

## 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 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 large biomass of Atlantic salmon to the treatment for a defined period of time.

Two studies were conducted at GIFAS large-scale facilities in Rossøya in October 2013 using Brønnbåten Brudanes (Havtrans AS) to perform the first study and Brønnbåten Romaster the second study. A pre-lice count from 30 fish was undertaken to establish present infection levels for both studies.

For the first study, 3004 Atlantic salmon with a mean weight of 5.06 kg (15.2 T) were pumped into a well containing freshwater. The fish were maintained in freshwater for a period of between 120 and 190 minutes. For the second study, approximately 22000 Atlantic salmon with a mean weight of 5.06 kg (110.4 T) were pumped into a well containing freshwater for between 35 and 135 minutes. For each study, after exposure 30 fish were taken from the wells and any attached sea lice were recorded.

For study one, the percentage reduction for *chalimus*, pre-adult and mature female stages of *L. salmonis* was 98%, 90% and 91% respectively giving an overall reduction for all stages of 92%. For study two, the percentage reduction for *chalimus*, pre-adult and mature female stages of *L. salmonis* was 100%, 78% and 86% respectively giving an overall reduction for all stages of 87%.

The most sensitive stages of *L. salmonis* appear to be the attached stages with percentage reductions of between 98 and 100% attained

The percentage reduction attained from both studies for all infective stages of sea lice found on Atlantic salmon exposed to freshwater (92% for study one and 87% for study two ) 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). The lower percentage levels of reduction recorded for study two may be attributed to a shorter exposure time to freshwater compared to fish exposed in study one (between 35 and 135 minutes for study two and 120 and 190 minutes for study one). The shorter exposure time was due to the study being terminated earlier than planned as the fish were showing signs of acute stress possibly due to potentially lowering pH levels. Results from previous studies undertaken at Gifas have shown that exposure times of between two and three hours have given excellent percentage reductions and this compares to the results recorded for study one.

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.

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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 associated with farming Atlantic salmon. 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.

Chemotherapeutant 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 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 those currently in use thus it is essential that alternative control measures are investigated. A potential treatment that merits further investigation is the use of freshwater to reduce lice infestations. In the past, a number of laboratory and field-based studies appeared to indicate that lower levels of salinity acted to reduce sea lice levels (Heuch 1995, Tucker *et al.* 2000, Bricknell *et al.* 2006, Bravo *et al.* 2008a Heuch *et al.* 2009) and recently several studies undertaken at Gifas support the sensitivity of sea lice when exposed to freshwater (Reynolds 2012, 2013). 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.

## Aims and Objectives

This is the third 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 study (report title: **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**) was the first study undertaken under semi-commercial conditions using using Brønnbåten Havtrans (Havtrans AS) to perform the study. A total of 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)). 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 *chalmus*, 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 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 biomass of Atlantic salmon to the treatment for a defined period of time.

## 2.0 Methods

### 2.1 Experimental design

Both studies were conducted at GIFAS large-scale facilities at Rossøya in October 2013 using Brønnbåten Brudanes (Havtrans AS) to perform the first study and Brønnbåten Romaster the second study.

#### 2.1.1 First study

For the first study, the well-boat had two wells: one filled with freshwater (0.16 ppt) and one filled with seawater (33.7 ppt). Freshwater was pumped into one well (325 m<sup>3</sup>) from Sundsfjord smolt the previous evening/early morning before arriving at the farm. A total of 3004 Atlantic salmon with a mean weight of 5.06 kg (15.2 T) were used in the study. The fish were pumped into the freshwater well from a 90m polar circle onto the well-boat (Picture 1). As the fish were pumped into the well, excess seawater was removed via a grid to ensure that the salinity of the freshwater was maintained during the study period.

The time required to transfer the fish into the well was recorded and transfer from first to last fish in took one hour and ten minutes (10:05 to 11:15am). Efficacy of treatment was assessed by undertaking a lice count at 13:15pm thus all fish were exposed to freshwater for between two hours and 3 hours 10 minutes. A lice count was performed on 30 fish which were netted directly from the well.



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

### 2.1.2 Second study

For the second study, the well-boat Romaster had two wells: one filled with freshwater (0.28 ppt) and one filled with seawater (33.7 ppt). Freshwater was pumped into one well (1500 m<sup>3</sup>) from Sundsfjord smolt during early morning before arriving at the farm. Approximately half the biomass in the cage (22000 Atlantic salmon with a mean weight of 5.06 kg) (110.4 T) were used in the study. The fish were pumped into the freshwater well from a 90m polar circle onto the well-boat (Picture 2). As the fish were pumped into the well, excess seawater was removed via a grid to ensure that the salinity of the freshwater was maintained during the study period.

