FINAL REPORT - Norwegian Research Council Project 184997/S40 (2008-2011)

NORSMOLT

Optimal smolt production and post smolt performance in the High North - Seawater intermixing, low temperatures and intensive rearing –

Project leader: Hilde Toften, Nofima

Project members[1]: Børge Damsgård, Sigurd Handeland, Torstein Kristensen, Helene Mikkelsen, Bjørn Olav Rosseland, Brit Salbu, Sigurd Stefansson, Hans-Christian Teien

A cooperation between Nofima Marin, University of Bergen, Norwegian Institute for Water Research and Norwegian University of Life Sciences

Summary

Water quality is essential for a successful smolt production. In this initiative we have been focusing on seawater intermixing, low temperatures and intensive rearing with the intent to propose solutions that can make Northern-Norway more self-sufficient with Atlantic salmon (*Salmo salar* L.) smolts and contribute to improved survival, growth, health and welfare in farmed salmon. Five experiments have been conducted since the project started in April 2008.

In September 2008 the SALIPARR experiment was conducted with juvenile salmon exposed to freshwater (control) or to two different salinities (0.5 and 10 ‰) for a short time. The goal was to reveal possible underlying regulatory mechanisms that can be influence by early exposure to brackish water. The freshwater had no source of aluminium or other metals which could be mobilized by the mixing of seawater. The results showed minor changes in physiological status and no indications of major changes in regulatory mechanisms. The welfare of the fish did not seem compromised by short term exposure to brackish water. The strategy of applying seawater during the pre-smolt stages of production seems feasible.

At the end of 2009 the SALISMOLT experiment was conducted. A control group in the same freshwater as in SALIPARR was compared with three treatment groups exposed to salinities of about 5, 10 and 15 ‰ for 6 weeks during the smoltification period. We found some differences in physiology, but none of these seems to have had any negative influence on the health and welfare of the fish. On the contrary, all the groups exposed to brackish water showed higher feed intake and growth than the control group in fresh water. After transfer to full strength sea water there were no differences in growth between the groups. Neither were there any differences in susceptibility to the winter ulcer bacteria *Moritella viscosa*. These results indicate that seawater intermixing (up to 14 ‰) can be a feasible strategy before the fish is fully smoltified in areas without problems with metals in the raw water source.

During spring 2010, the experiments COLDSMOLT I and II were conducted. Low temperatures may prevent the fish from responding to the increasing photoperiod by limiting the endocrine responsiveness of key endocrine tissues and target organs. In COLDSMOLT I we investigate how basic molecular mechanisms and smolt quality of wild Atlantic salmon smolts is affected by temperature during spring. The results showed that the groups completed parr-smolt transformation at different times according to their temperature regimes. Our findings are therefore in accordance with our hypothesis that the rate of change in parr-smolt transformation is controlled by temperature following photoperiod stimulation. In COLDSMOLT II we investigated how temperature controls the rate of acclimation to seawater. Low temperatures may limit the ability of the fish to responding to an increase in salinity by limiting the endocrine responsiveness of key endocrine tissues and target organs. The results showed that the groups responded to the increase in salinity at different rates according to their temperature regimes. Our findings are therefore in accordance with our hypothesis that the rate of seawater adaptation is controlled by temperature following completion of parr-smolt transformation.

In the last experiment COMBISMOLT (October 2010 to January 2011), the goal was to test which combinations of intensities, seawater intermixing and temperature would give an optimal production in terms of high survival, growth, health, and low risk of winter ulcer in farmed salmon. Preliminary results show better growth during the treatment period, but lower survival rates in the disease challenge test in the brackish water groups than in the freshwater groups. This indicates that seawater intermixing can allow a smolt production with higher intensity than pure freshwater, if no negative effects like remobilization of metals occur when mixing freshwater with seawater.

In addition, we have used experimental data from the current and previous projects to develop biological input to a production model for Atlantic salmon smolt under Arctic conditions. The initial model development based on industry data shows some interesting results, indicating small effects of production intensity in the freshwater stage on subsequent growth and mortality. Compiled experimental data will be analyzed and compared with these results to shed further light on this issue.

[1] In alphabetic order

Background and goals

In this project we aimed to provide more knowledge on how to increase and optimise the production of Atlantic salmon (*Salmo salar* L.) smolts in the northern areas of Norway. The project have been focusing on seawater intermixing, low temperatures and intensive rearing and intent to propose solutions that can make North-Norway more self-sufficient with smolts and contribute to improved survival, growth, health, and low risk of winter ulcer outbreaks in farmed salmon, both during hatchery and sea cage stages based on raw water low of TOC and metals, especially aluminium (Al) and iron (Fe).

The project has been divided in four work packages (WPs). In WP1, two tasks were formulated. In the first task, the aim was to monitor growth and physiological performance in juvenile salmon during exposure to different salinities. In the second task, we wanted to examine growth, smolt physiology, health and susceptibility to *Moritella viscosa* in salmon smolts after exposure to different salinities. In WP2 we wanted to investigate the effect of low temperatures on development of basic hypo-osmoregulatory mechanisms in salmon smolts. In WP3 the main aims were to examine interaction effects of salinity, temperature and intensity on performance and health of salmon smolts and to implement results from the NORSMOLT project and other research projects into a model. At last in WP4 the main aims were dissemination of results to the research community and stakeholders.

WP 1: SEAWATER INTERMIXING IN LAND BASED PRODUCTION OF ATLANTIC SALMON

Task 1.1: SALIPARR: Physiological performance and regulation in Atlantic salmon parr exposed to different salinities

Task leader: Torstein Kristensen, NIVA

Introduction

The use of seawater addition to production water during the freshwater life stages of Atlantic salmon is common in Norway. About 25% of juvenile and 45% of smolt production sites uses seawater, ranging in salinity from 0.2-10 ‰ to 0.2 to 33 ‰, respectively. In the low salinity range, the concentration of the major divalent ions Ca and Mg changes dramatically. Polyvalent cation receptor proteins (CaRs) are salinity receptors in fish, and these receptors respond strongly to changes in levels, and ratio between major cations. Salinity level employed on juveniles may therefore carry both short and long term effects on the expression, activity and development of ion regulatory enzyme systems. The goal of the experiment was to study effects of salinity on gene expression, enzyme activity and whole body ionoregulatory status. Atlantic salmon parr were exposed to two salinity levels (0.5 and 10 ‰) in duplicate tanks for two weeks, with a recovery period of two weeks in freshwater. No major changes in design from the original proposal occurred.

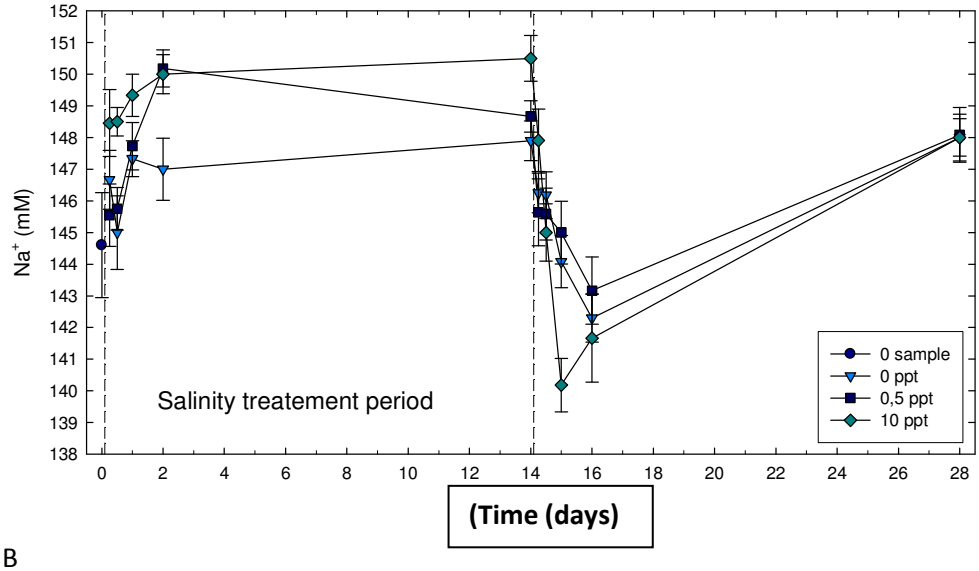
Material and Methods

The sampling and analytical methodology is described under WP3 in this document. Fish (n=12 pr treatment) were sampled at 0, 6, 12, 24, 48 and 336 hours after initiation and termination of salinity exposure. No major problems occurred during execution of the experiment or subsequent analysis.

Results

The salinity exposure caused small but significant changes in ion (5-7 %) and acid base balance, with consequent alteration upon return to freshwater to levels that in some cases differed from freshwater values prior to exposure (Fig 1A and B).

A



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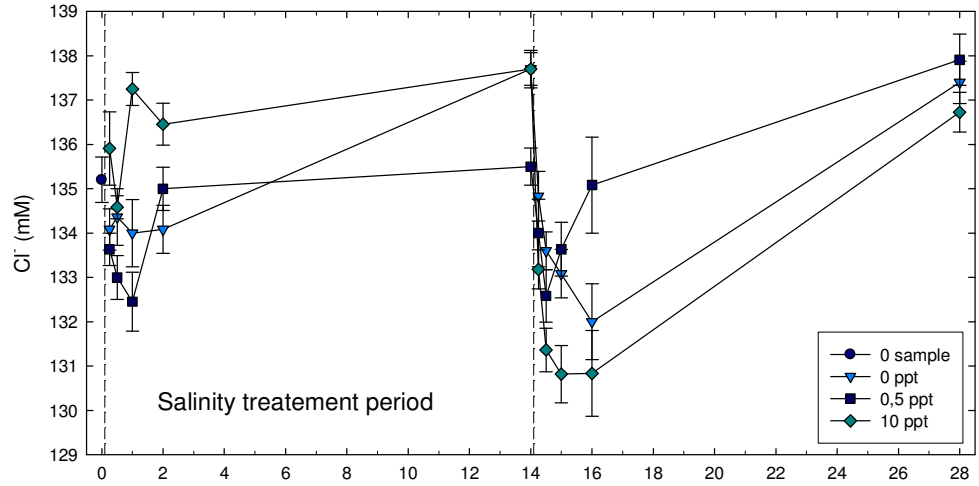

Figure 1. *Blood levels of sodium (A) and chloride (B) during and after salinity exposure. Values differ significantly from control in the 10 ‰ group after 12 hours of salinity exposure and onwards for both Na and Cl. In the 0.5 ‰ group, Na was significantly elevated after 12 hours and Cl after 336 hours. All groups showed a gradual reduction of ion levels in the post treatment sampling period until 24 to 48 hours.*

The gill Ca:Mg ratio was significantly lowered in the 10 ‰ treatment (Fig 2), indicating increased Mg content in the gills.

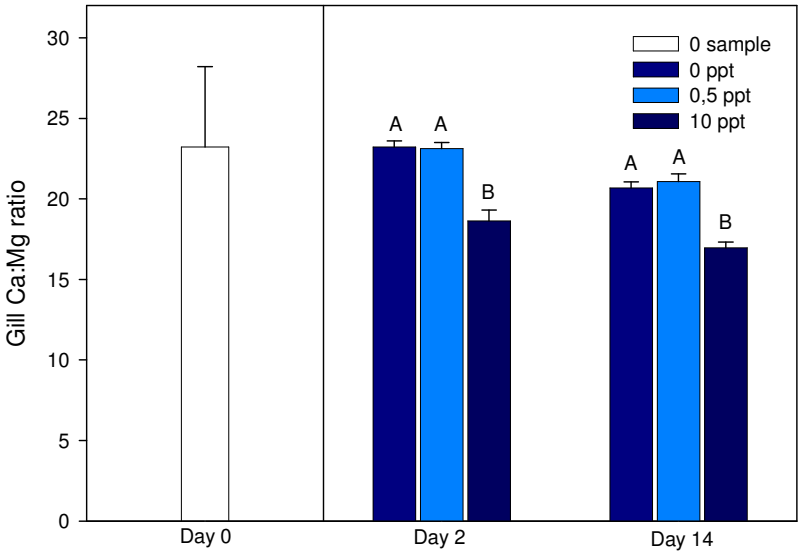

Figure 2. *Gill Calsium:Magnesium ratio at initiation of experiment and day 2 and 14 of salinity exposure.*

Gill Na^+ K^+ ATPase (NKA) enzyme activity was typical of Atlantic salmon parr, and showed a gradual reduction throughout the exposure and post-exposure period.

