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Salmon lice or suboptimal water quality — Reasons for reduced postsmolt survival?

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Abstract

Salmon populations on the western coast of Norway may experience both moderately acidified rivers and salmon lice (*Lepeophtheirus salmonis* Krøyer) attacks. The present study addresses the question of interactive effects of acidification and salmon lice infestation on postsmolt survival. Three groups each of approximately 1500 one-year old Atlantic salmon smolts of the Imsa strain, South-Western Norway, were exposed to one of three suboptimal water qualities (high acid, moderate acid and episodic acid) and experienced acidic water (pH 5.6–5.9 and 7–45 μg Ali/l) with different exposure duration (3 to 10 days). A fourth group exposed to pH > 6.6 and < 9 μg Ali/l acted as control (reference group). After freshwater exposure, smolts (n = 150) from each group were moved into tanks containing brackish water (16‰) and after 8 h they were given full strength seawater (33‰) and given 1 day of recovery before being infected with salmon lice copepodids. Four non-infected groups (n = 100) from the same exposures acted as controls. Over a 42 day period, postsmolts were regularly inspected and sampled for mortality, lice density and physiological status in seawater. The lice per smolt density were highest in the episodic acid group, followed by the high acid, moderate acid and the reference groups. Mortality was low in the four non-infected control groups, and significantly elevated in the lice infected groups (high acid > moderate acid > episodic acid > reference). Plasma chloride levels were within the normal range in the non-infected groups, while fish in the infected high acid and moderate acid groups had elevated plasma chloride levels. High gill aluminium was seen in the three exposure groups in freshwater. Year to year variations in acidification pressure and salmon lice densities can singularly and in combination explain some of the year to year variations in postsmolt survival and hence the variations in Atlantic salmon year–class strength in Norwegian rivers.

Keywords: Atlantic salmon; *Salmo salar*; Acid rain; Aluminium; Salmon lice; *Lepeophtheirus salmonis*; Susceptibility; Physiology

1. Introduction

High levels of H^+ and aluminium (Al) are lethal to Atlantic salmon (*Salmo salar* L.) smolts (Rosseland and Staurnes, 1994; Gensemer and Playle, 1999). Water toxicity is related to increased concentrations of H^+ (reduced pH) and inorganic monomeric aluminum (Ali) in freshwater. At lethal concentrations, H^+ acts primarily on the permeability of the cell membrane disrupting ionoregulation, whereas aluminum exerts its toxic properties by accumulation on and in the gill tissue,

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disrupting ionoregulation and impairing respiration. At lower concentrations, Al can still affect population traits by affecting growth, swimming performance, immune defence, behaviour and seawater tolerance (Rosseland and Staurnes, 1994; Staurnes et al., 1995, 1996; Kroglund and Staurnes, 1999; Ytrestøyl et al., 2001; Kroglund and Finstad, 2003).

A series of laboratory studies have revealed the effect of salmon lice (*Lepeophtheirus salmonis* Krøyer) on Atlantic salmon physiology, and the number of lice required to cause mortality has been estimated (reviewed by Tully and Nolan, 2002; Heuch et al., 2005). Physiological disturbances, including high levels of plasma cortisol and glucose, reduced osmoregulatory ability and reduced non-specific immunity in the host occur when the lice develop from the sessile chalimus 4 stage to the mobile first preadult stage. Sublethal effects, including reduced growth, swimming performance and impaired immune defenses have also been reported (Bjørn and Finstad, 1997; Nolan et al., 1999, 2000; Finstad et al., 2000; Wagner et al., 2003) and effects on populations have been described (Bjørn et al., 2001; Gargan et al., 2003). Salmon lice epidemics on farmed fish have chronologically followed the pattern of the salmon aquaculture industry development (MacKinnon, 1997). Similar epidemics have occurred subsequently on wild salmonids in all countries where a major salmon aquaculture industry has been developed (Pike and Wadsworth, 1999; Heuch et al., 2005).

Fish that are sublethally stressed may be more sensitive to disease and parasite attacks and can have a reduced tolerance to additional stressors (Iversen et al., 2005). Salmon populations on the western coast of Norway may experience both moderately acidified rivers (Kroglund et al., 2002) and salmon lice (Birkeland and Jakobsen, 1997; Holst et al., 2001; Heuch et al., 2005) attacks. Therefore, year to year variations in acidification pressure and salmon lice densities can singularly and in combination explain some of the year to year variation in postsmolt survival and hence the variation in Atlantic salmon year–class strength in Norwegian rivers.

The present study addresses the question of interactive effects of acidification and salmon lice infestation on postsmolt survival. Four groups each of one-year old Atlantic salmon smolts of the Imsa strain, South-Western Norway, were exposed to one of three suboptimal water qualities (high acid, moderate acid and episodic acid) from 3 to 10 days. After freshwater exposure, smolts from each of the 4 exposure groups were moved into tanks containing seawater and infected with salmon lice copepodids while four non-infected groups from the same exposures acted as controls. Over a 42 day period, postsmolts were regularly inspected and sampled for mortality, lice density and physiological status in seawater.

