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Temperature influence on the development and loss of seawater tolerance in two fast-growing strains of Atlantic salmon

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Abstract

Development of hypo-osmoregulatory ability, gill Na^+,K^+-ATPase activity, condition factor and growth in Atlantic salmon during parr–smolt transformation was studied in a 2×3 factorial design with three temperatures (12.0, 8.9 °C and ambient, 2.4–11.9 °C, mean: 6.0 °C) and two farmed strains of smolts (Mowi and AquaGen). The development of hypo-osmoregulatory ability and gill Na^+,K^+-ATPase activity were significantly influenced by freshwater temperature. In smolts raised at 12.0 °C, maximum gill Na^+,K^+-ATPase activity was reached in late April, compared with late May and mid-June in the 8.9 °C and ambient groups, respectively. In all groups, peak gill Na^+,K^+-ATPase activity was seen 350 degree days (d °C) after the onset of the smolt-related increase in enzyme activity (30 March) The period of high enzyme activity (>90% of maximum) lasted approximately 250 d °C. No distinct peak level in gill Na^+,K^+-ATPase activity was seen in the AquaGen strain at ambient temperature. Elevated temperatures also accelerated the loss of hypo-osmoregulatory capacity. In all groups, gill Na^+,K^+-ATPase activity reached pre-smolt levels approximately 500 d °C after the calculated peak level. Growth rate in freshwater was influenced by strain, temperature and their interaction, with the Mowi strain showing a higher growth rate than the AquaGen strain at 8.9 °C and ambient temperatures. Following transfer to seawater, a higher growth rate was recorded in smolts from the Mowi strain than the AquaGen strain from the ambient temperature regime. Temperature

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influences the development and loss of smolt characteristics in both strains, and has long-term effects on post-smolt performance in seawater.

Keywords: Atlantic salmon; Strains; Temperature; Smoltification; Osmoregulation; Gill Na^+,K^+-ATPase; Growth

1. Introduction

A high degree of genetic variation in growth performance has been reported among and within strains of Atlantic salmon (Gunnes and Gjedrem, 1978; Jørstad and Nævdal, 1996). Consequently, a large-scale breeding program for cultured Atlantic salmon is in progress in Norway to select salmon for economically important traits such as growth, age at first maturation, disease resistance and flesh quality (Gjedrem et al., 1991). The AquaGen and Mowi strains represent the most common strains used in Norwegian aquaculture production. Both strains have been used in commercial aquaculture since the early 1970s (Gjedrem et al., 1991). However, studies focusing on the relationship between growth and development of seawater tolerance have shown that, within a population, the fastest growing individuals completed smolting earliest (Metcalf and Thorpe, 1990; Økland et al., 1993). Taken together, this may indicate the artificial selection influence on important physiological traits in cultured Atlantic salmon smolts.

The parr–smolt transformation pre-adapts the juvenile salmon for a transition into the marine environment (Boeuf, 1993; McCormick et al., 1998). Among the most important processes involved in this transformation is the increase in hypo-osmoregulatory ability (McCormick and Saunders, 1987), which is the result of several physiological and anatomical changes in gut, kidney and gills, including higher gill Na^+,K^+-ATPase activity. Gill Na^+,K^+-ATPase is believed to be the primary enzyme for excretion of excess Na^+ and Cl^- from the body fluids, and is found in high concentrations in the chloride cells of the gills (Borgatti et al., 1992; McCormick, 1995).

The completion of the parr–smolt transformation takes place under an increasing daylength and the prevailing hypothesis is that changes in photoperiod serve to entrain an endogenous circannual rhythm, which controls the smolting process (Duston and Saunders, 1990). Although photoperiod is known to play a key role in the control of the parr–smolt transformation (McCormick et al., 1987; Saunders et al., 1989; Duston and Saunders, 1990; Saunders and Harmon, 1990), there is evidence that temperature influences the rate of change in smolt characters in salmonids (Adams et al., 1972; Zaugg and McLain, 1976; Johnson and Saunders, 1981; Soivio et al., 1989; Solbakken et al., 1994; McCormick et al., 1999; Shrimpton et al., 2000). In Atlantic salmon smolts, an early increase in temperature from 5 to 12 °C has been shown to advance the development of seawater tolerance compared with controls raised at ambient water temperature (5–6 °C, Solbakken et al., 1994). Staurnes et al. (1994) even observed the development of typical smolt characters, e.g. marked silvering, high seawater tolerance and high Na^+,K^+-ATPase activity, in groups of Atlantic salmon reared at constant long day and seasonally changing water temperature that increased during spring, suggesting an increase in temperature to be

important for development of hypo-osmoregulatory ability even in absence of appropriate photoperiod stimuli. Temperature has also been shown to be strongly correlated with downstream migration of Atlantic salmon smolts and may play an important role in triggering migratory behavior (Jonsson, 1991; Hvidsten et al., 1995). In river Imsa, Jonsson and Ruud-Hansen (1985) concluded that timing of the smolt migration was triggered by a combination of temperature increase during spring and actual temperature.

If salmon smolts are prevented from reaching seawater, they lose salinity tolerance and the underlying hypo-osmoregulatory mechanisms (Duston et al., 1991; Shrimpton et al., 2000; see also Hoar, 1988). This loss, often referred to as de-smoltification or parr-reversion, has been described in several studies (McCormick and Saunders, 1987; Duston et al., 1991). In Atlantic salmon, the loss of hypo-osmoregulatory ability is clearly influenced by temperature (Duston et al., 1991; Stefansson et al., 1998; McCormick et al., 1999) with elevated temperatures accelerating the loss of seawater tolerance. Although several studies have described effects of temperature on salmon parr–smolt transformation, few papers describe the combined effect of temperature on changes in hypo-osmoregulatory ability during both smoltification and de-smoltification processes in selected fast-growing salmon.

