Gildeskål Forskningsstasjon a.s

Ferskvannsavlusing i brønnbåt

The use of freshwater to control infestations of the sea louse
Lepeophtheirus salmonis K on Atlantic salmon *Salmo salar* L.

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Abstract/Summary

The study was conducted at GIFAS small-scale facilities in Langholmen in April 2013 using Brønnbåten Havtrans (Havtrans AS) to perform the study.

5200 Atlantic salmon with a mean weight of 4.4 kg were distributed into two wells (one with freshwater (0.16 ppt) and one with seawater (33.7 ppt)). The fish were maintained in both tanks for a period of between three and four hours after which, 100 fish from each well were pumped into two separate 5x5x5m cages. After exposure, the fish were sedated and any attached sea lice present were recorded. The average number of attached sea lice was compared to a pre-count undertaken prior to the study.

Results from the study showed that there was a very clear reduction in all stages of lice which parasitize Atlantic salmon after exposure to both seawater and freshwater. Salmon exposed to seawater had a percentage reduction in the average number of *chalimus*, pre adults and mature females of 47%, 59% and 31% respectively compared to the pre count. The overall reduction of all stages of infective lice was calculated to be 51%. Whereas salmon exposed to freshwater had a higher percentage reduction in the average number of chalimus, pre adults and mature females of 100%, 97% and 92% respectively compared to the pre count. The overall reduction of all stages of infective lice was calculated to be 96%.

The reduction recorded from salmon exposed to seawater was attributed to the effects of the fish being transferred from the polar circle and over the sorting machine. Similar percentage reductions were observed from the previous study undertaken at Gifas where it was shown that transferring fish from one cage to another or crowding the fish resulted in reduction of up to 40% compared to pre-count levels of infestation. Given this, the levels of reduction observed from fish exposed to the well containing freshwater were significantly lower for all stages of infective lice compared to fish maintained in the well containing seawater. The significantly lower reduction of all attached lice stages from fish exposed to freshwater compared to fish exposed to seawater must be attributed to the exposure to freshwater.

This present study clearly shows that freshwater has the potential to be used as an effective delousing method on farmed Atlantic salmon under more realistic commercial scale conditions. However, more work is required before the method can be used within the present industry. Further studies are required to further upscale the methods used in the present study so larger populations of salmon can be exposed to freshwater.

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1.0 Introduction

The salmon louse (*Lepeophtheirus salmonis*) is an ectoparasitic copepod feeding on skin, mucous and blood from farmed Atlantic salmon (*Salmo salar* L) and in Norway, the aquaculture industry is facing a worrying increase in the number of sea lice. The annual losses due to sea lice were estimated at 1000 million kroner in terms of mortality, lost growth and downgrading. Less quantifiable are the costs associated with secondary infections, an increased lice infestation on wild salmon and a negative market impact.

Chemotherapeutent treatment is then main method of control and there are several chemical bath-treatments available to reduce lice infections. Most bath treatments are expensive, labour intensive and some can be stressful to the salmon. Nearly all compounds are selective and only efficient against some of the lice's life-stages. After a treatment the lice levels tend to be low, but the levels will often build up again, and treatments must be repeated. Experience has shown that even low numbers of lice will reduce the salmon's appetite and growth. Several alternative methods, including a vaccine, in feed treatment are currently being investigated but chemotherapeutant treatment is costly to the industry, has other environmental and human health implications, and is itself closely regulated in the Northern Hemisphere. These constraints, in addition to concerns of the development of resistance through treatment over-use, and the increased reliance on a single in-feed treatment, SLICE® (emamectin benzoate), presents real risks and also places practical limits on how farms can manage sea lice infestations in a sustainable manner. To date, there are no new classes of chemotherapeutant treatments on the worldwide market that are in a position to succeed SLICE®. Should resistance to SLICE® develop and become widespread, the industry would apparently have no option but to revert to older control agents (e.g. organophosphates, pyrethroids, chitin inhibitors, peroxide). It is thus essential that alternative control measures are investigated.