2. Materials and methods

2.1. Fish stocks and rearing conditions

Offspring of Atlantic salmon were derived from spawning first-generation adult sea-ranched salmon collected from the River Imsa, South-Western Norway, in the fall of 2002. The offspring of these salmon were considered native River Imsa stock. The eggs and subsequent juvenile fish were reared under hatchery conditions with a naturally simulated light regime. During this period the fish were fed ordinary commercial dry diet, according to temperature and fish size. Mean weight and length (±S.E.) of the fish at start of the experiment was 86.2±14.5 and 21.2±1.3, respectively.

2.2. Experimental design

The experiment was performed at the NINA Research Station at Ims from 27 April to 6 May 2004 (freshwater period) and from 7 May to 17 June (seawater period). Four groups each of approximately 1500 one-year old Atlantic salmon smolts were exposed to one of three water qualities differing with respect to pH, Ali and exposure duration. Three groups (high acid, moderate acid and episodic acid) experienced acidic water (pH 5.6–5.9 and 7–45 μg Ali/l, see Table 1). A fourth group exposed to pH>6.9 and<9 μg Ali/l acted as control (reference group). See Kroglund et al. (2007-this volume) for description of water treatments. After freshwater exposure, smolts (n=150) from each of the 4 exposure groups were moved into tanks containing brackish water (16‰) for 8 h before receiving full strength seawater (33.4‰) within 4 h and were thereafter artificially infected with salmon lice. Each fish was exposed to an average of 2.4 copepodids per gram fish weight. The infection procedure was carried out as described by Grimnes and Jakobsen (1996) and Bjørn and Finstad (1997). Four non-infected groups (n=100) acted as controls. Seawater temperature on 7 May was 6.2 °C, increased gradually and reached 8.1 °C at the end of the experiment 17 June (mean temperature 7.7 °C (±0.7)). Salinity was 33.4‰ (±1.3) during the experimental period.

2.3. Fish sampling and analyses

At the end of the freshwater exposure (3–10 days), 10 fish per group were sampled for determination of plasma chloride concentration, gill Na^+, K^+-ATPase activity and total gill aluminium concentration. After transfer to seawater, 15 fish were dip netted out of each fish tank at regular intervals, anaesthetized separately in buckets by use of clove oil (0.5 ml/l) (Anderson et al., 1997), killed by a blow to the head before blood samples were withdrawn from the caudal vein using 1-ml heparinized syringes. Fish were thereafter kept separate in plastic bags for later lice analyses. The blood was transferred to 2-ml eppendorf tubes, and plasma was separated by centrifugation

Table 1
Water chemistry in the four tanks prior to transfer of fish to seawater

Group	pH	Ali (μg l^{-1})	Gill Al (μg g^{-1} dry weight)	Period of exposure (days)
Reference	6.95 (6.63–6.98)	6 (<5–9)	8.26±1.94*	
High acid	5.67 (5.33–5.85)	29 (18–45)	110.65±25.78**	10
Moderate acid	5.97 (5.63–5.95)	14 (7–34)	29.67±7.94***	10
Episodic acid	5.67 (5.33–5.85)	26 (21–31)[a]	184.18±47.21	3.3

Exposure to high acid and moderate acid was initiated April 27 at 16:30 pm and lasted until May 6 18:00 pm. Episodic acid was initiated May 4 at 10:30 and lasted until May 6 18:00 pm. For pH and Ali the means are values/concentrations per time. Labile or inorganic monomeric species of Al (Ali) represents the toxic form of Al. Asterisk (*) denotes significant differences ($p<0.05$) between all groups, ** denotes significant differences between all groups and *** denotes significant differences between all groups.
[a] Two measurements.

(3000 ×*g* for 5 min). Blood plasma was then stored at −28 °C. The second gill arch on the right hand side of each fish was dissected out and frozen in pre-weighed, acid washed polyethylene vials for analysis of total gill aluminum (gill-Al) content, while the second gill arch on the left hand side of each fish was dissected out and frozen in 2 ml eppendorf tubes in a SEI buffer solution (Zaugg, 1982). Measurements for gill Na^+, K^+-ATPase activity were only carried out in the non-infected groups. Over a 42 day period, postsmolts were regularly inspected and sampled for mortality.

