Gildeskål Forskningsstasjon a.s

Ferskvannsavlusing i brønnbåt: Study 4. Water quality.

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

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Gildeskål Forskningsstasjon a.s

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Abstract/Summary

The aim of this study was to assess the potential of using a buffering agent to maintain safe levels of pH when treating a large biomass of salmon in freshwater in a well boat for a define period of time. A secondary aim was to assess and compare the potential of freshwater and water with a salinity of 7ppt to remove the infectious stages of L. salmonis from Atlantic salmon with a mean weight of 1kg. Fish were pumped into each of the two wells simultaneously from a 90m polar circle cage. The freshwater well received approximately 30.3 tonnes of fish whilst the well with 7.0 ppt received approximately 39.7 tonnes of fish. Lice counts were undertaken prior to treatment and transfer to establish present infestations levels. Efficacy of treatment was assessed by undertaking a lice count at 18:05pm thus all fish were exposed to freshwater for between one hour and 3 hours 20 minutes. A lice count was performed on 30 fish which were netted directly from each of the wells.
A prepared solution of 50% Sodium hydroxide (NaOH) with a pH of 14.0 was used to help maintain safe pH levels throughout the study period. The amount of NaOH added to each well varied throughout the treatment period.
Results from the study showed that there were clear reductions in all stages of lice which parasitize Atlantic salmon after short-term exposure to freshwater and to a lesser extent after exposure to water with a salinity of 7.0 ppt. The percentage reductions in the infectious attached stages attained after the fish were exposed to freshwater (*chalimus*, pre-adult and mature females stages of L. salmonis) was 72% whilst for fish exposed to water with a salinity of 7.0 ppt was found to be 47.0%. The lower than expected reduction attained from the freshwater exposure may be due to differences in the grading system used and/or fish size. The low clearance rates attained after exposure to water with 7.0 ppt shows that it appears not to be an effective treatment medium however; further research is required to fully elucidate this.

Results from the study show that initially there was a steady but small decrease in pH in both wells once fish transfer had been complete and prior to the addition of NaOH. The addition of NaOH commenced approximately I hr. and 30 minutes after the fish had been transferred to both wells at a rate of 0.25 l/hr. The decline in pH slowed after the addition and in the well containing freshwater even increased slightly after 10 minutes post-addition. The decrease in pH levels continued however, as the rate at which NaOH was increased there were corresponding small increases in pH in both wells. This present study showed that there is potential for NaOH to be used as a buffering agent to control pH in wells filled with freshwater. However, further research is required to elucidate flow rates and how much to add to maintain safe levels throughout a desired treatment period of approximately three hours.

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1.0 Introduction

This is the fourth study in a suite assessing the potential use of freshwater as a delousing agent under commercial conditions. 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.

The second and third studies (report tilte; **Ferskvannsavlusing i brønnbåt. The use of freshwater to control infestations of the sea louse *Lepeophtheirus salmonis* K on Atlantic salmon *Salmo salar* L. April 2013 & October 2013**) were undertaken under more realistic commercial conditions.

These studies showed that a significant biomass of Atlantic salmon (up to 110 T) could be successfully deloused with freshwater. However, the studies also highlighted the need to maintain water quality parameters such as dissolved oxygen and particularly pH in order for the attached sea lice to be exposed to freshwater for sufficient time in order to be affected.

During the studies undertaken in October 2013, it was found that a super oxygenation system can maintain safe levels of dissolved oxygen. Saturation levels decreased from 124.0% at the start of the exposure study to 84.0% at which point oxygen was added and levels increased to 101.0% quickly thereafter. However, pH levels steadily decreased to 6.08 ppt during the exposure period At this point the fish were showing signs of acute stress and it was decided at this point to start pumping in seawater to safeguard the large biomass of fish and to ensure the welfare of the fish. Carbon dioxide readings on board the well boat (ranging from 19.1 to 68.4ppt) were based on pH levels and were not measured in real time. Readings from hand-held instruments measured CO_2 between 16.0 and 17.0 ppt at the later end of the study.

For carbon dioxide the safe criterion used for the Norwegian production of Atlantic salmon smolts is 15 mg L^{-1} (Fivelstad, S. 2013) provided dissolved oxygen concentrations are high. However, constant fish respiration can raise carbon dioxide levels high enough to interfere with oxygen intake by fish, in addition to lowering the pH of the water. If the cause of the stress noted in the fish was attributed to lowering of pH and/or an increased carbon dioxide concentration then some form of buffering agent may alleviate this problem.

A potential option to prevent swings in pH is to add Sodium hydroxide (NaOH), also known as caustic soda, lye/lut solution or Sodium Hydrate solution. It is a highly caustic metallic base and alkali salt which is available as a prepared solution at a number of different concentrations. Sodium hydroxide forms an approximate 50% (by weight) saturated solution with water. It is commonly used at smolt facilities which use recirculation systems to help maintain safe pH levels throughout production.

Aims and Objectives

Primary objective:

The main objective was to assess the potential of using a buffering agent (NaOH) to maintain safe levels of pH when treating a large biomass of salmon in freshwater for a define period of time.

Secondary objective:

To assess and compare the potential of freshwater and water with a salinity of 7ppt to remove the infectious stages of L. salmonis from Atlantic salmon with a mean weight of 1kg.