One such control measure which has received much attention, is the use of wrasse species placed in net pens to control sea lice infestations. Another potential treatment that merits further investigation is the use of freshwater to reduce lice infestations. A number of laboratory and field-based studies appear to indicate that lower levels of salinity act to reduce sea lice levels (Heuch 1995, Tucker *et al*. 2000, Bricknell *et al.* 2006, Bravo *et al*. 2008a Heuch *et al* 2009). In the long term, an effective environmental delousing method could reduce reliance on chemotherapeutants. This will also contribute to slowing resistance development against currently available treatments.

At the strategy meeting to the Fishery and Aquaculture Industry Research in June 2010 it was determined that one of the main priorities in the coming years was implementation of an integrated control strategy against lice, for a profitable and wealth-creating aquaculture industry based on sustainable production. The introduction of procedures that reduce stress in animals produced for human consumption have a positive marketing aspect. Several of the salmon industry's major customers in Europe require increased focus on fish welfare and the use of environmentally friendly production methods to the greatest extent possible. It will be positive for producers to be able to document environmentally friendly and sustainable practices that will satisfy the increased demand by consumers for environmentally friendly and non-chemical methods of production

Aims and Objectives

This study is a continuation of assessing the potential use of freshwater as a delousing agent. The first study (report title: **The use of freshwater to control infestations of the sea louse *Lepeophtheirus salmonis* K on Atlantic salmon *Salmo salar* L. September 2011**) initially assessed the potential for using freshwater to remove attached sea lice from infected Atlantic salmon. The study showed that exposing infected salmon to freshwater resulted in a significant reduction of both mature male and female lice after three hours and results from freshwater bioassays undertaken at the same time during the first study showed that after 1 hour exposure to freshwater, 10% of mature females were found to be dead whilst 90.9% of mature males had died as a result to exposure to freshwater.

These initial small-scale studies showed that there is potential in using freshwater to delouse infected Atlantic salmon.

Aims and Objectives

The aim of this study was to assess this potential under more realistic commercial conditions by using a well-boat filled with freshwater and exposing a larger number of Atlantic salmon to the treatment for a defined period of time.

2.0 Methods

2.1 Experimental design

The study was conducted at GIFAS small-scale facilities in Langholmen in April 2013 using Brønnbåten Havtrans (Havtrans AS) to perform the study. The well-boat had two wells: one filled with freshwater (0.16 ppt) and one filled with seawater (33.7 ppt).

5200 Atlantic salmon with a mean weight of 4.4 kg were used in the study. The fish were pumped from a 60m polar circle onto the well-boat (Figure 1). Approximately, half of the fish were transferred into each of the two wells. The fish were pumped on board and were transferred into each of the two wells via the sorting machine on the boat. This allowed for the removal of the seawater present during pumping. The time required to transfer each group of fish was recorded and after the last fish was transferred into each well, each group was maintained in both wells for approximately three hours. Transfer took one hour for each well thus each group was exposed to either freshwater or seawater for between 3 and 4 hours in total. After the desired exposure time was attained, 100 fish from each well were pumped back out of the well-boat into two 5x5x5m cages with 100 fish from the seawater group and 100 fish from the freshwater group in each cage.

Figure 1.The well-boat used in the study and fish being pumped from the polar circle into the two wells on board.

2.2 Registration of sea lice levels

For the fish being used in the study, a lice count was undertaken to assess the lice burden seven days prior to treatment with freshwater.

From the cage being used for the first study, 100 fish were sedated and any lice present were recorded. After counting has been complete, any lice remaining in the container was also recorded.

The 100 fish from each treatment transferred in each of the 5x5x5m cages were immediately sedated and any attached sea lice present were recorded. After counting has been complete, any lice remaining in the container was also recorded.

Lice were registered in 4 categories:

- *Lepeophtheirus salmonis*: Adult female
- *Lepeophtheirus salmonis*: Preadult and males
- *Lepeophtheirus salmonis*: Chalimus
- *Caligus elongatus*

2.3 Water Quality

Oxygen saturation and salinity within each well was monitored throughout the exposure test.

2.4 Statistics

Statistical significance of differences were computed from one-way or two-way analysis of variance (ANOVA) using Minitab™ statistical software (Ryan & Joiner, 1994). The normality and homogeneity of the variance of all data sets was tested prior to parametric statistical analysis. Normality was tested by graphic examination of probability plots and the Anderson-Darling test. Significant differences between treatments were determined by Tukey's multiple range test ($p < 0.05$). Differences in mean abundance of attached sea lice were detected after log transformation of the data.