In the laboratory the fish were thawed and salmon lice were examined according to Bjørn and Finstad (1998). The fish were measured to the nearest mm (fork length) and weighed to nearest 0.1 g (UWE, HGS-3000). Bush et al. (1997) have recommended the ecological terms for lice used in this study; prevalence and abundance. Plasma chloride was analyzed by use of a Radiometer CMT10 chloride titrator. Gill Na^+, K^+-ATPase activity was analyzed using the method of McCormick (1993). Briefly, gill tissue was homogenised in 150 μl SEID (SEI buffer containing 0.1% deoxycholic acid) and centrifuged at 5000 ×*g* for 30 s. Ten microliters of supernatant were added in duplicate wells of a 96-well microplate containing 200 μl assay medium, with and without 0.5 mM ouabain, and read at 340 nM for 10 min at 25 °C. Na^+, K^+-ATPase activity was determined as the ouabain sensitive fraction of the enzymatic coupling of ATP dephosphorylation to NADH oxidation, expressed as μmol ADP mg $protein^{-1}$ h^{-1}. Gill aluminium content were analysed according to Kroglund et al. (2001a,b). Water chemistry was analyzed at the Norwegian Institute for Water Research laboratory according to standard protocols. Aluminium was fractionated using the PCV method (see Kroglund and Finstad, 2003; Kroglund et al., 2007-this issue). *In situ* fractionating of Al in the field did not show any significant differences between species distribution of Al in and out of the tanks (Teien et al., 2006). Based on this we assume that Al-speciation did not change within the tanks.

2.4. Statistical analysis

Statistical tests were done using SPSS 13 for Windows. A Shapiro–Wilks test for normality combined with normal plots and de-trended normal plots were used to evaluate departure from normality. Due to lack of normal distribution, non-parametric tests were chosen for analysis of statistical differences in infection parameters and physiological parameters. A two-tailed Mann–Whitney *U*-test was used for testing significant differences between the groups with respect to plasma chloride, gill aluminium and gill Na^+, K^+-ATPase activity and a chi-square test was used to test significant differences between groups with respect to mortality and lice levels. A level of $p<0.05$ was considered as significant and values in figures and table are means (±S.E.).

3. Results

3.1. Water chemistry and gill aluminium

Water chemistry in the four tanks prior to transfer of fish to seawater is given in Table 1. Both pH (6.95), levels of inorganic monomeric aluminium (6 μg l^{-1}) and gill aluminium (8.26μg g^{-1} dry weight) were within the normal range for the reference group during the treatment phase from April 27 to May 6, representing values for non-acidified waters (Kroglund and Finstad, 2003). Both for high acid and episodic acid groups, mean pH was 5.67 and inorganic monomeric aluminium varied from 18–45μg l^{-1} during the experimental period lasting from April 27 to May 6 and from May 4 to May 6, respectively. Gill aluminium was significantly higher than the reference group for both the high acid, episodic acid and moderate acid groups (Mann–Whitney *U*-test, $p<0.001$). For the moderate acid group, pH had a mean level of 5.97 and inorganic monomeric aluminium had a mean level of 14μg l^{-1} during the exposure period which lasted from April 27 to May 6. Gill aluminium for this group was significantly lower when compared to the high acid and episodic acid group (Mann–Whitney *U*-test, $p<0.001$) but significantly higher than the reference group (Mann–Whitney *U*-test, $p<0.001$).

3.2. Lice development

On day 12 post infection (p.i.) only chalimus stages (CH1) were found (Fig. 1). At day 28p.i. stages containing chalimus 3 and 4 (CH3), preadult males 1 and 2 (PM1 and PM2) and preadult females 1 (PF1) were found. At day 42p.i., stages from preadult males (PM2) to adult females (ADF) were found with a highest percentage of preadult females 2 (PF2) and adult males (ADM) on 43.7 and 33.0%, respectively.

A pooled mean number of lice in the groups sampled for physiological analyses (grey bars) and in the moribund (white