The aim of the present study was, therefore, to characterise long-term effects of different water temperatures on changes in hypo-osmoregulatory ability and growth in two selected strains of Atlantic salmon smolts (Mowi and AquaGen). To evaluate possible interactions between temperature, physiological response and growth during smolting and in the early post-smolts phase, groups of 70 fish from each strain and temperature combination were individually tagged and their growth monitored regularly during the experiment. Untagged fish were sacrificed to monitor physiological changes associated with smoltification and early seawater performance.

2. Materials and methods

2.1. Fish stock, rearing conditions and experimental design

The fish were potential 1+ smolts from Mowi and AquaGen strains ($n = 1590$ of each strain). The fish from both Mowi and AquaGen strains represent the seventh generation of the first line in two separate breeding programs organized by Hydro Seafood and AquaGen, respectively. In the AquaGen strain, the fish from the river Namsen originally constituted 11% of the brood line increasing to more than 70% after the fourth generation (Gjedrem et al., 1991). The AquaGen strain used in this study also includes elements from river Nidelva, Gaula, Driva and Rana (T. Gjedrem, pers. com.). AquaGen started their breeding program at Sunndalsøra and Averøy in 1971 (Gjedrem et al., 1991), whereas Mowi started selection experiments at Hitra in 1969 using a set of size selected wild caught individuals from one-generation wild salmon strains. It is assumed that fish mainly from river Vosso but also from river Dale and Suldal contributed in this first generation of the Mowi strain (T. Gjedrem and G. Nævdal, pers. com.).

The experiment was conducted outdoors at Marine harvest's hatchery in Glomfjord, northern Norway (67°N) between 12 January and 14 September 1999. Both strains were

hatched in Glomfjord, in early January 1998 and first feed in early March, at 500 degree days post-hatching, at constant light and in heated water (approximately 12 °C). Between March 1998 and 6 January 1999, the two strains were raised separately in two ordinary 5-m^2 rearing tanks (30 m^3) on natural photoperiod and in heated water (approximately 8–10 °C). During this period, the fish were fed a commercial standard dry diet according to temperature and size (NorAqua Supra, pellet size ranging from 1.2 to 2.5 mm, Austreng et al., 1987). On 7 January, 210 individuals from each strain were randomly selected and individually tagged (Carlin tags, McAllister et al., 1992) for individual growth measurements (MOWI strain, initial mean weight = 58.2, S.D. = 10.3 g, mean length = 16.2, S.D. = 1.0, cm; AquaGen strain, initial mean weight = 57.9, S.D. = 7.6 g, mean length = 16.3, S.D. = 0.8 cm). The adipose fin was removed in fish from the AquaGen strain allowing co-rearing of both stains in the experimental tanks. At the same time, all juveniles were randomly distributed into six 3-m^2 grey outdoor tanks (9400 l, water temperature: 10 °C, $n = 265$, total number including tagged fish) until the start of the experiment. Each tank was supplied with approximately 12.0 l min^{-1} of freshwater. O_2 saturation was measured daily in the effluent water and was kept above 80% at all times. The fish were fed a commercial dry diet (Supra 3.0, NorAqua Innovation, Dirdal, Norway) in excess (110%) from automatic feeders between 09:00 and 14:00 h every day and the amount of food was adjusted weekly according to fish size and temperature (Austreng et al., 1987). On 12 January, the fish were transferred from the holding temperature (10 °C) to the three experimental temperatures (Fig. 1) 12.0 °C (high temperature: mean: 12.0 °C,

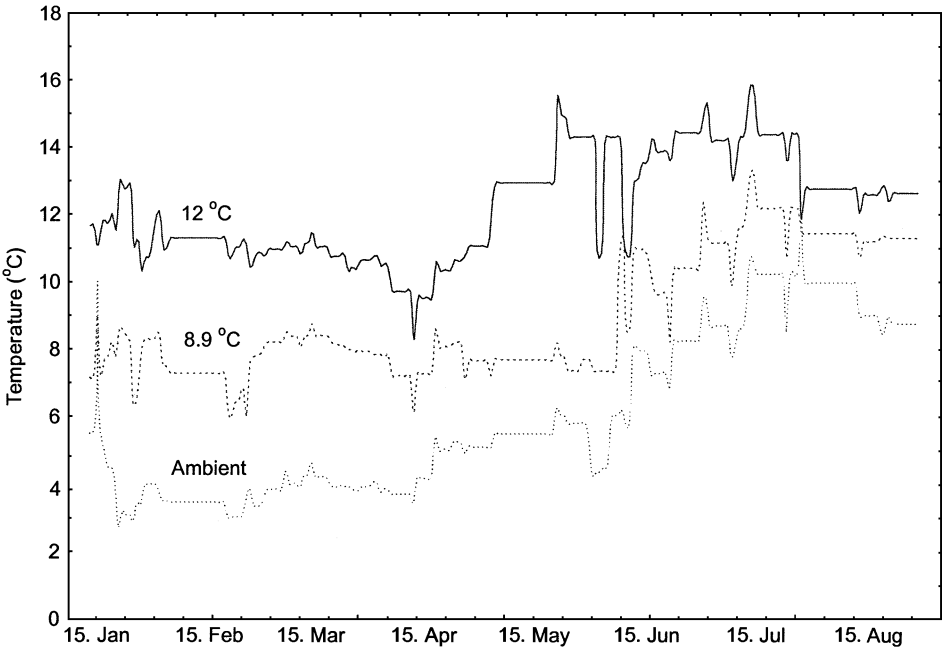

Fig. 1. Changes in freshwater temperature in the different experimental groups between 12 January and 29 July.

S.D. = 1.9, min: 8.2 °C, max: 15.9 °C), 8.9 °C (medium temperature: mean: 8.9 °C, S.D. = 1.9, min: 6.0 °C, max: 13.2 °C) and ambient temperature (mean: 6.0 °C, S.D. = 1.9, min: 2.4 °C, max: 11.9 °C) in a 2 × 3 factorial design including the two strains (Mowi and AquaGen), three temperatures (12.0, 8.9 °C and ambient) and two replicate tanks for each temperature.

