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Aquaculture 188 (2000) 189–201

Aquaculture

www.elsevier.nl/locate/aqua-online

Serum protein and IgM profiles in connection with the smolting and vaccination of out-of-season Atlantic salmon (*Salmo salar* L.)

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Received 23 April 1999; accepted 31 January 2000

Abstract

The concentrations of serum proteins and IgM were measured in vaccinated and untreated out-of-season smolts. Out-of-season (0 +) smolts were produced by exposing parr to continuous light (LD 24:0) until June 5 followed by a ''winter'' photoperiod of LD 12:12 for 6 weeks, and then continuous photoperiod (LD 24:0). The fish were vaccinated in the ''winter'' photoperiod using a commercially available quattro vaccine. The IgM levels remained low throughout the ''winter'' photoperiod, and increasing IgM levels were observed from the time of introducing the continuous photoperiod. The serum protein levels decreased at the start of the ''winter'' photoperiod, and increased at the beginning of the subsequent exposure to LD 24:0, but after 2 weeks the levels dropped again and remained low for the further 2 weeks before sea water transfer. For 1 + smolt, a synchronous drop in both serum proteins and IgM during smolting have been shown, indicating that 1 + and out-of-season smolt are not always comparable. Both serum IgM and protein concentrations increased after vaccination with an oil adjuvant quattro vaccine. The increase in serum proteins exceeded that of IgM. The sea water adaptability of the out-of-season smolt was affected by the vaccination as shown by an transient increase in serum chloride levels. Vaccination with oil adjuvant vaccines close to sea water transfer may therefore interfere with or delay the smolting process.

Keywords: Salmon; Serum proteins; IgM; Smolting; Vaccination; Out-of-season smolt

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0044-8486/00/$ - see front matter
PII: S0044-8486(00)00329-X

1. Introduction

In the last few years, there has been an increase in out-of-season smolt (0 +) production in Norwegian aquaculture, and at present they contribute about 30% of total smolt production. Producing out-of-season smolt could have impact on the fish physiology providing a smolt, which is different from a 1-year-old smolt (1 +). Most studies focus on 1 + smolt, and results are often directly transferred to out-of-season smolt without confirmatory investigations.

To produce 0 + smolts in autumn, growth must be accelerated using heated water, and the fish must receive appropriate photoperiod cues (Saunders et al., 1990, Gaignon and Quemener, 1992; Stefansson et al., 1992). Photoperiod is shown to be the major cue for the parr–smolt transformation, and the use of artificial photoperiods to alter the timing of smoltification has been documented (Clarke, 1989; Duston and Saunders, 1990), and is used in the production of out-of-season smolt (Thrush et al., 1994; Duston and Saunders, 1995).

The term smolt was first used for Atlantic salmon (*Salmo salar*), denoting a streamlined, silvery and active pelagic individual, physiologically adapted for a life in ocean waters (Hoar, 1976, 1988). In Atlantic salmon culture, a smolt is usually defined as a juvenile salmon that is able to survive and grow normally in sea water (Sigholt, 1997). Parr–smolt transformation or smolting should be seen as a result of many distinct and unrelated developmental events (Simpson, 1985). The pronounced changes include an increase in hypo-osmoregulatory ability, increased growth rate and metabolic changes, e.g. the reduction of body lipids resulting in reduction in condition coefficient (Farmer et al., 1978; Wedemeyer et al., 1980; Sheridan et al., 1983; McCormick and Saunders, 1987; Hoar, 1988).

Several authors have focused on gill tissue, with special emphasis on the chloride cells (de Renzis and Bornancin, 1984; Perry, 1997). Through the enzyme Na^+, K^+-ATPase, these cells play a major role in ionic and osmotic regulation in fishes (Foskett et al., 1983; de Renzis and Bornancin, 1984). Both the size and number of chloride cells increase during smolting, and their proliferation begins ahead of natural migration (Langdon and Thorpe, 1985). Gill Na^+, K^+-ATPase activity is frequently used as an indicator of enhanced hypo-osmoregulatory ability in salmon smolts, and during smolting the gill Na^+, K^+-ATPase activity increases, peaking during the final stages in fresh-water and entry into sea water (Zaugg and McLain, 1970).

In Norwegian Atlantic salmon farming, all smolts are vaccinated prior to sea water transfer. This could be done either several months or just a few weeks prior to sea water transfer, depending on size and water temperature. In recent years, smolts are also being produced for sea water transfer in autumn (August–October), using artificial light and temperature regimes. These smolts are usually vaccinated only a few weeks prior to sea water transfer (Eggset et al., 1999). Thus, vaccination is often performed after the parr–smolt transformation is initiated.

The endocrine-immune interaction during smolting is well documented, showing changes in immune cell distribution and function (Weyts et al., 1999). A decrease in serum immunoglobulin and total serum protein has been reported during smolting (Melingen et al., 1995a). In addition, the long-term specific antibody levels were

reduced in fish vaccinated during smoltification. In that study, vaccination with an aqueous vaccine seemed to have no negative impact on smoltification (Melingen et al., 1995b). However, results using oil adjuvant vaccine have indicated that vaccination close to the start of smoltification disturbed the smoltification process and caused a delay of approximately 2 weeks (Eggset et al., 1999).