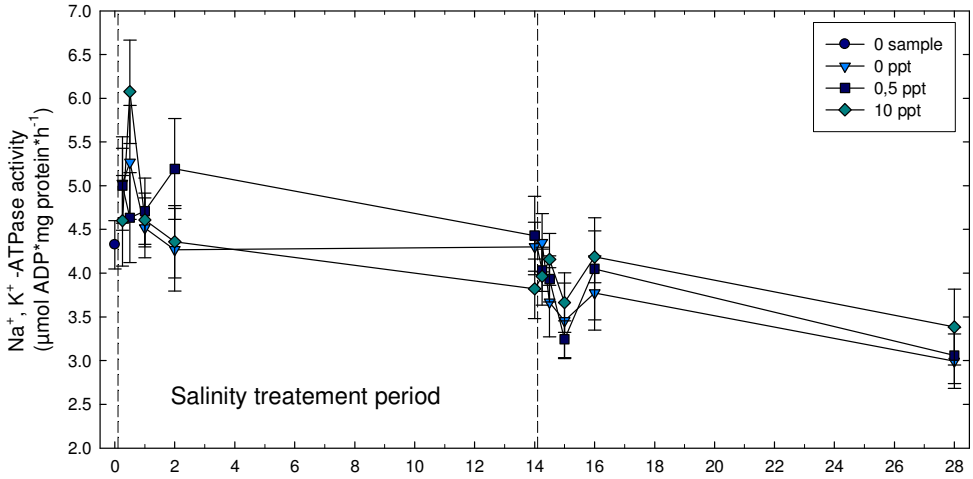

Figure 3. *NKA activity during and after salinity exposure. No significant difference between exposure groups was observed.*

Discussion/ conclusions

The results show minor changes in physiological status, but no indications of major changes in regulatory mechanisms. The welfare of the fish did not seem compromised by exposure or termination of exposure, although the reduction in NKA activity of 30 % after treatment. The strategy of applying seawater during the pre-smolt stages of production seem feasible, and may be underexploited in terms of the obvious water volume, temperature and water quality benefits that may be obtained, especially in areas where freshwater temperatures are low for prolonged periods of the year.

Task 1.2: SALISMOLT: Physiological performance and susceptibility to *M. viscosa* in smoltifying Atlantic salmon exposed to different salinities

Task leader: Hilde Toften, Nofima

Introduction

Seawater intermixing is a common practise in northern commercial smolt production. A production strategy with introduction of seawater prior to complete smoltification is used for a number of different reasons, and may carry both positive and negative aspects. Source of the added seawater is normally deep-water with a stable temperature of 6-8 ºC. Provided that added amount is sufficient, this will generate a substantial increase in temperature in the production water during winter conditions. Addition of 3% seawater (to 1‰) increases the buffering capacity of the production water substantially, as well as increasing the calcium (Ca) level to > 12 mg Ca L^{-1}. However, seawater addition is also shown to have a negative impact on water quality and fish by release of reactive, inorganic aluminium species (LAl). Ion-exchange of humic bound Al (Alo) with cations Na^+, K^+, Ca^{2+}, Mg^{2+} releases toxic LAl, which consequently binds to gill surface and cause ion regulatory and possibly also respiratory stress. Consequently, moderate seawater addition (today between 1 and 15 ‰) has not been recommended. However, in Northern Norway the general raw water quality may permit intermediate seawater addition as a more feasible strategy than in the south. The raw water quality in existing production sites in Northern Norway is generally good, with significantly higher pH, lower total organic carbon (TOC), lower total aluminium (Al) and lower total iron (Fe) compared to the other salmon producing regions in Norway (WQ 99-06).

Given the extensiveness of the seawater intermixing practice, there has been little scientific support for any beneficial effects for the fish. In a recent experiment we found that salmon reared at a salinity of 20 ‰ during the smoltification period showed reduced feed intake and growth, and became more susceptible to winter ulcers after transfer to sea. Whereas high salinities may have negative impact on the fish, lower salinities might be feasible or even beneficial for the fish, especially around the isosmotic 10 ‰ level. Thus, in this experiment we wanted to test salinities from 15 ‰ and below to find those levels that do not compromise the physiology, health and welfare status of Atlantic salmon smolts. There were no changes in design from the original proposal.

Material and Methods

Groups of individually tagged juvenile Atlantic salmon were held in eight tanks for six weeks at different salinities during the period of parr-smolt transformation. Sea water was intermixed with fresh water to obtain the different salinities. The control groups had freshwater (0 ‰), while the three treatment groups had average salinity levels of 4.8, 9.6 and 14.3 ‰. All groups had relatively low fish density (30 kg m^{-3}) and high specific water flow (1.3 L kg^{-1} min^{-1}) during the treatment period, but a small quantity of oxygen was added to maintain an O_2 level of above 85 % saturation in all tanks. Feed intake was recorded three times a week. Weight and length were registered at days 1-2, 43-44 and 84. Blood and tissue (gills, skin and muscle) for analysis of physiological and health status were sampled at day 0, 21 and 42. After the six weeks of treatment, the fish were transferred to full strength seawater and bath-challenged with *Moritella viscosa* or mock infected. In a prechallenge study three groups of fish were tested (30 fish (57 g) per group; dosage 8.9 $x10^6$, 8.9 $x10^5$ or 8.9 $x10^4$ cfu ml^{-1}; 7 ºC). Based on this a *M. Viscosa* dosage of 1.1 x 10^6 cfu ml^{-1} was used in the main challenge study. Two groups were established (control and treatment group) with about 280 fish (52 g) in each tank. Subsequently, the mortality was registered for another five weeks. Water quality was controlled and monitored throughout the experiment. No major problems occurred during execution of the experiment or subsequent analysis.

Results

Hypo-osmoregulatory ability and gill Na^+ K^+ ATPase activity

At the start of the experiment, the gill Na^+ K^+ ATPase (NKA) activity was low and there were no significant differences between any of the groups, but during the experiment, the activity increased in all groups to a range 6-7 µmol ADP mg protein^{-1} h^{-1} (Fig. 4a). At day 21 there were a significantly increased NKA activity in the group with the highest salinity, however at the end of the exposure period no differences were found. The plasma levels of Cl^- in the blood after 24 h seawater challenge were declining in all groups during the trial (Fig. 4b). The silvering index increased from 2 to 4 during the treatment period and there were no differences between the groups at any sampling date.

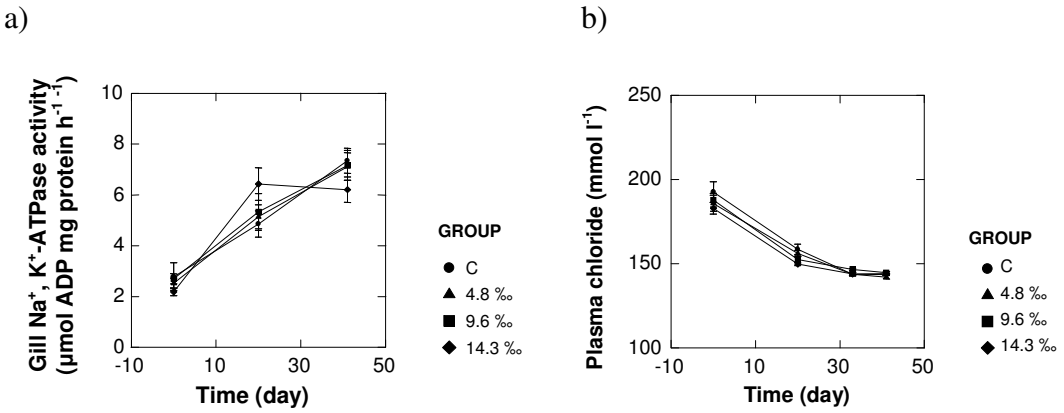

Figure 4. *a) Gill Na+, K+-ATPase activity; b) Plasma chloride after 24 h seawater exposure during different stages of smolt development in groups exposed to different salinities.*

Growth and feed intake

The average weight and length increased in all groups during the experiment, but not at the same rate. All the saline-acclimated groups had a higher growth rates than the control (Fig 5) during the treatment period. After transfer to full strength sea seawater, however, the growth was no longer significantly different (data not shown).

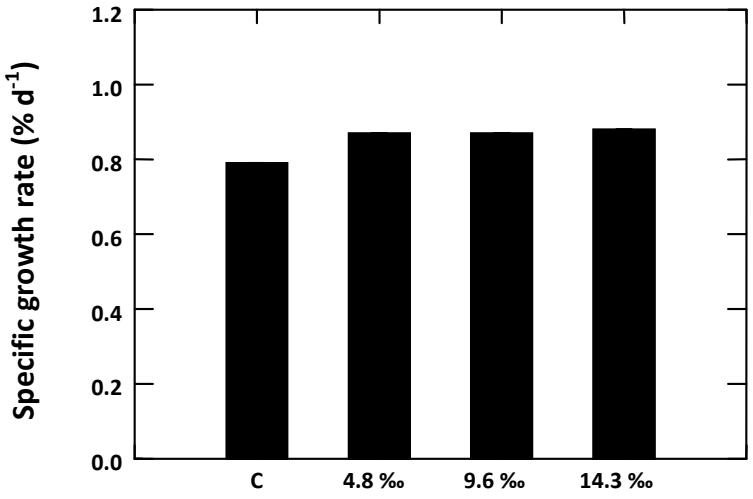

Figure 5. *Growth of fish groups exposed to different salinities during the parr-smolt transformation.*

The feed intake data confirms the growth data, both in the treatment period and after seawater transfer (Fig. 6). Fish subjected to increased salinity had higher feed intake during treatment. After transfer to full strength seawater, all groups showed increasing appetite.