2.2. Sampling procedures and analysis

Every second week between 12 January and 29 July, 12 untagged fish from each strain and temperature combination were collected and their hypo-osmoregulatory ability assessed by measuring plasma chloride levels after a 96-h seawater challenge test. The fish were transferred directly into 34.5 ‰ at 8 °C in 1-m tanks, as described and discussed in Handeland et al. (1998, 2000). 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. From fish in freshwater, gill filaments from the second gill arch were frozen in SEI buffer at − 80 °C and subsequently analyzed for Na^+,K^+-ATPase activity using the method of McCormick (1993). All fish were starved for 24 h prior to sampling or seawater exposure. All growth analyses are based on all the individually tagged fish (see Table 1). Fork length (L) from all individually tagged fish was measured to the nearest 0.1 cm and weight (W) to the nearest 0.1 g. Growth rate for individual fish between two dates was calculated as specific growth rate (SGR):

$$\mathrm{SGR} = (\ln W_2 - \ln W_1) * 100 / (T_2 - T_1)$$

where W_1 and W_2 are weights at days T_1 and T_2. Fultons condition factor (CF) was calculated from the formula:

$$\mathrm{CF} = 100 * W * L^{-3}$$

On 27 April, all smolts in the 12 °C group had typical morphological signs of smolting, i.e. dark fin margins, absence of parr marks, loose silvery scales and improved seawater tolerance. Hence, all the individually tagged smolts were separated from the untagged

Table 1
Individual growth rates during freshwater period and after transfer to seawater in two strains of Atlantic salmon smolts (Mowi and AquaGen) at three temperatures (ambient, 8.9 and 12.0 °C)

Individual growth rates (SGR % day^{-1}) in Mowi and AquaGen smolts at three temperatures						
Period	Mowi			AquaGen		
	Ambient	8.9 °C	12.0 °C	Ambient	8.9 °C	12.0 °C
12 January–27 April (FW)	0.17 (0.01)	0.51 (0.02)	0.73 (0.03)	0.09 (0.01)	0.37 (0.03)	0.76 (0.02)
Days 0–30 in seawater	0.30 (0.02)	0.40 (0.03)	0.32 (0.01)	0.12 (0.03)	0.37 (0.04)	0.38 (0.01)
29 July–14 September (SW)	0.85 (0.02)	0.87 (0.02)	0.71 (0.02)	0.78 (0.04)	0.85 (0.06)	0.84 (0.02)

The 12.0, 8.9 °C and ambient groups were transferred to seawater on 27 April, 28 May and 23 June, respectively (referred as day 0). Values are given as means ± S.E.

smolts and transferred to an identical 3-m^2 grey outdoors rearing tank with running ambient seawater (20 l min^{-1}, 33 ‰) for studies of post-smolt growth. The seawater was taken from 80 m depth and had a constant temperature at approximately 10 °C (S.D. = 1.19) during the experimental period. The untagged smolts remained in fresh water until 29 July. Correspondingly, all the tagged fish in the 8.9 °C and ambient groups were transferred to seawater on 28 May and 23 June, respectively. Following transfer to seawater, the fish were fed the same dry diet as described earlier. All tagged fish were measured again 30 days after transfer to seawater and finally on 14 September (Table 1).

2.3. Statistical analysis

All statistical analyses were performed with Statistica™ 5.1. Prior to statistical analysis, all data were tested for normality of distribution using the Kolmogornov–Smirnov test. The homogeneity of variances among the different groups was tested using the Hartley *F*-max test (Sokal and Rolf, 1995). Three-way nested ANOVA was applied to calculate the overall effects of temperature, strain and time on gill Na^+,K^+-ATPase activity and condition factor, whereas a three-way nested ANCOVA with length as covariate was used to calculate the overall effects of temperature, strain and time on changes in plasma chloride levels. Significant three-way ANOVA/ANCOVAs were followed by two-way ANOVA/ANCOVAs and a Student–Newman–Keuls multiple comparison test within each time point to determine differences among experimental groups (a significance level of 0.05 has been used unless noted in text). The growth rates (FW, days 0–30 and in SW) were tested in a two-way ANCOVA using geometric mean weight between T_1 and T_2 as covariate. No significant differences were seen among replicate tanks. A polynomial curve fit was used in Fig. 3 to illustrate the relationship between gill Na^+,K^+-ATPase activity and degree days (cumulative average daily temperature; d °C) from 30 March.

3. Results

3.1. Gill Na^+,K^+-ATPase activity

The development of gill Na^+,K^+-ATPase activity was affected by time ($p < 0.001$), temperature ($p < 0.001$), strain ($p < 0.05$), as well as the interaction between temperature and time ($p < 0.001$), and between strain and temperature ($p < 0.01$, Fig. 2A and B). In all groups, a slight, transient increase in enzyme activity was seen between 3 February and 26 February/30 March ($p < 0.05$), with the ambient groups reaching approximately 6.5–8 μmol ADP mg $protein^{-1}$ h^{-1} on 26 February, significantly higher than the 12.0 °C groups ($p < 0.05$). At 12.0 °C, a rapid increase in enzyme activity was observed between 30 March and 27 April in the Mowi strain ($p < 0.001$), and between 30 March and 11 May in the AquaGen strain ($p < 0.001$). Enzyme activity in the 12.0 °C groups subsequently decreased, reaching approximately 2 μmol ADP mg $protein^{-1}$ h^{-1} in late June. Similar changes in gill Na^+,K^+-ATPase activity were seen at 8.9 °C, but with peak levels occurring later (28 May) in both strains ($p < 0.001$). At ambient temperature, an increase in enzyme