2.0 Methods

2.1 Experimental design

The study was conducted at GIFAS large-scale facilities at Stivika in December 2013 using Brønnbåten Novatrans (Picture 1).
For the study, the well-boat had two wells: one filled with freshwater (0.0 ppt) and one filled with a blend of seawater and freshwater until a salinity of 7.0 ppt was attained.
Freshwater was pumped into each well from Sundsfjord smolt the previous evening/early morning before arriving at the farm site. Each well had a capacity of $600m^3$.
An approximate total of 70,000 Atlantic salmon with a mean weight of 1.00 kg (70.0 T) were used in the study. The fish were pumped into each of the two wells simultaneously from a 90m polar circle cage. The freshwater well received approximately 30.3 tonnes of fish whilst the well with 7.0 ppt received approximately 39.7 tonnes of fish.
As the fish were pumped into the wells, excess seawater was removed via a grid to ensure that the salinity in the two wells was maintained during the treatment period.
The time required to transfer the fish into the wells was recorded and transfer from first to last fish in took two hours and twenty minutes (14:45 to 17:05pm).
Efficacy of treatment was assessed by undertaking a lice count at 18:05pm thus all fish were exposed to freshwater for between one hour and 3 hours 20 minutes. A lice count was performed on 30 fish which were netted directly from each of the wells.

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

2.2 Buffering with NaOH

A prepared solution of 50% Sodium hydroxide (NaOH) with a pH of 14.0 was used to help maintain safe pH levels throughout the study period. The amount of NaOH added to each well varied throughout the treatment period.

2.3 Registration of sea lice levels

For the study, a lice count was undertaken the same day to assess the lice burden prior to exposure to freshwater and water with a salinity of 7.0 ppt. From the cage being used for the study, 30 fish were sedated with Bonzoak at a concentration of active substance of between 30-40mg / l (15-20ml Benzoak/100 liter) and any lice present were recorded. After counting has been complete, any lice remaining in the container were also recorded.

In addition, a lice count was immediately undertaken after the fish had been pumped onboard and excess seawater was removed and before they entered the wells to assess the mechanical/physical effects of pumping fish in removing attached stages of L. salmonis from infected fish. Thirty random fish destined for each well were sedated as described and all attached stages of L. salmonis were counted and recorded. After counting has been complete, any lice remaining in the container were also recorded.

A final lice count was undertaken after one hour from the last fish entering each of the two wells to evaluate the effects of reduced salinity on attached stages of L.salmonis. Thirty random fish from each well were netted directly and sedated as described and all attached stages of L. salmonis were counted and recorded. After counting has been complete, any lice remaining in the container were also recorded.

For all, lice were registered in the following 3 categories for all counts undertaken:

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

2.4 Water Quality

Oxygen saturation (%), salinity (ppt), CO_2 (mg/L), $CaCO_3$ and pH within each well was monitored periodically throughout the exposure period.

2.5 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 The effects of pumping and exposure to freshwater on attached stages of L.*salmonis.*

The results from the fish exposed to freshwater (0.0ppt) can be seen in figure 1. There was a significant reduction in all stages of sea lice counted after exposure to freshwater.

For *chalimus* stages there was no reduction in the average of 0.57 per fish immediately after the fish were pumped from the cage and passed over the grader on the well boat ($p > 0.05$). There was however, a significant reduction after exposure to freshwater for between one hour and 3 hours 20 minutes to an average of 0.17 per fish ($F_{2,\,87}$ 3.24; $p < 0.05$). For pre-adult stages, there was a significant reduction from an average of 9.7 per fish to 6.57 per fish recorded immediately after the fish were pumped from the cage and passed over the grader and a further significant reduction to an average 2.73 per fish once exposed to freshwater ($F_{2,\,87}$ 28.14; $p < 0.001$).

For mature female stages there was a reduction in the average of 0.93 to 0.73 per fish immediately after the fish were pumped from the cage and passed over the grader on the well boat but not significantly so ($p > 0.05$). There was however, a significant reduction after exposure to freshwater for between one hour and 3 hours 20 minutes to an average of 0.23 per fish ($F_{2,\,87}$ 6.99; $p < 0.01$).

There was a reduction for all stages of L. *salmonis* counted immediately after the fish were pumped from the cage and passed over the grader. The average number of all attached stages decreased from 11.2 per fish to 7.87 per fish however, this reduction was not found to be significant (p >

0.05). There was a significant reduction after exposure to freshwater with the average number of all stages of L.*salmonis* decreasing to an average 3.13 per fish ($F_{2, 87}$ 36.02; $p < 0.001$).