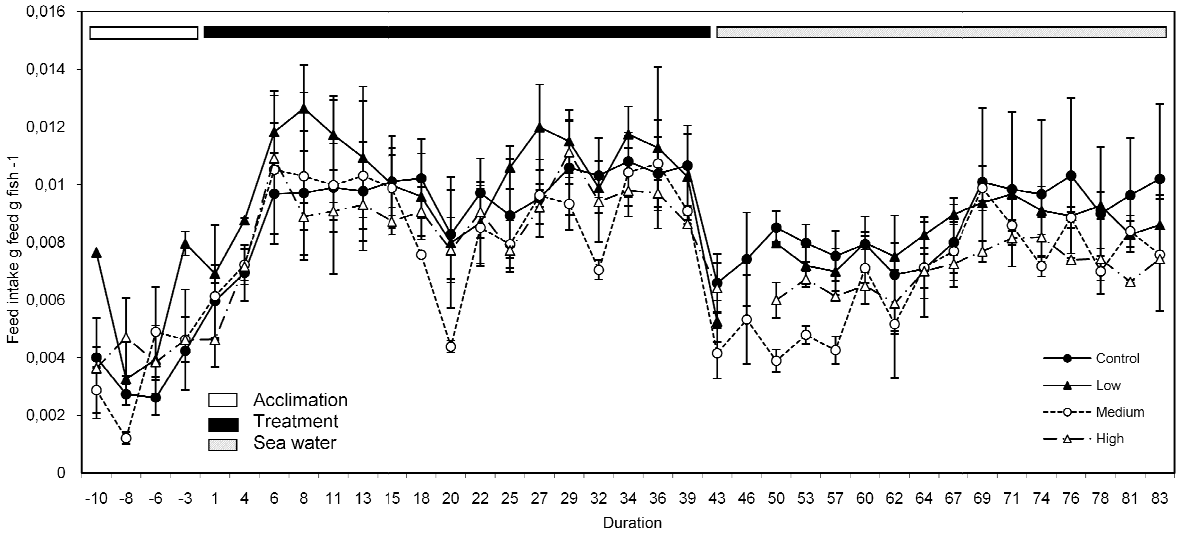

Figure 6. *Feed intake in groups of Atlantic salmon exposed to different salinities (4.8, 9.6 and 14.3 ‰) during three phases: acclimation; the smoltification period (treatment period); and after transfer to full-strength seawater.*

Physiological effects

Before the start of the treatment there were no differences in blood levels of Cl^-, Na^+, glucose, haematocrit, pCO_2, TCO_2, HCO_3 or pH between any of the groups. At day 21 plasma chloride levels are increased in all brackish water groups (Fig. 7) and the pCO_2, TCO_2, HCO_3 and pH in blood is positively correlated with the salinity (data not shown). At day 42 there is less differences between the different groups.

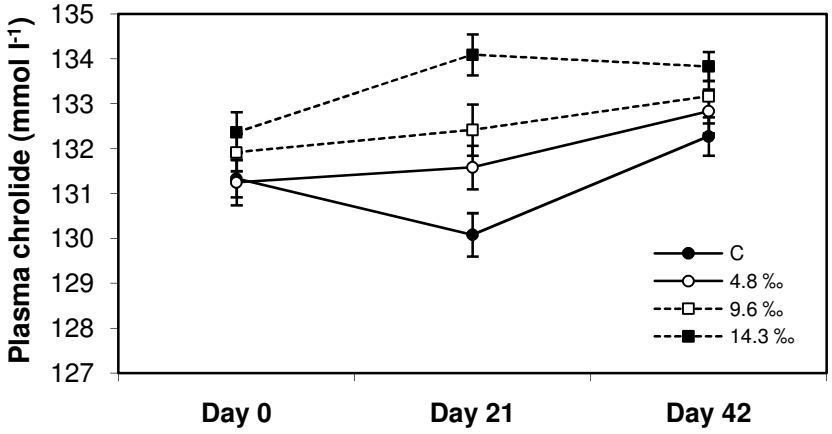

Figure 7. *Plasma chloride levels in groups exposed to different salinities.*

Survival after challenge with Moritella viscosa

In the challenge study, the survival did not differ between groups (Fig 8). The fish in all groups started to die at the same time and followed the same pattern throughout the trial.

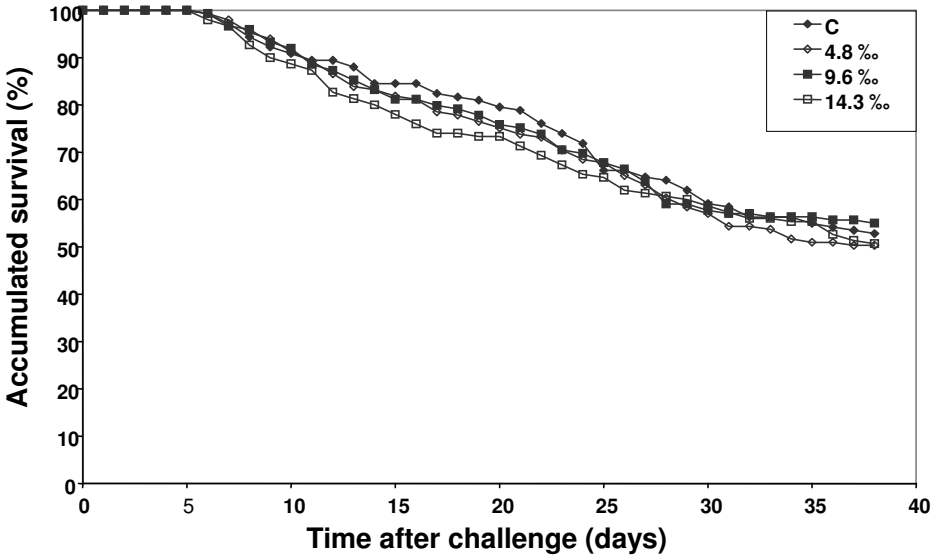

Figure 8. *Accumulated survival of Atlantic salmon post smolts (n= 590) after bath challenge with Moritella viscosa (1.3 x 10^6 cfu ml^{-1}) in seawater (salinity 34 ‰) at 7 ºC in groups previous exposed to different salinities during the parr-smolt transformation.*

Discussion and conclusions

The results show that salinities up to 14.3 ‰ are safe for juvenile salmon during the period of smoltification. We found some differences in osmoregulatory ability and blood physiology between the treatment groups and the control, but these seemed not to compromise the health and welfare of the fish. On the contrary, salinities from 5 to 14 ‰ lead to improved growth compared to the control reared in freshwater. These results indicate that seawater intermixing (up to ‰) can be a feasible strategy before the fish is fully smoltified.

WP 2: COLD SMOLT

Task 2.1: COLDSMOLT I: Low temperatures during smoltification and transfer to seawater: Osmoregulation and growth in wild Atlantic salmon smolts at different temperatures

Task leader: Sigurd Stefansson, UiB

Introduction

Atlantic salmon exhibit a highly developed smolt stage characterised by several physiological, morphological and behavioural events that pre-adapt the juvenile salmon for a life in the marine environment. This process, the parr-smolt transformation, occurs during spring and is controlled by seasonal changes in photoperiod. During smolting, photoperiod information is transformed via the light-pituitary axis, involving endocrine factors such as growth hormone, insulin-like growth factor I and cortisol, acting in concert with their respective receptors. Temperature is known to influence smoltification directly by controlling the rate of morphological and physiological development. In salmon smolts, an increase in temperature from 5 to 12 °C has been shown to advance the development of seawater tolerance compared with fish from a control group raised in ambient water. In river Bolstad, the lower part of river Vosso, a decrease in temperature caused by regulation of the river has resulted in a significant decrease in growth of the wild smolts. Hence, it has been speculated that the combination of low temperature and reduced fish size may influence on smolt development and timing of migration to sea in Vosso.

One of the most critical changes during parr-smolt transformation is the increase in hypo-osmoregulatory ability, including higher gill Na^+, K^+-ATPase activity (NKA). In freshwater, gill

NKA is the primary driving force for active uptake of Na^+ and consequently this enzyme is found in high concentrations in the chloride cells. Following transfer to seawater, the excess of monovalent ions is excreted by NKA and by Na^+, K^+, Cl^- co-transporter (NKCC) located in the basolateral membrane. The NKA enzyme consists of two obligatory polypeptide sub units, α and β. In Atlantic salmon recent studies have identified five different α (α1a, α1b, α1c, α2, α3) and four different β (β1a, β1b, β2 β3b) isoforms. Among these, α1a is linked to the chloride cell in freshwater whereas α1b is common in seawater adapted smolts. Several studies indicate that a shift in α-isoform expression enables the cells to preadapt to altered physiological conditions following seaward migration. The aim of the present work has been to investigate how basic transcriptional and translational changes in NKA α1a, α1b subunit isoforms, NKCC in gills of wild Atlantic salmon smolts originating from river Vosso is affected by temperature during spring. In addition, we monitored development of gill NKA enzyme activity, plasma Cl^- and growth to assess changes in overall smolt quality and to compare with the observed results of the basic molecular mechanisms in the fish.

Material and Methods

Fish stock and rearing conditions

The fish used were potential 1+ wild Atlantic salmon smolts of the Vosso strain reared at Voss Hatchery (Voss, Western Norway). From first feeding until end of August, the fish were maintained on natural photoperiod, ambient water and were fed a standard dry diet. On 1 September, all fish were transferred from Voss hatchery to Lake Evanger and located into a net pen. From this group, approximately 1500 pre-smolts were brought to the ILAB, Bergen on 28 December. Between 1 September and 27 December, the temperature in Lake Evanger decreased from 12 to 2.5 ºC. After arrival ILAB, the fish were put into in a $2m^2$ rearing tank supplied with running fresh water (approximately 4 ºC) and further exposed to natural photoperiod (60^oN).

Experimental set-up

On 2 March 2010, 1500 salmon parr (mean length 12.8 cm, s.d.= 0.6, mean weight 22.1 g, s.d.= 3.2), all showing high condition and visible parr-marks, were randomly distributed into 71 m^2 square fibre-glass tanks with a rearing volume of 500 l (n = 212 pr tank). Photoperiod was simulated natural photoperiod, 60^oN. Five days later the freshwater temperature was gradually altered from ambient (4.8 ºC) to temperature regimes referred to as 5.1 (s.d.= 0.42, 2 tanks), 8.1 (s.d.=0.21, 3 tanks) and 10.8 ºC (s.d.= 0.31, 2 tanks) over a period of one week. After this acclimation period, temperature was kept constant for the rest of the freshwater period. A sub population of 40 fish in each group was individually tagged on 5 March (Carlin tags) for observations of individual growth rates. On 20 May, all smolts in the 8.1 ºC group (3 tanks) showed normal morphological signs of smolting, i.e. dark fin margins, absence of parr marks and loose silvery scales.

Water quality

To obtain in formation of Al speciation and changes in Al speciation due to change in temperature in situ fractionation of water with respect to size (molecular mass) and charge was performed using membrane filtration (0.45µm) and ultra filtration (Amicon H1P10–20 hollow fibre) in combination with ion chromatography (Chelex 100).

Sampling procedures

At regular intervals random samples of 12 fish were taken from each group. All groups were starved 24 hours prior to sampling and the fish were killed by a blow to the head. From each fish, blood samples were drawn into a heparinised syringe from the caudal peduncle. At the same time, gill filaments were sampled, frozen in SEI buffer, RNA later or dry frozen at -80 ^oC. Samples frozen in SEI buffer were subsequently analysed for gill Na^+, K^+-ATPase activity.

RNA isolation, cDNA synthesis and real-time quantitative PCR

Gill NKA α-subunit isoforms (α1a, α1b), NKCC1a and elongation factor 1A (EF1α) mRNA levels were measured by Q-PCR assays using the ABI prism 7000 detection system platform (ABI; Applied Biosystems, Foster City, CA, USA). Briefly, for each assay, triplicate fivefold cDNA dilution series made from total RNA from different exposure groups were used to determine amplification efficiencies (E) calculated as the slope from the plot of log RNA concentration versus threshold cycle (Ct) values using the following formula: $E = 10^{(-1/\text{slope})}$. Expression is presented as relative to the endogenous reference gene EF1α. EF1α did not change over time or differ between treatments in present study.

Results

The temperature was different between treatments (5.2±0.3, 8.2±0.1 and 11±0.2) and pH ranged from 6.7 to 6.4, probably due to production of CO_2. However, the CO_2 concentration was low compared to critical levels for fish. The general water quality was similar for all temperature groups during the experimental period (Table 1 and 2), and the concentration of Al was about 48 µg/l, and mainly present as colloidal Al species (66 %) and 34% LMM Al species on none positively charged species, decreasing with increasing temperature. The Al concentration in gills was increased for fish in the low temperature water compared to the medium and high temperature water, being lower than background levels in gills for fish not exposed to Al (<10 µg/g).