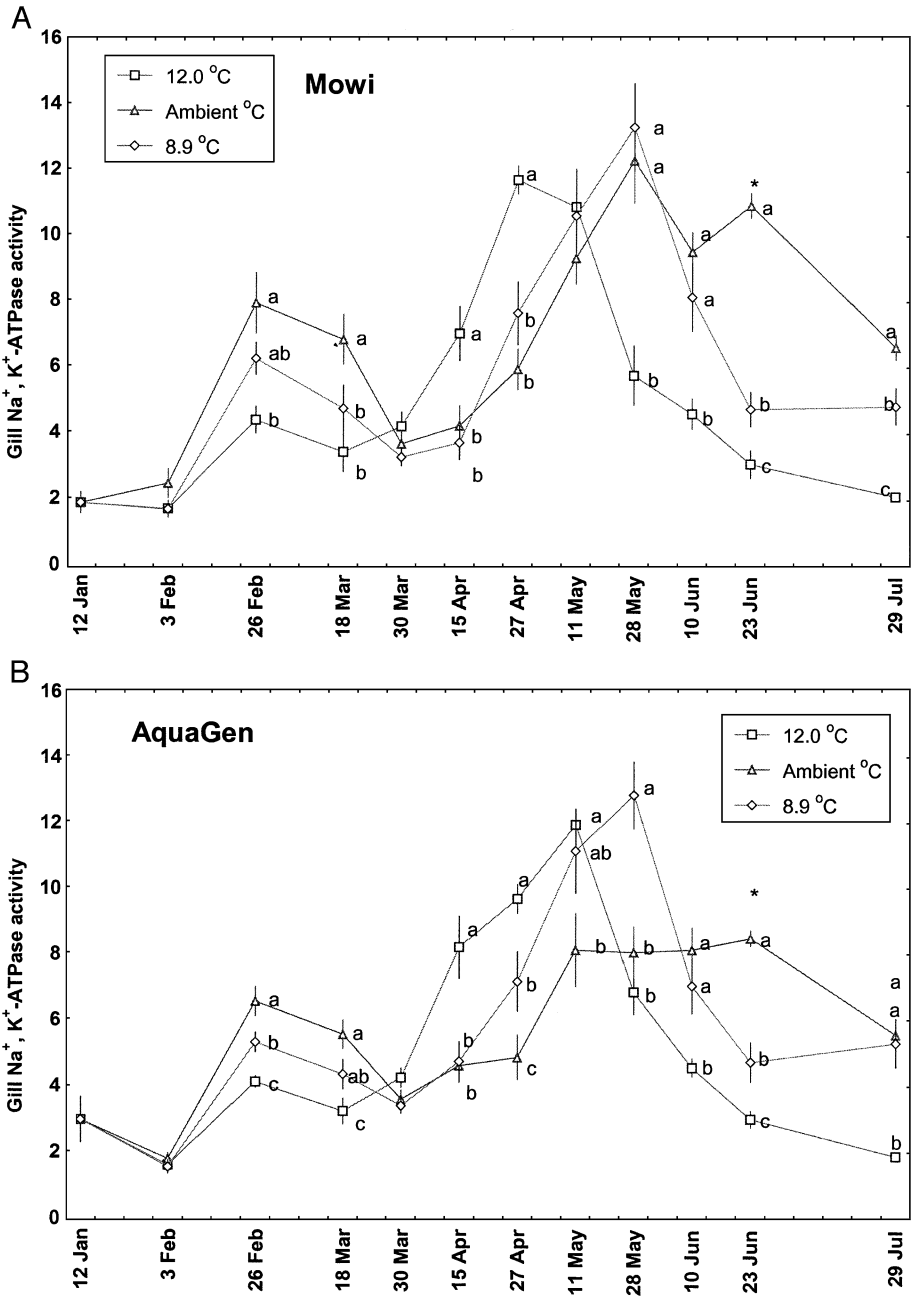

Fig. 2. A and B. Gill Na^+,K^+-ATPase activity in two strains of Atlantic salmon smolts (A: Mowi and B: AquaGen) at three temperatures (ambient, 8.9 and 12.0 °C) during the period between 12 January and 29 July. Values are given as means ± S.E. ($n = 12$). Significant differences among the different temperatures is indicated by different letters ($p < 0.05$). The asterisk indicates significant differences among strains ($p < 0.05$).

activity was seen in the Mowi strain between 30 March and 28 May ($p < 0.001$), and in the AquaGen strain between 30 March and 11 May ($p < 0.001$). In contrast to the other temperature/strain combinations, no distinct peak gill Na^+,K^+-ATPase activity was observed in the AquaGen strain at ambient temperature, and at 23 June gill Na^+,K^+-ATPase activity in the Mowi smolts was significantly higher than in the AquaGen smolts ($p < 0.05$). However, in both strains, gill Na^+,K^+-ATPase activity remained elevated until 23 July, followed by a significant decrease to 5–6 μmol ADP mg protein^{-1} h^{-1} ($p < 0.05$). At the end of the experiment, highest gill Na^+,K^+-ATPase activity in both strains was seen at ambient and 8.9 °C, significantly higher than at 12.0 °C.

Fig. 3 shows the relationship between gill Na^+,K^+-ATPase activity (all groups included) and degree days from the onset of the smolt-related increase in enzyme activity (30 March). The figure shows peak levels in gill Na^+,K^+-ATPase activity after approximately 350 d °C, i.e. when $d_{d\ °C}/d_{ATPase} = 0$, $F(\text{ATPase}) = 3.476031 + 0.401495*(\text{d °C}) - 0.007434*(\text{d °C})^2 + 0.000033*(\text{d °C})^3$, $r^2 = 44\%$. The same figure also indicates a period of enzyme activity >90% of maximum lasting for approximately 250 d °C, followed by a significant decrease in gill Na^+,K^+-ATPase activity reaching pre-smolt levels after approximately 860 or 510 d °C after the calculated peak level.

