atlantic salmon smolts is correlated to an increase in gill Na\(^{+}\),K\(^{+}\)-ATPase activity as the enzyme activity peaks in early spring (McCormick et al., 1987). Gill Na\(^{+}\),K\(^{+}\)-ATPase activity is correlated to both active ion uptake and excretion (Evans, 1982) and a general assumption is made that a significant increase in gill Na\(^{+}\),K\(^{+}\)-ATPase activity is necessary to maintain normal electrolyte and water balance in seawater (McCormick et al., 1987). The present data show that plasma chloride levels after 24 h of seawater exposure between March and April were regulated within the normal range of seawater acclimated smolts before any significant increase in enzyme activity. The development of hypo-osmoregulatory ability in advance of peak gill ATPase activity concurs with earlier studies of smolting Atlantic salmon (Saunders and Harmon, 1990; Solbakken et al., 1994; Berge et al., 1995) and contrasts with the assumption of a role for gill Na\(^{+}\),K\(^{+}\)-ATPase during smoltification. According to Potts et al. (1970), Atlantic salmon parr in seawater maintain low transepithelial salt fluxes and low permeability characteristics, whereas seawater-adapted smolts exhibit much higher fluxes, similar to marine fish. This suggests that the mechanisms by which parr osmoregulates differently from smolt and changes during smoltification may explain the observed disagreement between gill Na\(^{+}\),K\(^{+}\)-ATPase activity and plasma chloride levels. However, the observed increase in gill Na\(^{+}\),K\(^{+}\)-ATPase activity between April and May is in agreement with the results of McCormick et al. (1987), and show that the smolt transfer in this experiment was timed according to a maximum in gill Na\(^{+}\),K\(^{+}\)-ATPase activity.

According to Bath and Eddy (1979), the temporal pattern of seawater acclimation in rainbow trout (Oncorhynchus mykiss) can be separated into two different phases; initial adaptation (crisis period) and a subsequent stabilisation period. The initial phase lasts for about 4 days and includes rapid physiological changes, whereas the stabilisation period includes slower physiological changes and results in a new equilibrium with seawater within a period of 8–10 days (Bath and Eddy, 1979). During the initial phase, the fish have to cope with water loss and an increased salt load. In marine fish, the movement of chloride from plasma into the chloride cell is believed to be a secondary active transport energised by the co-transfer of sodium, and where the steep diffusion gradient is caused by the Na\(^{+}\),K\(^{+}\)-pump on the basal membrane of the cell (de Renzis and Bornancin, 1984; Greger and Schlatter, 1984). Chloride is further transported out of the chloride cell in one of two ways: passive movements through the apical membrane or by an active Cl\(^{-}\)–HCO\(_3^{-}\) exchange (de Renzis and Bornancin, 1984). Previous studies have shown that plasma chloride reflects the osmotic changes experienced by Atlantic salmon during
seawater acclimation (Virtanen and Oikari, 1984; McCormick et al., 1989; Sigholt and Finstad, 1990; Solbakken et al., 1994). In the present study, all groups exhibited a significant increase in plasma chloride levels and a dehydration of muscle tissue during the first 12 h of seawater exposure, which is in line with earlier findings on Atlantic salmon (Sigholt and Finstad, 1990; Handeland et al., 1996). No further changes were observed in plasma chloride levels, indicating that the fish quickly established effective hypo-osmoregulation in seawater.

In all groups, a prolonged period of tissue rehydration was observed compared with the quick stabilisation of plasma chloride levels. Rainbow trout transferred from freshwater to diluted seawater show a disproportionate distribution in salt load between extra- and intracellular compartments, with elevated levels of muscle ions after a stabilisation of plasma ion levels (Bath and Eddy, 1979; Finstad et al., 1988). Tissue moisture is known to reflect changes in both extra- and intracellular fluid volumes (Finstad et al., 1988; Sigholt and Finstad, 1990). A redistribution of salt load between extra- and intracellular compartments may thus explain the observed delay in the re-establishment of water balance in this experiment. Taken together, the results show that longer time is needed to regain the water balance of muscle tissue compared with the plasma ion levels. A delay in water balance of muscle tissue and a quick re-establishment of the plasma ion levels indicates therefore that the plasma chloride levels after 24 h of seawater exposure may be an insufficient estimator of osmotic disturbance in salmon smolts in seawater.

Increased plasma chloride levels and muscle dehydration during seawater exposure at low temperatures have been reported in Atlantic salmon (Virtanen and Oikari, 1984; Sigholt and Finstad, 1990) and rainbow trout (Finstad et al., 1988). In the present experiment, there was a significantly higher rate of tissue dehydration during the first 12–24 h in seawater and a prolonged period of tissue rehydration in the low temperature groups compared with the normal temperature groups (8°C, compare Fig. 3a and b). This indicates a temperature-dependent reduction in tissue moisture content and an increase in the period needed to complete the seawater acclimation at low temperatures.

The reduction in gill \( \text{Na}^{+},\text{K}^{+}-\text{ATPase} \) activity in groups at 8°C between 12 and 24 h of seawater exposure agrees with data presented by McCormick et al. (1989). The subsequent increase is concurrent with results from other studies (Madsen and Næmansen, 1989; McCormick et al., 1989; Berge et al., 1995). The overall reduction in gill \( \text{Na}^{+},\text{K}^{+}-\text{ATPase} \) activity in groups at 4°C during the first month in seawater is in apparent contrast with the general model linking gill ATPase activity with ion regulation and seawater survival (McCormick et al., 1987; Sigholt et al., 1995). However, unpublished results from our laboratory support these findings, suggesting a temperature-dependent role of gill \( \text{Na}^{+},\text{K}^{+}-\text{ATPase} \) in ion regulation in seawater.

Although osmoregulation is thought to demand a relatively small fraction of the basal metabolism (Kirschner, 1993, 1995), it has been argued that a temporary reduction in growth in salmonids after transfer to seawater is due to a high metabolic cost of osmoregulation (Clarke et al., 1981). If this cost is proportional to the osmotic gradient, a reduction in the osmotic gradient between the fish and the medium may be expected to decrease metabolic costs and, accordingly, increase growth. Within the salinity range used in this study (28 and 34%), only temperature was found to significantly affect the
growth (mean weight) during the first 2 months in seawater. These results are in agreement with the findings of Usher et al. (1991) and Duston (1994) who concluded that growth in Atlantic salmon smolts is independent of salinity, although effects at lower salinities can not be excluded. However, in the period between days 64 and 90, differences in growth rate among all groups were observed. At low temperature, the 34½% group had a significantly lower growth rate than the 28½% group and vice versa at higher temperature, suggesting a positive long-term effect of reduced salinity in groups of smolts reared at low temperatures.

5. Conclusion

The present study shows that the physiological response of salmon smolts acclimated to brackish water (28½%), e.g., in a fjord site, is not different from that of smolts acclimated to full strength seawater (34½%), e.g., in more exposed coastal areas. On the other hand, temperature significantly affected the hypo-osmoregulatory ability during the first month at sea, and indicated a longer acclimation time at low temperatures. During the first 2 months of seawater exposure, the growth patterns were affected by temperature only, whereas higher growth rates in brackish water at low temperature (4°C) in the period between days 64 and 90 indicate that a reduction in salinity may improve long-term growth in the sea.

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