3.0 Results

3.1 Sea lice infestation levels

The average number of Chalimus stages of *L. salmonis* recorded at the pre-count and after exposure to either freshwater or seawater can be seen in figure 2. After treatment, the average number recorded on fish exposed to seawater was found to have decreased to 0.1 per fish although not significantly so ($p > 0.05$) compared to 0.19 per fish recorded during the pre-count. For fish exposed to freshwater, there was a significant reduction to zero chalimus present on the 100 fish examined compared to the pre-count ($F_{2, 297}$ 8.50; $p < 0.001$).

The average number of pre adult stages (pre adults and mature males) of *L. salmonis* recorded at the pre-count and after exposure to either freshwater or seawater can be seen in figure 3. After exposure to both seawater and freshwater, the average number of pre adults per fish significantly decreased from 0.17 per fish at the pre-count to 0.29 and 0.02 per fish respectively ($F_{2, 297}$ 24.33; $p < 0.001$). Although there was a lower average number of pre adult stages on the fish exposed to freshwater compared to fish exposed to seawater, this difference was not found to be significant ($p > 0.05$).

The average number of the mature female stage of *L. salmonis* recorded at the pre-count and after exposure to either freshwater or seawater can be seen in figure 4. After exposure to seawater, the average number of mature females per fish decreased from 0.39 per fish at the pre-count to 0.27 per fish but not significantly so ($p > 0.05$). For the fish exposed to freshwater, there was a significant decrease to 0.03 per fish compared to the pre count ($F_{2, 297}$ 8.60; $p < 0.001$).

The average number of all stages of *L. salmonis* (chalimus, pre adults and mature females) recorded at the pre-count and after exposure to either freshwater or seawater can be seen in figure 5. After exposure to both seawater and freshwater, the average number of all stages per fish significantly decreased from 1.29 per fish at the pre-count to 0.63 and 0.05 per fish respectively ($F_{2, 297}$ 45.49; p < 0.001). There was also significantly less lice per fish for fish treated in freshwater compared to fish treated in seawater ($F_{2, 297}$ 29.45; p < 0.001).

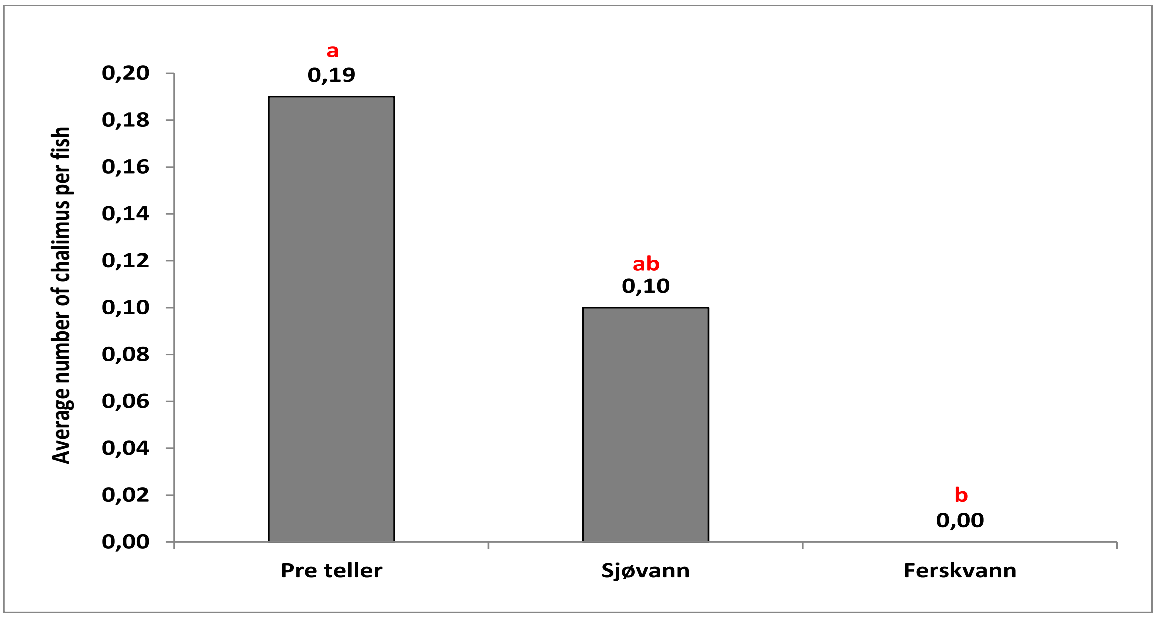

Figure 2 Average number of Chalimus stages of *L. salmonis* per fish recorded prior to treatment and after exposure to both seawater and freshwater. Values represent means ± S.D. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