Table 1. *Concentration of metals in gills of fish exposed to the different water qualities (mean ±SD, N=3-6).*

	Gill weight (g)	**Al (µg/g)**	**Cu (µg/g)**	**Fe (µg/g)**	**Mn (µg/g)**	**Zn (µg/g)**
Tank 1	0.010±0.003	14±21	1±2	195±129	13±2	463±115
Tank 2	0.011±0.003	55±117	1±2	245±131	15±5	453±119
Tank 3	0.013±0.003	3±2	2±1	204±75	15±4	422±92
Tank 4	0.013±0.004	2±1	2±1	195±88	15±4	457±144
Tank 5	0.013±0.003	4±6	2±0	209±96	14±3	423±134
Tank 6	0.012±0.003	3±4	2±0	191±94	15±10	364±121

Table 2. *General water quality in influent water and tank water of low, medium and high temperature (mean ±SD, N=3-6).*

		Influent-water	**Low Temp.**	**Mid. Temp.**	**High Temp.**
Temp.	**°C**	**ND**	**5.2±0.3**	**8.2±0.1**	**11±0.2**
pH (in situ)		6.8±0	6.5±0.1	6.4±0.1	6.4±0.1
Conductivity	mS/m	3.8±0.1	3.8±0.3	3.7±0.1	3.7±0.1
Turbidity	FNU	0.5±0.4	0.3±0.3	0.3±0.1	0.4±0.1
TOC	mg/L	1.8±0.3	1.8±0	1.7±0.1	1.7±0
Colloidal DOC	mg/L	ND	1.3±0	1.2±0.1	1.2±0.1
LMM DOC	mg/L	ND	0.5±0.1	0.5±0	0.5±0
NH4-N	µg N/L	39±24	54±21	61±11	51±7
CO_2	mg/L	ND	4.9±1.5	4.5±0.6	5.3±0.9
Cl	mg/L	5.5	5.6±0.1	5.6±0	5.5±0
NO_3	mg/L	0.1	0.2±0	0.2±0	0.2±0
SO_4	mg/L	2.91	2.7±0	2.7±0	2.7±0
F	mg/L	<0,04	<0,04	<0,04	<0,04
Ca	mg/L	1.8	1.8±0	1.8±0	1.8±0.6
Mg	mg/L	0.4	0.4±0	0.4±0	0.4±0
K	mg/L	1.8	2.3±0.5	2.5±0.3	2.1±0.2
Na	mg/L	3.7	3.8±0.1	3.8±0.1	3.8±0.1
Si	mg/L	2.2	2.3±0.1	2.3±0.1	2.2±0.1
Al total	µg/L	39	47±10	48±9	47±9
Colloidal Al	µg/L	29.2	31±12	33±9	35±9
LMM Al	µg/L	9.9	16±3	14±1	13±3
Alcation	µg/L	2.8	1±1	2±1	2±3

Growth rate reflected differences in temperature. Overall, SGR on 5.1 °C was 0.1 % day^{-1}, 0.28 % day^{-1} on 8.1 °C and 0.53 % day^{-1} on 10.8 °C, with significant differences between groups ($p<0.001$).

Plasma chloride levels in all groups were significantly influenced by time and temperature ($P<0.001$, Fig 9). In the 5.1 °C group, a decrease in the plasma chloride level was seen between 2 March and 7 April. In the 8.1 and 10.8 °C groups, a decrease and stabilization in plasma chloride levels was recorded between 7 April and 4-18 May ($P<0.05$).

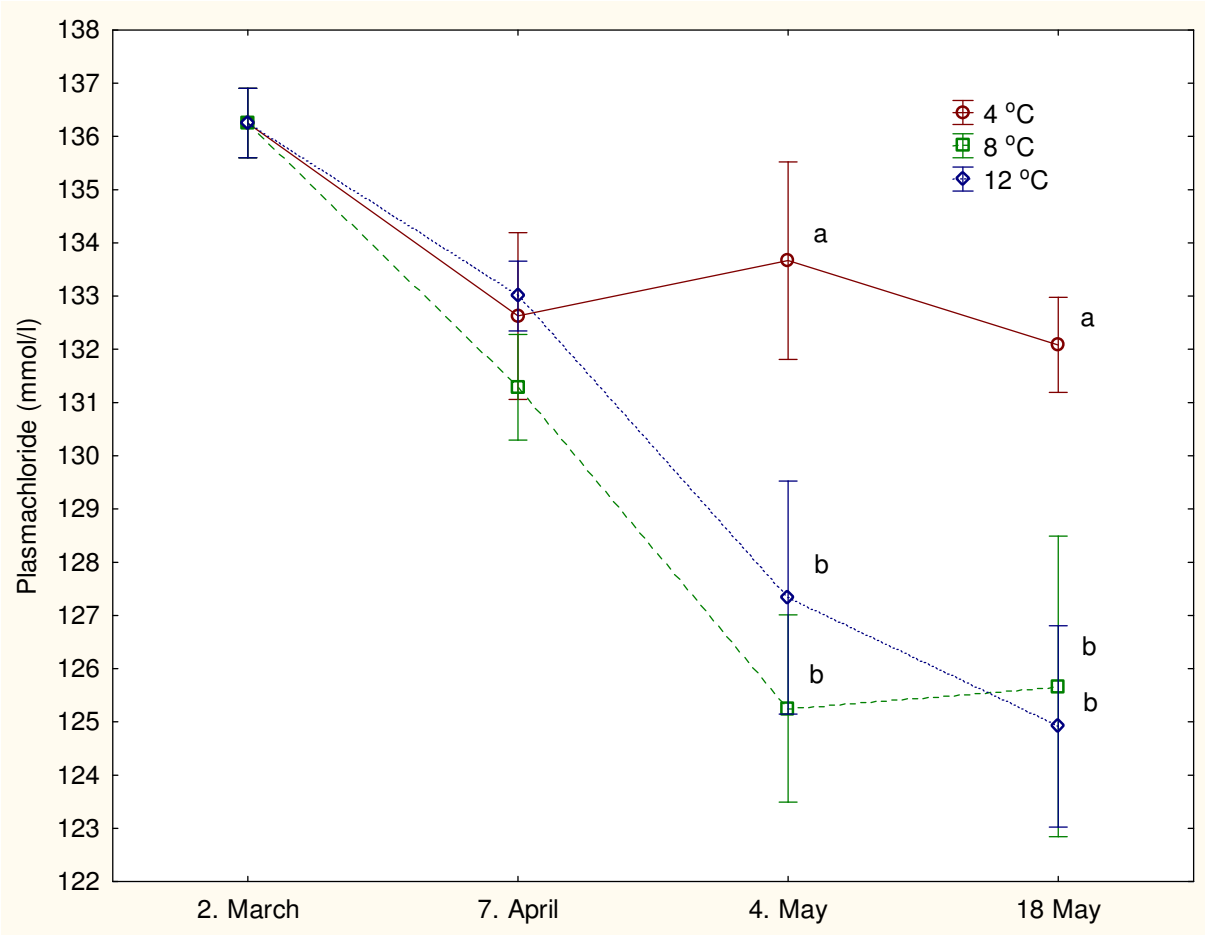

Figure 9. *Plasma chloride levels in salmon smolts at three temperatures (5.1, 8.1 and 10.8 °C)*

Overall, gill NKA α1a expressions levels increased in all groups between 2 March and 4 May (Fig. 10a), this increase was significant in the 5.1 and 10.8 °C groups ($p<0.01$). Overall, there was a significant effect of both time and temperature on gill NKA α1b expression ($p<0.05$, Fig. 10b). Expression levels of the 10.8 °C groups decreased significantly between 7 April and 18 May, while the levels of the 8.1 °C group peaked on 7 April, followed by a significant decrease on 4 May. Expression levels of the 5.1 °C group increased to a peak between 2 March and 4 May. Hence on 4 May, levels in the 5.1 °C group were significantly higher than in the 8.1 and 10.8 °C groups.

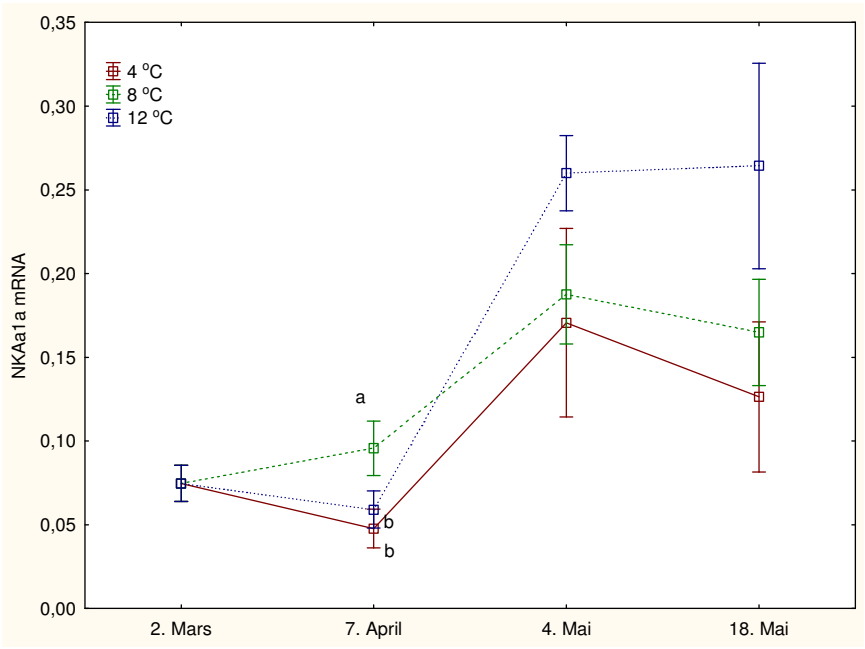

Figure 10a. *Relative gene expression of NKA α1a in salmon smolts at three temperatures (5.1, 8.1 and 10.8 °C)*

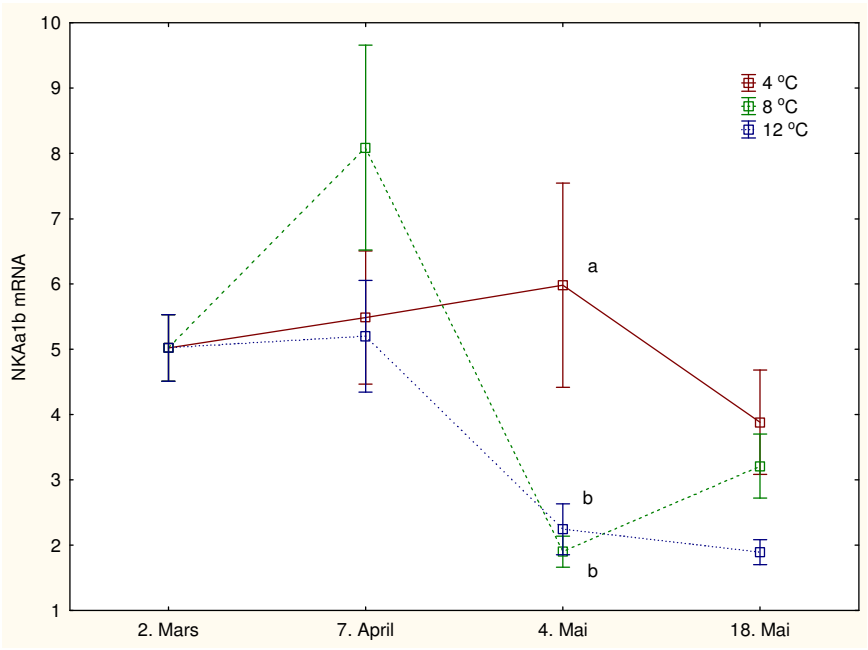

Figure 10b. *Relative gene expression of NKA α1b in salmon smolts at three temperatures (5.1, 8.1 and 10.8 °C)*

There was an overall effect of both time and temperature on gill NKCC1a expression levels ($p<0.05$, Fig. 11). At 5.1 °C expression levels increased significantly to a peak on 4 May, significantly higher than the 8.1 and 10.8 °C groups ($p<0.05$). Further, expression levels in 10.8 °C decreased between 4 and 18 May, and were significantly below levels in the 5.1 and 8.1 °C groups ($p<0.05$) at termination of the study.