The aim of this study was to examine if the profile of the serum IgM and serum proteins differed for the out-of-season smolt compared to 1 + smolt observed in earlier studies. The IgM level would give an indication of immune functions during the smolting period for out-of-season smolt.

2. Materials and methods

2.1. Fish stock and rearing conditions

Atlantic salmon (*S. salar* L.) of genetic origin from NLA-Bolaks were used. The fish were untreated and licensed by veterinary control as free from diseases as well as being without any previous history of infectious diseases. The salmon weighed 16.3 ± 1.8 (mean $\pm$ SD) g in June at the start of the experiment. The experiment was carried out at Ewos Research Station Lönningdal, Bergen, Norway. The fish were reared in 6-m grey fibreglass tanks with water level 170 cm. All fish tanks had light proof covers (HH-products), and the photoperiod (one metal-halogen headlight, 250 W per fish tank) was adjusted by a cycle timer. The oxygen level was adjusted by a process controlled system (MCp18R), which supplied the inlet water with a certain degree of supersaturated oxygen level, and added oxygen through diffusers directly into the fish tanks. The oxygen supply was regulated to maintain 80% oxygen saturation in the outlet water. A commercial dry diet (Ewos Vextra) with the manufacturers recommended pellet size was dispensed from automatic feeders to all groups. The fish were reared under continuous photoperiod LD 24:0 at 14°C until June 5, followed by a ''winter'' photoperiod of LD 12:12 for 6 weeks, and then continuous photoperiod (LD24:0) for another 4 weeks before sea water transfer. After sea water transfer, the fish were reared under continuous photoperiod. Due to the risk of introducing diseases, non-vaccinated fish (later referred to as untreated) were not transferred to sea, and thus no samples exist for these fish after sea water transfer.

2.2. Vaccination

A commercial vaccine against cold water vibriosis, vibriosis, furunculosis and infectious pancreas necrosis virus — Lipogen Quattro (Aqua Health) — was used. According to the distributors instructions, 0.2 ml bacterin/fish was injected intraperitoneally on July 9 1998 (vaccinated group). A vaccinated group ($n = 400$/group) was compared with a matching untreated group ($n = 300$/group). The fish were starved for 24 h and anaesthetised using metacaine (50 mg/l) prior to vaccination.

2.3. Sera

Sera were collected from each group according to regimes shown in Fig. 1. Fifteen serum samples were collected from each group at every sampling throughout the experiment if not specified otherwise. All fish were starved for 24 h and anaesthetised in metacaine before collecting the blood samples from the caudal vein. The blood coagulated at 4°C over night before centrifugation at $1000 \times g$ for 5 min. The serum was then stored in aliquots at −20°C for 24 h before transfering to −80°C. Rabbit antiserum to Atlantic salmon IgM was prepared as described by Håvarstein et al. (1988).

2.4. Total serum protein determination

Total protein concentration in serum was quantified using the Biuret Protein Assay, with Bovine Serum Albumin (BSA) as standard protein. Ten microliters undiluted serum and 300-μl Biuret reagent were added to 96 well polystyrene plates (Nunc Maxi Sorb), mixed well and incubated in darkness for 30 min at room temperature. Optical density (OD) was read at 540 nm. Serum protein levels were recorded as mg/ml (mean ± SD).

2.5. Purification of salmon IgM

The purification of salmon IgM was performed using the Pharmacia FPLC chromatographic system (Pharmacia, Uppsala, Sweden) as described by Håvarstein et al.(1988). Salmon IgM was used as a standard for the quantification of serum IgM.

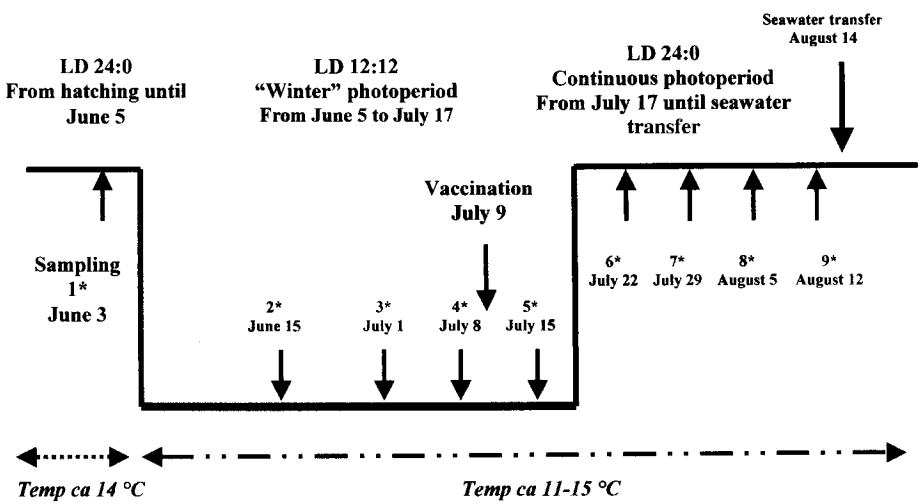

Fig. 1. Experimental design and sampling. The fish were reared under continuous photoperiod (LD 24:0) from hatching until start of the "winter" photoperiod (LD 12:12) on June 5. Six weeks later, the continuous photoperiod (LD 24:0) was introduced, and the fish were transferred to sea water 4 weeks later. During the freshwater period, sampling was performed at nine different times as indicated (*). In addition, three samplings were performed from vaccinated groups after sea water transfer (August 19, August 26 and November 11). For all samplings, $n = 15$ per group if not specified otherwise.

