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Contents

1. Summary

The present project documents both fish welfare and effect of thermal de-licing (Thermolicer®). Thermal de-licing is a new non-medicinal method representing an alternative to established medicines. Thermal de-licing should be used together with other measures as part of an integrated anti-lice strategy. There is a considerable need for development of effective non-medicinal anti-lice treatments which maintain acceptable levels of fish welfare if aquaculture is to remain sustainable.

The results show that thermal de-licing results in a significant reduction in the number of mobile and adult lice. Calculated reductions in lice burden vary between approximately 75 – 100%. Although not statistically significant, a reduction in number of attached lice stages was registered following treatment. Several participating farms recorded similar levels of lice three weeks post-treatment compared to pre-treatment levels. It is considered likely that infection from other cages and neighbouring farms as well as development of attached stages to mobile stages will affect the situation.

The project was executed simultaneously with development and optimisation of the method. Results from de-licing using the latest version of the machine on rainbow trout show that this method is acceptable in terms of fish welfare. No significant acute injuries were observed, the fish fed well shortly after treatment and mortality levels were low. The greatest challenges experienced during the project were related to crowding and pumping fish. Modification of the pumping system together with other improvements to the machine resulted in continual improvement throughout the project. Water analyses revealed that adequate water flow through the system is important. From experience gained during the project it became clear that 'gentle' crowding methods should be used when possible, and that treatment of sick fish may result in an increased risk of mortality.

2. Introduction

There is a general aim in the industry to reduce the amount of medicinal treatment against salmon lice. Uncritical use of pharmaceutical substances has led to development of resistance and lice with reduced susceptibility or resistance are now widely distributed along the Norwegian coast (Grøntvedt et al. 2014).

There is currently widespread interest in development of non-medicinal treatments against lice. These include use of cleaner fish, physical prevention (screens) and other methods which remove/kill the lice.

Thermal de-licing is based upon inactivation of the lice, which subsequently detach from the fish, following short term exposure to moderately heated water. It has been shown that salmonid fish can tolerate water temperatures of 20-34^{o}C for shorter periods (30 min for *S. trutta;* Elliott et al. 1981). It is considered likely that the upper temperature limit for salmon lice will lie around the same level, but that the difference in size between the two organisms will result in a shorter survival time for lice at suboptimal temperatures. This is supported by Steinviks own findings and previous work at Gildeskål research station (Brunsvik et al. 1996).

Thermal de-licing equipment was, following introductory studies, mainly tested on farmed salmon in Chile in 2013. This equipment is now in commercial use in Chile. Treatment effect on lice (*Caligus rogercresseyi*) is reported to be in the region of 99% on adult lice and around 60% on juvenile lice. Post treatment three day mortality levels are low. The Chilean equipment has a capacity of approximately 25 tons fish per hour. For use on a commercial scale in Norway, the equipment must be of a larger dimension than that used in Chile.

Two new Thermolicer® machines designed for Norwegian use were built by Steinsvik in 2014. According to Aquaculture legislation § 20, new methods and technical solutions must be tested and documented as acceptable in relation to fish welfare before they can be taken into use. This project was performed to fulfil the legislatory requirements for such documentation and to document the anti-lice effect.

3. Project organisation and aims

Organisation
The project for documentation of fish welfare and effect on lice was financed by the Norwegian Seafood Research Fund (FHF).

Project participants
Bremnes Seashore AS
Kobbevik og Furuholmen AS
Blom fiskeoppdrett AS
Bolaks AS
Steinsvik AS (previously Ocea)
Norwegian Veterinary Institute

Project group
Ida-Katrin G. Nerbøvik, Bremnes Seashore AS
Solveig Nygaard, Fiskehelse og Miljø AS
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Atle Lillehaug, Norwegian Veterinary Institute

Steering group
Geir Magne Knutsen, Bremnes Seashore AS,
Tore Laastad, Steinsvik AS
Ingebrigt Landa, Kobbevik og Furuholmen AS
Jan Helge Bildøy, Blom fiskeoppdrett AS
Randi Grøntvedt, Norwegian Veterinary Institute, secretary
Kjell Maroni, FHF responsible

Parallel to the project on documentation of fish welfare and effect on lice, Steinsvik AS have performed and financed continual development of Thermolicer® in cooperation with the farming companies involved in the project.

Aims
The documentation project had two aims:
1. Document fish welfare during use of thermal de-licing
2. Evaluate the methods effect against salmon lice

4. Execution and methods

De-licing with two Thermolicer® machines was performed on either research facilities or test farms. On four research facilities (farms A, B, C and D), extended welfare registrations and lice counting were performed in one cage (selected cage) per facility. For these trials, permission was obtained from the Norwegian experimental animal research panel. Table 1 summarises de-licing trials performed on research facilities. In advance of initial experimental treatments and between experimental treatments in research facilities, additional de-licing treatments were performed. This was due to simultaneous development of the Thermolicer® equipment. Use of research facilities in addition to test farms has been important in the process of testing out the various improvements to the system, for execution of detailed welfare screening and lice counting. The project has been allowed access to data from test farms to ensure a large data base. Dispensation from compulsory de-licing regulations was awarded by the Norwegian Food Safety Authority.

Sampling of fish for welfare screening was performed prior to de-licing in cages where the fish were crowded for pumping into the Thermolicer, and in the cage immediately following de-licing. No sampling was performed from other compartments of the system e.g. following pumping, before or after treatment with heated water.

Sampling of fish was performed at four different points in time from early to late during de-licing in an effort to spread the sampling between fish having been crowded for different lengths of time.

Table 1: Summary of de-licing in selected cages on four different research facilities

Research facility	Fish	Number fish	Cage size	Machine nr	Date
A	Salmon (ca 2 kg)	46185	24x24 cage	1	11.11.2014
B	Salmon (ca 2 kg)	45558	24x24 cage	1	25.11.2014
C	Salmon (< 2 kg)	125491	160 circle	2	27.01.2015
D	Rainbow trout (ca 2.5kg)	50694	24x24 cage	2	26.03.2015

The remaining cages on the research facilities were treated at different time points and on research facility A cages were treated in two different periods. On research facility C only a single selected cage was treated.

Thermal de-licing – technical description and modifications

The Thermolicer® is placed in a transport container. This container is placed on a barge. The container contains a treatment chamber, circulation pump, sensors, heating element and equipment for aeration and oxygenation of treatment water. In addition a rotating filter is placed external to the Thermolicer®. This filter continually filters fallen lice from the treatment water.