The remaining fish were pumped into the well containing seawater for delousing with hydrogen peroxide.



Picture 2. The well-boat used in the second study and fish being pumped from the polar circle into the freshwater well on board.

The time required to transfer the fish into the well was recorded and transfer from first to last fish in took one hour and forty minutes (14:00 to 15:40pm). Efficacy of treatment was assessed by undertaking a lice count at 16:15pm thus all fish were exposed to freshwater for between 35 minutes and 2 hours 15 minutes. A lice count was performed on 30 fish which were netted directly from the well.

## 2.2 Registration of sea lice levels

For both studies, a lice count was undertaken the same day to assess the lice burden prior to treatment with freshwater

From the cage being used for the first study, 50 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 was also recorded. For the second study, 30 fish were sedated and all lice counted prior to exposure to freshwater. For both studies, immediately after exposure to freshwater, a lice count was undertaken on thirty fish which were netted directly from the well. . In addition, a lice count was undertaken on thirty fish immediately after pumping and removal of seawater to assess the mechanical effects of pumping fish in removing attached stages of *L. salmonis* from infected fish

For both studies lice were registered in 4 categories for all lice counts:

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

## 2.3 Water Quality

Oxygen saturation (%) and salinity (ppt) within each well was monitored throughout for both studies. In addition, for the second study, pH, CO<sub>2</sub>, NH<sub>3</sub>, NH<sub>4</sub> and total ammonia (TAN) were recorded periodically 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<sup>TM</sup> 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 Study 1

The results from the first study can be seen in figure 1. There was a significant reduction in all stages of sea lice counted. For *chalimus* stages there was a reduction from an average of 1.62 per fish to 0.03 per fish after exposure to freshwater for between 120 and 190 minutes ( $F_{1, 78} 51.74$ ;  $p < 0.001$ ). For pre-adult stages, there was a significant reduction from an average of 3.4 per fish to 0.33 per fish recorded ( $F_{1, 78} 87.60$ ;  $p < 0.001$ ) and for mature females a reduction from an average of 2.18 per fish to 0.2 per fish ( $F_{1, 78} 67.51$ ;  $p < 0.001$ ) when exposed to freshwater. The total



reduction for all stages of *L. salmonis* counted was from an average of 7.2 per fish to 0.57 per fish ( $F_{1,78} 230.82$ ;  $p < 0.001$ ).

