Gildeskål Forskningsstasjon a.s

Ferskvannsavlusing i brønnbåt:

Elucidation of the effects of physical handling in removing attached sea lice from infested Atlantic salmon.

September 2015

FHF Project number: 901006

Preface

This study is part of the FHF funded project "*Bruken av ferskvann for å kontrollere infeksjoner av lakselus Lepeophtheirus salmonis K på atlantisk laks Salmo salar.*"

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A study was undertaken at Gifas facility Langholmen 2014 to fully elucidate the effects on physical removal of sea lice as fish are pumped/transferred from a cage to a well containing freshwater and back to the cage. If sea lice levels can be reduced by up to 40% due to the effects of physical contact before exposure to freshwater then short-term exposure to freshwater may be sufficient if the fish are pumped back into the cage after exposure using the same method. The aim of this study was to determine these physical effects over a range of exposure times from 15 minutes up to one hour.
The results from this study show clear reductions of all present infectious stages of L.*salmonis* after first and secondary handling. Results may also indicate that fish may be exposed to freshwater under commercial-scale conditions for shorter time periods than previous studies had shown as long as the fish are passed over grader systems before and after exposure to freshwater if the results are partially attributed to the stress of freshwater exposure on sea lice physiology.
Based on these results, this present study will attempt to fully elucidate the effects of physical handling in removing attached stages of L *salmonis* and whether reducing the exposure time in freshwater is practical under more realistic commercial-scale conditions by using well-boats.

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Abstract/Summary

The aim of this study was to fully elucidate the effects of physical handling in removing attached stages of L *salmonis* and whether reducing the exposure time in freshwater is practical under more realistic commercial-scale conditions by using well-boats. The study was conducted at GIFAS large-scale facilities at Leirvika in September 2015 using Brønnbåten Steigen (Norsk Fisketransport). Freshwater was pumped into one well from Sundsfjord smolt the previous evening/early morning before arriving at the farm site. Each well had a capacity of $450m^3$.
A total of 2616 Atlantic salmon with a mean weight of 4.17 kg (10.9 T) were used in the study. The fish were pumped into the freshwater well from a 90m polar circle cage. The freshwater well received all of the fish. Sea lice counts were undertaken during specific occasions on sedated Atlantic salmon.

The results from the study showed clear reductions of all stages of L.*salmonis* and C. *elongates* during the study period. Results indicate that pumping fish from a cage and/or passing fish over grader systems on well-boats has an independent physical effect in removing attached sea lice from Atlantic salmon compared to effects observed when exposed to freshwater. Overall reductions of 86.2% for all infectious stages of L.*salmonis* and 92.3% for C. *elongatus* were recorded after physical perturbation and three hours exposure to freshwater.
In addition, results indicate that some stages of L.*salmonis* and all stages of C. *elongatus* are sensitive when exposed to freshwater for up to three hours. Further, results indicate that overall reductions observed during the study period are directly related to a combined effects of both physical perturbation and exposure to freshwater.
The percentage reduction attained for all infective stages of sea lice found on Atlantic salmon exposed to freshwater (86.2%) would be considered to be a successful treatment outcome and infection levels would be below treatment thresholds imposed under Norwegian legislation.

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1.0 Introduction

A series of studies have been undertaken at Gifas to assess the potential for using freshwater to remove attached sea lice from infected Atlantic salmon. The studies started in 2011 and four studies have now been completed.

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.

The fourth study (report title: **Ferskvannsavlusing i brønnbåt: Study 4. Water quality. December 2013)** had the aim of assessing 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.

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.

The present studies have shown clear reductions in all infectious stages of *L. salmonis* from Atlantic salmon. However, the studies have also highlighted the need to fully elucidate the primary causal factors which contribute to these reductions.

A study was undertaken at Gifas facility Langholmen 2014 to fully elucidate the effects on physical removal of sea lice as fish are pumped/transferred from a cage to a well containing freshwater and back to the cage. If sea lice levels can be reduced by up to 40% due to the effects of physical contact before exposure to freshwater then short-term exposure to freshwater may be sufficient if the fish are pumped back into the cage after exposure using the same method. The aim of this study was to determine these physical effects over a range of exposure times from 15 minutes up to one hour. The results from this study show clear reductions of all present infectious stages of L.*salmonis* after first and secondary handling. Results may also indicate that fish can be exposed to freshwater under commercial-scale conditions for shorter time periods than previous studies had shown as long as the fish are passed over grader systems before and after exposure to freshwater if the results are partially attributed to the stress of freshwater exposure on sea lice physiology.

Based on these results, this present study will attempt to fully elucidate the effects of physical handling in removing attached stages of L *salmonis* and whether reducing the exposure time in freshwater is practical under more realistic commercial-scale conditions by using well-boats.

2.0 Methods

2.1 Experimental design

The study was conducted at GIFAS large-scale facilities at Leirvika in September 2015 using Brønnbåten Steigen (Norsk Fisketransport) (Picture 1). For the study, the well-boat had two wells: one filled with freshwater (0.7 ppt) and one filled with seawater. Freshwater was pumped into one well from Sundsfjord smolt the previous evening/early morning before arriving at the farm site. Each well had a capacity of 450m^3.

Picture 1. Brønnbåten Steigen (Norsk Fisketransport) used during the study.

A total of 2616 Atlantic salmon with a mean weight of 4.17 kg (10.9 T) were used in the study. The fish were pumped into the freshwater well from a 90m polar circle cage. The freshwater well received all of the fish.
As the fish were pumped into the well, excess seawater was removed when the fish passed over the grader to ensure that the salinity in the freshwater well 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 one hours and twenty minutes (10:47 to 12:07pm).

2.2 Registration of sea lice levels

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 study, 30 fish were sedated with Benzoak 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.
Sea lice were counted on 30 fish at the following points during the study period:

1. Immediately after the fish had been pumped from the cage and before they passed over the grader.
2. Immediately after the fish had passes over the grader and before transfer to the freshwater well.
3. Separate lice counts were undertaken after 1 hour, two hours and three hours exposure to freshwater.
4. At three hours post-freshwater exposure and immediately after the fish had passed over the grader for a second time before being transferred back to the cage.

After counting has been complete on each of these occasions, any lice remaining in the container was also recorded.
All lice present on the fish were registered in the following categories for all lice counts:

- *Lepeophtheirus salmonis*: Adult female
- *Lepeophtheirus salmonis*: Adult males
- *Lepeophtheirus salmonis*: Preadult stages

- *Lepeophtheirus salmonis*: Chalimus stages
- *Caligus elongatus* (combined all stages).

2.3 Fish welfare

All fish were continually monitored throughout the experimental period for signs of stress using cameras situated inside the wells.