2.6. Serum IgM concentration

IgM in serum was determined using a single radial immunodiffusion assay (SRID). A melted 1% agarose (Bio Rad agarose, standard low — m_r) with 2% PEG-6000 in 0.01 M phosphate buffered saline, pH 7.2 (PBS) was pre-warmed to 55°C. Rabbit anti-salmon IgM was mixed with agar solution resulting in a final serum dilution of 1:250. The antiserum-agar mix (0.1 ml/cm^2) was poured on to a Gelbound film plate. Serum was applied to 4-mm wells in the gel, and incubated in a humid atmosphere for 48 h. The gel was squeezed and washed three times in PBS, and dried before staining with Coomassie Blue (10 min), and subsequent destaining (6 min). The standard curve was derived from four concentrations of purified salmon IgM. Protein concentration of purified IgM was determined by the Bio-Rad assay, using BSA as protein standard. Serum IgM levels were recorded as mg/ml (mean ± SD).

2.7. Weight and length measurements

The weight (BWT, g) and fork (FL, cm) (mean ± SD) of each fish group ($n = 15$) were measured at every sampling throughout the experiment. Fulton's condition coefficient was calculated as $CF = (BWT/FL^3) \times 100$. Prior to measurements, the fish were starved for 24 h and anaesthetised in metacaine.

2.8. Sea water challenge

At each sampling, 12 fish were collected and their hypo-osmoregulatory ability assessed by measuring serum chloride levels after 24 h sea water challenge test (33–34‰). Control fish (both vaccinated and untreated) were kept in fresh water.

2.9. Chloride and ATPase activity

Chloride levels were measured from serum samples using 20-μl samples in a Radiometer CMT titrator. Chloride levels were recorded as mM (mean ± SD). Gill filaments were frozen in SEI buffer (0.30 M sucrose, 0.02 M Na_2EDTA, 0.10 M imidazol, solution adjusted to final pH = 7.1 at 37°C using 1.00 M HCl) at −80°C and subsequently analysed for gill Na^+, K^+-ATPase activity using the method of McCormick (1993). Na^+, K^+-ATPase activity was recorded in μmol ADP/mg protein/h (mean ± SD).

2.10. Data processing and statistics

Arithmetic means were used throughout the experiments. Mean values within and between groups were compared by one-way and two-way ANOVA, respectively, followed by Newman–Keuls post-hoc test. Measurements were considered significant when $p < 0.05$.

3. Results

3.1. Total serum protein

The vaccinated group had a significant lower total serum protein concentration than the untreated group 1 week after vaccination ($p < 0.001$), but 1 week later the vaccinated group had a higher serum protein level than the untreated group ($p < 0.001$). From that time to the end of the experiment, the vaccinated group had a significantly higher serum protein level than the untreated fish ($p < 0.001$) (Fig. 2).

The total serum protein level decreased significantly from June 3 (64.5 ± 18.0 mg/ml) to June 15 (57.5 ± 5.5 mg/ml) ($p < 0.05$), which corresponded to the introduction of the "winter" photoperiod. The level also decreased significantly from June 15 until July 1 (46.1 ± 9.9 mg/ml) ($p < 0.001$), and remained low until the photoperiod changed from LD 12:12 to LD 24:0 on July 17 ($p > 0.05$). The level increased significantly for the vaccinated group from July 15 (48.7 ± 7.5 mg/ml) to July 22 (57.3 ± 3.3 mg/ml) ($p < 0.001$), and increased even more from July 22 to July 29 (76.3 ± 7.2 mg/ml) ($p < 0.001$). From July 29, the level decreased dramatically to August 5 (55.2 ± 6.4 mg/ml) ($p < 0.001$). For the same period, similar results were found for the untreated group, decreasing from July 29 (57.0 ± 6.3 mg/ml) to August 5 (42.6 ± 4.7 mg/ml) ($p < 0.001$). From August 5 to August 12, the serum protein level increased for both the vaccinated (61.8 ± 6.5 mg/ml) and the untreated group (49.1 ± 3.6 mg/ml) ($p < 0.001$). The fish were transferred to sea water on August 14, and due to