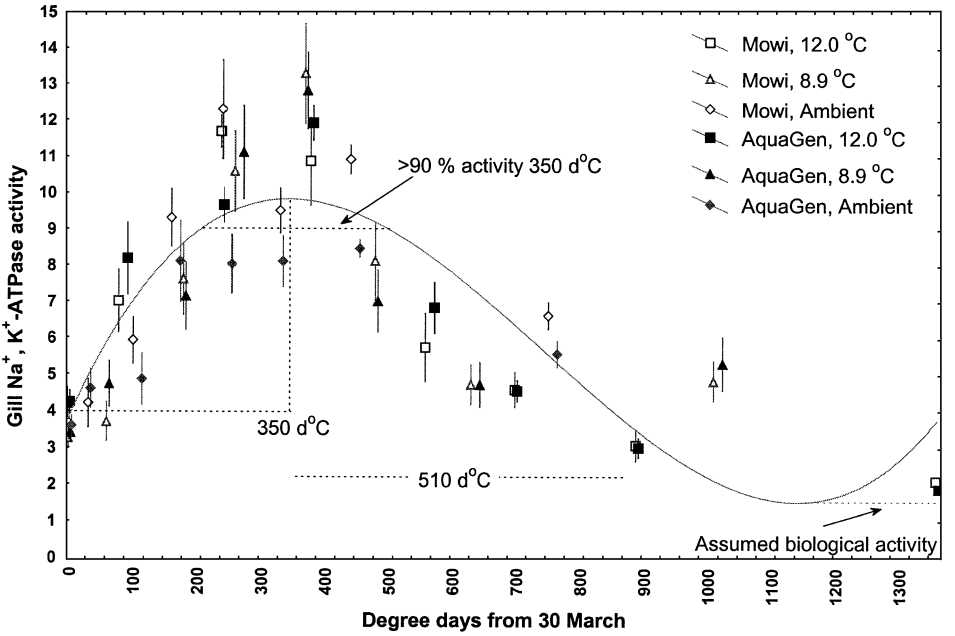

Fig. 3. The relationship between gill Na^+,K^+-ATPase activity (all groups included) and degree days (cumulative average daily temperature) from the onset of the smolt-related increase in enzyme activity (30 March). The figure shows a peak level in gill Na^+,K^+-ATPase activity after approximately 350 degree days (d °C), i.e. when $d_{d\ °C}/d_{ATPase} = 0$, $F(\text{ATPase}) = 3.476031 + 0.401495*(\text{d °C}) - 0.007434*(\text{d °C})^2 + 0.000033*(\text{d °C})^3$, $r^2 = 44\%$. The different groups were transferred to seawater after the following degree days (cumulative average daily temperature; d °C, counted from 30 March); 290 at 12.0 °C, 480 at 8.9 °C and 520 at ambient temperature.

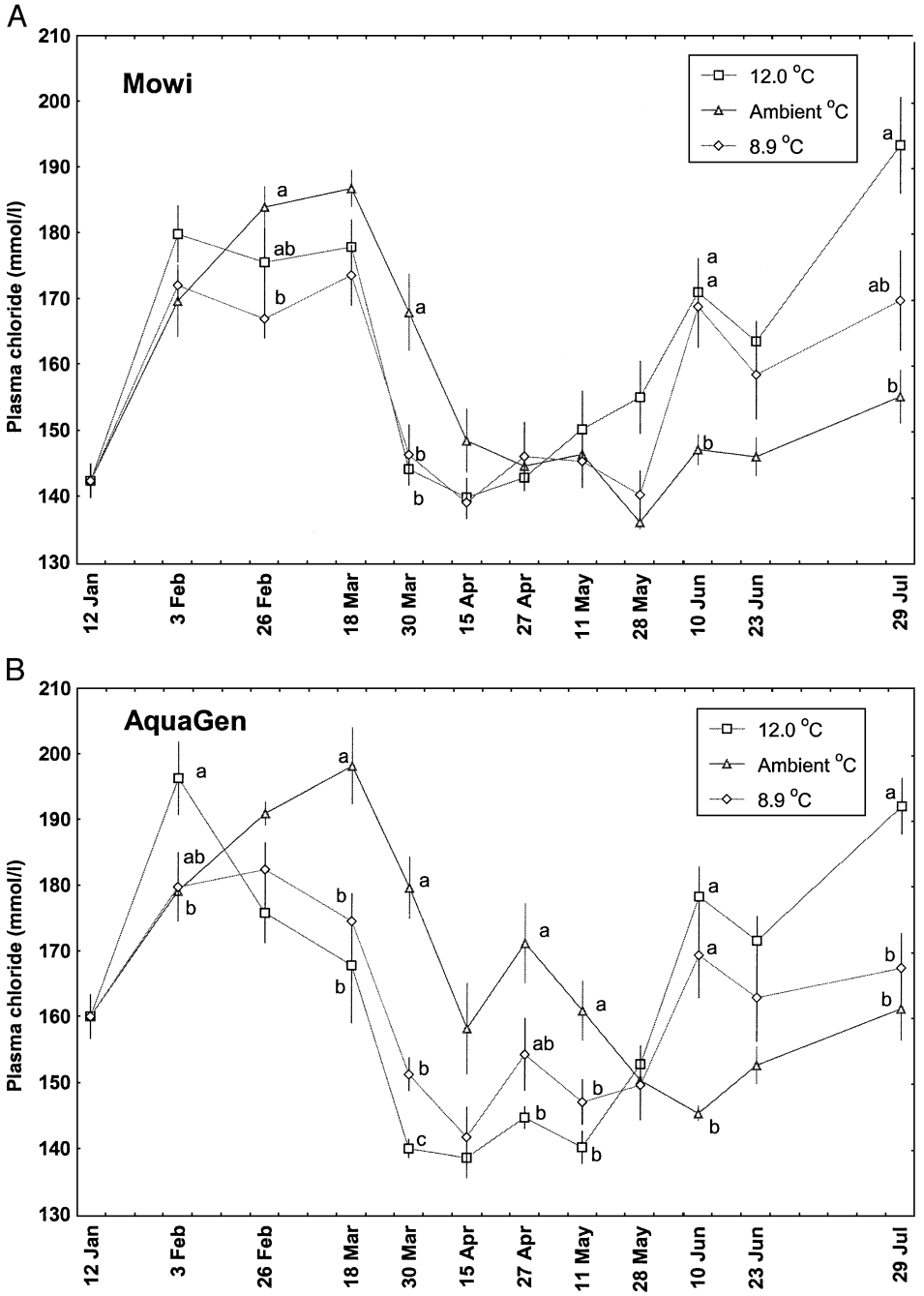

Fig. 4. A and B. Plasma chloride levels after a 96-h seawater challenge test in two strains of Atlantic salmon smolts (A: Mowi and B: AquaGen) at three temperatures (ambient, 8.9 and 12.0 °C) during the period between 12 January and 29 July (see Fig. 1 for other details).