2.4 Water Quality

Oxygen saturation (%), salinity (ppt), temperature (^{o}C) and pH within the freshwater tank was monitored routinely throughout for each exposure study.

2.5 Statistics

For lice counts statistical significance of differences were computed from one-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 Average number of *chalimus* stages of *L.salmonis* per fish.

The average number of *chalimus* stages of L.*salmonis* per fish recorded at each of the periods when lice counting was undertaken can be seen in figure 1. There were less *chalimus* stages recorded after the fish were pumped from the cage and before passing over the grader (average of 0.4 per fish) and after the fish had passed over the grader before entering the freshwater well (0.23 per fish) compared to values obtained prior to treatment (pre-count: average of 0.47 per fish). After one, two and three hour's exposure to freshwater, the average number of *chalimus* stages of L.*salmonis* per fish were calculated to be 0.27, 0.17 and 0.30 respectively. After freshwater exposure and immediately after the fish had passed over the grader prior to being transferred back to the cage, the average number of *chalimus* stages of L.*salmonis* per fish was found to be 0.10. At no time were the differences recorded significantly so ($F_{6, 203}$; 1.50 $p > 0.05$).

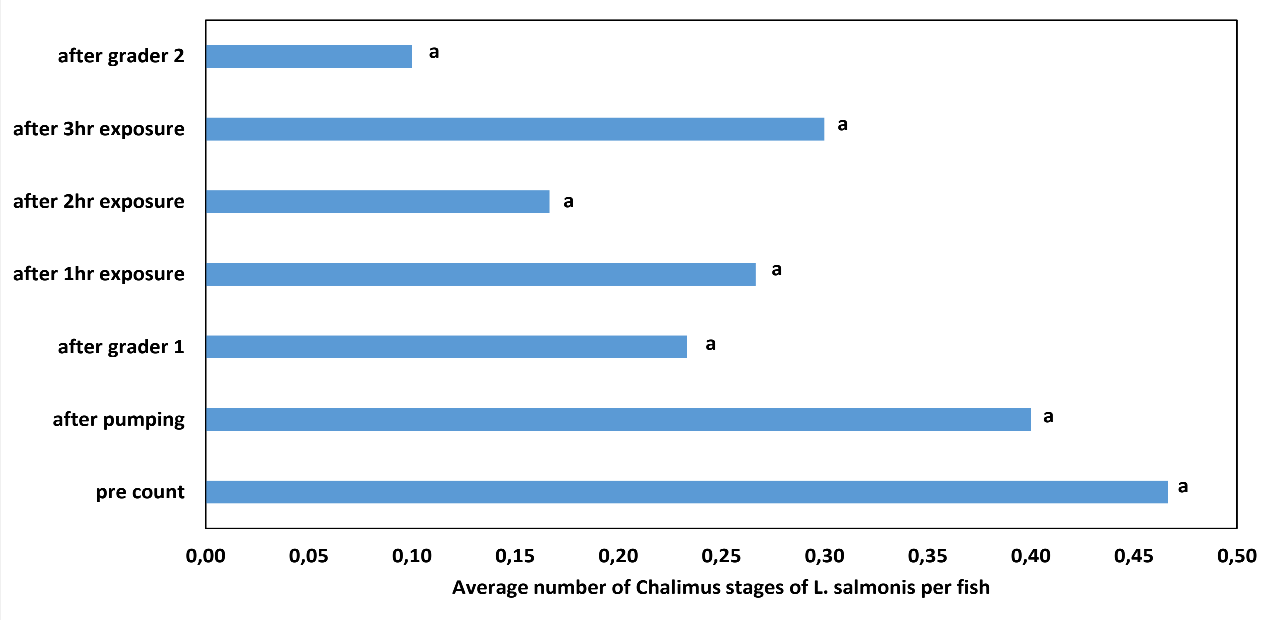

Figure 1 Average number of *Chalimus* stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken. Values represent means. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

The percentage differences in the average number of *chalimus* stages of L.*salmonis* per fish compared to the pre-treatment count at each of the periods when lice counting was undertaken can be seen in figure 2.there was a 14.3% reduction in the average number of *chalimus* stages immediately after the fish were pumped from the cage and a 50.0% reduction after the fish had passed over the grader compared to the pre-treatment count. After one, two and three hour's exposure to freshwater, percentage reductions compared to pre-treatment counts were calculated to be 42.9, 64.3 and 35.7%. After the fish had passed over the grader for a second time after three hours exposure to freshwater, the percentage reduction in the average number of *chalimus* per fish compared to the pre-treatment count was calculated to be 78.6%.

3.2 Average number of pre-adult stages of L.salmonis per fish

The average number of pre-adult stages of L.*salmonis* per fish recorded at each of the periods when lice counting was undertaken can be seen in figure 3 with percentage differences at each of the periods when lice counting was undertaken can be seen in figure 4. There was significantly less pre-adult stages at each of the periods when lice counts were undertaken compared to the pre-treatment count (3.70 per fish) ($F_{6, 203}$; 13.50 $p < 0.001$). After the fish were pumped from the cage and before passing over the grader, the average number of pre-adult stages per fish was found to be 60.4% less compared to the pre-treatment count (3.70 and 1.47 per fish respectively). After the fish had passed over the grader and before entering the freshwater well, there were 79.3% less pre-adult stages per fish compared to the pre-treatment count. The average number of pre-adult stages obtained after one, two and three hour's exposure to freshwater were found to be 0.57, 0.83 and 0.27 per fish. These values were 84.7, 77.5 and 92.8% less compared to the pre-treatment count. After the fish had passed over the grader for a second time after three hours exposure to freshwater, the percentage reduction in the average number of pre-adult stages per fish compared to the pre-treatment count was calculated to be 94.6% less (average number of pre-adult stages per fish of 0.2 compared to 3.7 per fish respectively).