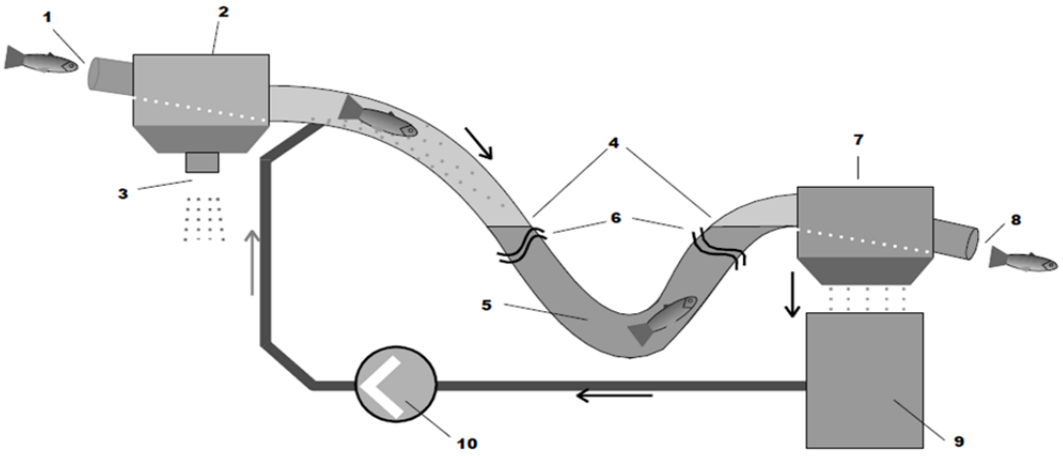


Figure 1: Schematic diagram of the Thermolicer®. 1. The fish enters via the fish pump. 2. Water strainer/removal of seawater. 3. Seawater is filtered and released from the system. 4. The fish are introduced to tepid water. 5. The fish move through the system filled with tepid water. 6. Water level in treatment chamber. 7. Tepid water removed. 8. Fish leave the system. 9. Tepid water led back to the heating tank for filtering, aeration and reheating. 10. Treatment water is pumped back to the treatment chamber.

During and parallel to the project Steinsvik has in cooperation with fish farmers performed continual development with introduction of various improvements and changes to the Thermolicer®. The following changes were made to machine 1:

1. Following initial de-licing in trial farms and prior to de-licing in research facilities (A and B):
    a. The water strainer was redesigned to reduce the amount of seawater entering the system
    b. The 'exit' system between the machine and the receiving cage was redesigned to avoid injury to the fish
2. Following de-licing on research facilities (A and B)
    a. Redesign of internal water strainer which had resulted in fin damage
    b. Additional heating element fitted
    c. Drum filter (150 µm) fitted to prevent release of lice from fish pump.
    d. Rotating filter fitted to improve water quality and filter lice in treatment chamber
3. Further planned improvements:
    a. Change of fish pump with adapted water strainer to increase capacity, reduce injury and crowding time

b. Run two parallel Thermolicer® machines to increase capacity, reduce injury and crowding time.

The following changes were made to machine 2:

1. Following de-licing on the first research facility (C) and prior to de-licing on research facility (D):
    a. The water strainer was redesigned to reduce the amount of seawater entering the system
    b. The 'exit' system between the machine and the receiving cage was redesigned to avoid injury to the fish
    c. Redesign of internal water strainer which had resulted in fin damage
    d. Fish counter changed to achieve better control of fish number
    e. Drum filter (150 µm) fitted to prevent release of lice from fish pump.
    f. Rotating filter fitted to improve water quality and filter lice in treatment chamber
    g. Change of fish pump with adapted water strainer to increase capacity, reduce injury and crowding time

Welfare documentation

A registration scheme developed by the Institute for Marine Research for evaluation of fish welfare on testing of new technology was used. Fish from selected cages were examined for acute external injuries on the gills, skin, eyes, fins and snout immediately before and after treatment and weekly until three weeks post treatment. The criteria for interpreting such injuries are detailed in appendix 1. Scoring was performed by a team comprising one fish health biologist and one veterinary surgeon.

Data collection

The following data was collected on all localities:

1. *Daily registrations in all cages on all localities in the period 2 weeks prior to and 3 weeks after treatment were:*
    - Number of fish
    - Number dead
    - Feed consumed
    - Unusual events which could indicate that fish health and welfare had been affected

2. *Weekly lice counting*
    - Lice counts from routine counting (according to legislatory requirements) were retrieved from farming companies registration systems for the 2 weeks prior to and 3 weeks following de-licing
    - Lice counts for all cages (20 fish per cage) immediately prior (1 week maximum) to treatment
    - Lice counts for all cages (20 fish per cage) immediately following (1 week maximum) treatment

In selected cages, lice counts and welfare parameters were recorded for 40 randomly selected fish immediately prior to and after treatment (on the day of treatment, 80 fish in total). The same process for 40 randomly selected fish was performed on day 7, 14 and 21 post treatment.

Statistical analysis

Welfare registrations

Acute injuries were analysed by comparison of the observed number of fish with a given welfare-score (Figure 2) before and after treatment using Pearson's Chi-square test with Monte Carlo simulation of p-value (based on 2000 replicates). Where few fish were observed in the highest score categories, score categories were combined.

For the welfare parameters gill pallor and cataract where eventual injury is likely to develop over time, pre-treatment scores were also compared with scores 1, 2 and 3 weeks post treatment. To summarise fin injuries, we used the sum of scores for all fins for each fish and compared the distributions of total fin-score pre- and post-treatment using a two-sided Wilcoxon test.

The analyses were repeated utilising only the first 15 fish sampled before and after treatment (to focus on the fish with the shortest crowding time). All analyses were performed separately for each site. P-values from the four sites were adjusted for multiple testing using the function p.adjust in R for each welfare parameter analysed.

Treatment time point varied for each cage on each of the four test farms. Daily dead fish counts were categorized as 'before' (0-3 days prior to treatment), 'after' (0-3 days following treatment), 'week 1' (day 4 -10 following treatment), 'week 2' (day 11-17 following treatment) and 'week 3' (day 18-24 following treatment) for descriptive presentation of mortality statistics (extended by several weeks before and after treatment). Routine lice counts were categorized in the same way.