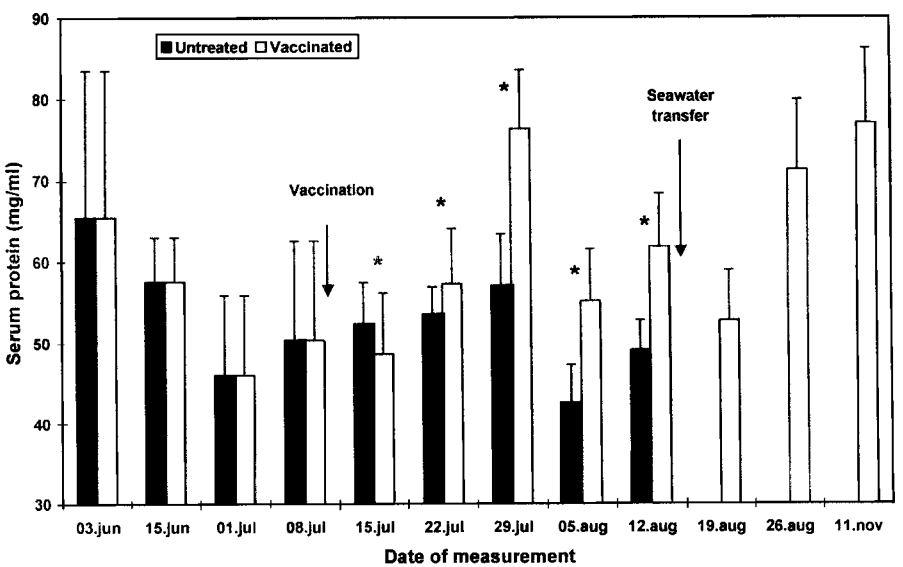

Fig. 2. Total serum protein concentration was measured by the Biuret Protein Assay (mean, bar indicates SD, $n = 15$) in vaccinated and untreated salmon throughout the experimental period. * Indicating significant difference ($p < 0.05$) between control and vaccinated groups.

the risk of diseases only vaccinated fish were transferred. Following the sea water transfer, the serum protein level decreased from August 12 to August 19 (52.7 ± 6.2 mg/ml) ($p < 0.001$), but 1 week later the level had increased to 71.2 ± 8.7 mg/ml ($p < 0.001$). After 3 months in sea water, the serum protein level had not changed significantly compared to that observed 2 weeks after sea water transfer ($p > 0.05$).

3.2. Total serum IgM

The total serum IgM concentration was measured in all serum samples. As for the total serum proteins, there were significant differences in serum IgM between the vaccinated and corresponding untreated fish some time after vaccination ($p < 0.05$) (Fig. 3).

The serum IgM levels were stable when changing from continuous photoperiod to the introduction of the "winter" photoperiod ($p > 0.05$), but increased from July 1 (0.5 ± 0.2 mg/ml) to July 8 (0.7 ± 0.2 mg/ml) ($p < 0.05$). One week after vaccination the vaccinated group had significant higher serum IgM levels than the untreated group ($p < 0.05$). Two weeks after vaccination the difference between the vaccinated group (1.2 ± 0.2 mg/ml) and the untreated group (0.9 ± 0.2 mg/ml) had increased ($p < 0.001$). The first 2 weeks after changing the photoperiod from LD 12:12 to LD 24:0, the serum IgM level was stable ($p > 0.05$). From July 29 to August 5, the level increased significantly for both the vaccinated and the untreated group($p < 0.001$), but remained stable until sea water transfer for the untreated group ($p > 0.05$) while increasing for the

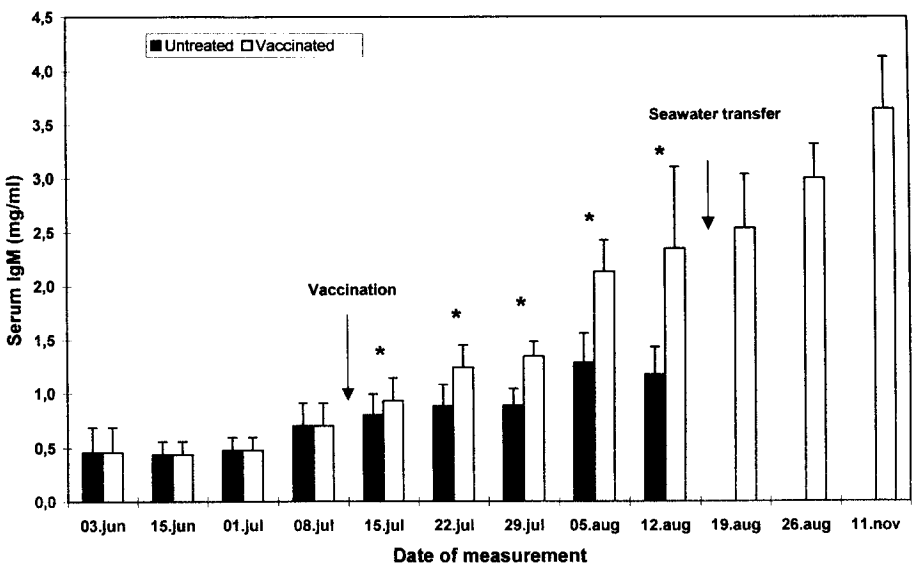

Fig. 3. Total serum IgM concentration measured by single radial immuno-diffusion assay (SRID) (mean, bar indicates SD, $n = 15$) in vaccinated and untreated salmon throughout the experiment. * Indicating significant difference ($p < 0.05$) between control and vaccinated groups.