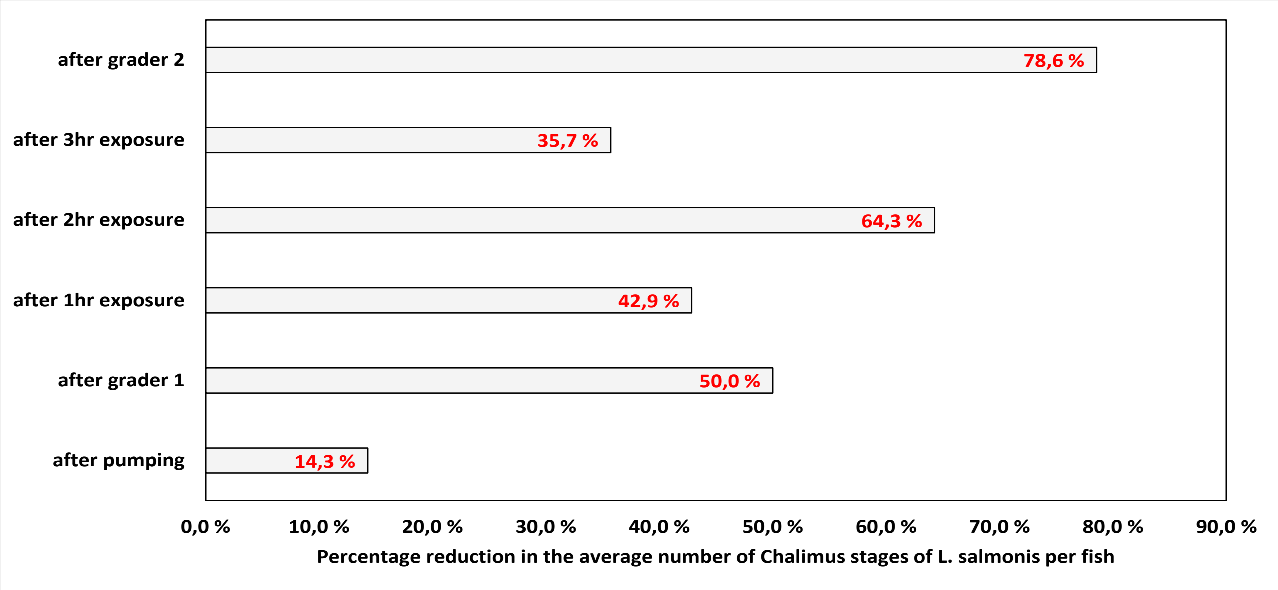

Figure 2 Percentage reduction in the average number of *Chalimus* stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken.

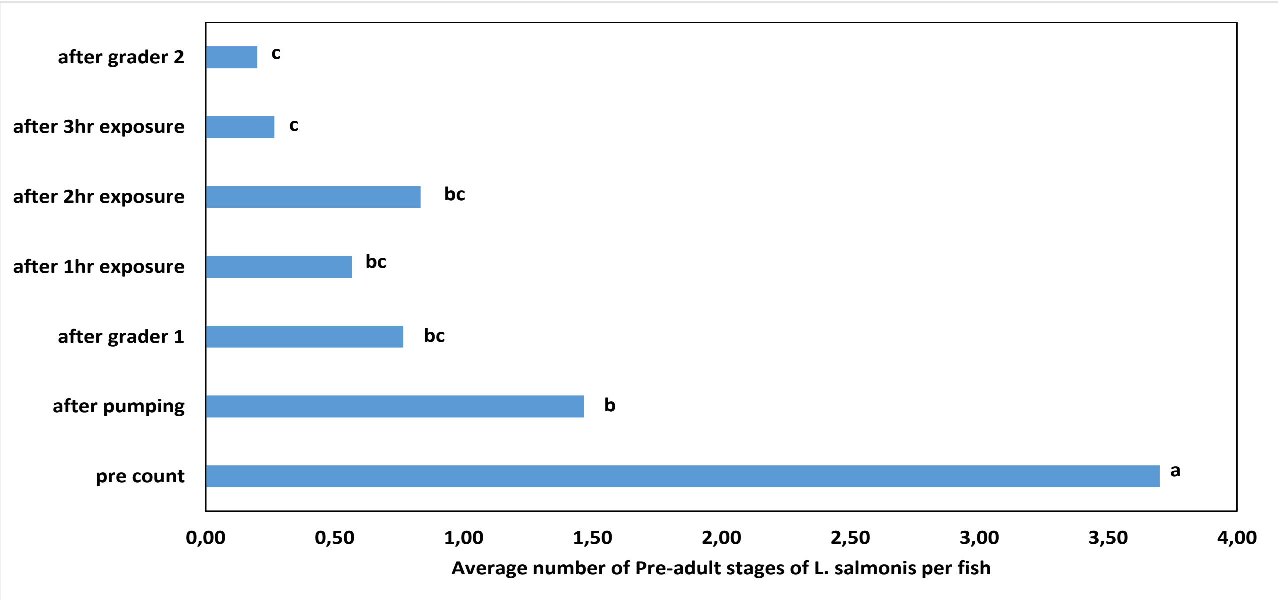

Figure 3 Average number of pre-adult stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken. Values represent means. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

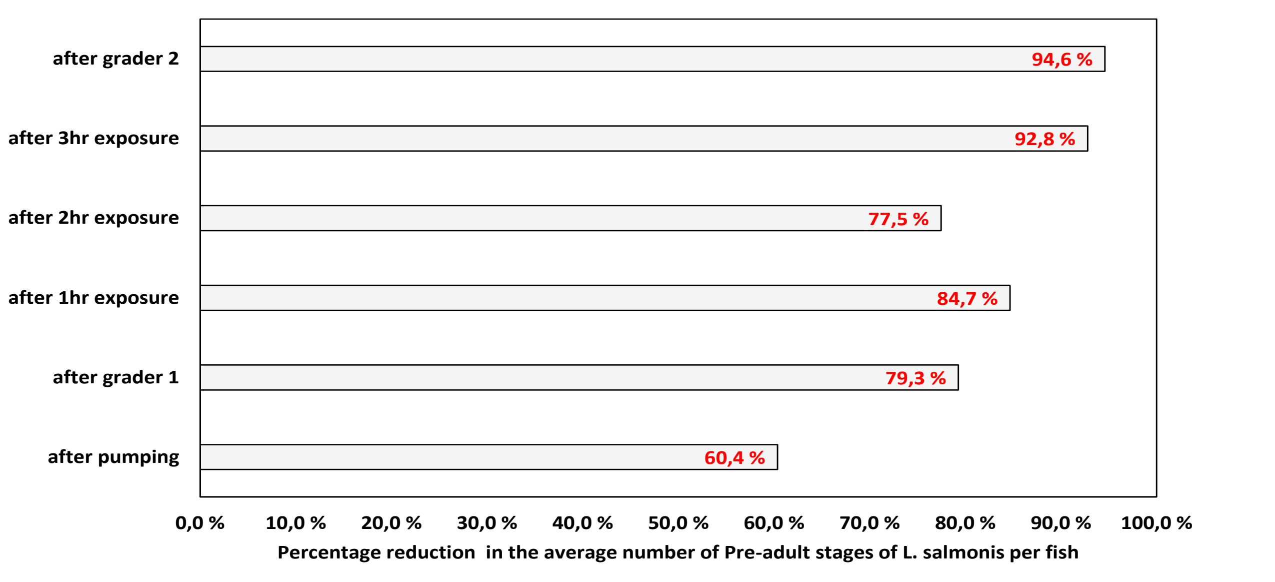

Figure 4 Percentage reduction in the average number of Pre-adult stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken.