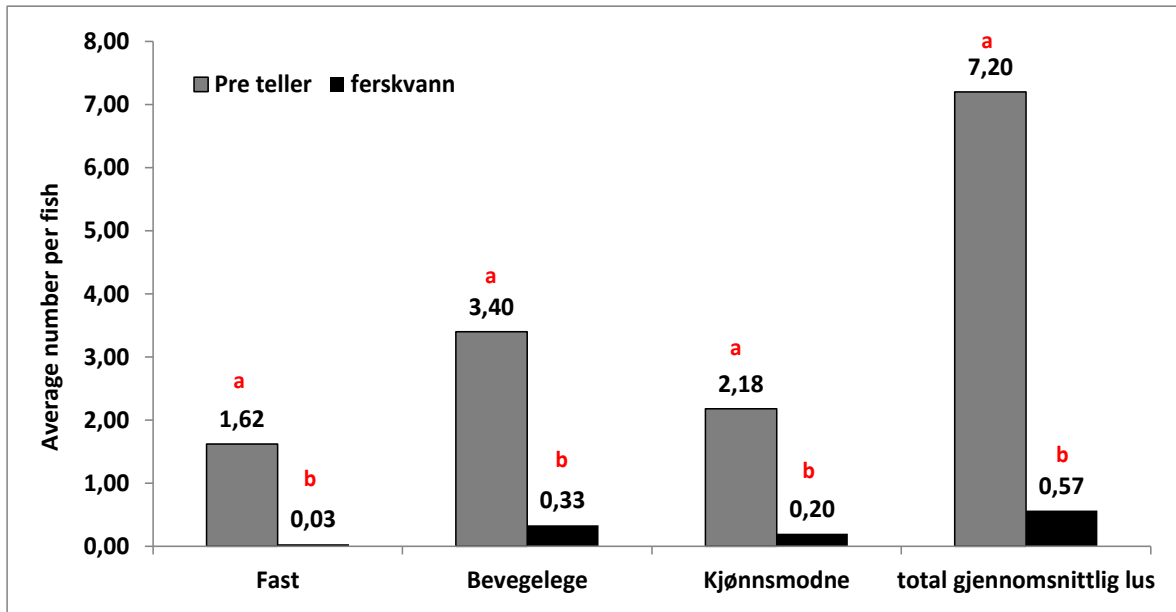


Figure 1 Average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after exposure to freshwater. Values represent means  $\pm$  S.D. Mean values which do NOT share a letter were found to be significantly different by 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 2. There was a 97.9% reduction in *chalimus* stages for fish exposed to freshwater. For pre adult stages and mature females exposed to freshwater there was a 90.2% and 90.8% reduction respectively. The percentage reduction in the total average number of all stages of *L. salmonis* was found to be 92.1% for fish exposed to freshwater compared to pre-count values.

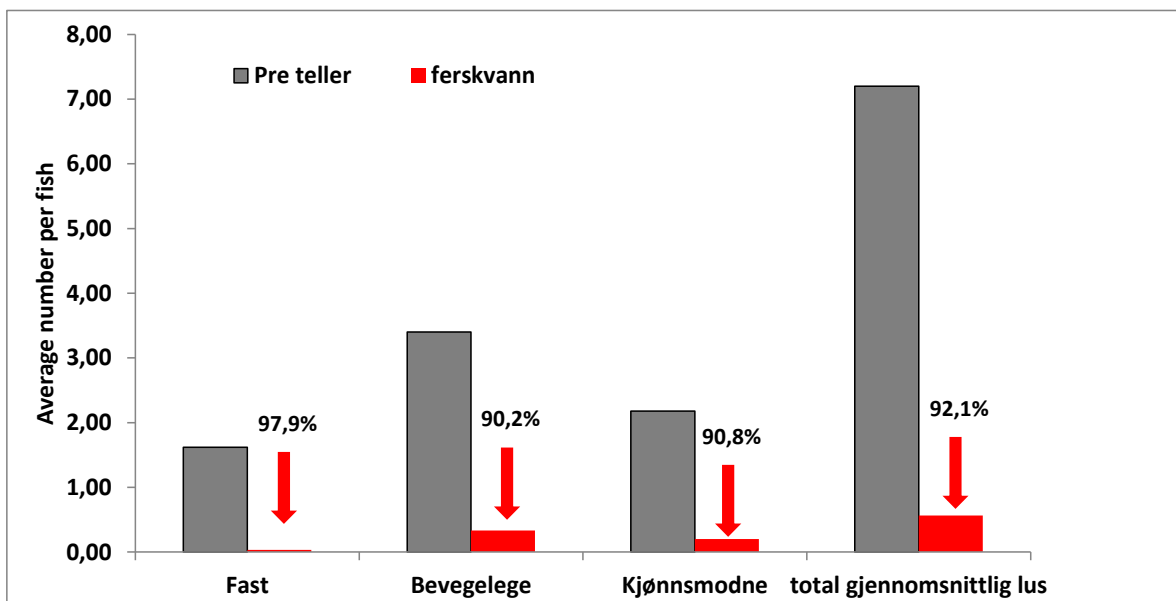


Figure 2 Percentage reduction in the average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment and after exposure to freshwater

### 3.2 Study 2

The results from the second study can be seen in figure 2. There were reductions in all stages of *L. salmonis* after pumping and transferring of fish before exposure to freshwater. For *chalimus* stages there was a significant reduction from an average of 2.03 per fish to 0.47 per fish after handling ( $F_{1,57} 21.33$ ;  $p < 0.001$ ). There were reductions in the average number of pre-adults and mature female stages (from 2.67 to 1.87 and from 2.17 to 1.87 respectively) but not significantly so ( $p > 0.05$ ). There was a significant reduction in the total average number of attached stages of sea lice after handling and pumping the fish from 6.87 per fish to 4.20 per fish ( $F_{1,57} 14.74$ ;  $p < 0.001$ ).

For fish exposed to freshwater, there were significant reductions in all stages of sea lice counted compared to infection levels prior to treatment. For *chalimus* stages there was a reduction from an average of 2.03 per fish to 0.0 per fish after exposure to freshwater for between 35 and 135 minutes ( $F_{1,50.03} 51.74$ ;  $p < 0.001$ ). For pre-adult stages, there was a significant reduction from an average of 2.67 per fish to 0.60 per fish recorded ( $F_{1,57} 29.97$ ;  $p < 0.001$ ) and for mature females a reduction from an average of 2.17 per fish to 0.3 per fish ( $F_{1,57} 47.41$ ;  $p < 0.001$ ) when exposed to freshwater. The total reduction for all stages of *L. salmonis* counted was from an average of 6.87 per fish to 0.90 per fish ( $F_{1,57} 118.25$ ;  $p < 0.001$ ).