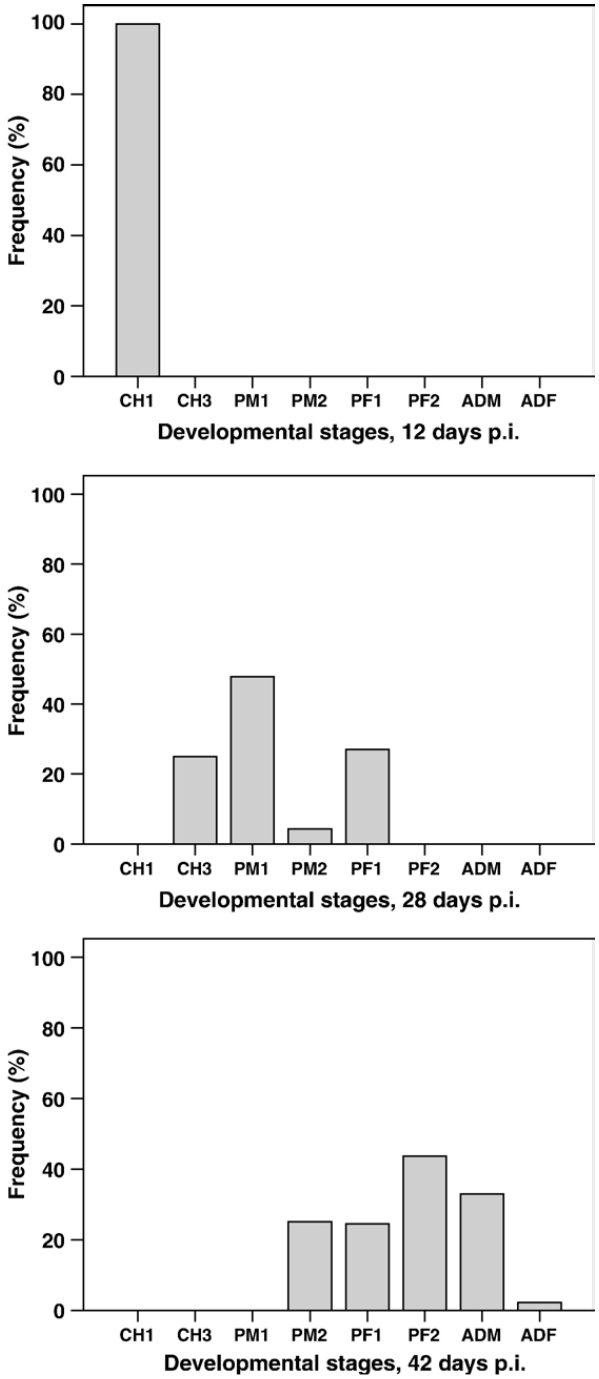

Fig. 1. Frequency distribution (%) of developmental stages of salmon lice on infected Atlantic salmon smolts sampled at 12 (18 May), 28 (3 June) and 42 (17 June) days post infection (p.i.). CH1: First and second chalimus stage combined; CH3: Third and fourth chalimus stage combined; P1M: First preadult male; P2M: Second preadult male; P1F: First preadult female; P2F: Second preadult female; ADM: Adult male; ADF: Adult female.

bars) groups are given in Fig. 2. There were significant higher levels (chi-square test, $p<0.05$) for the moribund (reference) group compared to all the other 7 groups. Further, there were significant differences (chi-square test, $p<0.05$) between the moribund episodic acid group and all sampled and high acid (moribund) groups and significant differences between the moribund groups moderate acid and high acid (chi-square test, $\chi^2=7.184$, $df=1$, $p=0.007$). Reference groups (moribund and sampled) had the highest and lowest mean lice loads, respectively.

3.3. Cumulative mortality

Mortality was low in the four non-infected groups (Fig. 3) and significantly elevated in all lice infected groups compared to non-infected groups. Cumulative mortality in lice infected groups high acid, moderate acid and episodic acid groups was significantly higher compared to the reference lice infected group (chi-square tests, $\chi^2=26.797$, $df=1$, $p=0.001$; $\chi^2=7.364$, $df=1$, $p=0.05$; $\chi^2=4.333$, $df=1$, $p=0.05$, respectively) at the end of the experiment. Therefore, lice infection caused highest mortality in the group with low pH and high Al (high acid), followed by the moderate acid group (slightly higher pH and Al) and followed by the episodic acid group (same dose as acid group but with shorter exposure time (3.3 days) as the two former groups (10 days). Reference and episodic acid groups had significantly lower mortality than the high acid and moderate acid groups from 10 May (4 days p.i.) (chi-square test, $\chi^2=5.332$, $df=1$, $p=0.01$) and this remained so during the whole experiment except for episodic acid group at June 13 (38 days p.i.). A significant segregation (chi-square test, $\chi^2=5.332$, $df=1$, $p=0.05$) between high acid and moderate acid groups was observed at 14 June (39 days p.i.).

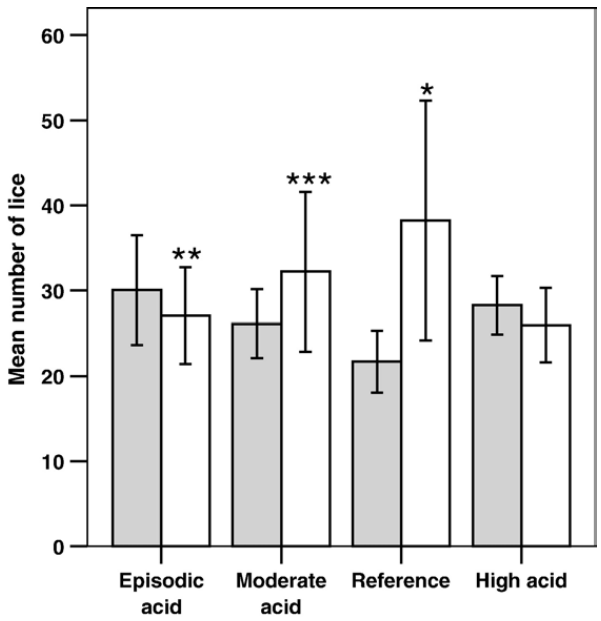

Fig. 2. Total mean number of lice from lice infected groups during the whole experiment. Grey bars are mean numbers of lice from sampled fish while open bars are mean numbers of lice from moribund fish. Values are given as means±S.E. Asterisk (*) denotes significant differences ($p<0.05$) from the other groups; ** significant differences from all sampled groups and high acid (moribund group), *** significant difference from high acid (moribund group).