3.3 Average number of mature male stages of L.salmonis per fish

The average number of mature male stages of L*.salmonis* per fish recorded at each of the periods when lice counting was undertaken can be seen in figure 5 with percentage differences at each of the periods when lice counting was undertaken can be seen in figure 6.

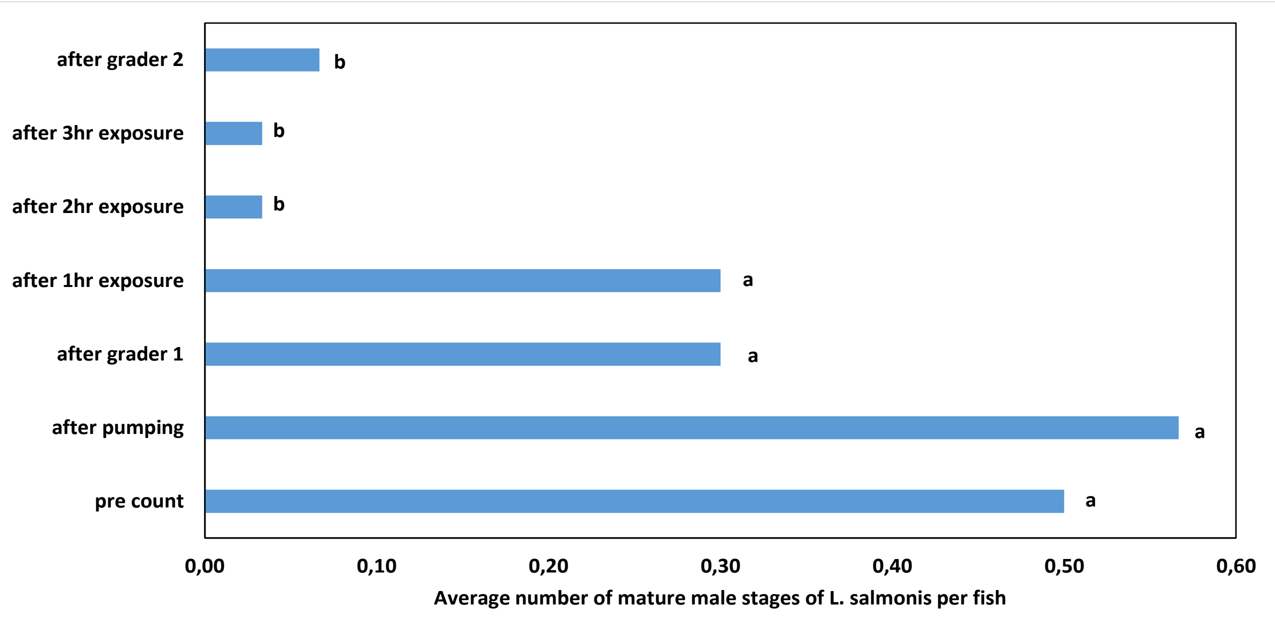

Figure 5 Average number of mature male stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken. Values represent means. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

There were no significant differences the average number of mature male stages of L. salmonis after the fish had been pumped from the cage (0.57 per fish), after passing over the grader (0.3 per fish) and after one hour exposure to freshwater (0.3 per fish) compared to pre-count values (0.5 per fish) ($p > 0.05$). There were no percentage reduction after pumping and 40% reduction both after passing over the grader and after one hour exposure. There were significantly less mature male lice present on the fish after two and three hours exposure to freshwater (0.03 per fish) ($F_{6, 203}$; 2.64 $p < 0.05$) compared to pre-treatment values. This represents a reduction of 93.3% compared to the pre-treatment count. There were significantly less mature male lice per fish after freshwater exposure and passing over the grader for a second time ($p < 0.05$) compared to the pre-treatment count but more compared to values obtained after two and three hours exposure to freshwater. The overall percentage reduction in mature male lice per fish at the end of the treatment was calculated to be 86.7% compared to the pre-treatment value.

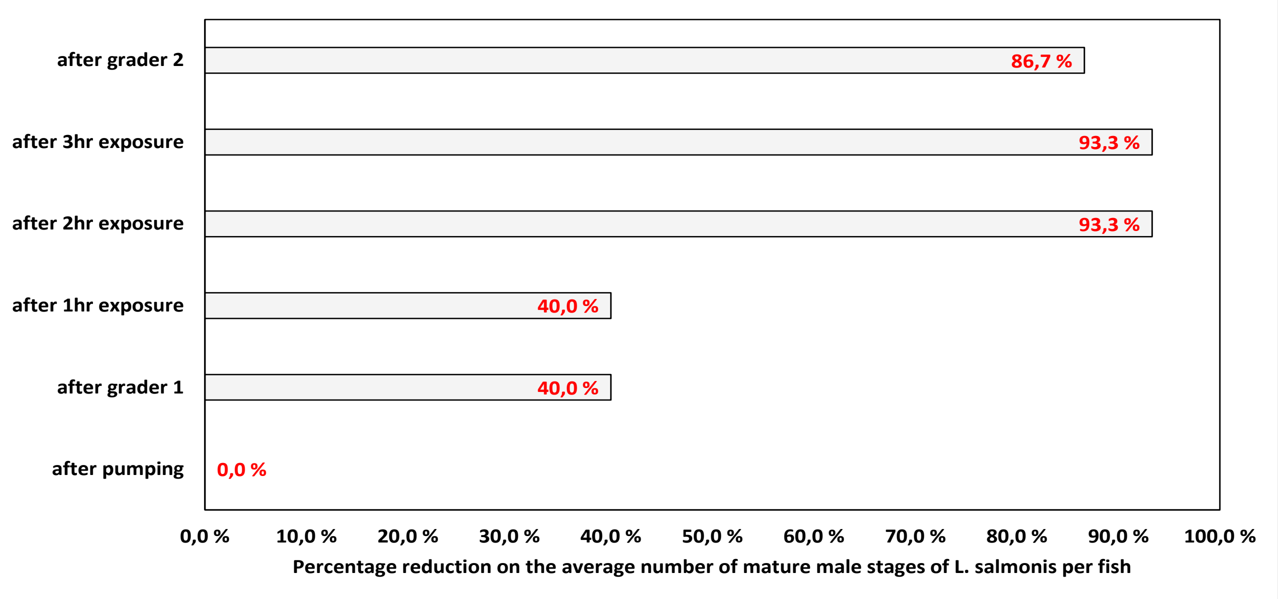

Figure 6 Percentage reduction in the average number of mature male stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken.