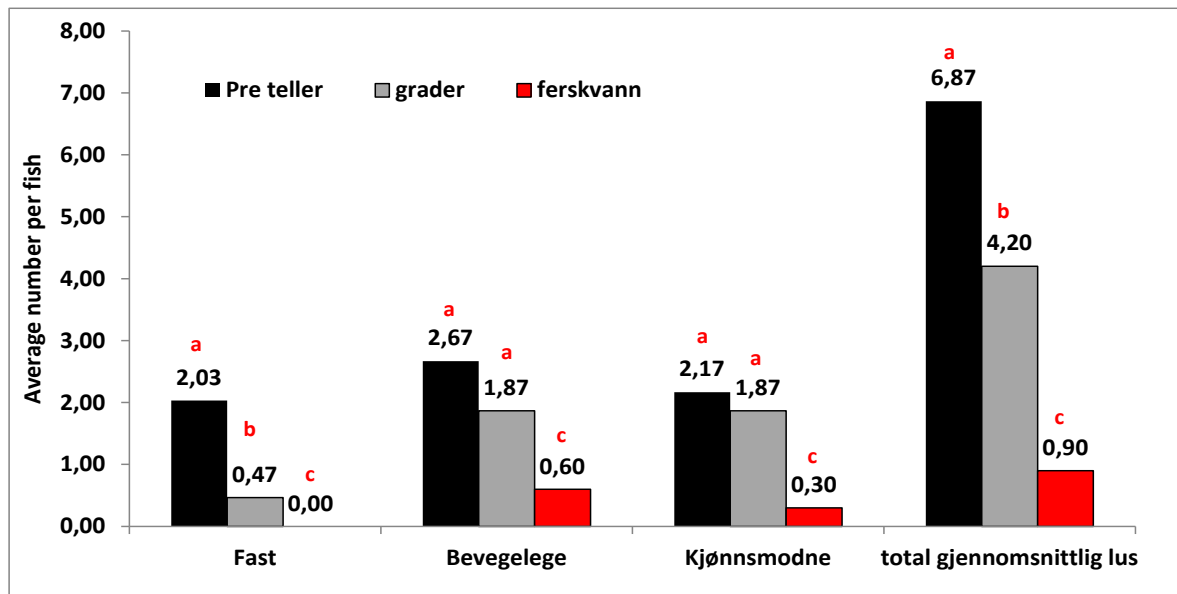


Figure 3 Average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment, immediately after pumping and after exposure to freshwater. Values represent means  $\pm$  S.D. Mean values which do NOT share a letter were found to be 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 77% reduction in *chalimus* stages immediately after pumping/handling. For pre-adult stages and mature female lice there was a reduction of 30% and 14% respectively after pumping/handling. The total reduction for all attached stages of lice counted after pumping/handling was calculated to be 39%.

The percentage reduction in the average number of *chalimus* stages after exposure to freshwater was 100% compared to pre-count values whilst reductions of 78% and 86% were recorded for pre-adult stages and mature female lice after freshwater exposure. The total percentage reduction in all attached stages for fish exposed to freshwater was calculated to be 87%.