Lice counts

Box plots were used to provide a descriptive presentation of lice counts prior to and following treatment. For selected cages in which lice counts were available for individual fish, a negative binomial regression model was performed to test for significant differences in lice numbers immediately before and after treatment, before and after 1 week, 2 weeks and 3 weeks. For sites A and B lice numbers for individual fish in all cages were available. These cages were de-liced at different time points over an extended period. For these sites an equivalent model was performed in which 'cage' was included as a random effect (to correct for the fact that lice numbers are more similar within a cage than between cages). We have currently insufficient data to model lice number development with time as a function of internal infection pressure in a particular site and infection pressure from neighbouring sites.

We estimated time taken for surviving attached larvae to develop to motile lice given site specific sea temperatures in the weeks following treatment. Kristoffersen et al. (2014) estimated a development time of 155 degree days from attached larvae to motile louse (based on temperature independent salmon louse demography reported by Stien et al. (2005)). For the present study we estimated that attached lice surviving a thermal treatment had already spent 50 degree days attached to the fish. The number of additional days required to reach a total of 155 degree days was then calculated.

All descriptive and statistical analyses were performed in the program R. The R package lme4 was used for negative binomial regression with cage as the random effect.

Histology

On research facilities gill samples from 5 fish were taken at each time point at which welfare parameters were registered. The Norwegian Veterinary Institute's routines for processing histological sections were followed. Sections were examined using light microscopy. Findings from routine diagnostic investigation of formalin fixed gills were summarized. Quantitative analysis of histological findings was not performed.

Water analysis

Three water samples were taken during de-licing of the four selected cages. Two water samples were tapped directly from the Thermolicer® treatment chamber (Figure 1). One sample was taken early and one taken late during the de-licing process. One control sea water sample was taken in the immediate proximity of the fish pump intake. Water samples were sent to the Norwegian Institute of Water Research for analysis of alkalinity (NS-EN ISO 9963-1:1996), ammonium (internal method), nitrite and nitrate (NS-EN ISO 4745:1991), pH (NS-EN ISO 10523.2012) and turbidity (internal method).

5. Results

Welfare parameters

The results of welfare parameter registration in fish from each selected cage in each of the four research facilities (A, B, C and D) are summarized in Figure 2. During statistical analysis of welfare parameters, focus was placed on acute injuries resulting from the thermal treatment on the day of treatment. An equivalent analysis for acute injuries in weeks 1, 2 and 3 following treatment was not performed.

Gill and cataract scores were included to identify effects on gill and eye health over time. For these welfare parameters focus was placed on both immediate effect and development over time.

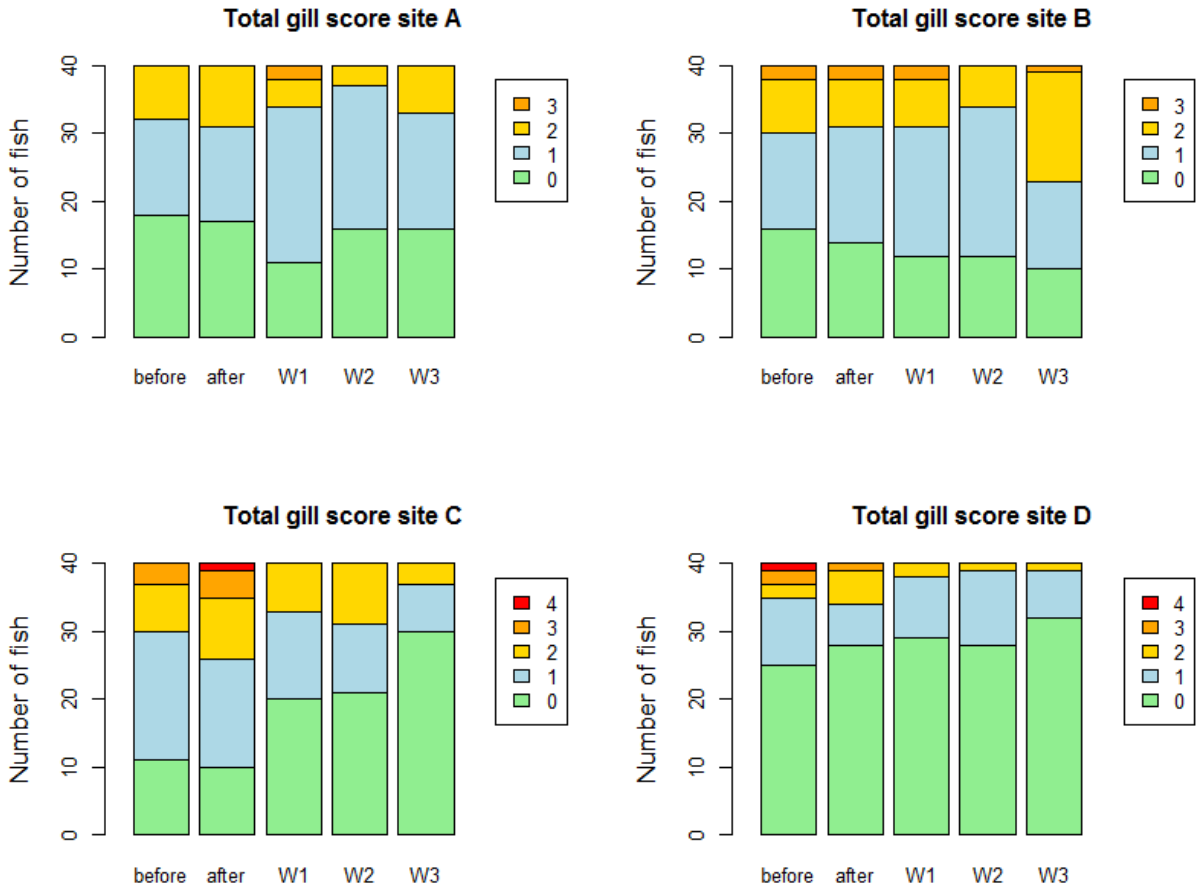


Figure 2 a): Total gill score in fish from the four research facilities. 40 fish were examined at each time point. The colour code indicates the number of fish in each category.