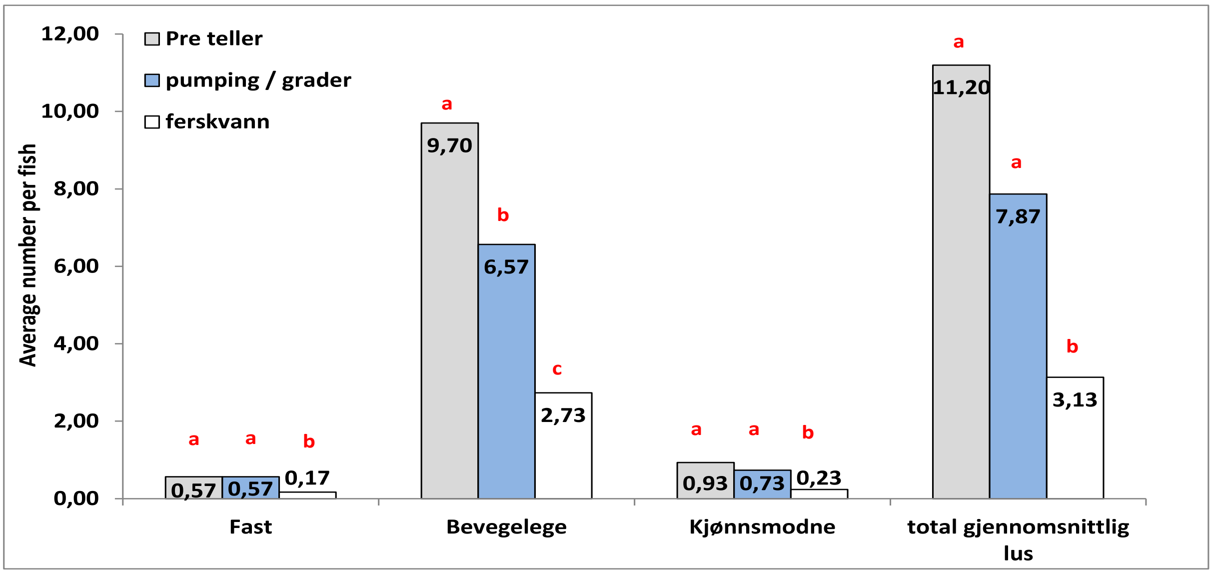

Figure 1 Average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after pumping and exposure to freshwater. Values represent means ± S.D. Mean values which do NOT share a letter were found to be significantly different 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 after pumping and exposure to freshwater can be seen in figure 2. There was a 0% reduction in *chalimus* stages immediately after the fish were pumped from the cage and passed over the grader and a 70.6% reduction in the average number after exposure to freshwater. For pre-adult stages, there was a 32.3% after pumping and a 71.8 % reduction after exposure to freshwater. For mature female stages, there was a 21.4% reduction after pumping and a 75.0% reduction in the average number present after exposure to freshwater. The percentage reduction for all combined stages of L.salmonis was 29.8% after pumping and 72.0 % after exposure to freshwater.

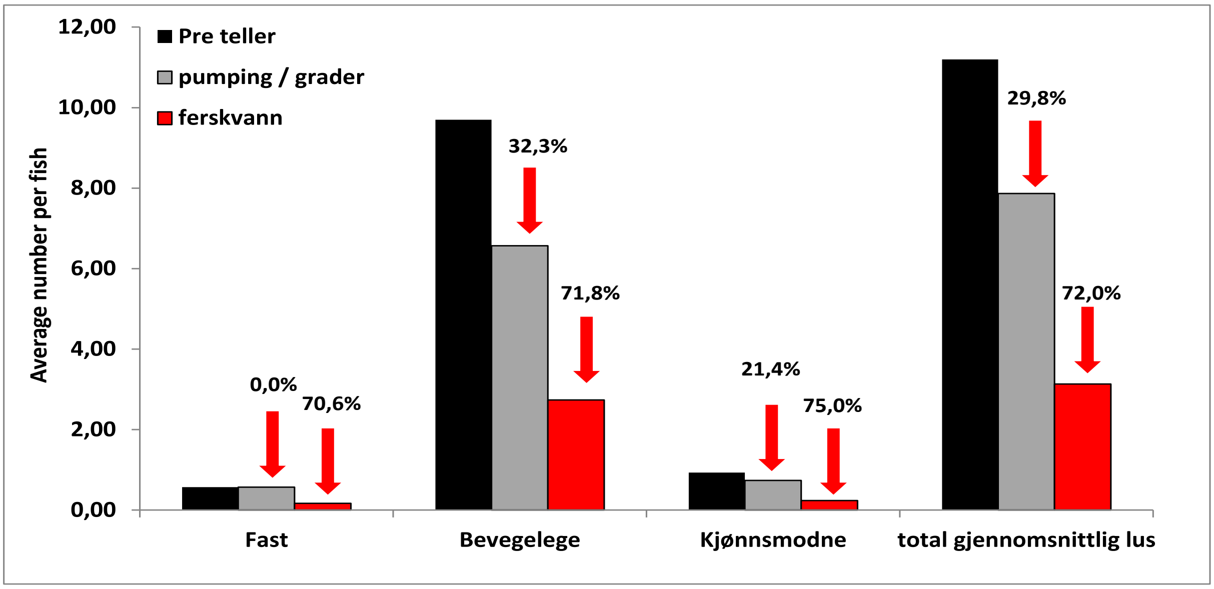

Figure 2 Percentage reduction in the average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after pumping and exposure to freshwater.

3.2 The effects of pumping and exposure to water with a salinity of 7.0 ppt on attached stages of L.*salmonis.*

The results from the fish exposed to water with a salinity of 7.0 ppt can be seen in figure 3. For *chalimus* stages there was a small but not significant reduction in the average of 0.57 per fish to 0.5 per fish immediately after the fish were pumped from the cage and passed over the grader on the well boat ($p > 0.05$). A similar trend was recorded after exposure to water with a salinity of 7.0 ppt for between one hour and 3 hours 20 minutes with a decrease in the average number per fish to 0.33 ($p > 0.05$). For pre-adult stages, there was a significant reduction from an average of 9.7 per fish to 5.77 per fish recorded immediately after the fish were pumped from the cage and passed over the grader and a further significant reduction to an average 4.97 per fish once exposed to freshwater ($F_{2, 87}$ 9.73; $p < 0.001$).