3.2. Hypo-osmoregulatory ability

Changes in hypo-osmoregulatory ability between 12 January and 29 July were significantly affected by freshwater temperature ($p < 0.05$) and time ($p < 0.001$) as well as their interactions ($p < 0.001$). No differences were seen between the two strains (Fig. 4A and B). Plasma chloride levels after seawater challenge increased significantly and remained high in all groups between 12 January and 18 March Hypo-osmoregulatory ability improved significantly in both strains at 8.9 and 12.0 °C between 18 March and 15 April ($p < 0.05$). A similar but delayed decrease in plasma chloride levels was seen in the ambient groups between 18 March and 28 May in the Mowi smolts ($p < 0.001$), and between 18 March and 10 June in the AquaGen smolts ($p < 0.001$). In both strains, plasma chloride levels were significantly lower at ambient temperature than at 8.9 and 12.0 °C on 10 June ($p < 0.05$). A significant increase in plasma chloride levels was recorded in the 12.0 °C groups between 15 April and 29 July ($p < 0.001$), reaching approximately 190 mmol/l. In the ambient and 8.9 °C groups, a similar loss of seawater tolerance was seen between 28 May and 29 July ($p < 0.05$ in the Mowi strain), although plasma chloride levels in these groups never reached the same high levels as observed at 12.0 °C.

3.3. Growth and condition factor

Growth rate in fresh water was affected by temperature ($p < 0.001$), strain ($p < 0.001$) and their interactions ($p < 0.05$, Table 1), with the Mowi smolts showing an overall higher growth rate during the freshwater phase compared with AquaGen smolts ($p < 0.001$). Highest growth rate was observed at 12.0 °C, significantly higher than at 8.9 °C ($p < 0.001$), whereas the ambient groups showed the lowest growth rate ($p < 0.001$). No differences in growth rate were observed among strains at 12.0 °C during the freshwater phase. However, at ambient temperature and at 8.9 °C, higher growth rates were recorded in Mowi smolts compared to AquaGen smolts ($p < 0.05$).

Following transfer to seawater (days 0–30, Table 1), lowest growth rate was recorded in the ambient AquaGen group, significantly below all other groups ($p < 0.001$). No significant differences in seawater growth were observed in the period between 29 July and 14 September. Final length and weight of the fish are given in Table 2.

A transient decrease in condition factor was seen in the AquaGen smolts at ambient temperature between 12 January and 30 March ($p < 0.05$, Fig. 5A and B). In the 8.9 and 12.0 °C groups, condition factor decreased significantly between 15 April and 29 July

Table 2
Final length, weight and condition in two strains of Atlantic salmon smolts (Mowi and AquaGen) at three temperatures (ambient, 8.9 and 12.0 °C)

Period	Mowi			AquaGen		
	Ambient	8.9 °C	12.0 °C	Ambient	8.9 °C	12.0 °C
Length (cm)	21.8 (0.20)	26.5 (0.31)	27.6 (0.21)	20.9 (0.28)	25.4 (0.52)	28.4 (0.26)
Weight (g)	119.0 (3.44)	207.1 (7.23)	227.6 (7.94)	103.3 (3.95)	189.0 (13.16)	256.3 (7.18)
Condition	1.12 (0.01)	1.08 (0.01)	1.06 (0.01)	1.10 (0.01)	1.11 (0.01)	1.1 (0.01)

Values are given as means ± S.E.