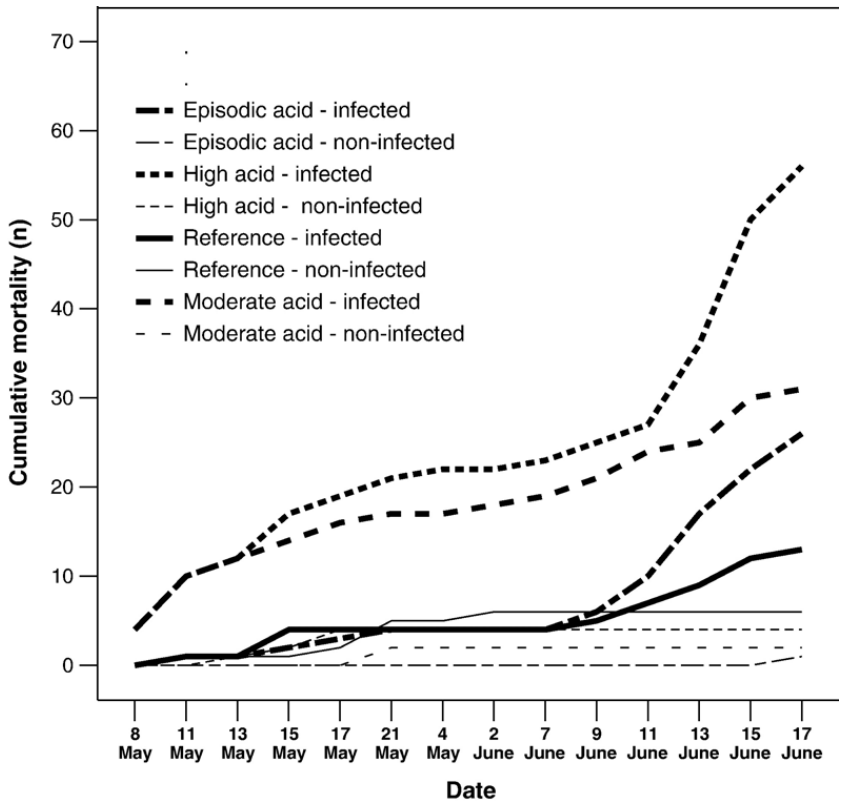

Fig. 3. Cumulative mortality in smolts exposed to high acid, moderate acid and episodic acid water for 3 to 10 days and transferred from freshwater to seawater and infected with salmon lice or held as non-infected groups. ———: reference group; - - - - - -: high acid group; __ __ __ __ : moderate acid group and ____ __ ____: episodic acid group. Infected groups are given by bold lines and control groups (non-infected) by thin lines.

3.4. Hydro-mineral balance

The gill Na^+, K^+-ATPase activity measured at the end of the freshwater exposure (6 May) showed that the high acid group had significantly lower activity (Mann–Whitney *U*-test, $p<0.05$) than the other groups. An increasing activity was seen in episodic acid, moderate acid and reference groups but no significant differences were seen among these groups (Fig. 4). On 7 May, after one day in seawater, gill Na^+, K^+-ATPase activity was elevated in reference, moderate acid and episodic acid groups while levels in high acid groups were still significantly lower (Mann–Whitney *U*-test, $p<0.05$) compared to the three other groups. At the last sampling date (3 June) gill Na^+-K^+-ATPase activity had increased in all groups and reached a level of approximately 20 μmol ADP mg protein^{-1} h^{-1}.

Plasma chloride in the non-infected groups and infected groups are shown in Fig. 5A and B. For both figures values for 6 and 7 May are used as starting reference points. On 6 May, episodic acid groups had the lowest values of plasma chloride on 125 mM and this value was significantly lower than the reference group (Mann–Whitney *U*-test, $p<0.01$). Further, both the moderate acid and high acid groups had significantly lower (Mann–Whitney *U*-test, $p<0.01$) plasma chloride values than the reference group at 6 May. After transfer to seawater on 7 May, the high acid group had significantly