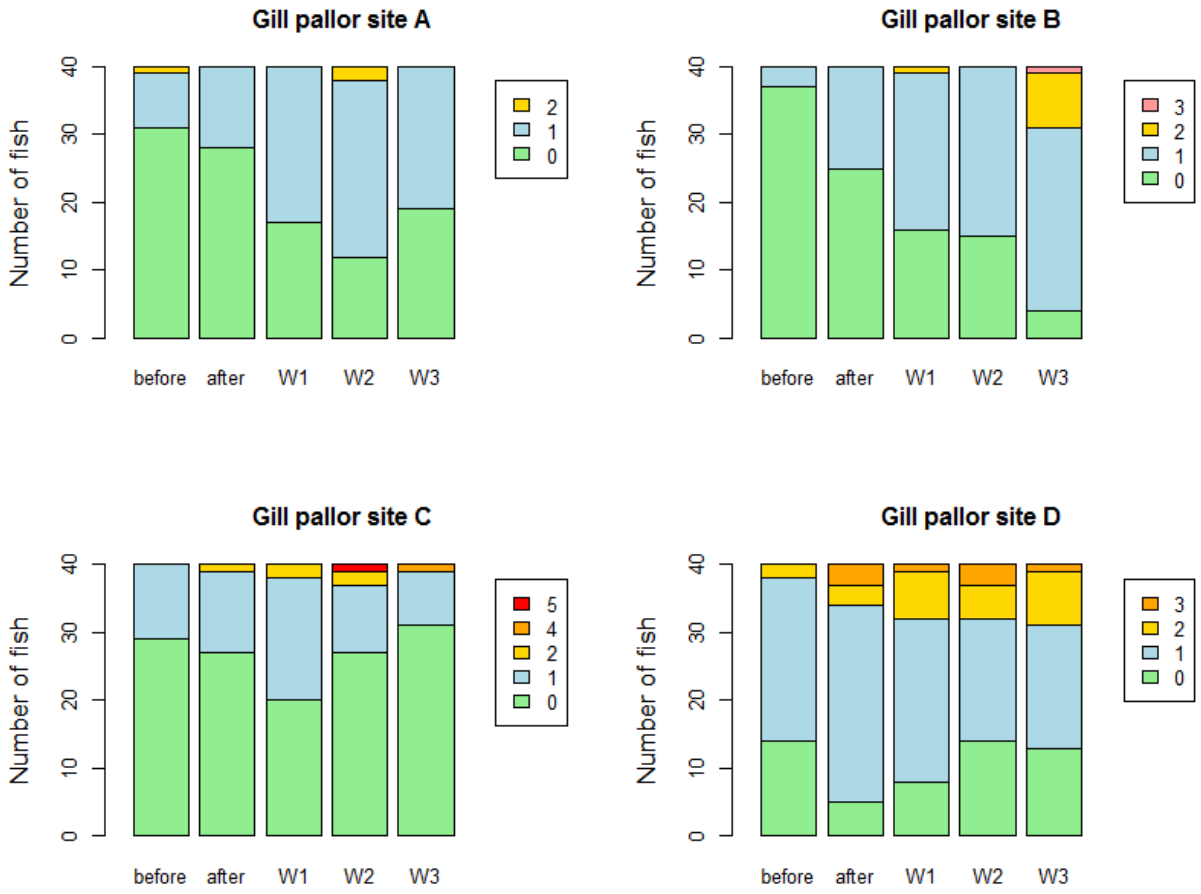


Figure 2 b): Evaluation of gill pallor in 40 fish per time point at each of the four research facilities. Colour codes indicate category in which O = normal. There is a significant change between before and after treatment in fish in site B with an increasing proportion of fish in category 1 (p = 0.002, adjusted p = 0.008) and fish from site D displayed a tendency towards an increased proportion in category 3 (p = 0.04, adjusted p = 0.11). Site B showed a significant increase in gill pallor score between before and all weekly time points after treatment (all adjusted p-values < 0.01). For site A a significant increase in gill pallor score was registered between before and week 1 (adjusted p = 0.04) to week 2 (adjusted p <0.01).