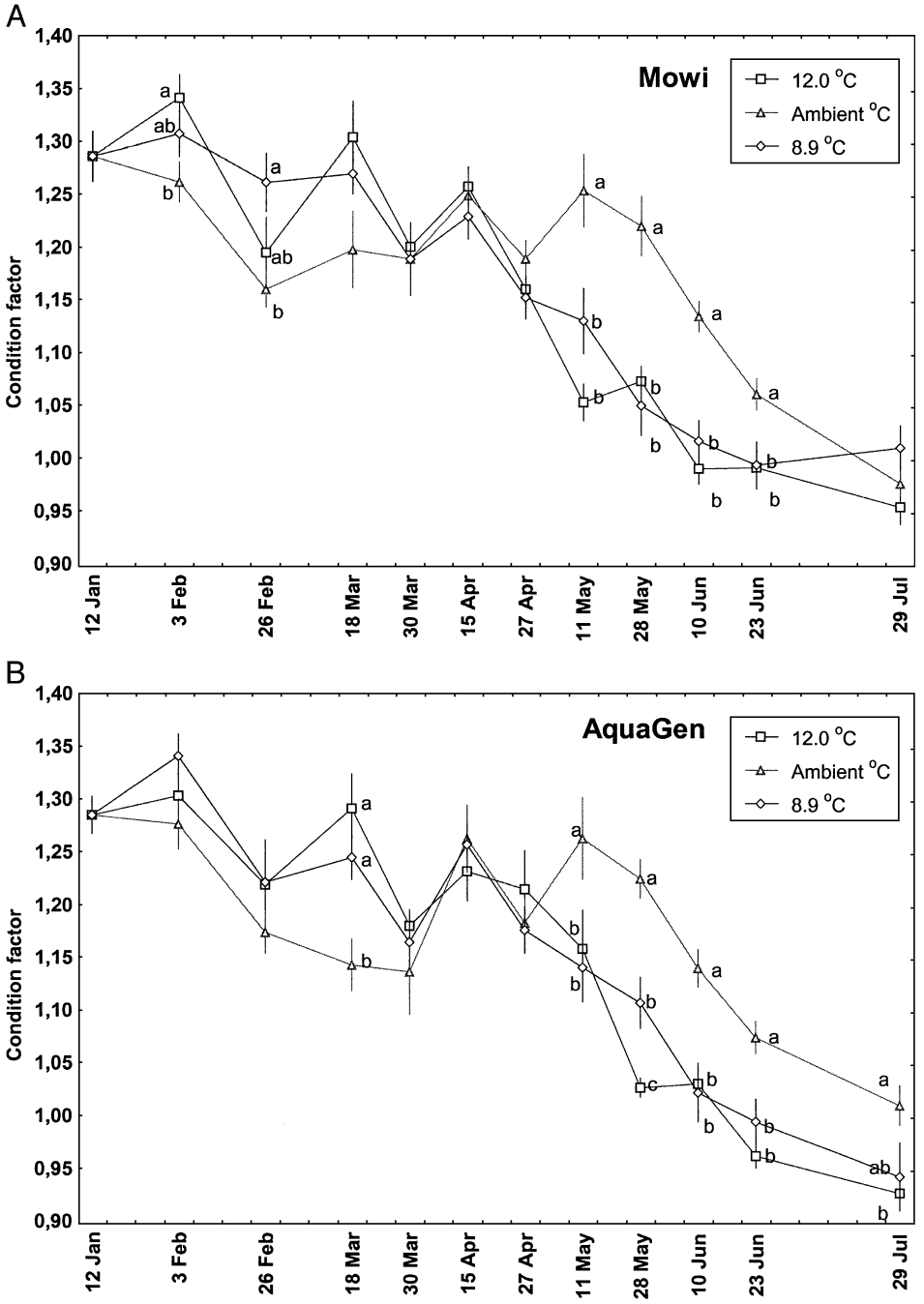

Fig. 5. A and B. Changes in condition factor in two strains of Atlantic salmon smolts (A: Mowi and B: AquaGen) at three temperatures (ambient, 8.9 and 12.0 °C) during the period between 12 January and 29 July (see Fig. 1 for other details).

($p < 0.001$). A similar fall in condition factor was seen in the ambient groups between 11 May and 29 July ($p < 0.001$), reaching levels comparable to the 8.9 and 12.0 °C groups at 29 July. Between 11 May and 23 June, condition factor in the ambient groups was significantly higher than in the other groups ($p < 0.05$).

4. Discussion

The development of hypo-osmoregulatory ability and increase in gill Na^+,K^+-ATPase activity during smolting were significantly influenced by freshwater temperature, with earlier development of seawater tolerance in salmon smolts at higher temperatures. In smolts raised at 12.0 °C, maximum gill Na^+,K^+-ATPase activity was reached in late April, compared to late May and mid-June in the 8.9 °C and ambient groups. Our results are similar to those of Solbakken et al. (1994), McCormick et al., 2000 and Shrimpton et al. (2000) who concluded that an increase in rearing temperature during late winter and spring accelerated the parr–smolt transformation in Atlantic salmon. These results further correspond to those reported for coho and chinook salmon by Zaugg and McLain (1976) and Clarke et al. (1981). In contrast, no effect of a temperature rise from ambient to 10–11 °C during spring on smolt development was reported by Dickhoff et al. (1989) and Duston and Saunders (1995). Differences in strain, body size and rearing conditions may explain the observed differences in smolt development and growth following transfer to seawater.

The present findings correspond with the results of Schwarzbaum et al. (1991) showing higher levels of gill Na^+,K^+-ATPase following cold acclimation in freshwater-acclimated Arctic char (*Salvelinus alpinus*) and Roach (*Rutilus rutilus*). It has been suggested that increased gill Na^+,K^+-ATPase activity at low temperatures is an adaptation to compensate for lower transport capacity of enzymes (McCormick et al., 1996). It should be noted, however, that this is not a universal finding, as Atlantic salmon juveniles acclimated to 2 °C in winter had lower gill Na^+,K^+-ATPase activity relative to fish acclimated to 10 °C (McCormick et al., 2000).

In separate studies, Staurnes et al. (1994) and Solbakken et al. (1994) reported the development of typical smolt indices, i.e. high seawater tolerance, elevated gill Na^+,K^+-ATPase activity and marked body silvering, in salmon smolts reared at constant photoperiod and seasonally changing temperature. Hence, Staurnes et al. (1994) suggested temperature can act as a cue for smolt development in absence of a photoperiod stimulus. The present design does not permit a detailed resolution of these findings. However, if smolting was strictly temperature-dependent, we would expect the number of degree days from the onset of smolting to the observed peak levels in gill Na^+,K^+-ATPase activity to be constant. In a review, McCormick et al. (1996) calculated the number of degree days during smolting (starting 1 January) and concluded that at warmer temperatures more degree days were required to achieve an advanced timing in peak levels of gill Na^+,K^+-ATPase activity. However, another approach is to examine the effect of temperature on the development of hypo-osmoregulatory ability from the onset of the smolt-related increase in gill Na^+,K^+-ATPase activity (30 March). From this point of view, the present data show a peak level in gill Na^+,K^+-ATPase activity after 350 degree days. Hence, our findings

suggest temperature to act as an overall rate-controlling factor of the smolting process in Atlantic salmon smolts driven by changes in photoperiod.

Our findings are in line with the overall conclusions from previous studies (Soivio et al., 1989; Gaignon and Quemener, 1992; see also McCormick et al., 1996) that completion of smolting is advanced at elevated temperature. Duston and Saunders (1992) reported an increase in salinity tolerance concomitant with an increase in growth rate in Atlantic salmon smolts raised under out-of-phase photoperiod cycles of 6, 12 and 18 months. Accordingly, Solbakken et al. (1994) reported a rapid increase in salinity tolerance following an increase in temperature, as well as increased growth rate, advanced peak gill Na,K-ATPase activity and reduction in condition factor at constant 12 °C. These findings are in apparent contrast to earlier studies of steelhead trout indicating that high temperatures may prevent the development of high ATPase levels (Adams et al., 1972). However, different sampling frequencies may provide different resolutions of the changes in ATPase activity. In Atlantic salmon, smolts exposed to a temperature increase from 6 to 11 °C, Björnsson et al. (1989) reported a five-fold increase in circulating GH levels, concurrent with improved seawater tolerance. Taken together, our present data and those of Björnsson et al. (1989) and McCormick et al. (2000) suggest that an increase in temperature may affect smolting partly through elevated GH levels, which may explain the accelerated development of seawater tolerance, gill Na^+,K^+-ATPase activity and condition factor.