vaccinated group ($p > 0.05$). Following sea water transfer, the serum IgM level increased from August 12 (2.4 ± 0.8 mg/ml) to August 19 (2.5 ± 0.5 mg/ml) ($p < 0.05$), and by August 26 (3.0 ± 0.3 mg/ml) the serum IgM had increased even more ($p < 0.05$). After 3 months in sea water (November 11), the serum IgM level (3.7 ± 0.5 mg/ml) had increased by about 20% compared to levels just after sea water transfer ($p < 0.001$).

3.3. Growth and condition coefficient

At the start of the experiment on July 3, the fish weighed 16.3 ± 1.8 g, and at the time of vaccination (June 9) the fish weighed 38.2 ± 5.3 g. On August 14, at sea water transfer, the fish weighed 57.7 ± 8.4 g, while the weight had increased to 310.3 ± 46.8 g by November 11($p < 0.001$).

The CF was stable throughout the "winter" photoperiod ($p > 0.05$) but increased from August 5 (1.04 ± 0.02) to August 12 (1.14 ± 0.04) ($p < 0.05$). One week after sea water transfer the CF had decreased to 0.93 ± 0.03 ($p < 0.01$).

3.4. Serum chloride levels

The serum chloride level was measured throughout the experiment. At all samplings, except for one, the fish exposed to sea water for 24 h had a significantly higher serum

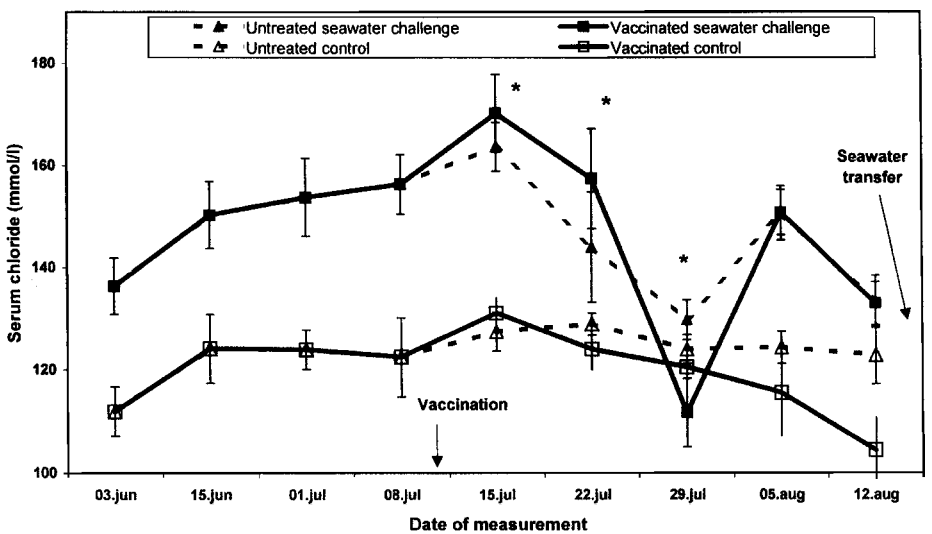

Fig. 4. Mean serum chloride levels (mean, bars indicate SD, $n = 15$ for each control fish group (vaccinated and untreated), and $n = 12$ for each sea water-challenged group (vaccinated and untreated)) throughout the freshwater period. * Indicating significant difference ($p < 0.05$) between control and vaccinated groups for sea water challenge.

chloride level compared to their corresponding freshwater control fish ($p < 0.05$) (Fig. 4). The chloride level increased for both the sea water challenged fish and the control fish from June 3 to June 15 ($p < 0.001$). From June 15 until July 8, the chloride level was stable for the control fish ($p > 0.05$), while the level increased for the sea water challenge fish ($p < 0.001$). The fish were vaccinated on July 9, and 1 week later the chloride level had increased for the vaccinated control fish ($p < 0.001$) but no significant change was observed for the control fish ($p > 0.05$). For the sea water-challenged fish, there were similar observations. The chloride level increased for both the vaccinated and the untreated fish 1 week after vaccination ($p < 0.001$) but the vaccinated fish had a significantly higher level than the untreated fish ($p < 0.01$). Following the change in photoperiod from LD 12:12 to LD 24:0, the serum chloride level decreased for the sea water challenge fish ($p < 0.001$), and 2 weeks after introduction of the continuous photoperiod the untreated sea water-challenged fish (129.7 $\pm$ 3.9 mM) had almost the same level as the corresponding control fish (123.9 $\pm$ 2.8mM) ($p < 0.05$). At the same time, the vaccinated sea water-challenged fish (111.6 $\pm$ 6.6 mM) actually had a lower chloride level than the corresponding control fish (120.4 $\pm$ 6.4 mM) ($p < 0.01$). This was the lowest level observed, and at sea water transfer on August 14, the sea water-challenged fish had increased serum chloride levels to 132.9 $\pm$ 4.4 mM, while the levels in corresponding control fish were 104.3 $\pm$ 6.4 mM.