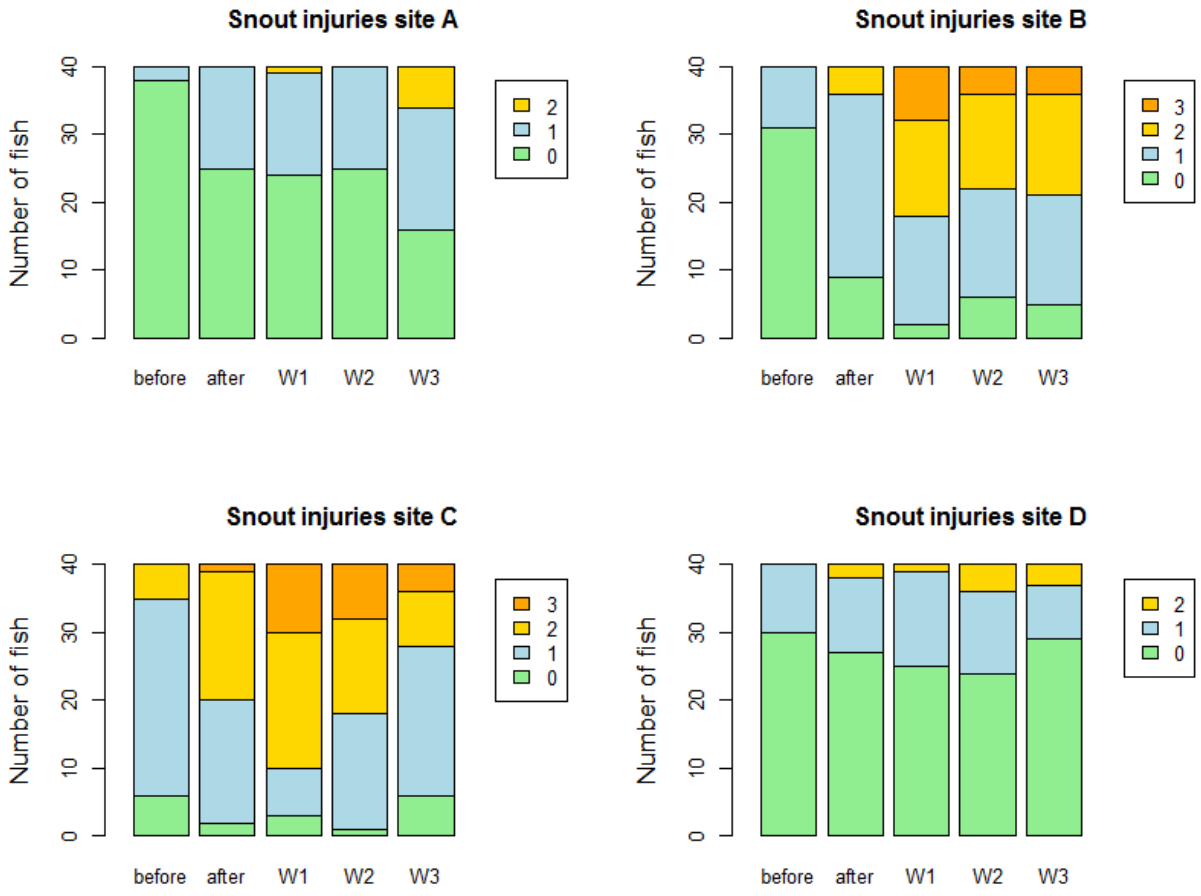


Figure 2 c): Evaluation of snout injuries in 40 fish per time point in research facilities. The various categories are colour coded with 0 = normal. Significant changes were registered before and after treatment in site A (adjusted p = 0.003) with an increase in category 1, while an increase in category 1 and 2 was registered in site B (adjusted p = 0.002) and an increase in category 2 and 3 in site C (adjusted p = 0.003). No increase in snout injury was registered in site D.

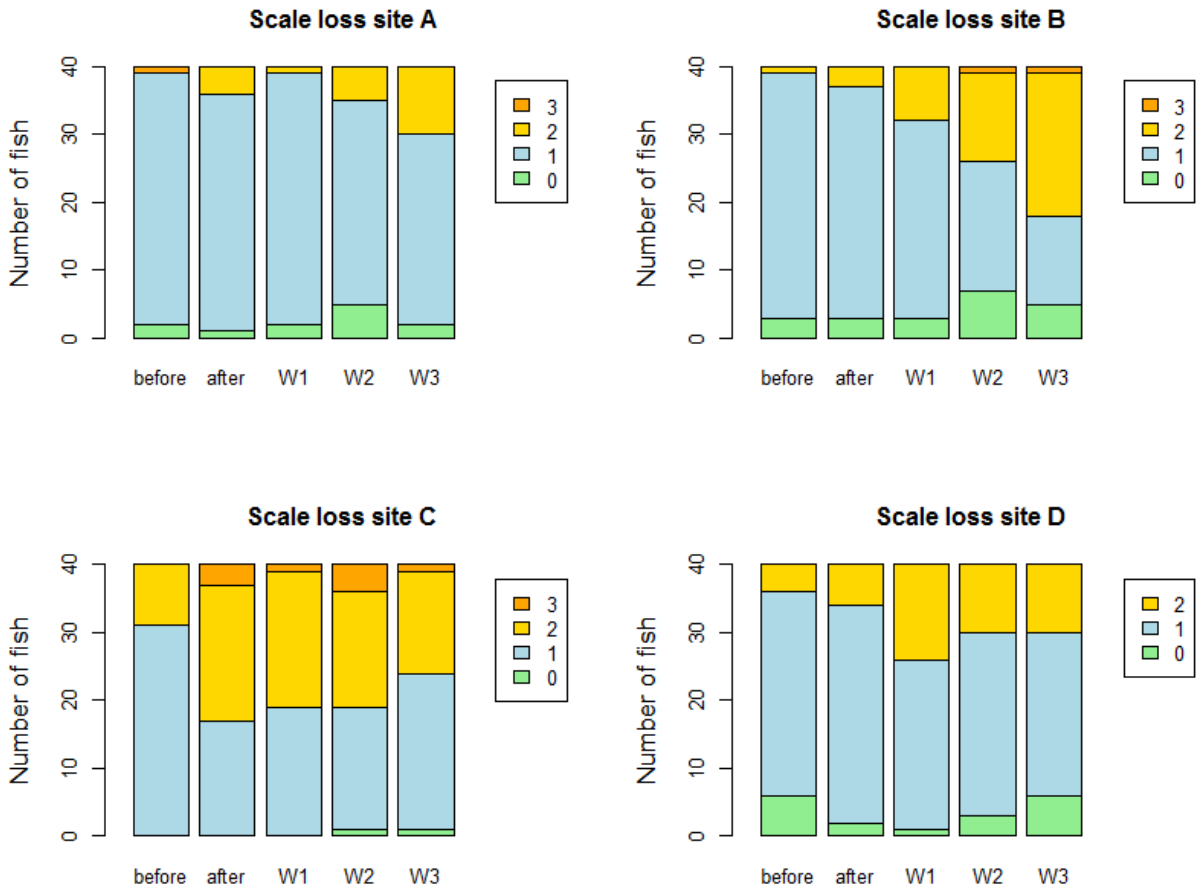


Figure 2 d): Evaluation of scale loss on 40 fish per time point on each of the four research facilities. The various categories are colour coded with 0 = normal. A significant change was registered following treatment in site C (adjusted p = 0.01) with an increase in category 2 and 3 fish. No significant change in scale loss was registered following treatment in the remaining sites.