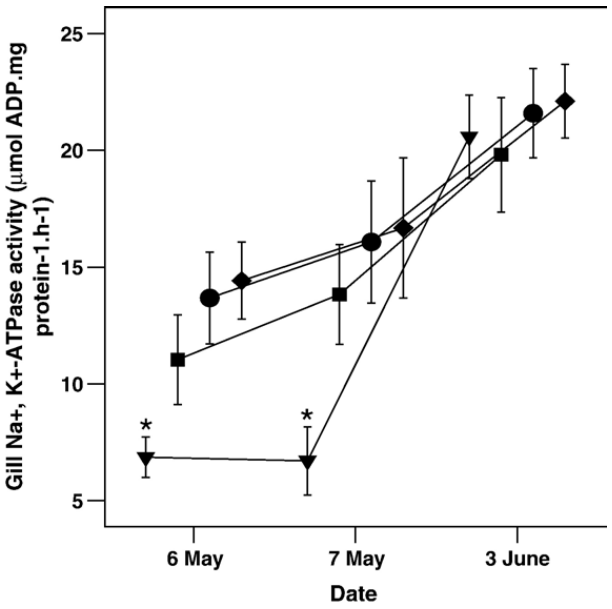

Fig. 4. Gill Na^+, K^+-ATPase in smolts exposed to high acid, moderate acid and episodic acid water for 3 to 10 days and transferred from freshwater to seawater. ♦: reference; ▼: high acid; ●: moderate acid and ■: episodic acid. Samples taken 6 May are from freshwater while the other samples are from seawater. Values are given as means ± S.E. ($n=10$). Asterisk (*) denotes significant differences between groups at each sampling date ($p<0.05$).

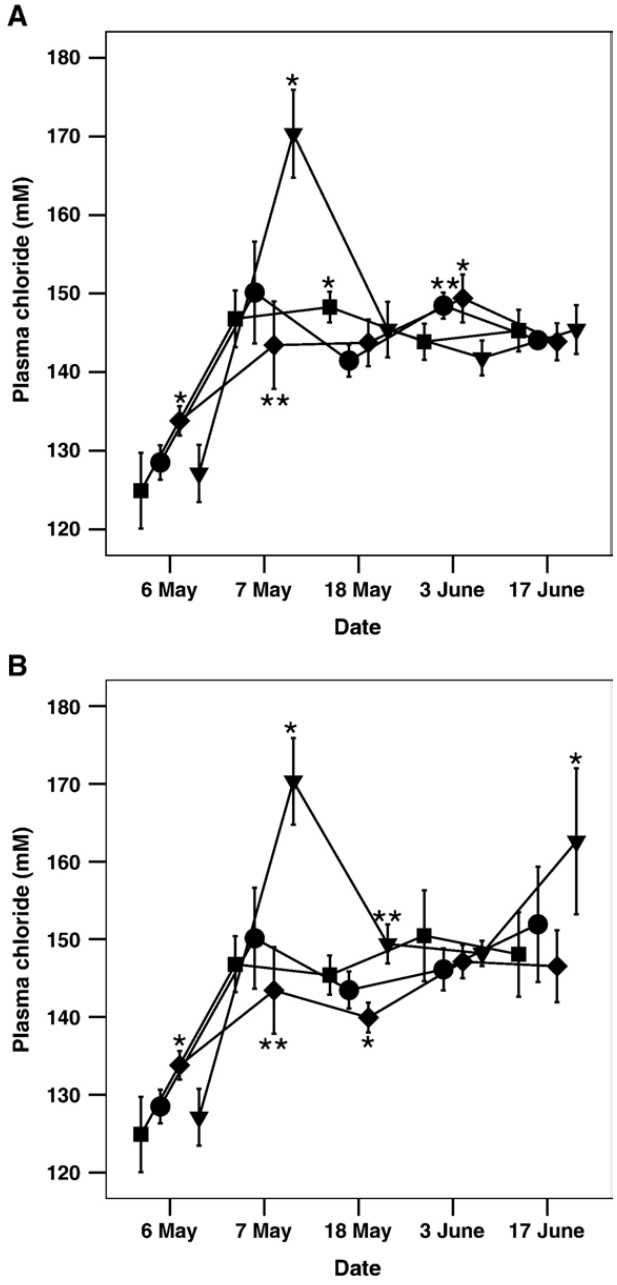

Fig. 5. A and B. Plasma chloride in *non-infected* (5A) and *lice infected* smolts (5B) exposed to high acid, moderate acid and episodic acid water for 3 to 10 days and transferred from freshwater to seawater. ♦: reference; ▼: high acid; ●: moderate acid and ■: episodic acid. Samples taken 6 May are from freshwater while the other samples are from seawater. Values are given as means±S.E. ($n=15$). Asterisk(s) (*, **) denotes significant differences between groups at each sampling date ($p<0.05$). For Fig. 5A: **— 3 June no significant difference between reference and moderate acid group and for Fig. 5B: **— 18 May no significant difference between high acid and episodic acid group.

higher plasma chloride levels (170 mM, Mann–Whitney *U*-test, $p<0.001$) than the reference, episodic acid and moderate acid groups. The reference group had also significantly lower levels on 143 mM (Mann–Whitney *U*-test, $p<0.01$) than the other three groups at this date. For the non-infected groups on 18 May (Fig. 5A), the episodic acid group had higher plasma chloride levels (148 mM, Mann–Whitney *U*-test, $p<0.05$) than the other three groups and on 3 June, moderate acid and reference groups had higher levels (Mann–Whitney *U*-test, $p<0.01$) than the episodic acid and high acid groups. Despite significant differences, these levels were within the normal range. At the last sampling date (17 June), there were no differences between groups.