3.4 Average number of mature female L.salmonis per fish

The average number of mature female stages of L.*salmonis* per fish recorded at each of the periods when lice counting was undertaken can be seen in figure 7 with percentage differences at each of the periods when lice counting was undertaken can be seen in figure 8.

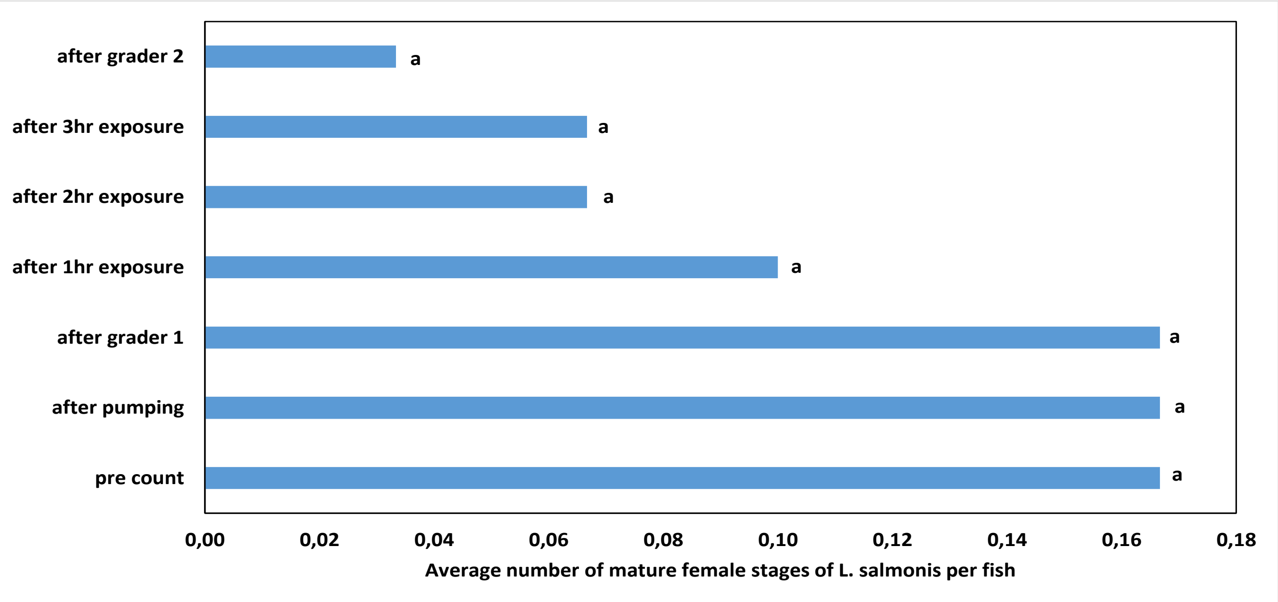

Figure 7 Average number of mature female stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken. Values represent means. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

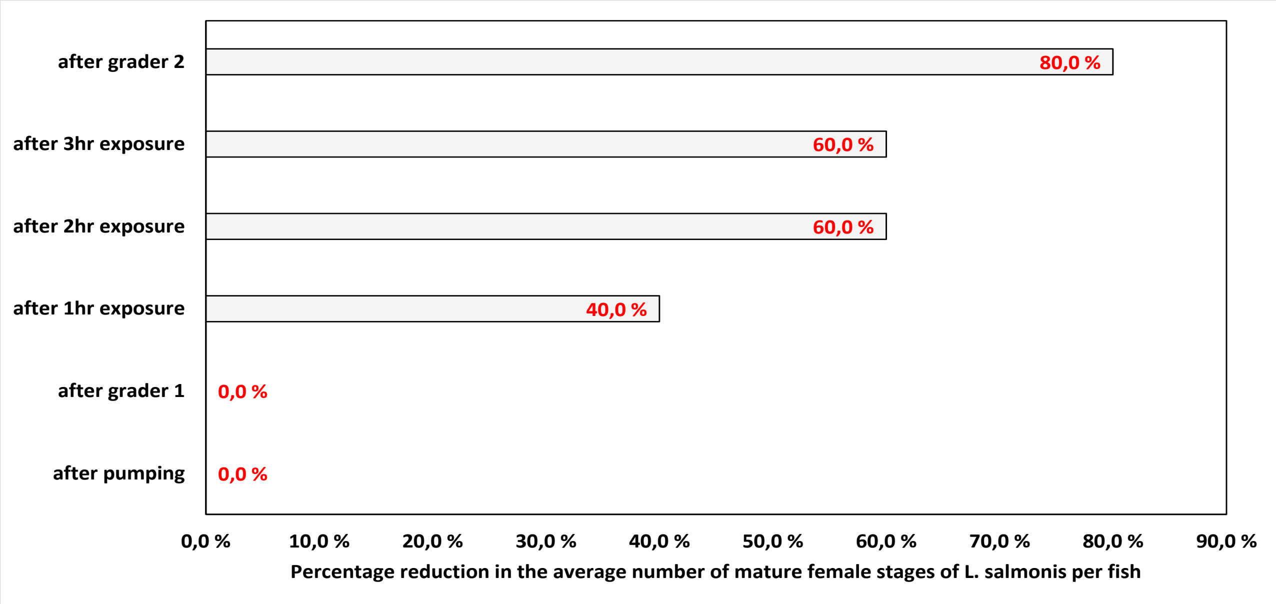

Figure 8 Percentage reduction in the average number of mature female stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken.

There were no significant differences the average number of mature female stages of L. salmonis after the fish had been pumped from the cage (0.17 per fish), after passing over the grader (0.17 per fish) and after one hour exposure to freshwater (0.10 per fish) compared to pre-count values (0.17 per fish) ($p > 0.05$). The values calculated after one hour exposure to freshwater represented a difference of 40.0% compared to the pre-count value.
There were less average numbers of mature female lice per fish after two and three hour's exposure to freshwater (0.07 per fish) compared to the pre-count value but not significantly so ($p > 0.05$). After the fish had passed over the grader at the end of the exposure period the average number of mature female L. salmonis per fish was calculated to be 0.03 per fish which was 80.0% less compared to the pre-count value (0.17 per fish).