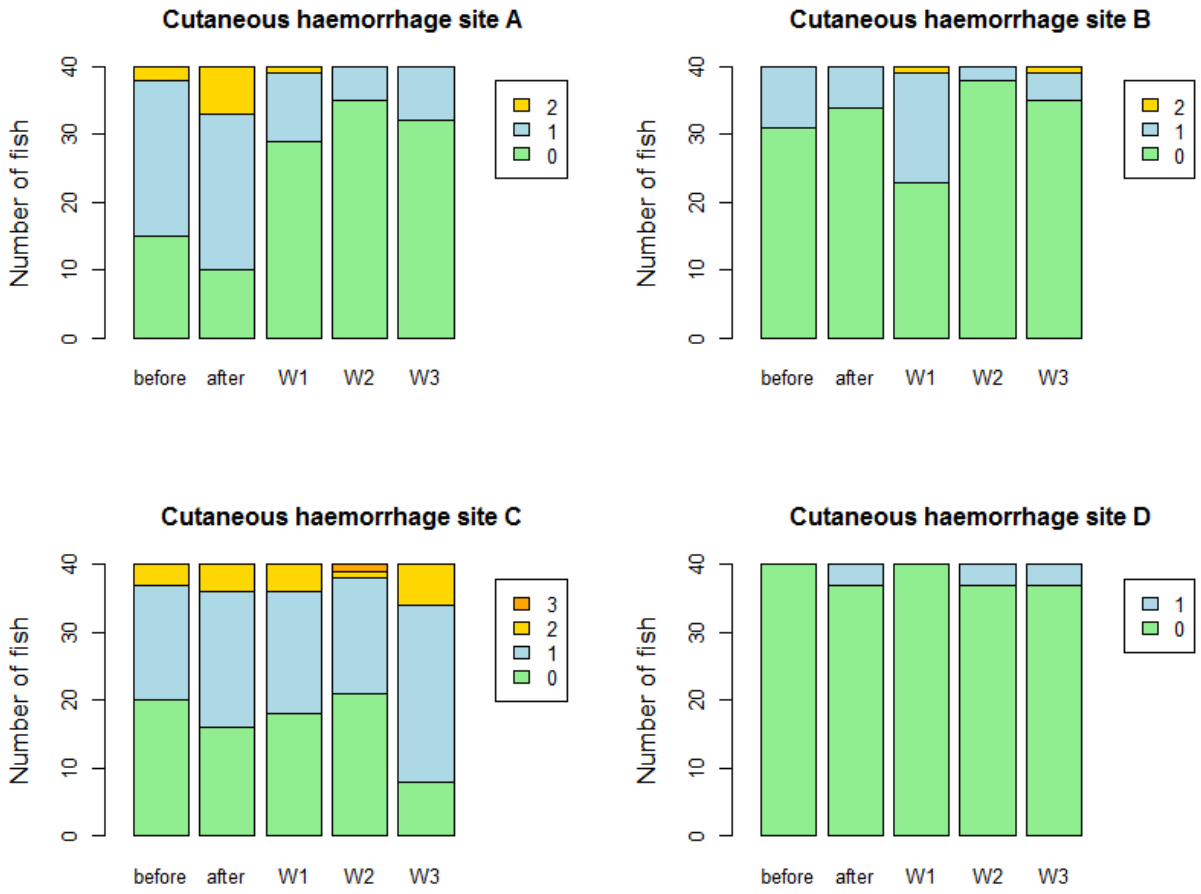


Figure 2 e): Evaluation of cutaneous haemorrhage per 40 fish from each of the four research facilities. The various categories are colour coded with O = normal. No significant changes were identified following treatment in any of the four sites.

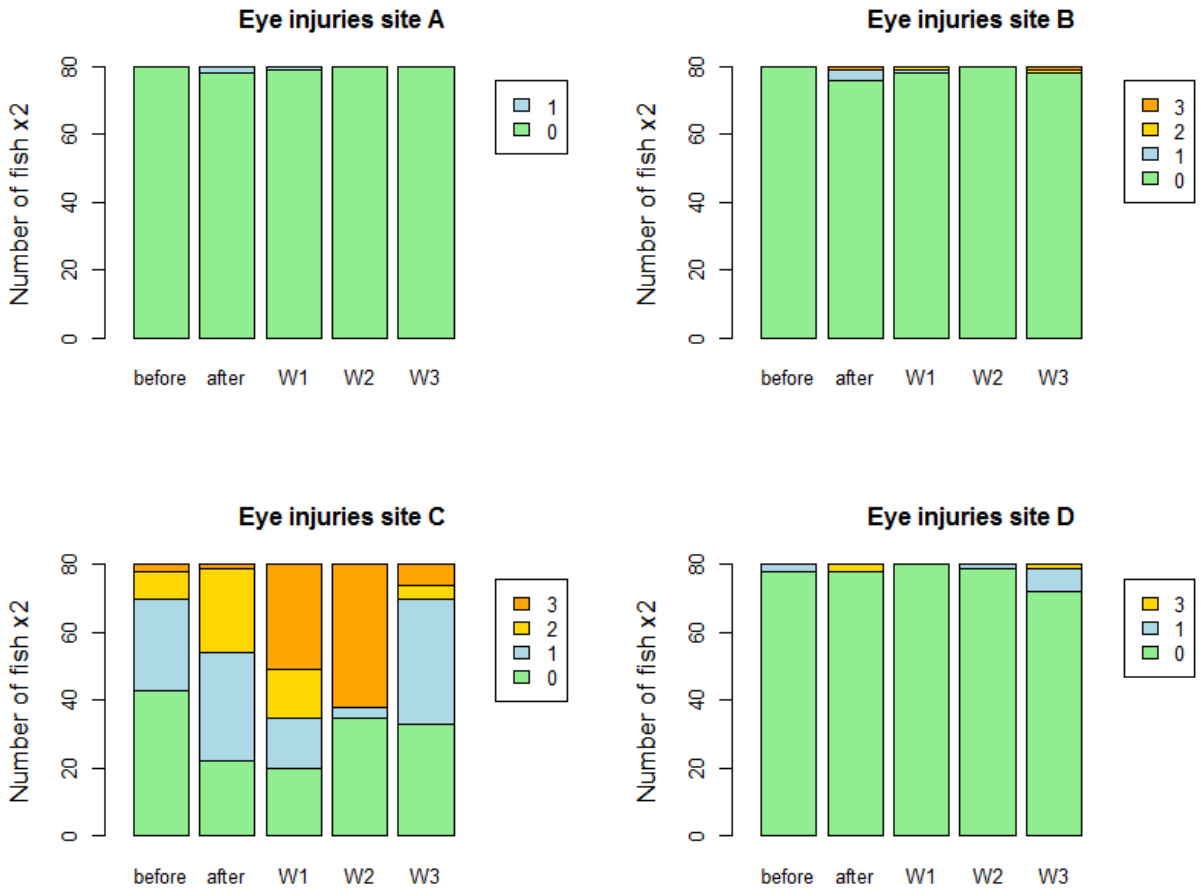


Figure 2 f): Evaluation of eye injuries per time point (40 fish; 80 eyes in total) for each of the four research facilities. No significant changes were identified following treatment in any of the four sites.