For mature female stages there was no reduction in the average of 0.93 per fish immediately after the fish were pumped from the cage and passed over the grader on the well and a small but not significant reduction the average number of mature females (0.63 per fish) after exposure ($p > 0.05$).

There was a significant reduction for all stages of L. *salmonis* counted immediately after the fish were pumped from the cage and passed over the grader. The average number of all attached stages decreased from 11.2 per fish to 7.2 per fish. In addition, there was a significant reduction after exposure to water with a salinity of 7.0 ppt for between one hour and 3 hours 20 minutes with the average number of all stages of L.*salmonis* decreasing to an average 5.93 per fish ($F_{2, 87}$ 11.00; $p < 0.001$).

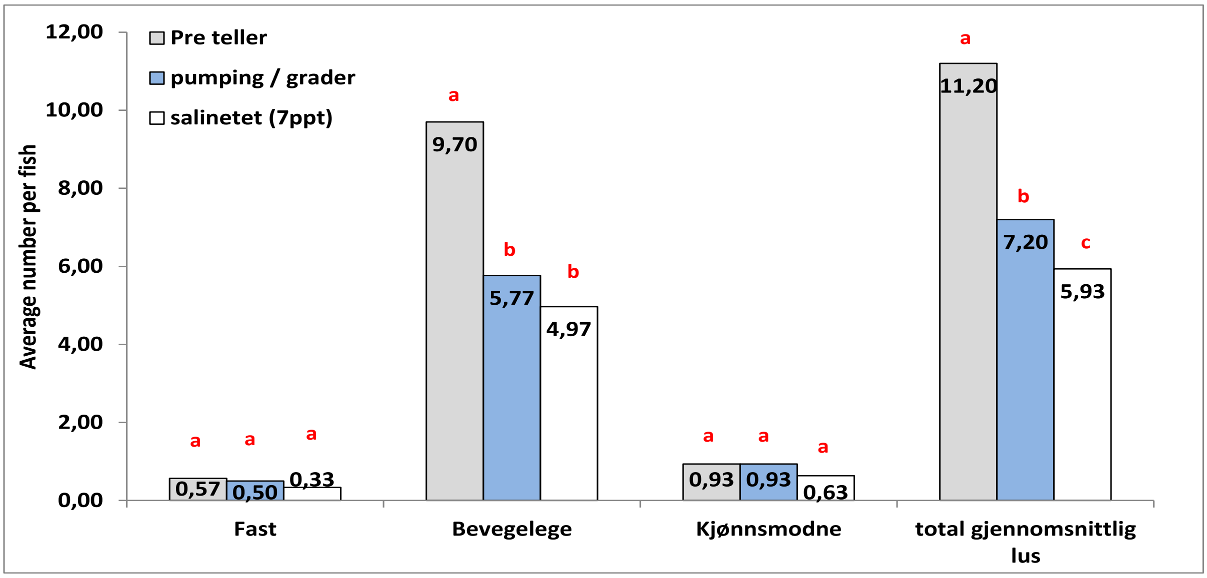

Figure 3 Average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after pumping and exposure to water with a salinity of 7.0 ppt. Values represent means ± S.D. Mean values which do NOT share a letter were found to be 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 4. There was a small reduction of 11.8% in *chalimus* stages immediately after the fish were pumped from the cage and passed over the grader and a 41.2% reduction in the average number after exposure to water with a salinity of 7.0 ppt. For pre-adult stages, there was a 40.5% after pumping and a 48.8 % reduction after exposure. For mature female stages, there was no reduction after pumping and a 32.1% reduction in the average number present after exposure to water with salinity of 7.0 ppt. The percentage reduction for all combined stages of L.salmonis was 35.7% after pumping and 47.0 % after exposure.