3.5 Average number of all stages of *L.salmonis* per fish

The average number of all stages of L.*salmonis* per fish recorded at each of the periods when lice counting was undertaken can be seen in figure 9 with percentage differences at each of the periods when lice counting was undertaken can be seen in figure 10.
The average number of L. salmonis per fish calculated after the fish were pumped from the cage and before passing over the grader was significantly less (2.6 per fish) compared to the pre-count value (4.83 per fish) ($F_{6, 203}$; 11.0 $p < 0.001$). This represents a reduction of 46.2%.
There were significant continual reductions ($p < 0.001$) after the fish had passed over the grader (1.47 per fish); after one hour exposure to freshwater (1.23 per fish) and after two hours exposure to freshwater (1.10 per fish) compared to the pre-count value. These values represent percentage reductions of 69.7%, 74.5% and 77.2% compared to the pre-count value. After three hours exposure to freshwater the average number of L.*salmonis* per fish was calculated to be 0.67 which represents a percentage reduction of 86.2% compared to the pre-count value.
At the end of the treatment period when the fish had passed over the grader for a second time, the average number of sea lice per fish was calculated to be the same as for the three hour exposure count (0.67 lice per fish).

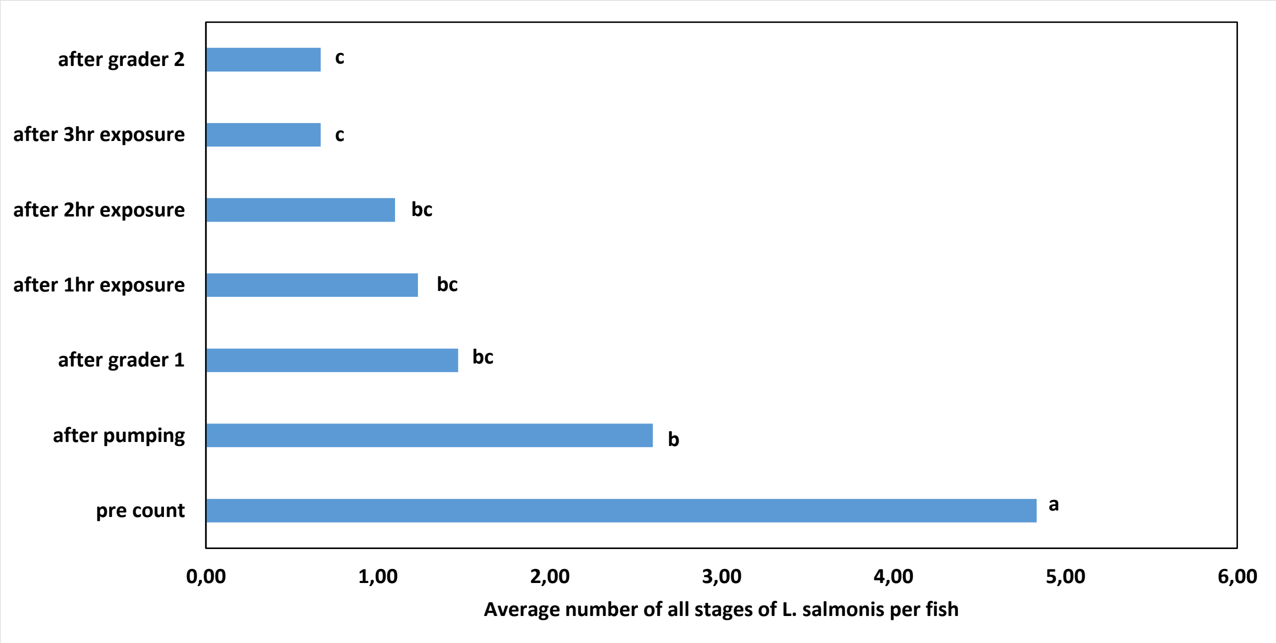

Figure 9 Average number of all stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken. Values represent means. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

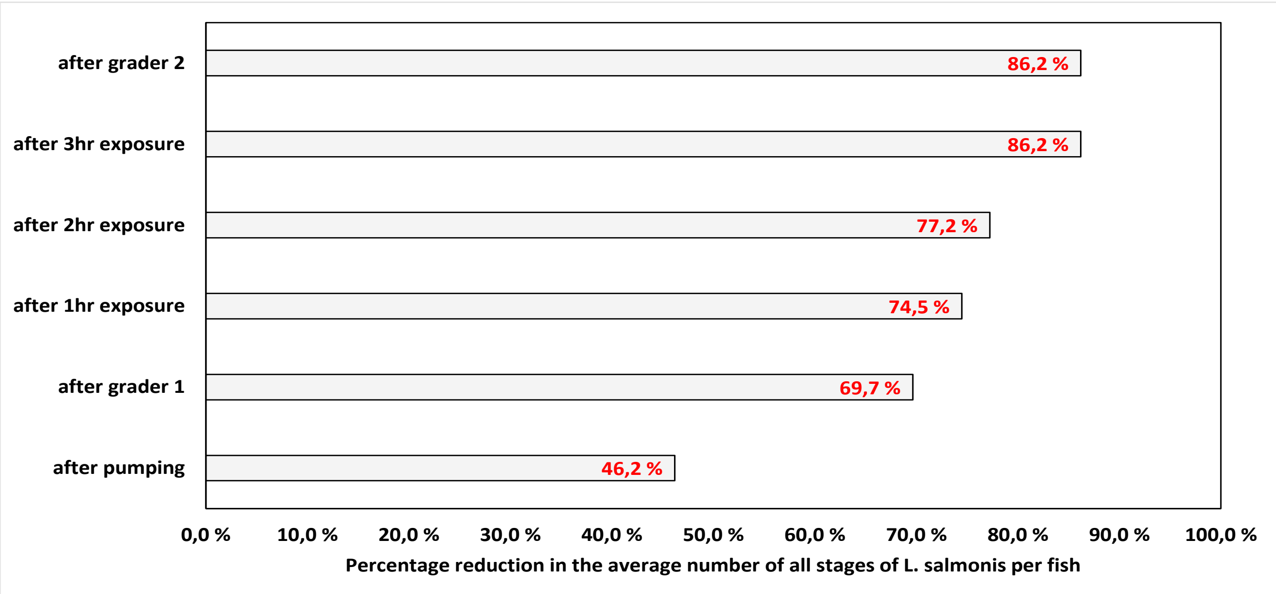

Figure 10 Percentage reduction in the average number of all stages of *L. salmonis* per fish recorded at each of the described time points where lice counting was undertaken.

3.6 Average number of all stages of *C. elongatus* per fish

The average number of all stages of C. *elongatus* per fish recorded at each of the periods when lice counting was undertaken can be seen in figure 11 with percentage differences at each of the periods when lice counting was undertaken can be seen in figure 12.