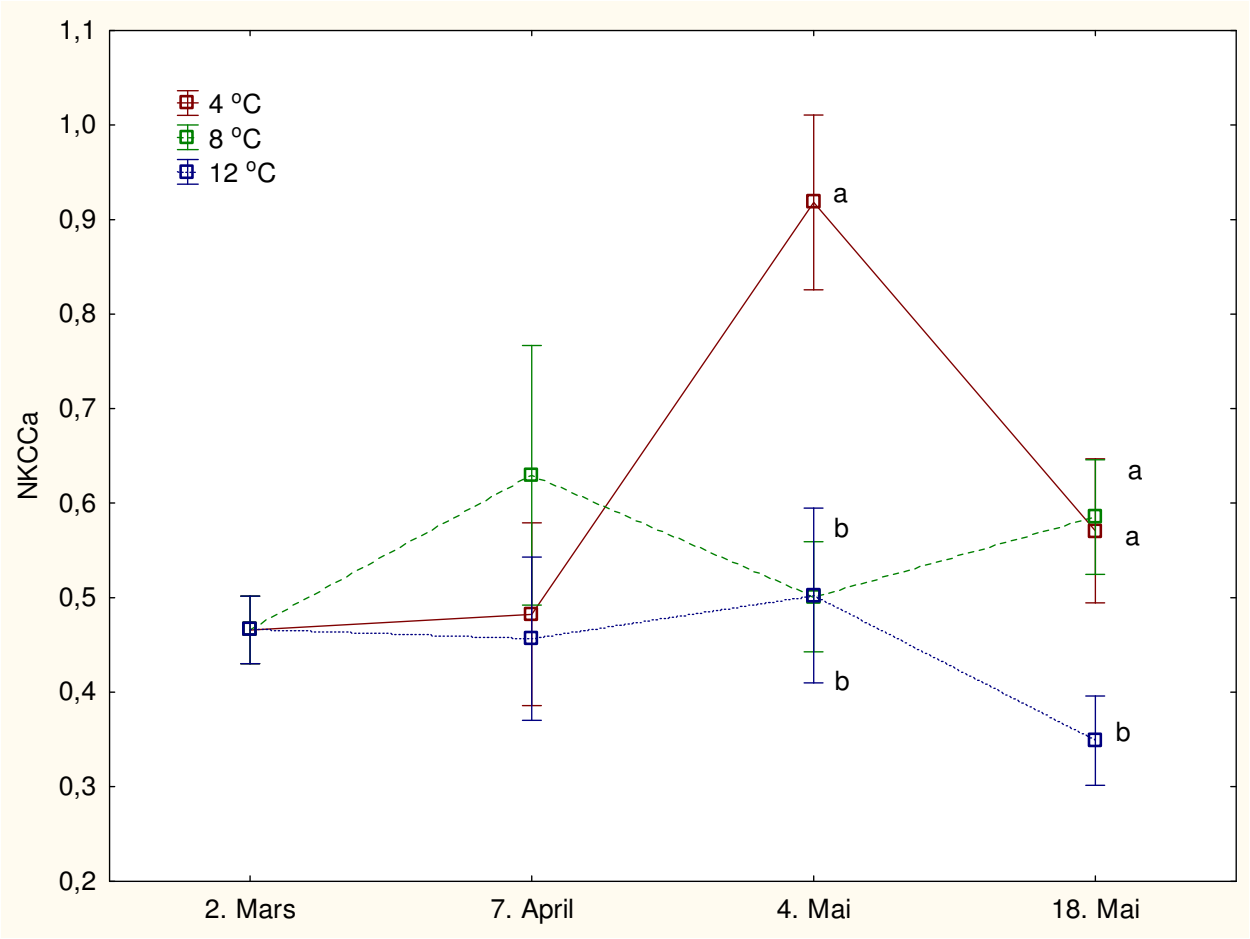

Figure 11. *Relative gene expression of NKCC1a in salmon smolts at three temperatures (5.1, 8.1 and 10.8 °C).*

The gill Na^+, K^+-ATPase activity was significantly affected by time, temperature and their interactions ($P<0.001$, Fig 12). During the course of the freshwater period, a significant increase in gill Na^+, K^+-ATPase activity was observed in all groups between 2 March and 7 April. While the gill Na^+, K^+-ATPase activity in the 5.1 °C group continued to rise significantly and reaching peak level on 18 May ($P<0.001$), the level in the 8.1 °C group stabilised after 7 April at approximately 11.5 µmol ADP mg protein $^{-1}$ h $^{-1}$. Contrary, the gill Na^+, K^+-ATPase activity in the 10.8 °C group declined significantly ($P<0.001$) after 7 April. On the last sampling in freshwater, the gill Na^+, K^+-ATPase activity in the 5.1 °C group was significantly higher than that in both the 8.1 ($P<0.05$), and the 10.8 °C groups ($P<0.05$).

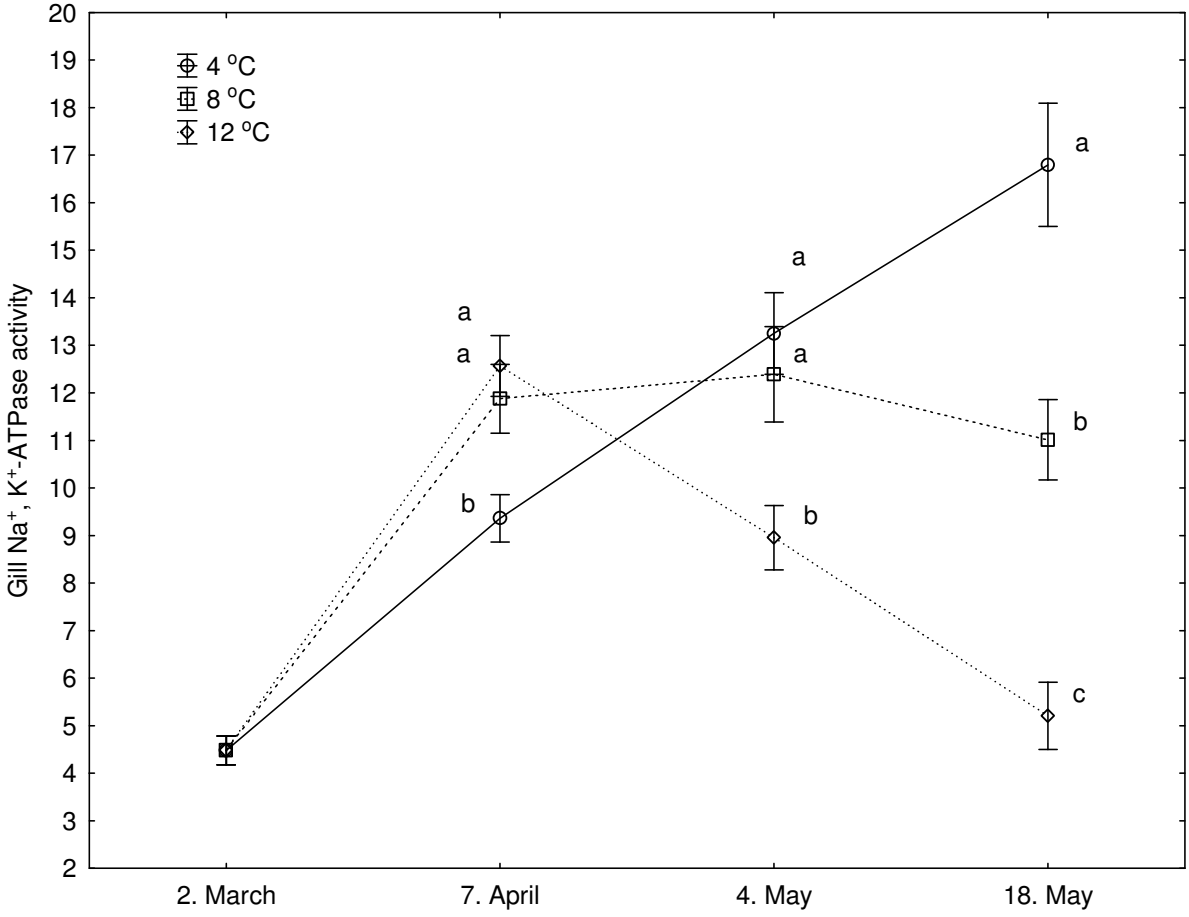

Figure 12. *Gill Na^+, K^+-ATPase activity in salmon smolts at three temperatures (5.1, 8.1 and 10.8 °C).*

Conclusions

Previous studies have estimated a period of approximately 350 degree days (dd) between the onset of the smolt-related increase in NKA activity of Atlantic salmon and the peak in enzyme activity, providing a useful model for the prediction of completion of smolting under various rearing temperatures. In a similar manner, temperature influences the duration of the period of peak smolt characters, e.g. high seawater tolerance, often referred to as the 'smolt window'. Smolt characters are lost sooner when smolts are held in freshwater at higher temperatures, and this de-smoltification eventually prevents the smolts from being able to acclimate to seawater.

Low temperatures may prevent the fish from responding to the increasing photoperiod by limiting the endocrine responsiveness of key endocrine tissues and target organs. In the present study, the groups completed parr-smolt transformation at different times according to their temperature regimes. This is documented by the changes in NKA activity, NKA α isoform expression and NKCC1a expression. Our findings are therefore in accordance with our hypothesis that the rate of change in parr-smolt transformation is controlled by temperature following photoperiod stimulation.

WP 3: INTERACTIONS BETWEEN SALINITY, TEMPERATURE AND INTENSITY

Task 3.1: COLDSMOLT II: Salinity and temperature interactions: Osmoregulation and growth in wild Atlantic salmon smolts at different temperatures in seawater

Task leader: Sigurd Stefansson, UiB

Introduction

Temperature is known to influence smoltification directly by controlling the rate of morphological and physiological development. In river Bolstad, the lower part of river Vosso, a decrease in temperature caused by regulation of the river has resulted in a significant decrease in growth of the wild smolts. Hence, it has been speculated that the combination of low temperature and reduced fish size may influence on smolt development and timing of migration to sea in Vosso. One of the most critical changes during parr-smolt transformation is the increase in hypo-osmoregulatory ability, including higher gill Na^+, K^+-ATPase activity (NKA). In freshwater, gill NKA is the primary driving force for active uptake of Na^+ and consequently this enzyme is found in high concentrations in the chloride cells. Following transfer to seawater, the excess of monovalent ions is excreted by NKA and by Na^+, K^+, Cl^- co-transporter (NKCC) located in the basolateral membrane.

The aim of the present work has been to investigate how temperature controlled the rate of acclimation to seawater, with emphasis on mechanisms of ion regulation in the gills of wild Atlantic salmon smolts originating from river Vosso. We monitored the development of gill NKA enzyme activity, plasma Cl^- and growth.