In Atlantic salmon, a decrease in seawater tolerance will occur if the smolts are prevented from reaching seawater (McCormick and Saunders, 1987). Previous studies have shown that both loss of seawater tolerance and reduction in gill Na^+,K^+-ATPase activity occur faster at higher temperatures (Duston et al., 1991; Stefansson et al., 1998). This is also confirmed in the present study in which the high-temperature groups had a more rapid loss of seawater tolerance and gill Na^+,K^+-ATPase activity compared to the ambient groups. Furthermore, studies on loss of seawater characteristics in Atlantic salmon have suggested a relationship between degree days experienced by the smolts and the observed decrease in gill enzyme activity (Stefansson et al., 1998; see also McCormick et al., 1996). McCormick et al. (1999) reported a period of stability, or "smolt window", lasting for approximately 200 degree days after the observed peak levels in gill Na^+,K^+-ATPase activity followed by a rapid decrease within approximately 500 degree days. This is in agreement with the present results in which the smolt window was calculated to be 250 degree days and was followed by a decrease in enzyme activity reaching pre-smolt levels approximately 510 degree days after the peak in enzyme activity.

Under natural conditions, the seasonal changes in day length have been identified as a major environmental factor driving the smoltification process (see Hoar, 1988). Although the downstream migration of smolts is assumed to be correlated with the completion of the smolting (McCormick and Björnsson, 1994), the annual variation in timing of downstream migration indicates that this migration cannot be controlled by photoperiod alone. Hence, in several studies discharge, water quality, turbidity and water temperature have all been implicated as important environmental factors that influence the annual timing of smolt run (Jonsson and Ruud-Hansen, 1985; Jonsson, 1991; Greenstreet, 1992; Hvidsten et al., 1995). For instance, Jonsson and Ruud-Hansen (1985) concluded that timing of the annual smolt run in the river Imsa was triggered by a combination of temperature increase during

spring and absolute temperature. Bohlin et al. (1993) reported that timing of smolt migration in anadromous Baltic brown trout (*Salmo trutta*) was positively associated with the number of degree-days during spring, changes in water level, temperature change and average smolt size. Further, a positive correlation between gill Na^+,K^+-ATPase activity and smolt migration has been documented in wild stocks of Atlantic salmon (Birt et al., 1990; Whitesel, 1993; McCormick and Björnsson, 1994), rainbow trout (*O. mykiss*, Zaugg and Wagner, 1973), coho salmon (Rodgers et al., 1987), chinook salmon (Buckman and Ewing, 1982) and brown trout (Aarestrup et al., 2000). Combined with the present data, showing that the development of gill Na^+,K^+-ATPase activity is partly controlled by temperature, we suggest a link between water temperature, physiology and the timing of smolt run during spring. This link may contribute to an explanation of the reported variations in timing of smolt runs between years, i.e. a warm spring is likely to be associated with an earlier smolt run (Bohlin et al., 1993; McCormick et al., 1998).

Overall growth rate was highest at 12.0 °C in both strains. However, at 8.9 °C and ambient temperature, growth was higher in fish from the Mowi strain. At least two factors may explain the present observations: (1) genetic differences in the founding populations of the two strains and/or (2) differences in the artificial selection regimes imposed during the breeding program. The genetic origin of the AquaGen strain was based on wild caught salmon from river Namsen, Nidelv, Gaula, Driva and Rana (B. Gjedrem, pers. com.). These rivers are located in mid-Norway, which is a colder area compared to the southwestern parts in which River Vosso, Dale and Suldal are located, the origin area for the Mowi strain. According to Conover and Present (1990), many species with a large north–south distribution area show a larger growth potential in the northern compared to the southern populations (Imsland, 1997). This would suggest a higher growth potential in smolts from the AquaGen strain. However, in the present study, no differences among strains were observed at 12.0 °C, whereas a higher growth potential was recorded in the Mowi strain at ambient and low temperatures. Hence, our data contradict the predictions of the counter-gradient hypothesis (Conover and Present, 1990). However, both Mowi and AquaGen strains have been under selection for several generations (Gjedrem et al., 1991). A main goal in the breeding program for the Mowi strain has been to select individuals showing high growth during the freshwater period, compared to the AquaGen breeding program, which has selected for improved growth in seawater (B. Gjedrem, pers. com.). Taken together, this suggests that differences in the artificial selection regime probably best explains the observed differences in growth during the freshwater period.

The higher growth rate of the Mowi strain at low temperatures may be of interest for salmon hatcheries as most smolt producers raise their fish in ambient water with low temperatures during the winter. Following transfer to seawater (days 0–30), a lower growth rate was recorded in the ambient AquaGen group compared to the Mowi group. Poor post-smolt growth is generally correlated with poor smolt status (Solbakken et al., 1994). In a recent study, Handeland and Stefansson (2001) reported that survival and growth in off-season salmon smolts were positively correlated with gill Na^+,K^+-ATPase activity. The same authors further suggested gill Na^+,K^+-ATPase activity to be the most reliable indicator of smolt quality. The observed low enzyme activity in the AquaGen smolts at seawater entry may therefore partly explain the poor growth in this group following transfer to seawater.

The present results can be used to predict optimal time of seawater transfer to avoid osmoregulatory problems (Stagg et al., 1989; Handeland et al., 1998), growth depression (Björnsson et al., 1988; Duston, 1994) and high mortalities (Duston, 1994) associated with poor smolt status. Our results indicate that even relatively small differences in the extent and timing of smolt development caused by environmental manipulation are associated with a substantial difference in subsequent growth performance in seawater.

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