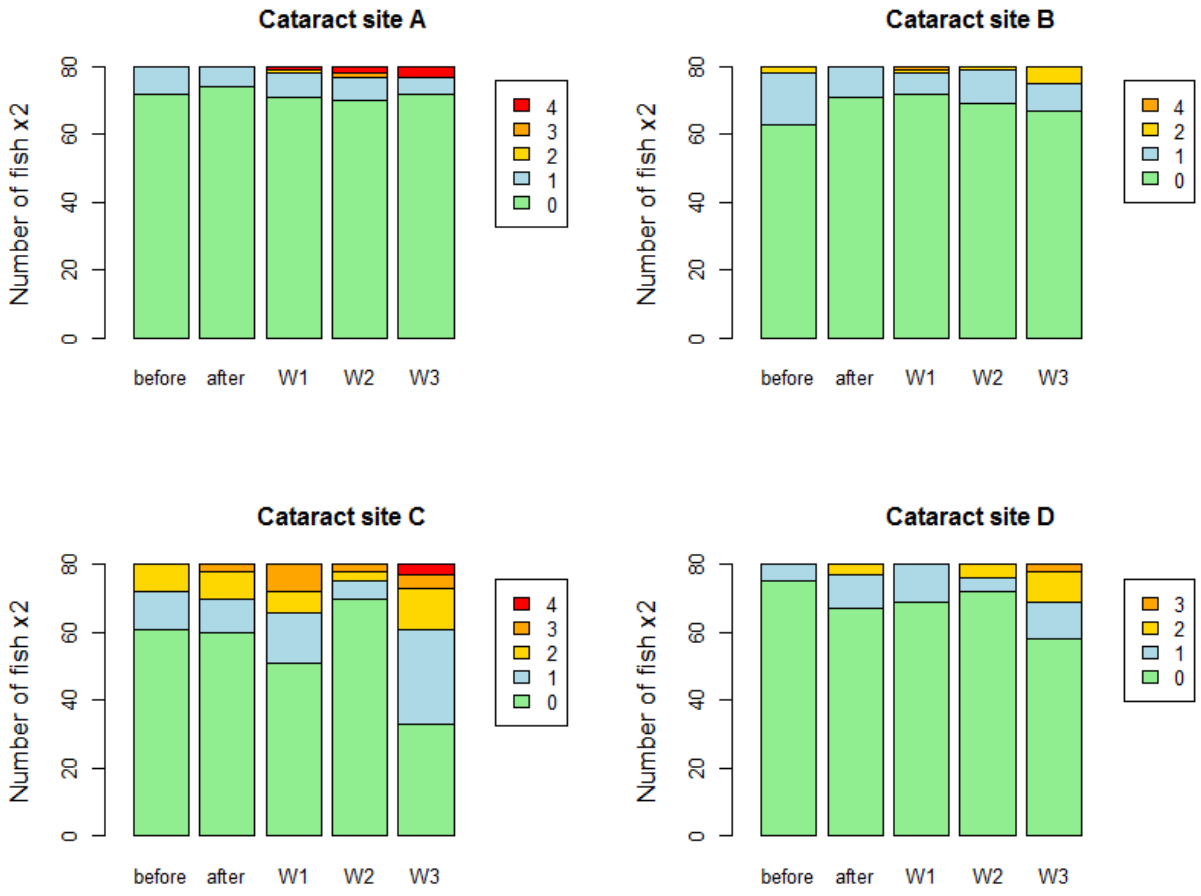


Figure 2 g): Evaluation of cataract per time point (40 fish; 80 eyes in total) in each of the four research facilities. No significant changes were identified immediately following treatment in any of the four sites. However, at 3 weeks post treatment a significant change was identified in site C (adjusted p = 0.01) and a tendency towards change identified in site D (p = 0.01, adjusted p = 0.12)

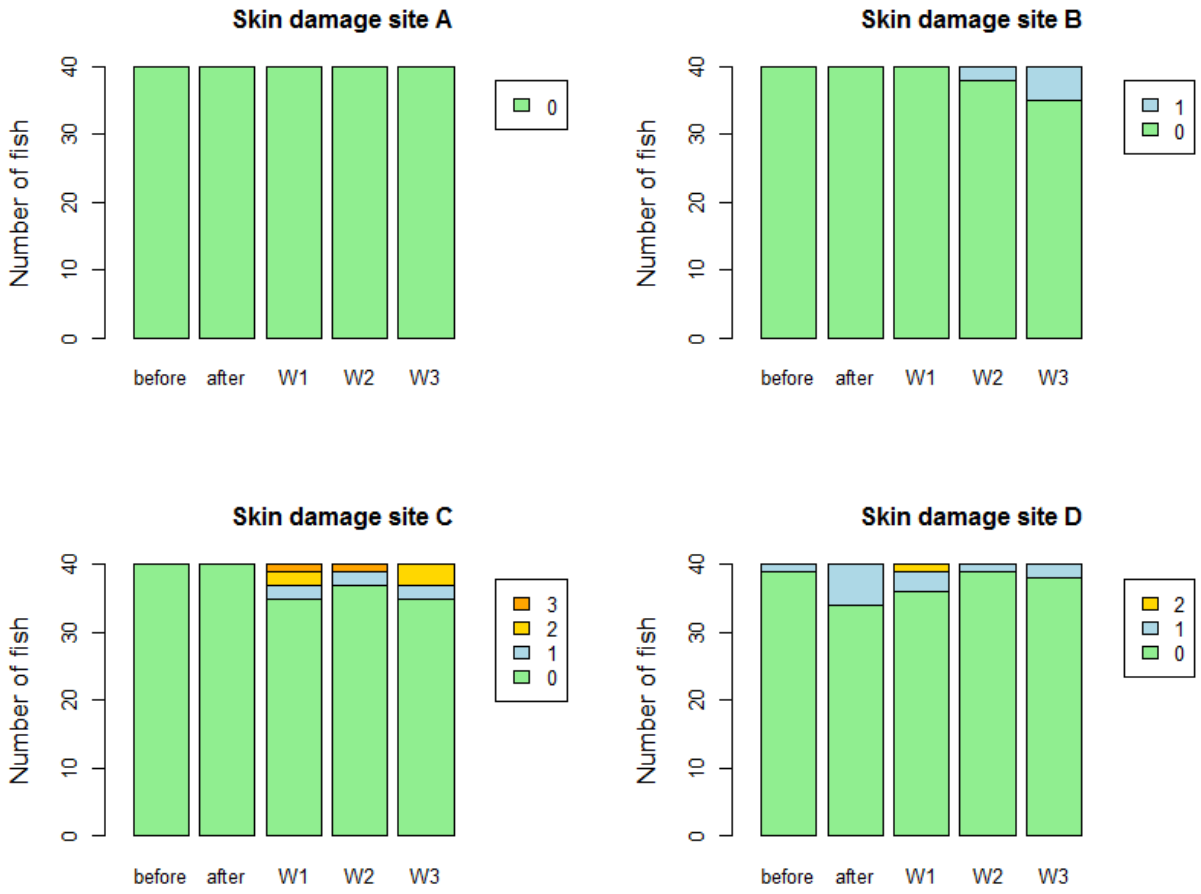


Figure 2 g): Evaluation of skin damage in 40 fish in each of the four research facilities. No significant changes were identified following treatment in any of the four sites. A tendency towards significant change was identified in site D with an increase in the number of fish in category 1 ($p = 0.12$), particularly in fish which had been crowded for the longest period.