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

No significant differences in gill Na^+, K^+-ATPase activity between the vaccinated and the untreated fish was observed from the start of the experiment and until the last comparing measurement ($p > 0.05$). Just after the introduction of LD 12:12 photoperiod, the Na^+, K^+-ATPase activity dropped from 13.3 $\pm$ 6.0 μmol ADP/mg protein/h to 5.5 $\pm$ 4.1 μmol ADP/mg protein/h in 2 weeks ($p < 0.0005$). The level remained stable until 2 weeks after vaccination, when the untreated group had decreased from 4.7 $\pm$ 1.6 μmol ADP/mg protein/h to 2.9 $\pm$ 1.4 μmol ADP/mg protein/h ($p < 0.01$). In the same period, the level was stable for the vaccinated group. One week prior to sea water transfer the Na^+, K^+-ATPase activity increased for the untreated group from 3.3 $\pm$ 1.2 μmol ADP/mg protein/h to 6.2 $\pm$ 1.9 μmol ADP/mg protein/h ($p < 0.05$), while the level remained stable for the vaccinated group ($p > 0.05$). This was the only measurement that was significantly different between the vaccinated (4.8 $\pm$ 1.1 μmol ADP/mg protein/h) and the untreated (6.2 $\pm$ 1.9 μmol ADP/mg protein/h) groups ($p < 0.0001$).

4. Discussion

For the out-of-season smolt (0 +), the profiles of both the serum proteins and IgM differed from that reported for 1 + smolt (Melingen et al., 1995a). In 1 + smolt, the serum IgM and serum proteins had similar profiles, with a 24% reduction in the smolting period followed by an increase after sea water transfer. For out-of-season

smolt, the serum IgM levels remained low throughout the ''winter'' photoperiod, and increasing IgM levels were observed from the time of introducing the continuous photoperiod, which is the time of smoltification. For these fish, the serum IgM levels still increased until the last sampling 3 months after sea water transfer. For 1 + smolt, the serum protein profile was similar to that of IgM (Melingen et al., 1995a). For out-of-season smolt, the serum protein level profile was different from the serum IgM profile, with a decrease in serum protein levels after the start of the ''winter'' photoperiod. The serum protein levels increased from introduction of the continuous photoperiod, but after 2 weeks the level dropped until sea water transfer. After sea water transfer, the serum protein concentration increased for 2 weeks, then remained stable for the next 3 months. These results indicate that one should be careful not to directly transfer results from traditional to out-of-season smolts without full investigation.

For out-of-season smolt, serum protein concentration and serum IgM levels increased after vaccination with an oil adjuvant quattro vaccine. In an earlier experiment, no changes in these parameters were found for 1 + smolt using a monovalente aqueous vaccine (Melingen et al., 1995a). Others, using a Freund adjuvant *Vibrio anguillarum* vaccine have reported a decrease in IgM in sea bass (Coeurdacier et al., 1997). The increase in serum proteins observed in the present experiment exceeded that of IgM. The underlying mechanisms or specific proteins causing this increase in serum IgM and proteins are not known in detail. However, it has been shown that B-lymphocytes are directly affected by cortisol, and reduced plasma IgM levels were found in rainbow trout plasma after cortisol administration (Hou et al., 1999). Nagae et al. (1994) showed that high levels of thyroxine enhanced IgM synthesis in Masu salmon, but also indicated the importance of higher water temperatures to enhance the immune response in fish. Growth hormone is also known to enhance the immune system (Berczi and Nagy, 1987). The increased IgM production in present experiment could also result from specific stimulation by the antigens in the quattro vaccine, a non-specific adjuvant stimulation, or a combination of both. The results indicate that vaccination in the ''winter'' photoperiod of out-of-season smolts stimulates high Ig levels, but this does not necessarily correlate with protective and long term immune responses. The difference in IgM could be further studied by FACS and ELISPOT analysis of B-cells. FACS analysis of leucocyte populations through the smolting period would provide information on presence of immune cells involved non-specific, specific and long-term effects of immune stimulation.

In producing out-of-season smolts, growth is accelerated by using heated water, and the fish receives artificial photoperiod cues. Faster physiological development is observed for the out-of-season smolt, but the timing of physiological events leading to characteristics of 1 + smolt may be different for out-of-season smolts. Therefore, differences in silvering, gill Na^+, K^+-ATPase activity and serum chloride levels might be present. If the gill Na^+, K^+-ATPase activity and serum chloride levels are used as indicators of when the smolt is adapted to sea water, the out-of-season smolt used in the present experiment would not be ready for sea water transfer when it actually took place (4 weeks after introducing the continuous photoperiod). The time for sea water transfer was based on the experience from Ewos Research Station Lönningdal (Vidar Hjartnes, personal communication). A ''square wave'' photoperiod was used in this experiment to

induce smoltification, based on the fact that an abrupt increase in daylength, following a period of short daylength, has been shown to elicit smoltification in Atlantic salmon (Björnsson et al., 1989; Duston and Saunders, 1990), provided that the short day period is longer than 1 month (Björnsson et al., 1989; Sigholt et al., 1995), and provided that the freshwater temperature is sufficiently high. According to experience from previous experiments (Eggset et al., 1997) and practical smolt production, the peak in sea water tolerance is reached about 6 weeks after a shift from the short winter daylengths to continuous light.