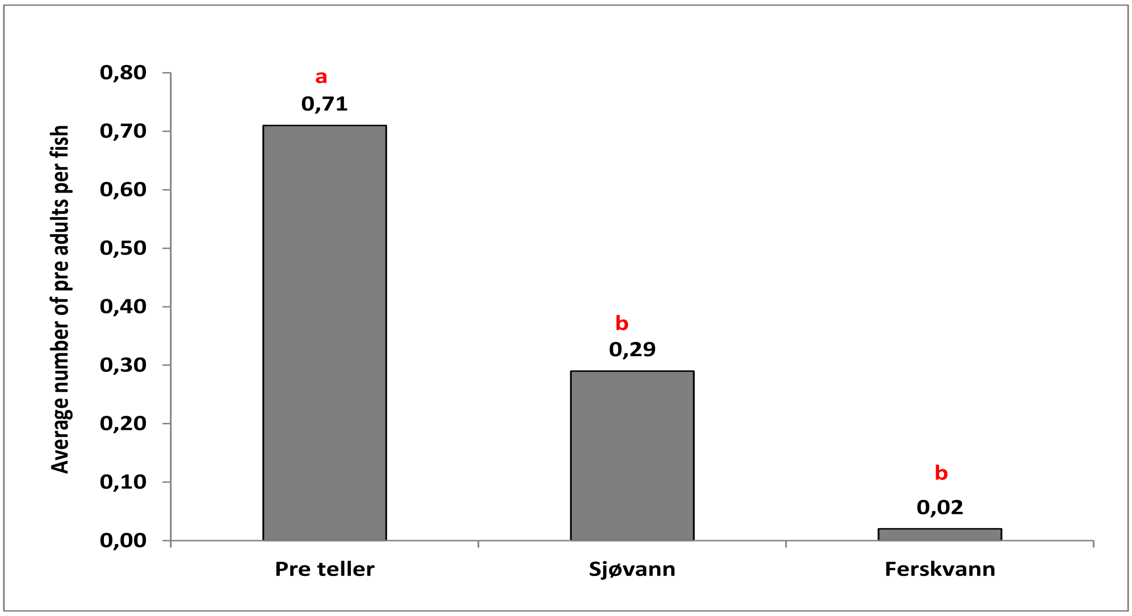

Figure 3 Average number of pre adult stages of *L. salmonis* per fish recorded prior to treatment and after exposure to both seawater and freshwater. Values represent means ± S.D. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