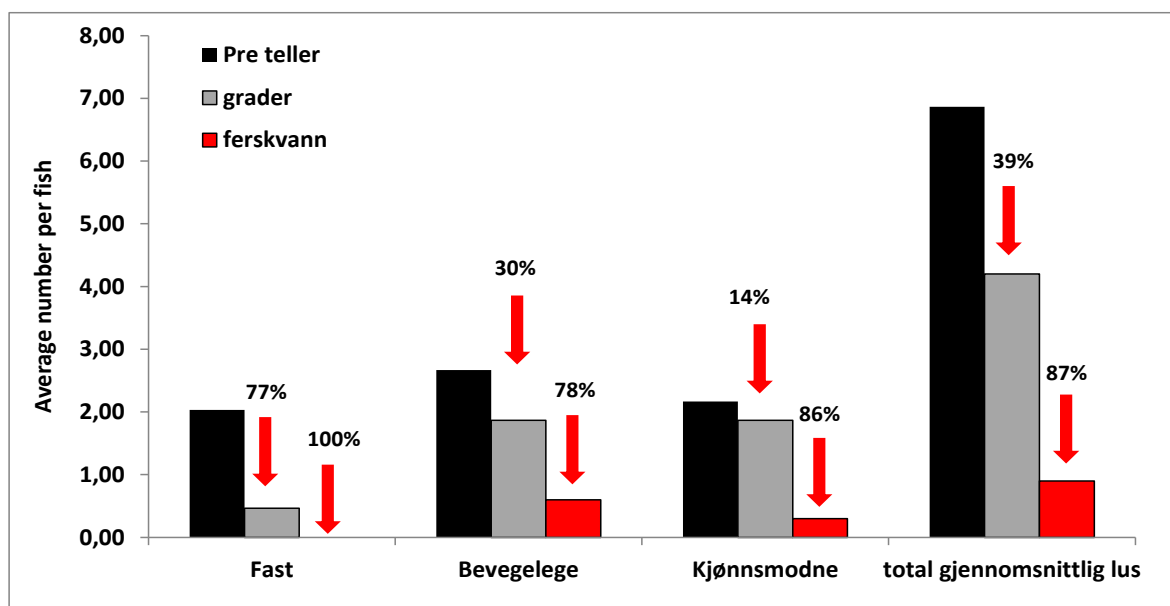


Figure 4 Percentage reduction in the average number of *Chalimus*, Pre-adult, mature female stages and all counted stages of *L. salmonis* per fish recorded prior to treatment, immediately after pumping and after exposure to freshwater.

### 3.3 Water quality

For study 1, Oxygen saturation (%) and salinity (ppt) were continually monitored by the crew of the well-boat throughout the study period (no data available). The temperature of the seawater was recorded at 10.0 °C and the temperature of the freshwater used in the well was recorded at 5.5 °C. The study was stopped at 13:10 as oxygen levels decreased to 48%. Fresh seawater was pumped into the well at this time.

For the second study, oxygen saturation, pH, CO<sub>2</sub> and ammonia levels were recorded at intervals throughout the exposure of fish in freshwater (pH, CO<sub>2</sub> and oxygen were dually monitored from both on-board and hand-held instruments whilst all others were recorded using hand-held monitoring equipment. All recorded data is summarized in table 1.

Table 1 Water quality parameters recorded during the second study.

Time	Notes	pH	pH (båt)	Oxygen (%)	CO <sub>2</sub> mg/L	CO <sub>2</sub> (båt) mg/L	Ammonia		
							NH <sub>3</sub>	NH <sub>4</sub>	TAN
13:45		7.1					0.25	0.27	0.21
14:00	Pumping starter av fisk								
14:25		7.15		124.0					
14:30							0.18	0.19	0.15
14:40		6.94		135.0					
14:42					1				
14:47							0.13	0.14	0.11
14:48		6.83		128.0					
14:51		6.92		119.0		19.1			
14:58		6.72		123.0					
15:16		6.44		109.0					
15:19	15,6 kg/h O2 added	6.3		96.0					
15:45			6.3	84.0		68.4			
16:00		6.06		101.0					

16:28		6.08		99.0				
16:30					16.0		0.46	0.49
16:35	Bunnventiler åpnet, Salinitet 8 promille							0.38
16:42					17.0			
16:48		6.42		81.0			0.45	0.48
								0.37

## 4.0 Discussion

### 4.1 Reduction in sea lice infestation levels

Results from both studies showed that there was a very clear reduction in all stages of lice which parasitize Atlantic salmon after short-term exposure to freshwater. In addition, these results are consistent with results from the first study using well-boats (Reynolds, May 2013). The most sensitive stages of *L. salmonis* appear to be the attached stages with percentage reductions of between 98 and 100% attained. In previous studies, 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 (Reynolds 2013). A similar trend was observed from a study in September 2011 where no *chalimus* were found on the fish after one hour 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). The results from this and previous studies suggest that *chalimus* stages of the sea lice life cycle are also extremely sensitive to short term exposure to freshwater. The data suggests that the earlier attached stages are more sensitive to freshwater exposure and any treatment that interrupts or removes a part of a parasite life cycle can be classed as a successful control agent.

There were also significant reductions in both pre-adult stages and mature females for both studies although not as high as the reduction recorded for attached stages. Although *chalimus* stages were shown to be more sensitive to freshwater, the percentage reductions recorded for both pre-adult and mature stages of *L. salmonis* show that freshwater exposure is also effective in removing these more developed stages. Indeed, results from the first study showed over 90% reduction for these stages and between 86 and 87% for the second study. Given the current problems with treatment failures noted for the bath treatments used presently, these percentage reductions may be classed as effective treatment clearance rates and freshwater can be used as an effective delousing agent on commercial-scale salmon farms.