Material and Methods

Fish stock and rearing conditions

The fish used were potential 1+ wild Atlantic salmon smolts of the Vosso strain reared at Voss Hatchery (Voss, Western Norway). From first feeding until end of August, the fish were maintained on natural photoperiod, ambient water and were fed a standard dry diet. On 1 September, all fish were transferred from Voss hatchery to Lake Evanger and located into a net pen. From this group, approximately 650 pre-smolts were brought to the ILAB, Bergen on 28 December. Between 1 September and 27 December, the temperature in Lake Evanger decreased from 12 to 2.5 ºC. After arrival at ILAB, the fish were randomly distributed into 3 1 m^2 square fibre-glass tanks with a rearing volume of 500 l (n = 212 pr tank). Light was controlled by a computer to simulate the natural photoperiod, 60 ^oN. A sub population of 40 fish in each group was individually tagged on 5 March.

Seawater experiment

On 20 May, all fish showed normal morphological signs of smolting, i.e. dark fin margins, absence of parr marks and loose silvery scales. Hence, the fish (mean length 14.1 cm, s.d.= 0.5, mean weight 28.5 g, s.d.= 4.5, condition factor 0.99, s.d.= 0.08) were distributed randomly into 6 1m rearing tanks. Beginning 21 May, the temperature was gradually altered from 8.1 to 4.1 (s.d.= 0.40, 2 tanks), 8.1 (s.d.= 0.36, 2 tanks) and 11.9 ºC (s.d.= 0.33, 2 tanks) over a period of one week. Finally on 26 May, salinity in all tanks was regularly increased and thereafter stabilized on a weekly basis, from freshwater (0 ‰) to a salinity of 14.3 ‰ between 26 May and 2 June, from 14.3 to 25.1 ‰ between 2 June and 8 June and from 25.1 to 33.7 ‰ from 8 June onwards.

Sampling procedures

Prior to transfer and at regular intervals in seawater random samples of 12 fish were taken from each group. All groups were starved 24 hours prior to sampling and the fish were killed by a blow to the head. From each fish, blood samples were drawn into a heparinised syringe from the caudal peduncle. At the same time, gill filaments were sampled, frozen in SEI buffer, RNA later or dry frozen at -80 ^oC. Samples frozen in SEI buffer were subsequently analysed for gill Na^+, K^+-ATPase activity.

Results

Following transfer to seawater, the development of plasma sodium levels were significantly influenced by time and temperature (P<0.001, Fig 13). In all groups, a rise in plasma sodium levels was observed. During the seawater period, the overall plasma sodium levels were seen to be higher in the 4 °C group, compared to the 8 and 12 °C groups, significantly higher on 16 and 23 June (P<0.05).

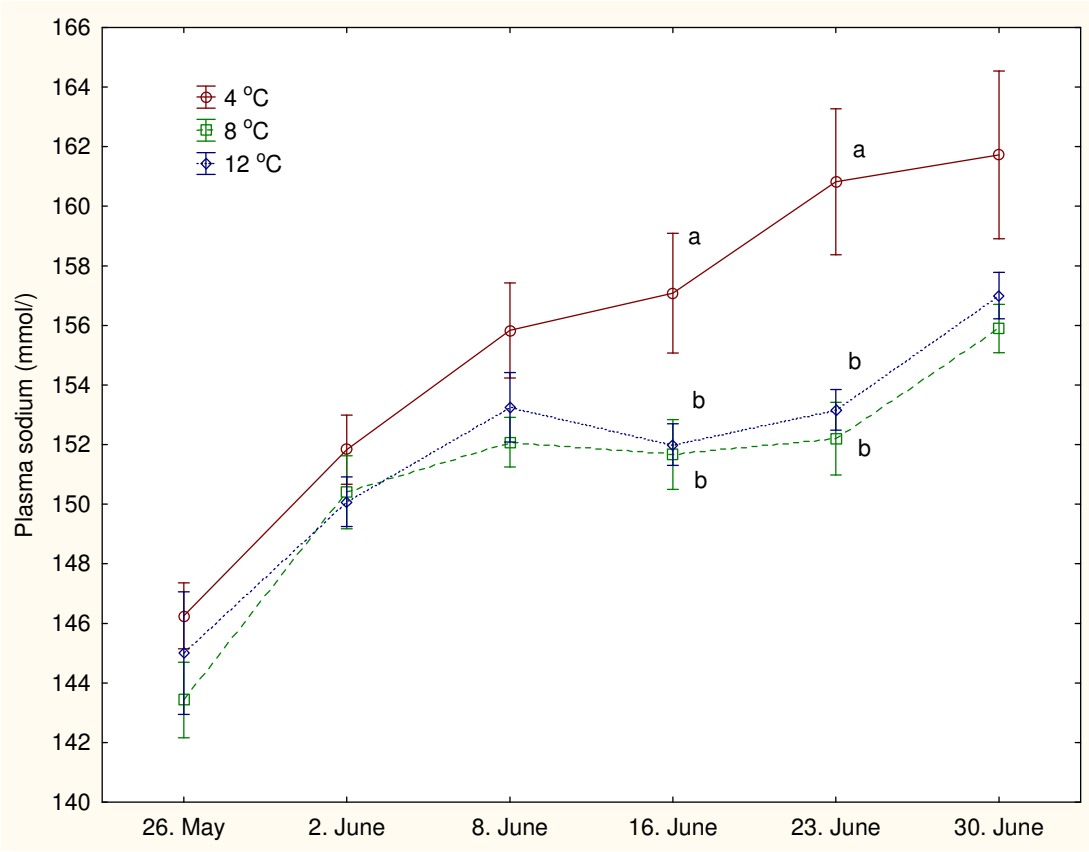

Figure 13. *Plasma sodium levels in salmon smolts following transfer to seawater at three temperatures (4.1, 8.1 and 11.9 °C).*

Development of gill Na^+, K^+-ATPase activity following transfer to seawater

Overall, gill NKA activity was significantly affected by time and temperature (P<0.001, Fig 14). In the 4 °C group, activity remained relatively stable at approximately 9 µmol ADP mg protein $^{-1}$ h $^{-1}$ until 16 June (33 ‰). Thereafter a gradual increase in gill NKA activity was observed, reaching peak level at 13 µmol ADP mg protein $^{-1}$ h $^{-1}$ on 30 June (P<0.001). In the other groups a similar, but advanced trend was seen. The 8 °C group showed increased gill NKA activity between 8 June (25 ‰) and 30 June (P<0.001), while in the 12 °C group the gill NKA activity increased between 2 June (15 ‰) and 30 June (P<0.001). On the last sampling, 30 June, the highest gill NKA activity was observed in the 8 and 12 °C groups, significantly higher than the 4 °C group (P<0.05).

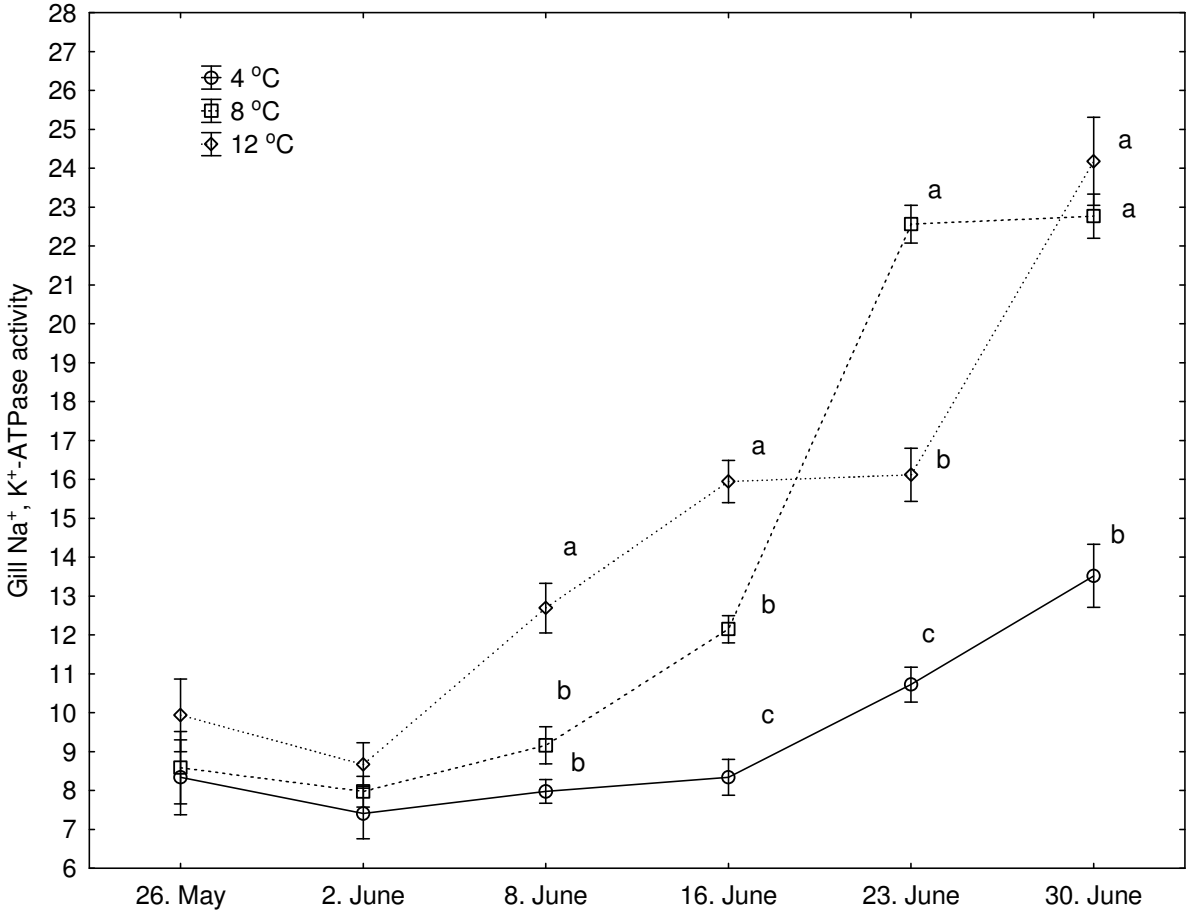

Figure 14. *Gill Na^+, K^+-ATPase activity in salmon smolts following transfer to seawater at three temperatures (4.1, 8.1 and 11.9 °C).*

Conclusions

Low temperatures may limit the ability of the fish to responding to an increase in salinity by limiting the endocrine responsiveness of key endocrine tissues and target organs. In the present study, the groups responded to the increase in salinity at different rates according to their temperature regimes. This is documented by the changes in NKA activity and the ability to maintain plasma ion levels. Our findings are therefore in accordance with our hypothesis that the rate of seawater adaptation is controlled by temperature following completion of parr-smolt transformation.

Data on gene expression of NKA α1a, α1b, CFTR-I and NKCC1a are being processed and will be published at a later time.

Task 3.2: COMBISMOLT: Interactions between salinity, temperature and intensive rearing

Task leader: Hilde Toften, Nofima

Introduction

Intensive rearing practices, including high fish densities, low water consumption and oxygen supplementation are now commonly used in many hatcheries around the world. These practices affect water quality by accumulation of metabolites (CO_2, NH_3/NH_4, organic material), changes in pH due to CO_2 accumulation, and consequent changes in metal speciation. When seawater addition is being used there will be an increase in pH due to an increased buffering capacity. Increased pH shifts equilibrium in the $CO_2 \leftrightarrow HCO_3$- reaction of the bicarbonate system towards HCO_3^-. Consequently, a larger proportion of CO_2 (g) will react and form HCO_3^- (aq) at higher pH, thus partly removing a major factor restricting growth and optimal performance in smolt production. Using seawater addition for this purpose requires caution, as increased pH also shifts the equilibrium of $NH_4^+ \leftrightarrow NH_3$, (pKa0: 9.245) towards more toxic NH_3. Data from an ongoing project shows a significantly higher ($p<0.001$) total ammonia nitrogen (TAN):CO_2 relationship in fish tanks with 20 ‰ salinity at pH 7.5 than in freshwater (pH 6.7) at low specific water flow (~ 0.14 L kg^{-1} min^{-1}). This indicates a reduction of effective CO_2 concentration and an increase in NH_3 when seawater addition is being applied. Thus, seawater intermixing may allow a smolt production with higher intensity than pure freshwater. In this experiment we wanted to test different intensities in combination with seawater intermixing at a relatively low temperature. Based on previous experimental results in the NORSMOLT project, we have been combining these intensities with a salinity and temperature which have been found to be safe. The original design was slightly changed. One of the intensity levels were changed and we used two salinities instead of one and one temperature instead of two giving the same amount of treatment groups, but a more interesting and industry relevant design.