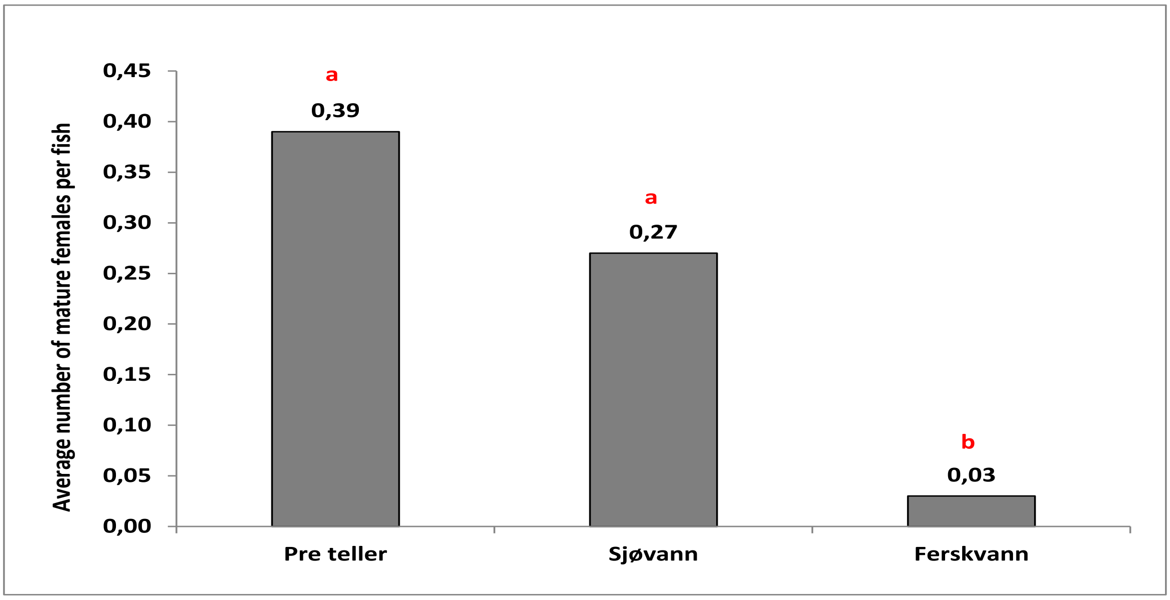

Figure 4 Average number of the mature female stage of *L. salmonis* per fish recorded prior to treatment and after exposure to both seawater and freshwater. Values represent means ± S.D. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

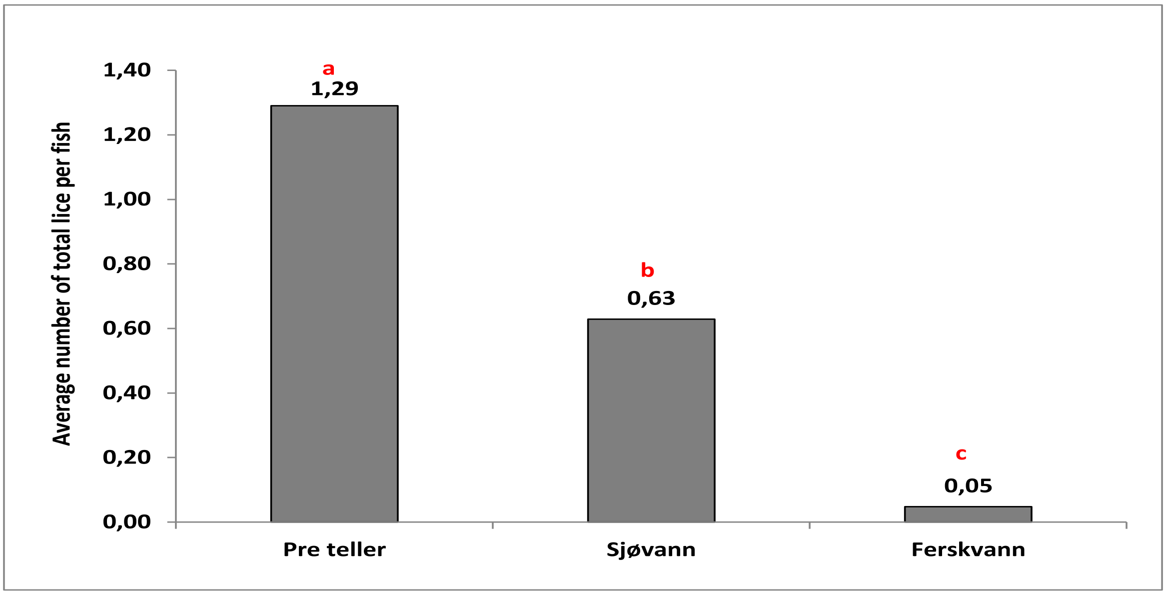

Figure 5 Average number of all stages of *L. salmonis* per fish recorded prior to treatment and after exposure to both seawater and freshwater. Values represent means ± S.D. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

The percentage reduction in the average number of chalimus, pre adult, mature females and total lice can be seen in figure 6. There was a 47% reduction in chalimus stages on fish transferred to the seawater well compared to the pre-count and a 100% reduction for fish exposed to freshwater. For pre adult stages, fish exposed to seawater had a percentage reduction of 59% and fish exposed to freshwater had a 97% reduction compared to average numbers recorded at the pre-count. A similar trend was observed for mature female stages of L. *salmonis* with a 31% and 92% reduction for fish treated in seawater and freshwater respectively compared to numbers recorded at the pre-count. The percentage reduction in the total average number of all stages of L *salmonis* was found to be 51% for fish exposed to seawater and 96% for fish exposed to freshwater compared to pre-count values.