For the infected groups on 18 May, plasma chloride values were significantly lower in the reference group (Mann–Whitney *U*-test, $p<0.01$) compared to the other groups with the high acid group still having the highest plasma chloride value (149 mM, Fig. 5B). At sampling date 3 June, no significant differences between groups were found, however, at the last sampling on 17 June, the high acid group had significantly higher plasma chloride values (163 mM, Mann–Whitney *U*-test, $p<0.05$) than the episodic acid, moderate acid and reference groups (148, 152 and 147 mM, respectively).

4. Discussion

4.1. Water chemistry and gill aluminium

A pH value as low as 5.4 is not in itself sufficient to reduce postsmolt seawater tolerance (Fivelstad et al., 2004), however, at similar or higher pH values, reduced seawater tolerance is observed when the smolts have been exposed to elevated concentrations of Al (Staurnes et al., 1993; Kroglund and Staurnes, 1999; Kroglund et al., 2001a; Kroglund and Finstad, 2003). In the present experiment, water chemistry and gill aluminium levels were within the normal range for acidification naïve fish at Ims (Kroglund et al., 2007-this issue). For the high acid, episodic acid and moderate acid groups, water quality was significantly reduced and Al levels were within the range considered suboptimal and even toxic for Atlantic salmon smolts (Kroglund and Staurnes, 1999; Kroglund et al., 2001a,b; Kroglund and Finstad, 2003). In a recent paper by Teien et al. (2006), a model describing the interaction between Ali and gill aluminium is presented. Although critical levels for aluminium accumulation on gills are debated (Lacroix et al., 1990; Peuranen et al., 1994) increased physiological responses in fish with increased aluminium accumulation in fish gills have been observed in Atlantic salmon (Kroglund et al., 2001a,b; Kroglund and Finstad, 2003). Increasing accumulation of aluminium was found in the present study on the gills of the episodic acid and acid groups and these levels are known to induce negative physiological responses (Kroglund and Finstad, 2003; Kroglund et al., 2007-this issue). For the moderate acid group, gill aluminium was also significantly higher than the reference group, reaching levels, which may induce

negative physiological responses (Kroglund and Finstad, 2003).

4.2. Lice development

The development of salmon lice infestations corresponded to previous reports for Atlantic salmon (Johnson and Albright, 1991; Grimnes and Jakobsen, 1996; Bjørn and Finstad, 1998; Finstad et al., 2000). In these experiments, seawater temperature in the experimental tanks was held at approximately 10 °C. In the present experiment, the seawater temperature was lower (range 6.2 °C–8.1 °C) and therefore the development from the attached chalimus stages 1–4 to the mobile preadult- and adult stages took longer than reported in experiments performed at higher temperatures. As seen from Fig. 1, preadult- and adult stages had developed at 42 days post infection (17 June) while there were still some stages of preadult males (PM2) and preadult females (PF1). In accordance with Johnson and Albright (1991), the present experiment verified that developmental time to adult males was quicker than the developmental time to adult females.

The mean number of lice was lowest in the sampled reference group and highest in the moribund reference group (Fig. 2). Stress causes immuno-suppression, which results in increased susceptibility for infectious diseases (Iwama et al., 1997; MacKinnon, 1998). Salmon lice infestations on salmonids have led to increased cortisol secretion followed by a reduced immune defence (Bjørn and Finstad, 1997; Finstad et al., 2000). Coho salmon (*Oncorhynchus kisutch*) are known to be more susceptible to salmon lice infections when artificially stressed by cortisol implants (Johnson & Albright, 1992a). Further, implantations of corticosteroids in fish have increased their susceptibility to a variety of parasitic diseases. Atlantic salmon seem to have a low effective immunity against sea lice, *Caligus elongatus*, (MacKinnon, 1998) and salmon lice, *L. salmonis* (Grayson et al., 1991; Johnson and Albright, 1992b). Thus, stress caused by salmon lice infestations will probably increase the host susceptibility for re-infection by salmon lice as well as secondary infections. There were no significant differences between the sampled reference group and the three other groups with respect to mean number of lice but the former group had lower lice load than the groups, which had experienced a suboptimal water quality previous to their seawater transfer. This might be a reason for the somewhat higher salmon lice infestations seen in these groups. Alternatively it may be speculated that the highest mean number of lice in the moribund groups was seen in the reference group because a higher lice load was needed to kill the "healthy" fish compared to the other groups which had experienced a suboptimal water quality prior to seawater transfer.