There was a 46.2% reduction in the average number C. *elongatus* per fish recorded after the fish were pumped from the cage and before passing over the grader (0.23 per fish) compared to the average number recorded at the pre-count (0.43 per fish) but not significantly so. There was a significant reduction (92.3%) after the fish had passed over the grader before freshwater exposure compared to the pre-count value ($F_{6,\ 203}$; 4.75 $p < 0.001$). Significant reduction in the average number of C. *elongatus* per fish were recorded after one hour (92.3%) and two hours (100%)

exposure to freshwater ($p < 0.001$) compared to the pre-count value. There were C. *elongatus* present on the fish counted after three hours exposure and after the fish had passed over the grader for the second time (0.07 and 0.03 per fish respectively). The overall percentage reduction after the treatment period was calculated to be 92.3% compared to the pre-treatment value.

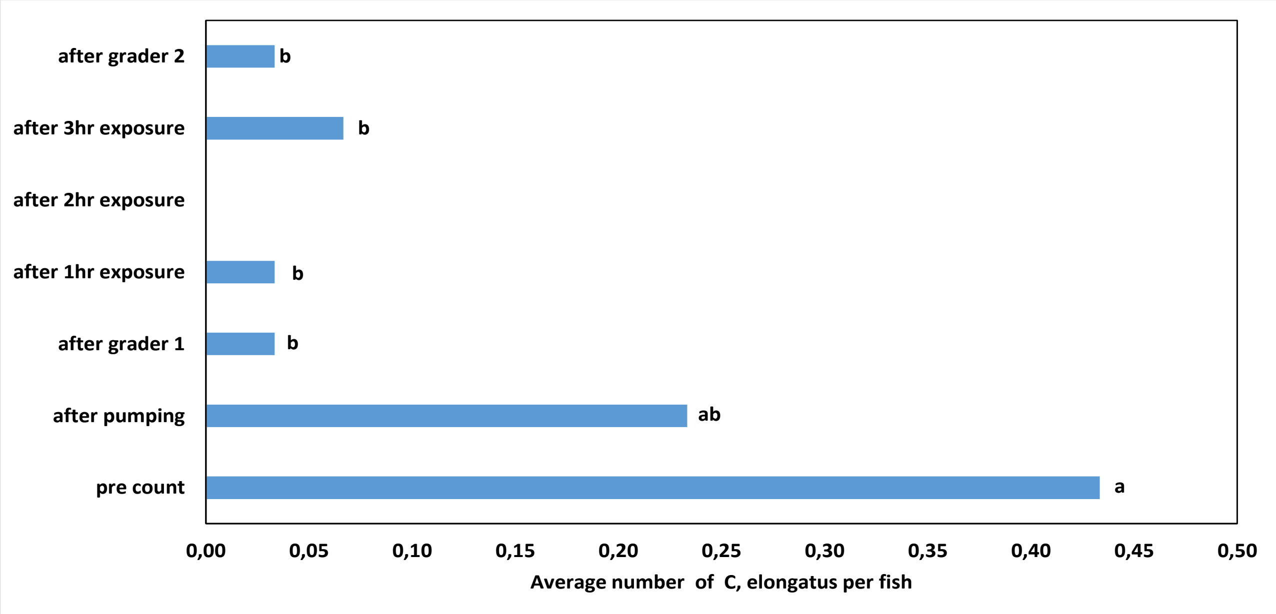

Figure 11 Average number of all stages of *C. elongatus* per fish recorded at each of the described time points where lice counting was undertaken. Values represent means. Mean values which do NOT share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test.

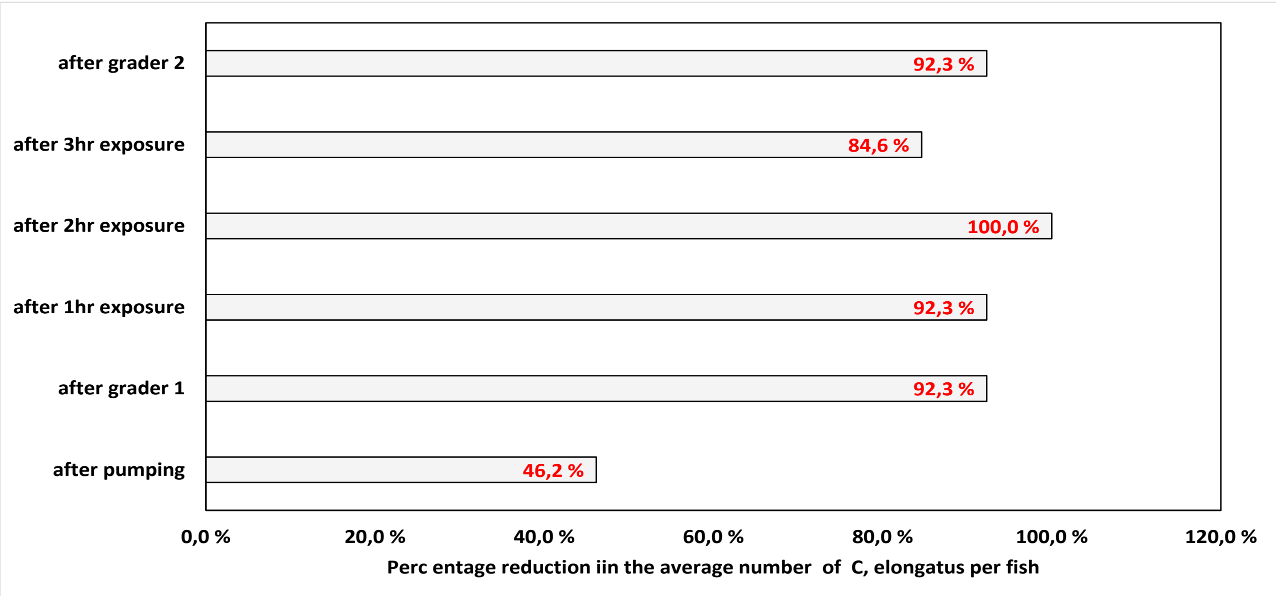

Figure 12 Percentage reduction in the average number of all stages of *C. elongatus* per fish recorded at each of the described time points where lice counting was undertaken.

3.7 Water quality

Water quality parameters recorded during the experimental period cane be seen in table 1. Oxygen saturation (%) decreased from 99.3% at the start to 81.4% at the end of the study period. Salinity increased slightly from 0.03 ppt at the start of the study to 0.08 ppt at the end. Water temperature remained constant throughout the study period (10.8 ^{0}C).