The gill Na^+, K^+-ATPase activity starts to increase at the end of the continuous photoperiod, i.e. after 4 weeks, which is when fish were transferred to sea water. The drop in serum chloride levels indicated that the fish were ready for sea water transfer as early as 10 days after the end of the ''winter'' photoperiod. This would be approximately 4 1/2 weeks earlier than recommended. Several Norwegian fish farmers have reported findings of a similar drop in serum chloride levels as shown in this experiment (Vidar Hjartnes, personal communication). Thrush et al. (1994) provided the most comprehensive study of the performance of out-of-season smolts and demonstrated that an early transfer to sea water enabled out-of-season smolts to gain a growth advantage, which was maintained during the entire growing cycle. Transfer of smolt to sea water 2–5 weeks earlier than recommended in Norwegian fish farming would give a considerable growth increase for out-of-season smolt compared to smolt kept in freshwater for the recommended period. Using the definition of a smolt in culture as a juvenile salmon able to grow and survive normally in sea water, this smolt is a fully adapted smolt 4 weeks into the continuous photoperiod. This is supported by the results showing the very good growth of the smolt, growing from approximately 58 g to more than 300 g in 3 months.

This experiment showed an increase in serum chloride levels just after vaccination, but 3 weeks later the levels were back to normal. An increase in serum chloride levels indicates a decreased sea water adaptability of the smolt, and therefore vaccination close to sea water transfer may delay the smolting process, and thereby time of sea water transfer. Results from this experiment support the finding of Eggset et al. (1999) indicating a 2-weeks delay in smoltification when vaccinating close to the start of smoltification. In both cases, an oil adjuvant vaccine was used, and other kinds of vaccines may not have this effect on smoltification.

In recent years, more antigens have been introduced into the vaccines used in aquaculture. In Norway, the most complex one is a Pentium vaccine containing antigens from Infectious pancreas necrosis virus, *V. salmonicida*, *V. anguillarum*, *V. viscosus* and *Aeromonas salmonicida* ssp *salmonicida*. Normally, a 0.2-ml i.p. dose is used, but when vaccinating out-of-season smolt, this volume may be too large since out-of-season smolts are generally smaller than 1 + smolts (Vidar Hjartnes, personal communication). Therefore, fish vaccine manufacturers have started to develop 0.1-ml dose vaccines. It is a challenge to identify the best time of vaccination with respect to obtaining the desired immune responses to the actual vaccine. There is a complex interaction between the immune and neuroendocrine systems during smoltification, and it is important to identify the best time for stimulation of out-of-season smolt as the fish vaccines contain several antigens in a small injection volume.

References

Berczi, I., Nagy, E., 1987. The effect of prolactin and growth hormone on hemolymphopoietic tissue and immune function. In: Berczi, I., Kovacs, K. (Eds.), Hormones and Immunity. MTP Press, Lancaster, pp. 147–171.

Björnsson, B. Th., Thorarensen, H., Hirano, T., Ogasaware, T., Kristinsson, J.B., 1989. Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypo-osmoregulatory ability of juvenile Atlantic salmon (*Salmo salar*) during parr–smolt transformation. Aquaculture 82, 77–91.

Clarke, W.C., 1989. Photoperiod control of smolting: a review. Physiol. Ecol. Jpn. 1, 497–502.

Coeurdacier, J.L., Pepin, J.F., Fauvel, C., Legall, P., Bourmaud, A.F., Romestand, B., 1997. Alterations in total protein, IgM and specific antibody activity of male and female sea bass (*Dicentrarchus labrax* L., 1758) sera following injection with killed *Vibrio anguillarum*. Fish Shellfish Immunol. 7, 151–160.

de Renzis, G., Bornancin, M., 1984. Ion transport and gill ATPases. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology vol. 10B Academic Press, London, pp. 65–104.

Duston, J., Saunders, R.L., 1990. Control of the timing of smoltification in Atlantic salmon: endogenous rhythms and environmental factors. Saunders, R.L. (Ed.), Proceedings of Can.-Nor. Finfish Aqua. Workshop. Can. Tech. Rep. Fish. Aquat. Sci. 1761, 99–105.

Duston, J., Saunders, R.L., 1995. Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. Aquaculture 135, 295–309.

Eggset, G., Mortensen, A., Johansen, L.-H., Sommer, A.-I., 1997. Susceptibility to furunculosis, cold water vibriosis, and infectious pancreatic necrosis (IPN) in post-smolt Atlantic salmon (*Salmo salar* L.) as a function of smolt status by sea water transfer. Aquaculture 158, 179–191.