4.3. Cumulative mortality

Mortality in the non-infected groups was low during the experiment. However, for the infected groups, mortality was highest in the following order: high acid group; moderate acid group; episodic acid group; reference group. Both high acid and moderate acid groups experienced lice induced mortality shortly after seawater transfer but mortality increased dramatically when the lice developed to preadult stages at approximately 28 days post infection. This is in accordance with previous findings (Grimnes and Jakobsen, 1996; Bjørn and Finstad, 1997; Finstad et al., 2000), but the present study shows for the first time that the combined effect of suboptimal water quality and salmon lice significantly increases mortality in Atlantic salmon postsmolts. Further, groups, which have been exposed to suboptimal water quality without salmon lice infestation experienced a significantly lower mortality. Therefore, the additive effect of both salmon lice and suboptimal water quality seems to be a significant reason for reduced postsmolt survival.

4.4. Hydro-mineral balance

Gill Na^+, K^+-ATPase activity was within the range shown to be normal for smolts ready for seawater transfer in the reference, moderate acid and episodic acid groups (Nilsen et al., 2003; Handeland et al., 2003). However, for the high acid group, gill Na^+, K^+-ATPase activity was significantly lower than in the other groups. An inhibition of gill Na^+, K^+-ATPase activity caused by acid water and aluminium has been shown in several previous studies (e.g. Staurnes et al., 1984; Staurnes et al., 1993; Kroglund and Staurnes, 1999; Magee et al., 2003). The low gill Na^+, K^+-ATPase activity shown for the high acid group on 6 May (FW) and 7 May (SW) was also reflected in the higher mortality in this group compared to the other groups after seawater transfer. However, after 28 days in seawater, gill Na^+, K^+-ATPase activity had increased to normal seawater values in accordance with Berge et al. (1995) and Handeland et al. (2000) in all groups including the high acid group.

Plasma chloride levels reflected the lower gill Na^+, K^+-ATPase activity in the high acid group. This was seen on 7 May when the fish were transferred to seawater and this group had mean plasma chloride

values well above what is acceptable for seawater tolerant smolts (Sigholt and Finstad, 1990). For the non-infected groups, plasma chloride levels during the rest of the experiments were far below 160 mM indicating a good seawater tolerance (Sigholt and Finstad, 1990). For the infected groups, plasma chloride levels in the high acid group were significantly higher than the other groups (mean values 163 mM) and were reflected in the highest mortality seen in this group.

4.5. Salmon lice and suboptimal water quality — population effects

It has long been debated as to whether the causes for reduced marine postsmolt survival are suboptimal water quality (acid rain/aluminium) or parasites acting within the marine environment (see review by Finstad and Jonsson, 2001). Acid rain has reduced several salmonid stocks in Norway (Hesthagen and Hansen, 1991; Kroglund et al., 2002). In addition, salmon lice have been identified as major population regulation factor (Heuch et al., 2005). Reduction in marine survival related to previous treatment of acid water and aluminium has been shown in a study by Kroglund and Finstad (2003) and Kroglund et al. (2007-this issue). As pointed out by Rosseland and Staurnes (1994); Magee et al. (2003) and Kroglund and Finstad (2003), the importance of measuring and assessing sublethal stresses in freshwater and the effect on subsequent marine survival should be highlighted. Further, treatment of fish by a protective substance EX giving fish protection for salmon lice attacks for up to 16 weeks have shown to be positive for marine survival compared to unprotected fish (see Finstad and Jonsson, 2001). In the present study we have shown for the first time that a combination of suboptimal water quality and salmon lice attacks increases marine mortality in Atlantic salmon postsmolts. The initial mortality observed in high acid and moderate acid groups from 7 May to 3 June can be attributed to poor hypo-osmoregulatory capacity killing the more sensitive fish. With increased time in seawater, gill Na^+, K^+-ATPase activity increased and plasma chloride levels decreased to levels normal observed for postsmolts in seawater. When the salmon lice developed into mobile preadult stages, the high acid and moderate acid smolts were still more sensitive to this additional stressor indicating compromised tolerance to lice infestations. These effects were not noticeable until 30 days, indicating delayed responses to the initial stressor, i.e. acid freshwater. Our results go some way to answering the question presented in the title: Salmon lice or suboptimal water quality — reasons for reduced postsmolt survival. Year to year variations in acidification pressure and salmon lice densities can singularly and in combination explain some of the year to year variations in postsmolt survival and hence the variations in Atlantic salmon year–class strength in Norwegian rivers.

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