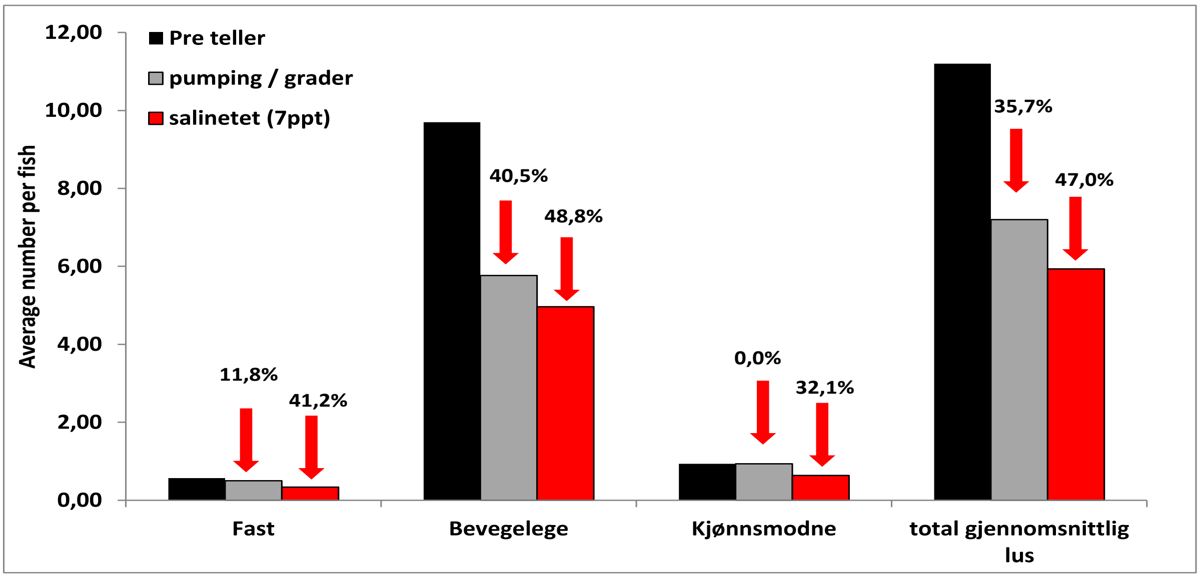

Figure 4 Percentage reduction in the average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after pumping and exposure to water with a salinity of 7.0 ppt.

3.3 Comparison of the percentage reduction in all attached stages of L.salmonis between after exposure to freshwater or water with a salinity of 7.0 ppt

The percentage reduction in the average number of *chalimus*, pre adult, mature females and total lice exposed to either freshwater or water at 7.0 ppt can be seen in figure 5.

For *chalimus* stages, there was a 41.2% reduction in the average number at 7.0 ppt whilst a 70.6% reduction was recorded in the average number for fish exposed to freshwater. For pre-adult stages, a similar trend was recorded with a 48.8% reduction for fish exposed to 7.0 ppt and a 71.8% reduction for fish exposed to freshwater. There was a lower percentage reduction in the average number of mature female lice per fish recorded for the fish exposed to 7.0 ppt compared to fish exposed to freshwater (32.1% and 75.0% respectively). For all stages of L.salmonis, there was an overall percentage reduction in the average number per fish of 47.0% for fish exposed to 7.0 ppt whilst fish exposed to freshwater attained a higher percentage reduction of 72.0% overall.

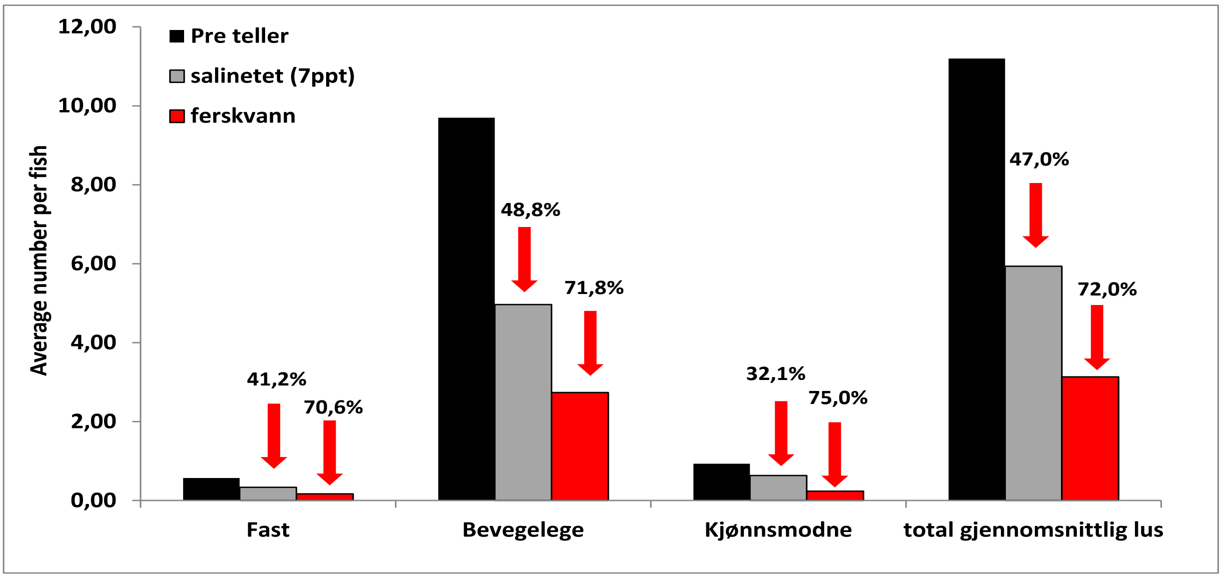

Figure 5 Percentage reduction in the average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after exposure to freshwater or water with a salinity of 7.0 ppt

3.4 Water quality

Water quality parameters recorded during the study are summarized in table 1.
During the study, oxygen saturation (%) and salinity (ppt) were periodically monitored in both wells once the fish had been transferred. Oxygen saturation decreased from 104.0% (recorded at thirty minutes after pumping had started) to 68.0% at 17:29 (thirty minutes prior to the end of the exposure time) in the well containing water at a salinity of 7.0 ppt. A similar reduction on oxygen saturation occurred in the well containing freshwater with saturation levels decreasing from 102.0% to 54.0% at the end of the exposure test period. The desired salinity levels were maintained in both wells throughout the whole exposure time period.
The temperature of the seawater was recorded at 6.7 ^{0}C and the temperature of both the water at 7.0 ppt and the freshwater was recorded at 5.4 and 5.2 ^{0}C respectively.
CO_2 levels increased from 0.0 mg/L in both wells to 17.0 and 12.0 mg/L in the 7.0 ppt and freshwater wells respectively at the end of the exposure period (18:05).
Calcium carbonate ($CaCO_3$) levels increased from 40.0 mg/L at 14:38 to 65.0 mg/L at 17:38 in the well containing 7.0 ppt and increased from 10.0 mg/L at 14:38 to 35.0 mg/L at 17.38 in the well containing freshwater.