Material and Methods

After three weeks of acclimation, six treatment groups (two replicates per group, 500 L tanks) of individually marked fish were formed (Table 3) by changing the salinity, water flow (L min^{-1}) and adding extra oxygen to different degrees: 1) Control (Fresh water (FW) and specific water flow (SWF) of 0.27 L kg^{-1} min^{-1}); 2) FW2 (FW and SWF of 0.15 L kg^{-1} min^{-1}); 3) FW3 (FW and SWF of 0.12 L kg^{-1} min^{-1}); 4) BW1 (Brackish water (BW) and SWF of 0.27 L kg^{-1} min^{-1}); 5) BW2 (BW and SWF of 0.15 L kg^{-1} min^{-1}); 6) BW3 (BW and SWF of 0.12 L kg^{-1} min^{-1}). The temperature was kept at about 8 ^oC (Table 3). All groups had similar and relatively high fish density (55-60 kg m^{-3}). Feed intake was recorded three times a week. Weight and length were registered at days 1-2, 43-44 and 79. Blood and tissue (gills, skin and muscle) for analysis of physiological and health status were sampled at day 0, 21 and 42. After the six weeks of treatment, the fish were transferred to full strength seawater and bath-challenged with *Moritella viscosa* or mock infected. A pre challenge experiment was performed with *M. viscosa* isolate LFI 5006 to determine the bath challenge doses that would result in 30 -50 % mortality in salmon. Based on the pre challenge test a *M. Viscosa* dosage of 7.8 x 10^5 cfu ml^{-1} was used in the main challenge study. Two days prior to the bacterial challenge, 420 fish from each experimental group were distributed into four 1800 litres tanks with full-strength seawater at 7 ºC. Two tanks were bath challenged with *M. viscosa* and two tanks were treated as the challenged fish, but were not exposed to the bacteria (mock infection). Subsequently, the mortality was registered for another five weeks. Water quality was controlled and monitored throughout the experiment. Unfortunately, a technical accident occurred just before the end of the treatment period (day 41) giving reduced oxygen level during the night in some of the tanks with brackish water. This resulted in about 7 and 19 % mortality (based on biomass) in group BW2 and BW3, respectively. All though there may be possible problems interpreting the result of these groups, the remaining fish were included in the disease challenge test.

Results

Water quality

The differences in salinity and specific water flow resulted in differences in the water quality between the groups (Table 3). With reducing specific water flow the level of CO_2 increased and the pH decreased and the change was larger in brackish water than in freshwater.

Table 3: *Average (± STDEV) salinity, specific water flow (SWF), temperature, carbon dioxide concentration (CO_2), pH and oxygen saturation (O_2) in outlet water from different treatment groups during the treatment period. The numbers in paraphrases are the number of sampling points included.*

Parameter	C (FW1)	FW2	FW3	BW1	BW2	BW3
Salinity (‰)	0 ±0.0 (70)	0 ±0.0 (70)	0 ±0.0 (70)	13.8 ±0.24 (70)	13.9 ±0.24 (70)	14.1 ±0.24 (70)
SWF (L $kg^{-1}min^{-1}$)	0.27	0.15	0.12	0.27	0.15	0.12
Temperature (℃)	7.9 ±0.21 (82)	8.0 ±0.22 (82)	8.1 ±0.22 (82)	7.7 ±0.21 (82)	7.7 ±0.22 (82)	7.8 ±0.22 (82)
CO_2 (mg L^{-1})	9.2 ±3.06 (14)	11.9 ±2.89 (14)	16.1 ±3.71 (14)	8.5 ±1.10 (14)	15.4 ±3.27 (14)	22.4 ±5.21 (14)
pH	6.28 ±0.05 (14)	6.18 ±0.05 (14)	6.15 ±0.08 (14)	7.01 ±0.04 (14)	6.78 ±0.05 (14)	6.63 ±0.13 (14)
O_2 (%)	88.9 ±11.0 (68)	94.2 ±18.9 (68)	95.2 ±24.3 (68)	87.3 ±13.3 (68)	99.9 ±24.0 (68)	98.1 ±23.9 (68)

Growth and feed intake

When the FW groups are compared there seems to be a tendency to reduced growth with reduced specific water flow (Fig 15) as we have seen in several previous experiments. The BW1 and BW2 groups seem to grow better than the FW groups. However, preliminary statistical tests indicate that none of these differences were significant. The BW3 group had the lowest growth of all the groups, probably because this group experienced more disturbances during the experiment than the other groups.

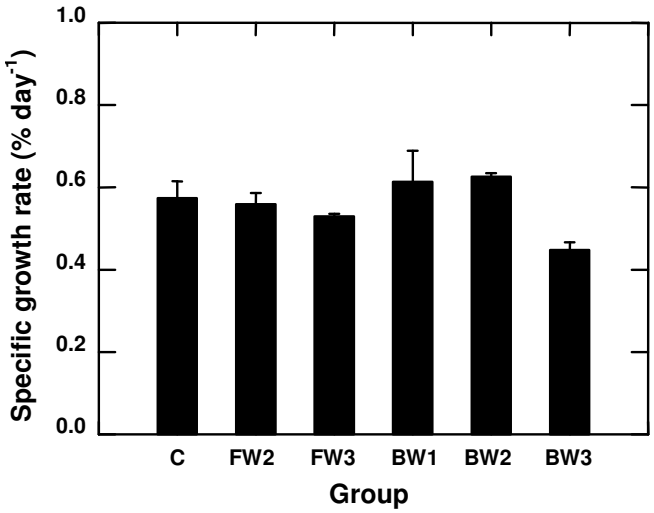

Fig 15. *Growth of fish groups exposed to different salinities (FW: 0 ‰ and BW: 14 ‰) and specific water flows (1: 0.27; 2: 0.15; 3:0.12 L kg^{-1} min^{-1}) during the parr-smolt transformation.*

During the next five weeks in seawater, the growth increased considerably in all groups (Fig 16). All though there were some differences in growth, none of these seems to be significant.

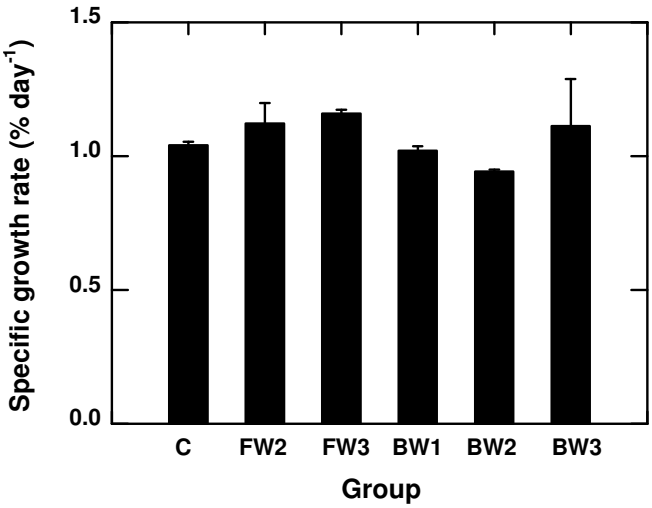

Fig 16. *Growth of post smolt groups during five weeks rearing in full-strength seawater that previous have been exposed to different salinities (FW: 0 ‰ and BW: 14 ‰) and specific water flows (1: 0.27; 2: 0.15; 3:0.12 L kg^{-1} min^{-1}) during the parr-smolt transformation.*

The feed intake data confirms the growth data (Fig. 17). Generally, the feed intakes in the BW groups were higher than the FW groups.

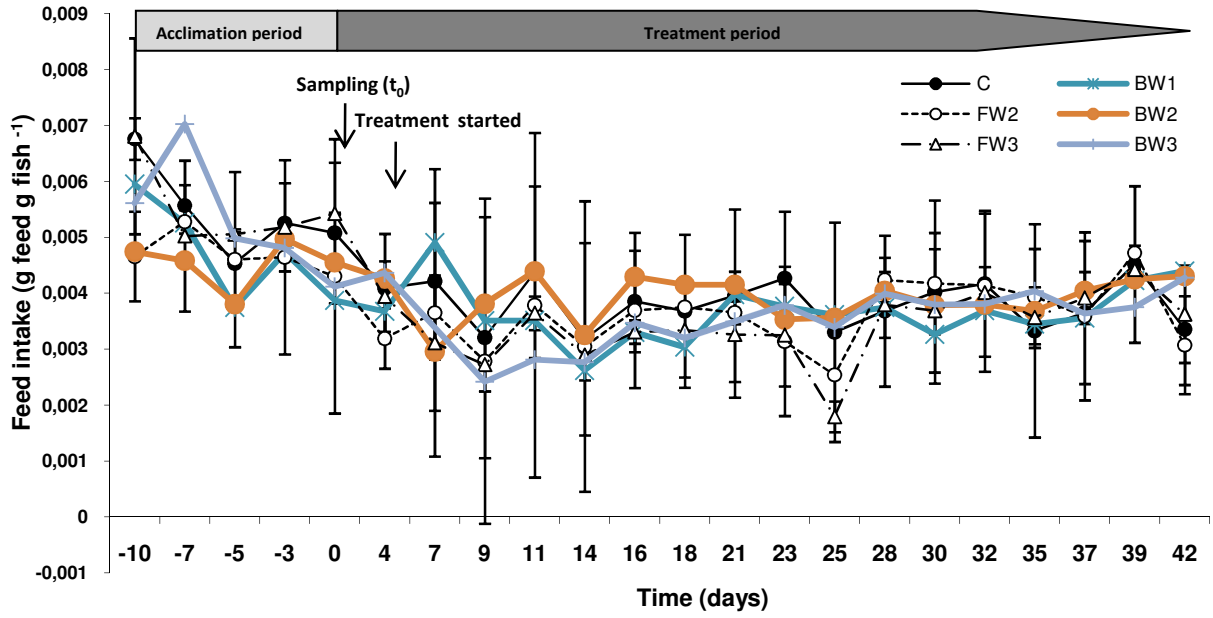

Figure 17. *Feed intake in groups of Atlantic salmon exposed to different salinities (FW: 0 ‰ and BW: 14 ‰) and specific water flows (1: 0.27; 2: 0.15; 3:0.12 L kg^{-1} min^{-1}) during the parr-smolt transformation.*

Challenge with *Moritella viscosa*

The mean survival after 33 days challenge was from 14-35 % in the groups (Table 5, Fig 18). The fish in brackish water groups started to die earlier than the freshwater groups and at day 10 after challenge (Table 4) there was a significantly lower survival in the BW groups than the FW groups. After 33 days, the survival is still tending to be lower in two of the BW groups compared to the FW groups, but only two of them are significantly different (Table 5).