Eggset, G., Mortensen, A., Löken, S., 1999. Vaccination of Atlantic salmon (*Salmo salar* L.) before and during smoltification; effects on smoltification and immunological protection. Aquaculture 170, 101–112.

Farmer, G.J., Ritter, A., Ashfield, D., 1978. Sea water adaption and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 35, 93–100.

Foskett, K., Bern, H.A., Machen, T.E., Conner, M., 1983. Chloride cells and the hormonal control of teleost fish osmoregulation. J. Exp. Biol. 106, 255–281.

Gaignon, J.L., Quemener, L., 1992. Influence of early thermic and photoperiodic control on growth and smoltification in Atlantic salmon (*Salmo salar*). Aquat. Living Resour. 5, 185–195.

Hoar, W.S., 1976. Smolt transformation: evolution, behaviour, and physiology. J. Fish. Res. Board Can. 33, 1233–1252.

Hoar, W.S., 1988. The physiology of smolting salmonids. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology vol. XIB Academic Press, New York, NY, pp. 275–343.

Hou, Y.Y., Suzuki, Y., Aida, K., 1999. Effects of steroid hormones on immunoglobulin M (IgM) in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 20, 155–162.

Håvarstein, L.S., Aasjord, P.M., Ness, S., Endresen, C., 1988. Purification and partial characterization of an IgM-like serum immunoglobulin from Atlantic salmon (*Salmo salar*). Dev. Comp. Immunol. 12, 773–785.

Langdon, J.S., Thorpe, J.E., 1985. The ontogeny of smoltification: developmental patterns of gill Na^+/K^+-ATPase, SDH and chloride cells in juvenile Atlantic salmon, (*Salmo salar* L.). Aquaculture 45, 83–96.

McCormick, S.D., Saunders, R.L., 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth and metabolism. Am. Fish. Soc. Symp. 1, 211–229.

McCormick, S.D., 1993. Methods for non-lethal gill biopsy and measurements of Na^+, K^+-ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658.

Melingen, G.O., Stefansson, S.O., Berg, A., Wergeland, H.I., 1995a. Changes in serum protein and IgM concentration during smolting and early post-smolt period in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.). Fish Shellfish Immunol. 5, 211–221.

Melingen, G.O., Nilsen, F., Wergeland, H.I., 1995b. The serum antibody levels in Atlantic salmon (*Salmo salar* L.) after vaccination with *Vibrio salmonicida* at different times during smolting and early post-smolt period. Fish Shellfish Immunol. 5, 223–235.

Nagae, M., Fuda, H., Hara, A., Saneyoshi, M., Yamauchi, K., 1994. Changes in serum concentrations of Immunoglobulin M (IgM), cortisol and thyroxine (T4) during smoltification in the Masu salmon, *Oncorhynchus masou*. Fish. Sci. 60 (2), 241–242.

Perry, S.F., 1997. The chloride cell: structure and function in the gills of freshwater fishes. Annu. Rev. Physiol. 59, 325–347.

Saunders, R.L., Duston, J., Harmon, P.R., Knox, D.E., Stewart, M.W., 1990. Production of underyearling Atlantic salmon smolts. Bull. Aquacult. Assoc. Can. 90 (4), 61–63.

Sheridan, M.A., Allen, W.V., Kerstetter, T.H., 1983. Seasonal variations in the lipid composition of steelhead trout, *Salmo gairdneri*, associated with parr–smolt transformation. J. Fish Biol. 23, 125–134.

Sigholt, T., Staurnes, M., Jakobsen, H.J., Åsgård, T., 1995. Effects of continuous light and short-day photoperiod on smolting, sea water survival and growth in Atlantic salmon (*Salmo salar*). Aquaculture 130, 373–388.

Sigholt, T., 1997. Control of parr–smolt transformation and sea water tolerance in farmed Atlantic salmon (*Salmo salar*). Thesis presented for the degree of Doctor philosophiae at NTNU Trondheim, Norway

Simpson, T.H., 1985. Epilogue. Aquaculture 45, 395–398.

Stefansson, S.O., Berg, A.E., Hansen, T., Saunders, R.L., 1992. The potential for development of salinity tolerance in underyearling Atlantic salmon (*Salmo salar*). World Aquaculture 23 (2), 52–55.

Thrush, M.A., Duncan, N.J., Bromage, N.R., 1994. The use of photoperiod in the production of out-of-season Atlantic salmon (*Salmo salar*) smolts. Aquaculture 121, 29–44.

Wedemeyer, G.A., Saunders, R.L., Clarke, W.C., 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42, 1–14.

Weyts, F.A.A., Cohen, N., Flik, G., Verburg-van Kemenade, B.M.L., 1999. Interactions between the immune system and the hypothalamo-pituitary–interrenal axis in fish. Fish Shellfish Immunol. 9, 1–20.

Zaugg, W.S., McLain, L.R., 1970. Adenosine triphosphatase activity in gills of salmonids; seasonal variation and salt water influence in Coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 35, 587–596.