3.4.1 Buffering with NaOH

NaOH at 50% solution was first added to the well containing 7.0 ppt at 16:20 (one hour 35 minutes after transferring of the fish had commenced. The 50% solution was added at a flow rate of 0.25 liters/hour. The pH of the water was recorded at 7.83 prior to commencement of pumping fish and had decreased to 7.58, 30 minutes after pumping the fish had started. The pH recorded when NaOH was first added to the well had decreased to 6.92.The rate of NaOH solution added to the well was increased to 10liters/hour at 17:35 (2 hours and 50 minutes after pumping had commenced). During the time between the two additions of NaOH solution, pH levels had slowly decreased from 6.92 to 6.41. The4 flow rate of NaOH was further increased to 15 liters/hour at 17:54 and the pH increased to 6.48 soon after.
For the well containing freshwater, NaOH solution (0.25 liters/hour) was first added at 16:24 (one hour 39 minutes after transferring of fish had commenced). The pH in this well had decreased from 7.92 prior to fish being introduced to 6.74 when the solution was first added to the well. There was a small decrease in pH two minutes after the solution was added followed by a small increase to 6.7 10 minutes after the addition of NaOH solution. pH slowly decreased from this point onwards to 6.48 when NaOH solution was added at an increased flow rate to 1 liter /hour at 17:16. The flow rate of NaOH solution was increased to 10 liters/hour at 17:35 followed by a further increase to 15 liters /hour at 17:54. During this period pH increased from 6.43 to 6.48.

Table 1 Water quality parameters recorded during the study period for each of the two wells.

Time	Notes	Styrbord rom – 7.0 promille					Babord rom - 0 promille				
		pH	Oxygen (%)	CO_2 mg/L	$CaCO_3$ mg/L	Salinity (ppt)	pH	Oxygen (%)	CO_2 mg/L	$CaCO_3$ mg/L	Salinity (ppt)
14:06		7,83	-	-	-	-	7,92	-	-	-	-
14:23		-	-	0.0	-	-	-	-	0.0	-	-
14:30		-	-	-	-	-	-	-	-	-	-
14:31		-	-	-	-	-	-	-	-	-	-
14:38		-	-	-	40.0	-	-	-	-	10.0	-
14:44	Tempratur styrbord: 5,4 ;Temp babord: 5,2	-	-	-	-	-	-	-	-	-	-
14:45	oppstart av fiskepumping inn i rommene	-	-	-	-	-	-	-	-	-	-
15:15		7,58	-	-	-	-	7,33	-	-	-	-
15:16		-	104.0	-	-	-	-	102.0	-	-	-
15:37		-	-	0.0	-	-	-	-	0.0	-	-
15:40		-	-	-	-	-	7,28	-	-	-	0.0
15:44		-	103.0	-	-	-	-	-	-	-	-
15:45		-	-	-	-	-	7,18	-	-	-	-
15:48		-	-	-	-	6,9	-	98.0	-		-
15:52		-	-	-	-	-	7,04	-	-	-	-
16:01		-	-	-	-	-	6,88	98.0	-	-	-
16:06	lagt lut slangen oppi lufter uten å slå på	-	-	-	-	-	6,92	-	-	-	-
16:20	kjørt 0,25l/h lut i 5 min i SB	6,92	-	-	-	-	-	-	-	-	-
16:21		-	-	-	-	-	-	97.0	-	-	-

16:24	**lut 0,25/h til BB**	**-**	**-**	**-**	**-**	**-**	**6,74**	**-**	**-**	**-**	**-**
16:26		**-**	**-**	**-**	**-**	**7,1**	**6,65**	**96**	**-**	**-**	**-**
16:34		**6,70**	**94.0**	**3.0**	**-**	**-**	**6,70**	**-**	**3.0**	**-**	**-**
16:42		**6,69**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
16:44		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
16:47		**-**	**-**	**-**	**-**	**-**	**6,62**	**88.0**	**-**	**-**	**-**
17:05		**-**	**-**	**-**	**-**	**-**	**6,48**	**76.0**	**-**	**-**	**-**
17:16	**lut 1/h til BB**	**6,53**	**-**	**-**	**-**	**-**	**-**	**68.0**	**-**	**-**	**-**
17:20		**6,54**	**77.0**	**13.0**	**-**	**-**	**-**	**-**	**11.0**	**-**	**-**
17:29		**-**	**68.0**	**-**	**-**	**-**	**-**	**63.0**	**-**	**-**	**-**
17:35	**Økt lut til 10 l/h**	**6,41**	**-**	**-**	**-**	**-**	**6,43**	**-**	**-**	**-**	**-**
17:38		**-**	**-**	**-**	**65.0**	**-**	**-**	**-**	**-**	**35.0**	**-**
17:40		**-**	**-**	**-**	**-**	**-**	**6,46**	**-**	**-**	**-**	**-**
17:44		**6,41**	**-**	**14.0**	**-**	**-**	**6,46**	**59.0**	**12.0**	**-**	**-**
17:50		**-**	**-**	**-**	**-**	**-**	**6,48**	**57.0**	**-**	**-**	**-**
17:54	**Økt lut til 15 l/h**	**-**	**-**	**-**	**-**	**-**	**-**	**56.0**	**-**	**-**	**-**
18:05	**Åpnet Bunnluker**	**6,48**	**-**	**17.0**	**-**	**-**	**6,48**	**54.0**	**12.0**	**-**	**0,4**