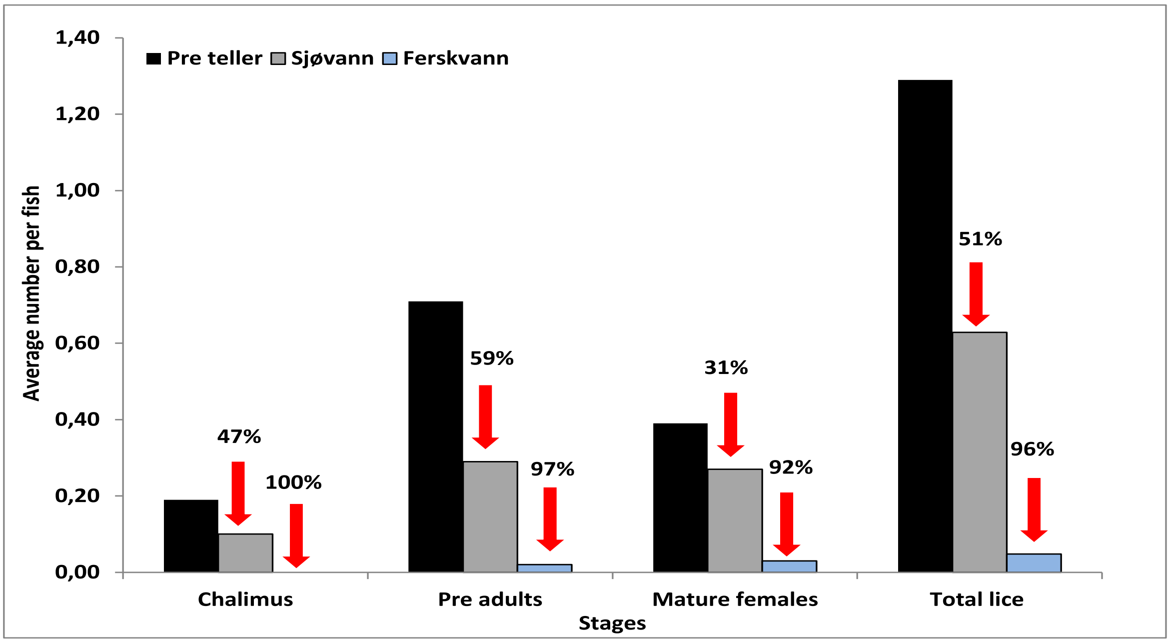

Figure 6 Average number of chalimus, pre-adults, mature females and total lice recorded for the pre-count and after exposure to seawater and freshwater. Values represent the average recorded for 100 fish (n = 100; N = 300). The red arrows and percentage values represent the percentage reduction of each stage of sea lice compared to the pre-count values.

3.2 Water quality

Oxygen saturation (%) and salinity (ppt) recorded in each well can be seen in table 1. For the freshwater well, oxygen saturation levels decreased from 95,3 % at time 0 to 62,5% at 180 minutes whilst salinity increased from 0.16 ppt at time zero to 0.37 ppt at 45 minutes with no further increases recorded thereafter. For the seawater well, oxygen saturation decreased from 104.3 at time zero to 89.9 at 180 minutes and salinity remained stable throughout ranging from 33.4 ppt to 34.0 ppt.

Table 1 Salinity and Oxygen saturation recorded in each well during the study.