Table 4. *Cumulated survival (%) 10 days post bath challenge of Atlantic salmon with Moritella viscosa (7.8 x 10^5 cfu ml^{-1}).*

Group	Dead	Survivals	Total	% Survival at day 10	Significant level
C	3	137	140	100	a
FW2	2	138	140	100	ab
FW3	4	136	140	99	b
BW1	45	96	141	89	c
BW2	34	105	139	88	c
BW3	22	118	140	93	c

Table 5. *Cumulated survival (%) 33 days post bath challenge of Atlantic salmon with Moritella viscosa (7.8 x 10^5 cfu ml^{-1}).*

Group	Dead	Survivals	Total	% Survival at day 33	Significant level
C	101	39	140	28	a
FW2	108	32	140	23	ab
FW3	91	49	140	35	bc
BW1	123	17	140	12	bc
BW2	120	20	140	14	b
BW3	106	34	140	24	c

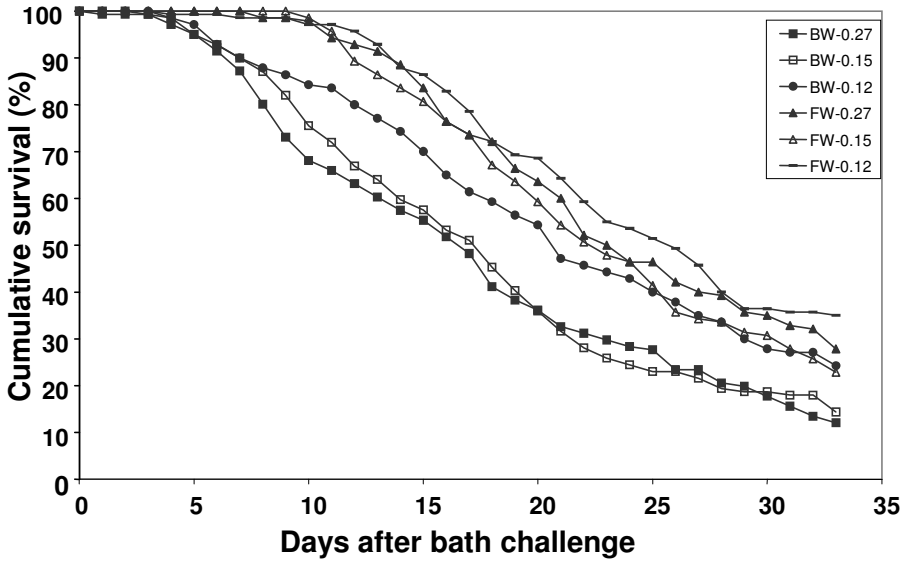

Figure 18. *Accumulated survival of Atlantic salmon, S. salar L., post smolts (n= 840) after bath challenge with Moritella viscosa (7.8 x 10^5 cfu ml^{-1}) in seawater (salinity 34 ‰) at 7 ºC in groups of salmon reared at freshwater (FW) or brackish water (SW) with salinity 14 ‰ with specific water flow of 0.27, 0.15 and 0.12 L kg^{-1} min^{-1}.*

The amount of sores was evaluated of the surviving fish and most fish of them had sores, and there were no difference amongst groups.

Discussion and conclusions

Our preliminary results show better growth during the treatment period, but lower survival rates in the disease challenge test in the brackish water groups than in the freshwater groups. This indicates that seawater intermixing can allow a smolt production with higher intensity than pure freshwater, if no remobilization of metals occur when mixing freshwater with seawater.

Task 3.3: Input to production models for Atlantic salmon smolt in Arctic areas

Task leader: Torstein Kristensen, NIVA

Introduction

The goal of the task was to use experimental data from the current and previous projects to develop biological input to a production model for Atlantic salmon smolt in Arctic conditions. The model will incorporate results on effects of environmental conditions on biological performance, defining the limits for safe production. Data on the environmental factors: light; temperature, salinity and production intensity, and the interactions between these factors on growth and immune system/ ion-balance performance. The biological data will serve as input to production models. The major change in design was to first apply the model approach to collected industrial data prior to application on experimental data. The experimental data will then upon finalization (2011) be compared to the results from the industrial data.

Material and Methods

A dataset from commercial Atlantic salmon producers on production intensity and production strategies in smolt tanks (n=63-94) was obtained during 1999-2006 through the WQ-project. The effects of production intensity on subsequent fish mortality and growth during the early sea phase (90 days) was examined by principal component analysis (PCA) and subsequent generalized linear model (GLM) analysis. Levels of accumulated metabolites (CO_2, total ammonia nitrogen (TAN) and NH_3), and information provided by producers (production density (kg fish m^{3-1}), specific water use (L kg $fish^{-1}$ min^{-1}) and oxygen drop (mg L^{-1}) from tank inlet to tank outlet), was used as predictor variables. However, the quality of the data sets will vary due to different methods used to monitor water quality in laboratories and farms. In addition, several other welfare relevant variables such as disease history; temperature during freshwater and sea stage; season (S1) or off-season (S0) smolt production, and the use of seawater addition during the freshwater stage were analyzed.

Results

No strong intensity effects on mortality or growth were found. CO_2 levels alone ($p<0.001$, $R^2=0.16$), and in combination with specific water use ($R^2=0.20$) had the strongest effect on mortality (Fig. 19). In both cases mortality decreased with increasing density. For growth, the intensity model with the most support ($R^2=0.17$) was O2 drop, density and their interaction effects, resulting in the best growth at low and high intensity, and poorer growth at intermediate levels. Documented viral disease outbreaks (Infectious pancreatic necrosis (IPN) and two cases of pancreas disease (PD)) in the sea phase resulted in significantly higher mortalities at 90 days compared with undiagnosed smolt groups, although mortalities were highly variable in both categories (Fig. 20). The temperature difference between the freshwater stage and seawater had a small, but significant, effect on growth with the best growth in groups stocked to warmer seawater ($p=0.04$, $R^2=0.06$). S0 and S1 smolt groups did not differ significantly in growth, but mortality was significantly ($p=0.02$) higher in S1 groups. Seawater addition as a categorical variable had no significant effects, but when analysed within the seawater addition group, intermediate salinities (15-25 ‰) gave the best results on growth ($p=0.04$, $R^2=0.15$).

Discussion/ conclusions

Production intensity had small explanatory power on subsequent seawater performance in the analyzed smolt groups. If anything, the analysis shows a beneficial effect of intensive production strategies on subsequent performance. Analysis of the various production strategies indicates better survival of S0 compared with S1 smolt groups, improved growth when stocked in seawater warmer than freshwater, and a negative effect of viral disease outbreaks on survival. The results clearly demonstrate the difficulty of extrapolating results from experimental work on fish welfare and production intensity parameters to commercial production. On the other hand, the presented results may simply demonstrate that the traditional fish welfare criteria growth and mortality may not

suffice to evaluate welfare consequences of suboptimal water quality or production strategies in the aquaculture industry. Further work on experimentally obtained datasets will hopefully help to elucidate this issue.

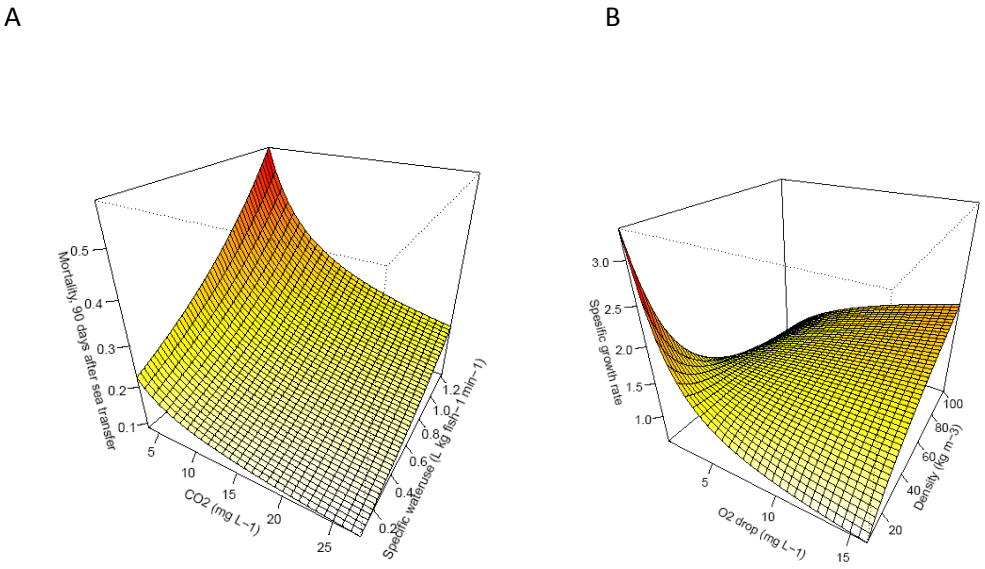

Figure 19. *A: Estimated combined effects of CO_2 and specific water use in the final freshwater stage on mortality rates at day 90 post-sea transfer in commercial smolt groups. B: Estimated combined effects of O_2 drop and density in the final freshwater stage on specific growth rate (% bw day^{-1}) at day 90 post-sea transfer in commercial smolt groups.*

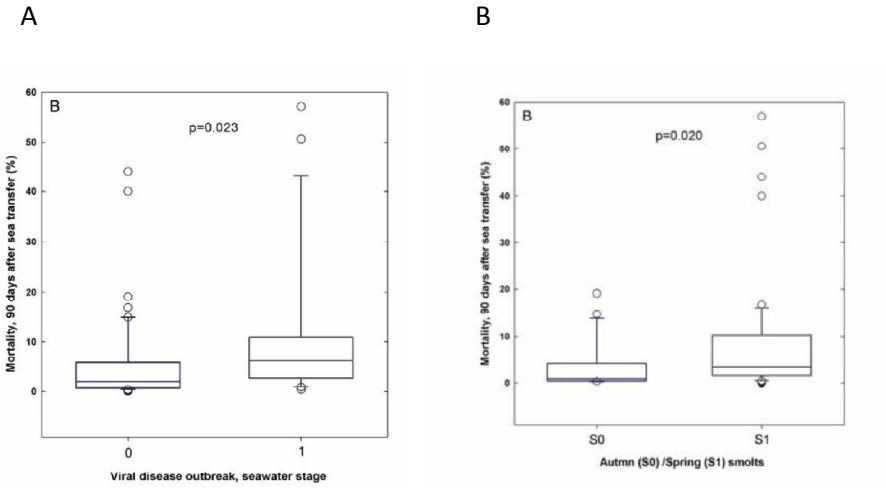

Figure 20. *Median, 25-75 percentile (box) and 5-95 percentile (bars) values mortality (%) (B) during the first 90 days after sea transfer for commercial smolt groups. A: The categorical variable used for comparison was viral disease outbreak during seawater stage. B: The categorical variable used for comparison was smolt groups stocked as either S0 (autumn) or S1 (spring) smolts (one way Anova).*

WP 4: KNOWLEDGE MANAGEMENT

WP leader: Hilde Toften, Nofima

Task 4.1: Networking

We wanted to form a stakeholder network group with representatives from the producers in the High North region. Initiatives were made, but since some of the experiments were postponed, we felt that we needed more results to present before such a group was established. Besides, several of the project members have been very active in communicating the results in existing network groups.

Task 4.2: Stakeholder seminar

Our goal was to communicate the results from the different experiments at a stakeholder seminar at the end of the project period. This has been done at several seminars arranged by different organisations and companies (see list of presentations below).

Task 4.3: Popular publications

We wanted to publish a series of popular articles in relevant magazines and on internet. This has been done for the experiments carried out early in the project period (see list below). For the later experiments plans are being made to develop popular publications in connection with AQUA NOR 2011.

Task 4.4: Scientific publication

Our intention was to present our results at relevant international meetings and publish them in relevant international peer-reviewed scientific journals. Several oral presentations have been done in the period (see list of presentations below). Six manuscripts have been worked on (see list of papers under preparation) and two of them will be submitted during summer 2011 and two of them are planned to be submitted during autumn 2011.