4.0 Discussion

4.1 The effects of pumping and exposure to reduced salinities on attached stages of L.*salmonis*

Results from the study showed that there were clear reductions in all stages of lice which parasitize Atlantic salmon after short-term exposure to freshwater and to a lesser extent after exposure to water with a salinity of 7.0 ppt.

The percentage reductions in the infectious attached stages attained after the fish were exposed to freshwater (0.0 ppt), 71%, 72%, 75% for *chalimus*, pre-adult and mature females stages of L. salmonis respectively were lower compared to previous studies undertaken at Gifas. The previous study undertaken in October 2013 involved the use of two different well-boats. For the first, 15.0 T of Atlantic salmon were exposed to freshwater whilst for the second, 110.0 T of salmon were treated. The percentage reductions attained during the first study were 98%, 91%, 91% for *chalimus*, pre-adult and mature females stages of L. salmonis respectively giving an overall reduction of 92% for all attached stages. Whilst for the second, the percentage reductions attained during the study were 100%, 78%, 86% for *chalimus*, pre-adult and mature females stages of L. salmonis respectively giving an overall reduction of 87% for all attached stages (Reynolds October 2013). In addition, the previous studies undertaken at Gifas had shown that *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 (Reynolds May 2013). However, the percentage reduction in the average number of *chalimus* stages after exposure to freshwater was calculated to be only 71.0% during the present study. This percentage reduction was found to be similar for pre-adult and mature female stages exposed to freshwater (72% and 75% respectively). The reason for these lower than expected reductions are unclear but may be partially explained by examining the differences between this study and those previous undertaken at Gifas.

One of the main differences between this present study and previous studies is the size of the fish used. This present study exposed 1 kg Atlantic salmon to freshwater whilst all previous studies have involved larger fish between 4 and 5 kg. It has been shown from previous studies that as the fish are being pumped from the polar circle to the well on the boat that there was a reduction in the average number of infectious stages immediately after pumping and before exposure to freshwater. The percentage reductions recorded for chalimus, pre-adult and mature female stages (Reynolds October 2013) were 77%, 30% and 14% respectively, giving a total reduction for all stages of 39%. Similar percentage reductions were observed from previous studies 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). The reductions in attached stages recorded immediately after the fish were pumped from the cage and before exposure to freshwater from these previous studies can be attributed to mechanical perturbation. Physical contact from crowding, contact with the inner surface of the pipes used to pump the fish and contact with the grading platform. The percentage reduction in infectious stages recorded after pumping and the fish passing over the grader during the present study were calculated to be 30.0% for all stages for the fish being transferred to the freshwater well and 36.0% for the fish being transferred to the 7.0 ppt well. These lower percentage reduction attributed to mechanical/physical effects of transferring the fish to the well boat may partially explain the lower than expected reductions recorded after exposure to freshwater. As the fish were smaller than those previously treated, they may have been subjected to less physical exposure to each other and also to the inner surfaces of the pipes and grader. Indeed, it was observed that the fish passing over the grader prior to entering the wells seemed to be subjected to much less physical perturbation compared to the larger fish used in the previous studies (picture 2). The grader systems used on commercial well-boats do vary in design and this may partially explain the differences recorded in infectious stages recorded during this study when compared to previous ones undertaken at Gifas.

(a)

(b)

Picture 2.Comarisson of two different grading systems used. (a) The grading system from the well-boat Romaster used during the studies undertaken in October 2013 and (b) the grading system used in present study.

It has been concluded from the previous studies that pumping and transferring the fish over the grader to the well plays an important role in the overall success of the treatment with freshwater. A percentage of the attached sea lice may be exposed to physical contact via the inner surfaces of the pipes, other fish and the surface of the grader resulting in damage and may be more susceptible to the reduced salinity conditions. It has been shown from the previous studies that that *chalimus* stages are more likely to be removed from salmon than the other later developed stages. This may

be partially explained due to the site of attachment as pre-adults and particularly mature female lice seem to preferentially choose attachment sites on areas where they are subjected to less mechanical and/or environmental perturbations (behind the dorsal, pectoral and anal fins). It may also be partially attributed to the fact that the later developed stages are more robust and can withstand greater mechanical stressors compared to *chalimus* stages. The result from this study showed little or no reductions in *chalimus* stages (0.0% for the fish being transferred to the freshwater well and 12.0% for the fish being transferred to the well containing 7.0ppt) after the fish had passed over the grader. These unexpected results may be explained to the point discussed above.
Another potential contributing factor may have been the density of the fish in the wells. There have been three well-boats used up to present during this series of studies with a varying biomass of fish used for each study. Table two shows the varying densities of fish for each of the different well-boats and corresponding parentage reductions attained after treatment. However, there does not appear to be any correlation between density of fish in the well and percentage reductions attained after treatment.