Table 1 Water quality parameters recorded during the study period

Time	Oxygen (%)	Salinity (ppt)	Temperature (^{0}C)
10.26	99.3	0.03	10.8
11.33	91.8	0.08	10.8
11.50	84.8	0.08	10.8
12.57	75.9	0.08	10.8
13.12	77.3	0.08	10.8
14.03	78.5	0.08	10.8
15.00	81.4	0.08	10.8

4.0 Discussion

Previous well-boat studies using a freshwater bath treatment undertaken at Gifas have shown clear reductions in all infectious stages of *L. salmonis* from Atlantic salmon. However, the studies had also highlighted the need to fully elucidate the primary causal factors which contributed to these reductions. The main aim of this study was to elucidate the effects of physical perturbation in removing attached sea lice from Atlantic salmon. The results from this study show clear reductions of all stages of L.*salmonis* and C. *elongates* during the study period.

Reductions were observed in the average number of *chalimus* (14.3%) and pre-adult stages (60.4%) of L. *salmonis* and 46.2% of C. *elongatus* immediately after the fish had been pumped from the cage and before they were passed over the grader system. However, there were no reductions recorded for mature male and female stages of L. *salmonis* after the fish had been pumped from the cage. Further reductions were recorded after the fish had passed over the grader and before exposed to freshwater. This secondary handling resulted in further reductions in the average number of *chalimus* stages (50.0%), pre-adult stages (79.3%) and C. *elongatus* (92.3%). Again there were no reductions in the average number of mature female L. *salmonis* recorded after during this period however, a 40% reduction in mature male stages was recorded. The clearance rates recorded after the second handling were generally higher for both species of sea lice and all stages of L. *salmonis* apart for mature females. 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 holding cage and before exposure to freshwater from these tests can be attributed to mechanical perturbation.: physical contact from crowding, contact with the inner surface of the pipes used to pump the fish, netting and contact with the grading system. The results from this study clearly show that pumping fish from a cage has an independent physical effect in removing attached sea lice from Atlantic salmon as further reductions were recorded after the fish had been passed over the grader before exposure to freshwater. The absence of reduction in the number of female stages recorded during these two physical processes may be explained due to the site of attachment as 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 maturely developed stages are more robust and can withstand greater mechanical stressors compared to *chalimus* stages as it was observed that there were no reduction in mature males recorded until the fish had passed over the grader. Further, the average number of mature female lice present was only 0.17 per fish prior to the study and any reductive effects caused by physical and/or mechanical perturbation would be difficult to assess due to the low level of infestation for this particular stage of L. *salmonis.*

There were further reductions in the number of *chalimus*, pre-adult and female stages of L. *salmonis* after being exposed to freshwater for one hour but not for mature male stages and C. *elongatus*. The reductions obtained for C. *elongatus* prior to freshwater exposure (0.03 per fish) may have been sufficiently low as to clearly assess further effects of freshwater exposure and more fish may have had to be counted to fully elucidate potential effects. The lack of reductions in the average number of mature male sea lice (0.3 per fish) may be partially explained as for C. *elongatus*. In addition, counting more fish at each sampling point would account for variation in the spread of infectious agents through host populations.

There were differences in the patterns of reduction observed for all stages of L. *salmonis* and C. *elongatus* after two and three hour's exposure to freshwater. For *chalimus* stages there was a further reduction recorded after two hours exposure (average of 0.17 per fish) but no reductions at three hours post-exposure to freshwater (average of 0.3 per fish). Previous studies have shown that *chalimus* stages of the sea lice life cycle were sensitive to short term exposure to freshwater with percentage reductions of between 98 and 100% attained after up to three hours treatment exposure. 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. 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). This is in contrast to findings from this study. There are no clear explanations for these observations and further research is required to fully elucidate these findings. There were further reductions recorded for pre-adult and mature male and females stages of L. *salmonis* after up to three hours exposure to freshwater (92.8%, 93.3% and 60.0% respectively). The results indicate that exposure to freshwater had a reductive effect on these stages of sea lice and these reductions in combination with the mechanical effects of pumping the fish from the cage and passing them over the grader compromises the health status of sea lice. The physical effects of handling followed by exposure to freshwater may have affected the lice attached to the fish and weakened them resulting in the loss or reduction in their attachment ability.

There were further reductions recorded after direct exposure to freshwater for three hours and an additional handling as the fish passed over the grader for a second time before transfer back to the cage. Total reduction in the average number of *chalimus*, male and female stages of L. *salmonis* were found to be 78.6%, 94.6% and 80.0% respectively. These reductions recorded as the fish had passed over the grader for a second time may have been caused by the effects of exposure to three hours in freshwater thus compromising the health status of the sea lice and reducing their ability to remain attached to the fish as they passed over the grader. It may be that the longer the fish remain in freshwater the more attached sea lice become compromised either through osmotic challenge or as appears more likely, the combined effects of physical perturbation and freshwater exposure. There were no further reduction recorded for mature male stages after the fish had been transferred over the grader for a second time. The average number of mature male stages per fish (0.03 after three hours exposure to freshwater and 0.07 after passing over the grader) was low at this stage of treatment and more fish would have had to be examined to fully elucidate any potential effects.

The overall reductions recorded for all stages of L. *salmonis* after three hours exposure combined with the fish passing over the grader for a second time (average of 0.67 per fish or 86.2% reduction) would be considered to be a successful treatment outcome as infection levels had been considerably reduced. The reduction attained during this treatment has been shown to be higher compared to chemical bath treatments as used presently in many commercial farms.

There was an attempt to count sea lice from the fish as they were being pumped from the well-boat back to the cage after the treatment period. However, the volume of fish being discharged back to

the cage was too great as to allow for the safe netting of the fish. Future studies if undertaken would have to account for this to allow for elucidation of the additional physical effects of this secondary pumping of the fish. A small 5m cage placed in the polar circle would allow for some fish to be gathered for lice counting at this stage.
Water quality parameters recorded during these tests showed that salinity levels in the freshwater well were maintained throughout the study period with only a small increase recorded. Similarity, water temperature remained constant throughout with no abrupt changes recorded. Dissolved oxygen levels were maintained above sub-lethal concentrations throughout. There was little difference between seawater (12.5^{0}C) and freshwater temperature in the well during the exposure period.

5.0 Conclusions

The results from this study show clear reductions of all stages of L.*salmonis* and C. *elongates* during the study period. Results indicate that pumping fish from a cage and/or passing fish over grader systems on well-boats has an independent physical effect in removing attached sea lice from Atlantic salmon compared to effects observed when exposed to freshwater.
In addition, results indicate that some stages of L.*salmonis* and all stages of C. *elongatus* are sensitive when exposed to freshwater for up to three hours. Further, results indicate that overall reductions observed during the study period are directly related to a combined effects of both physical perturbation and exposure to freshwater.
The percentage reduction attained for all infective stages of sea lice found on Atlantic salmon exposed to freshwater (86.2%) would be considered to be a successful treatment outcome and infection levels would be below treatment thresholds imposed under Norwegian legislation.

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6.0 References

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