For both studies, one of the aims of this project was to assess the potential for using freshwater under commercial-scale scenarios. For the first study, the total biomass exposed to freshwater was 15 tonnes whilst for the second study 110 tonnes of fish were exposed to freshwater. For the first study, the fish were sent for processing immediately after the study. There are was no accurate determination of mortality rates for this study however, reports from the processor suggest that fish mortality was as normally would be expected. For the second study which exposed a very large biomass of salmon to freshwater, mortality rates due to treatment effects were also difficult to determine as half the fish in the cage were treated with freshwater and the remainder treated with

hydrogen peroxide. Both groups were returned to the cage after treatment thus any mortalities recorded after cannot be attributed to either of the treatments. However, two days post-treatment there were approximately 100 mortalities removed from the cage. Given the total number of fish treated by both methods, total mortality can be assessed as less than 1% of the total population of fish.

#### 4.2 Mechanical and handling effects

The reductions in attached stages recorded immediately after the fish were pumped from the cage and before exposure to freshwater for study two 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 reductions recorded for *chalimus*, pre-adult and mature female stages 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). From the data, it can be seen 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.

#### 4.3 Water quality

For the first study, the method used to transfer the fish from the cage to the well containing the freshwater and removing excess seawater was successful in maintaining the low salinity of the freshwater 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 into the freshwater well as it did not have a super-saturation system on board. Oxygen concentration levels decrease to less than 50% three hours after transferring of the fish had begun. At this point the study was stopped and seawater was pumped into the well to re-oxygenate and exchange the freshwater in the well.

For the second study, the well-boat was fitted with a super oxygenation system and 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 (table 1). 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<sub>2</sub> 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<sup>-1</sup> (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 system may alleviate this problem. A potential option to prevent swings in pH is to add some type of carbonate buffering material such as calcium carbonate (CaCO<sub>3</sub>) or sodium bicarbonate (Na<sub>2</sub>CO<sub>3</sub>). Without a buffering system, free carbon dioxide will form large

amounts of a weak acid (carbonic acid) that may potentially decrease the pH level. Such additions will initially remove all free carbon dioxide and store it in reserve as bicarbonate and carbonate buffers. Storing carbon dioxide in the buffering system would prevent wide pH fluctuations.

Fish excrete ammonia and lesser amounts of urea into the water as wastes. Two forms of ammonia occur in aquaculture systems, ionized and un-ionized. The un-ionized form of ammonia (NH<sub>3</sub>) is extremely toxic while the ionized form (NH<sub>4</sub><sup>+</sup>) is not. Both forms are grouped together as "total ammonia. (TAN)" The recommended limit in Norway for the concentration that Atlantic salmon can tolerate is set at 2 milligram TAN per litre (Kolarevic *et al* 2012.). Concentrations of TAN recorded throughout the exposure period remained well below these recommended levels (ranging from 0.11 and 0.38 mg/L).

#### **4.4 General points**

The percentage reduction attained from both studies for all infective stages of sea lice found on Atlantic salmon exposed to freshwater (92% for study one and 87% for study two ) 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). The lower percentage levels of reduction recorded for study two may be attributed to a shorter exposure time to freshwater compared to fish exposed in study one (between 35 and 135 minutes for study two and 120 and 190 minutes for study one). The shorter exposure time was due to the study being terminated earlier than planned as the fish were showing signs of acute stress possibly due to potentially lowering pH levels. Results from previous studies undertaken at Gifas have shown that exposure times of between two and three hours have given excellent percentage reductions and this compares to the results recorded for study one.

There were differences between the temperature of the seawater (10.1 °C) and the freshwater contained within the well (5.5 °C) when study one was undertaken. This marked difference between seawater and freshwater may have contributed to the reduction in attached sea lice recorded. There is no current research that indicates that when the different stages of sea lice are exposed to abrupt changes in water temperature then survival is compromised. Further research is required to ascertain whether acute exposure to differing sudden changes in water temperature has an effect on sea lice survival.

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 realistic commercial scale conditions. 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 ‰ and that significantly high biomasses of salmon can be treated with well-boats filled with freshwater.

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 used for prolonged periods or re-used for treatment of several cages and a larger biomass of salmon at one given time-point.

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