Table 2 Comparison of the density of Atlantic salmon for each of the different well-boats used and corresponding percentage reductions attained after treatment.

Date	Brønnbåt used	Well capacity (m^3)	Biomass of fish (T)	Density of fish per m^3	Percentage reduction of all stages
October 2013	Brudanes	325	15.2	48.0 kg	92.0%
October 2013	Romaster	1500	110.4	73.6 kg	87.0%
December 2013	Novatrans	600	30.3	50.5 kg	72.0%
December 2013	Novatrans	600	39.7	66.2 kg	47.0%

The percentage reductions in the infectious attached stages attained after the fish were exposed to water with a salinity of 7.0 ppt (42%, 49%, 32% for *chalimus*, pre-adult and mature female stages of L. salmonis respectively were much lower compared to the reductions attained from the fish exposed to freshwater. The data suggest that for freshwater to be an effective treatment in removing attached/infectious stages of L.salmonis from Atlantic salmon then salinities of less than 1.0 ppt must be used. Previous research has 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). It may be that for water with a salinity of 7.0 ppt to be successful in removing infectious stages of sea lice then longer exposure times may be required. The optimal exposure time for the removal of *chalimus*, pre-adult and mature female stages of L. salmonis appears to be around three hours based on previous studies.

4.2 Water quality and buffering

Results from the present study show that the method used to transfer the fish from the cage to both wells was successful in maintaining the salinities in both throughout the exposure period. There were no significant increases in salinity. However, there was a steady decrease in oxygen saturation during the period. It was not possible for the well-boat to diffuse oxygen in to either well as it did not have a super-saturation system on board. Oxygen concentration levels decreases to less than 54% three hours and twenty minutes after transferring of the fish had begun (table 1). At this point the study was stopped and seawater was pumped into the well to re-oxygenate and exchange the water in both wells. Previous studies have shown that oxygen concentration levels can be maintained or increased if such a system was available.

Carbon dioxide readings on board the well boat increased from 0.0 mg/L 17.0 mg/L in the well containing the water at 7.0 ppt and to 12.0 mg/L in the freshwater well during the treatment period. For carbon dioxide the safe criterion used for the Norwegian production of Atlantic salmon smolts is 15 mg L^{-1} (Fivelstad, S. 2013) provided dissolved oxygen concentrations are high. However, constant fish respiration can raise carbon dioxide levels high enough to interfere with oxygen intake by fish, in addition to lowering the pH of the water

The main aim of this study was to assess the potential of NaOH in maintaining safe levels of pH when treating a large biomass of salmon in freshwater for a define period of time. Previous studies have shown that pH levels steadily decreased during the exposure period and fish have shown signs of acute stress as a result of this steady decrease in pH. Without a buffering system, free carbon dioxide will form large amounts of a weak acid (carbonic acid) that may potentially decrease the pH level. Results from the study show that initially there was a steady but small decrease in pH in both wells once fish transfer had been complete and prior to the addition of NaOH. The addition of NaOH commenced approximately I hr. and 30 minutes after the fish had been transferred to both wells at a rate of 0.25 l/hr. The decline in pH slowed after the addition and in the well containing freshwater even increased slightly after 10 minutes post-addition. The decrease in pH levels continued however, as the rate at which NaOH was increased there were corresponding small increases in pH in both wells. The results from the study indicate that the addition of NaOH can assist in maintaining safe levels of pH throughout the treatment period but must be added much sooner at a higher rate to prevent pH levels falling too low. The total amount of NaOH added to each well was approximately 6.0 litres throughout the treatment period. Only one pump was used for both wells thus preventing complete control of the pH levels in each well. Further studies should involve the use of one pump per well and maximum flow rates of the pumps used should be up to 30l/hour to maintain good buffering potential. It is proposed that pH levels of between 6.8 and 7.0 should be maintained if possible throughout the treatment period which should be in the range of around three hours to achieve good clearance rates from the infectious stages of L.salmonis.

4.3 General points

The percentage reduction attained from both studies for all infective stages of sea lice found on Atlantic salmon exposed to freshwater (72%) 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) as there were an average of 0.25 mature female lice remaining on the fish after exposure whereas the reductions attained after exposure to water at 7.0 ppt would constitute a treatment failure. The rational for using water with a slight increase in salinity was to assess the potential for using less freshwater but maintaining good clearance rates. Reducing the capacity of freshwater required would allow for well-boats to spend less time filling the wells at source and lessens the amount required. However, as seen from the results, water with a slight increase in salinity does not appear to provide an effective treatment but given the lower than expected clearance rates attained with fish exposed to freshwater then perhaps further research is required to further elucidate this.

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 showed that there is potential for NaOH to be used as a buffering agent to control pH in wells filled with freshwater. However, further research is required to elucidate flow rates and how much to add to maintain safe levels throughout a desired treatment period of approximately three hours.

The study also highlighted the potential for grading systems to influence clearance rates and to some extent fish size may also play a contributing factor.

Water with a salinity of 7.0 ppt appears not to be an effective treatment medium however; further research is required to fully elucidate this.

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