Time (minutes)	Freshwater well		Seawater well	
	Oxygen saturation (%)	Salinity (ppt)	Oxygen saturation (%)	Salinity (ppt)
0	95,3	0,16	104,3	33,50
15	97,3	0,17	101,3	33,70
30	97,4	0,30	97,1	33,90
45	95,0	0,37	95,4	34,00
60	85,6	0,37	62,4	34,00
90	79,8	0,37	89,8	33,70
120	72,3	0,37	84,2	33,40
150	65,4	0,37	81,9	34,00
165	63,5	0,37	80,2	33,40
180	62,5	0,37	79,3	33,70

4.0 Discussion

Results from the study showed that there was a very clear reduction in all stages of lice which parasitize Atlantic salmon after exposure to both seawater and freshwater. Salmon exposed to seawater had a percentage reduction in the average number of *chalimus*, pre adults and mature females of 47%, 59% and 31% respectively compared to the pre count. The overall reduction of all stages of infective lice was calculated to be 51%. Whereas salmon exposed to freshwater had a higher percentage reduction in the average number of chalimus, pre adults and mature females of 100%, 97% and 92% respectively compared to the pre count. The overall reduction of all stages of infective lice was calculated to be 96%.

The reduction recorded from salmon exposed to seawater was attributed to the effects of the fish being transferred from the polar circle and over the sorting machine. Similar percentage reductions were observed from the previous study undertaken at Gifas where it was shown that transferring fish from one cage to another or crowding the fish resulted in reduction of up to 40% compared to pre-count levels of infestation (Reynolds 2011). Given this, the levels of reduction observed from fish exposed to the well containing freshwater were significantly lower for all stages of infective lice compared to fish maintained in the well containing seawater. The significantly lower reduction of all attached lice stages from fish exposed to freshwater compared to fish exposed to seawater must be attributed to the treatment effect as the previous small-scale study showed that exposing infected salmon to freshwater resulted in a significant reduction of both mature male and female lice by 82.4% and 67.7% respectively after three hours.

The *chalimus* stages of L *salmonis* showed the highest sensitivity to freshwater exposure with none being found on 100 fish after the three to four hour treatment. A similar trend was observed from the first study where no *chalimus* were found on the fish after one hour exposure to freshwater. This suggests that this stage of the sea lice life cycle is also sensitive to short term exposure to freshwater.

A large proportion of the research undertaken on effects of salinity on sea lice have focussed on the free-swimming copepodid stages, Studies have shown that survival, planktonic development, settlement on the host and development on the host fish are adversely affected by low salinity. Low salinities appear to have a greater impact on the planktonic than on the parasitic stages (Pike *et al*, 1999). Newly hatched larvae do not survive below 15 parts per thousand (‰) and only negligible development to the infective copepodid occurs between 20 and 25‰ (Genna *et al,* 2005; Ritchie, 1997), (salinity of the open oceans varies from 33 to 38‰). Another study showed the survival of free-swimming copepodids was “severely compromised” by salinities below 29‰ (Bricknell *et al,* 2006). These results show that both survival and host infectivity of L. salmonis are severely compromised by short-term exposure to reduced salinity levels. This present study has clearly shown that infective stages of lice are susceptible to low salinity levels under 1 ‰.

Water quality parameters recorded during exposure showed that there was a slight but insignificant increase in salinity in the freshwater well (table 1). However, this small increase is attributed to small amounts of seawater entering the well during fish transfer and stopped after this time. Salinity at no time increased beyond 0.37 ppt as a result of most of the seawater being removed as the fish were pumped into the freshwater well. There was a steady decrease in oxygen saturation levels in both wells but not to the point where re-oxygenation would be required.

The percentage reduction attained for all infective stages of sea lice found on Atlantic salmon exposed to freshwater (96%) would be considered to be a successful treatment outcome and infection levels would be below treatment thresholds imposed under Norwegian legislation (0.5 sexually mature females per fish). Given the concerns of the development of resistance through treatment over-use and the fact that no new classes of chemotherapeutant treatments on the worldwide market are in a position to succeed the current range in use it is essential that alternative control measures are investigated. The previous and present studies have clearly shown that there is potential for freshwater to be used as part of an integrated approach to control sea lice infestations.

5.0 Conclusions

This present study clearly shows that freshwater has the potential to be used as an effective delousing method on farmed Atlantic salmon under more realistic commercial scale conditions. However, more work is required before the method can be used within the present industry. Further studies are required to further upscale the methods used in the present study so larger populations of salmon can be exposed to freshwater.

In addition, a repeat study would assist in confirming these present results and also assist in identifying and modifying the method used to transfer fish to and from commercial-scale cages. A more detailed study into water chemistry would also allow for further elucidation of changing parameters and may allow for the freshwater to be re-used for treatment of several cages and a larger biomass of salmon.

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