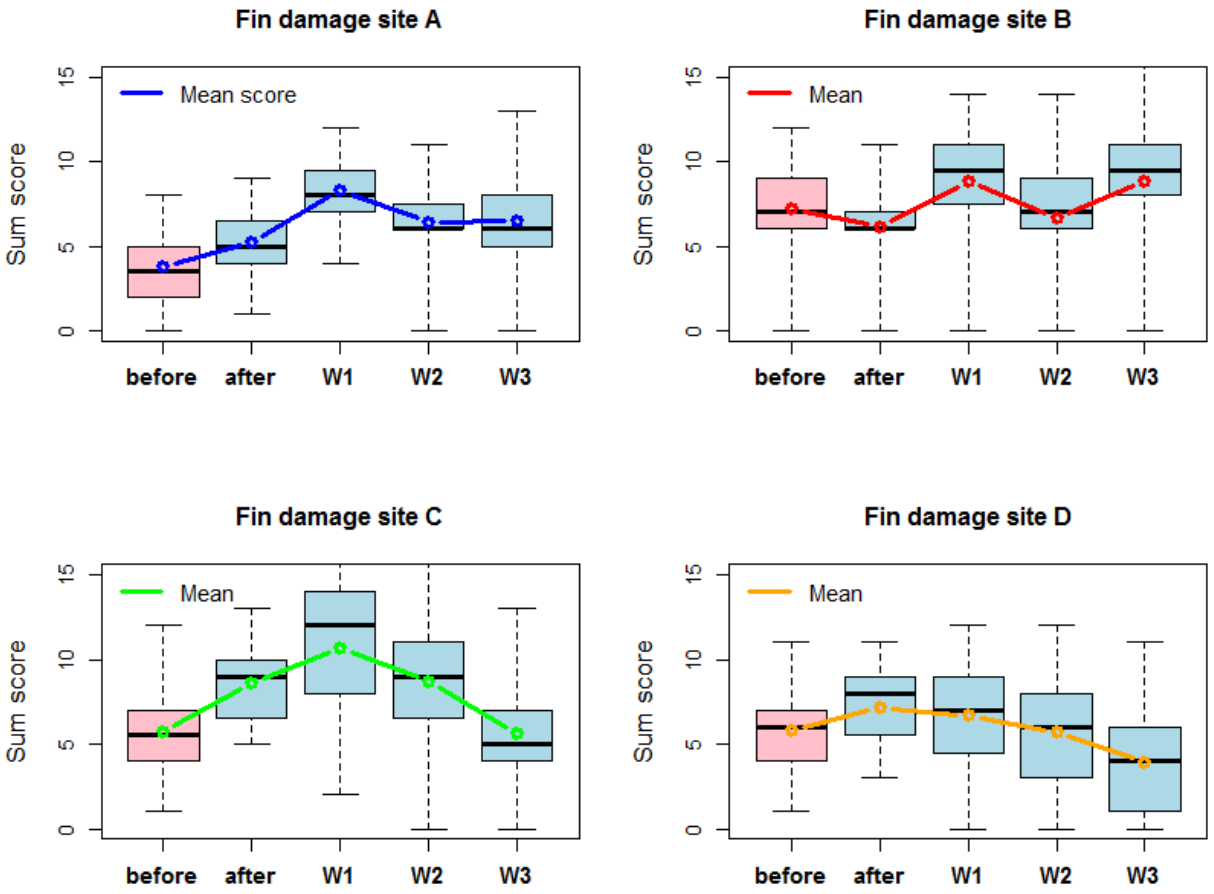


Figure 2 h): Evaluation of fin damage in 40 fish per time point in each of the four research facilities. The boxplot shows the distribution of the sum scores for fin damage with the median value (black line) and 25 and 75 percentiles shown as the box.

Following Wilcoxon-testing of fin damage (sum score of all fins minus anal fin) significantly greater levels of fin damage were identified following treatment on sites A and C, even when limiting the analysis to fish sampled early in the treatment process. Sites B and D displayed greater fin damage before treatment compared to after treatment, although this is non-significant when the analysis is limited to fish sampled early in the treatment process.

Mortality – Research facilities

Mortality records for each cage were collected for the two weeks before and for 4 weeks following treatment. Figure 3 shows the percentage mortality summarized weekly (the same cages selected for welfare documentation). Figure 4 shows weekly mortality for all cages in sites A, B and D. On site C only one cage was treated, and mortality registrations for all cages are not shown.

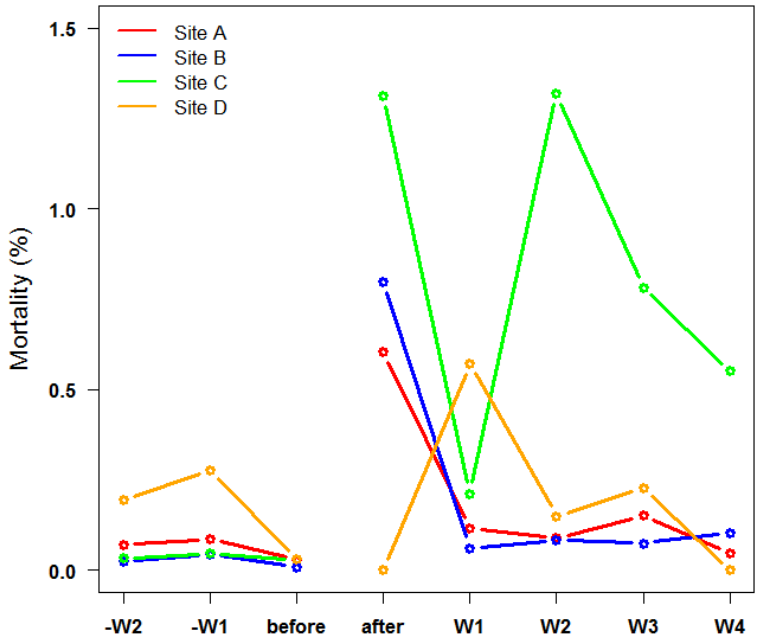
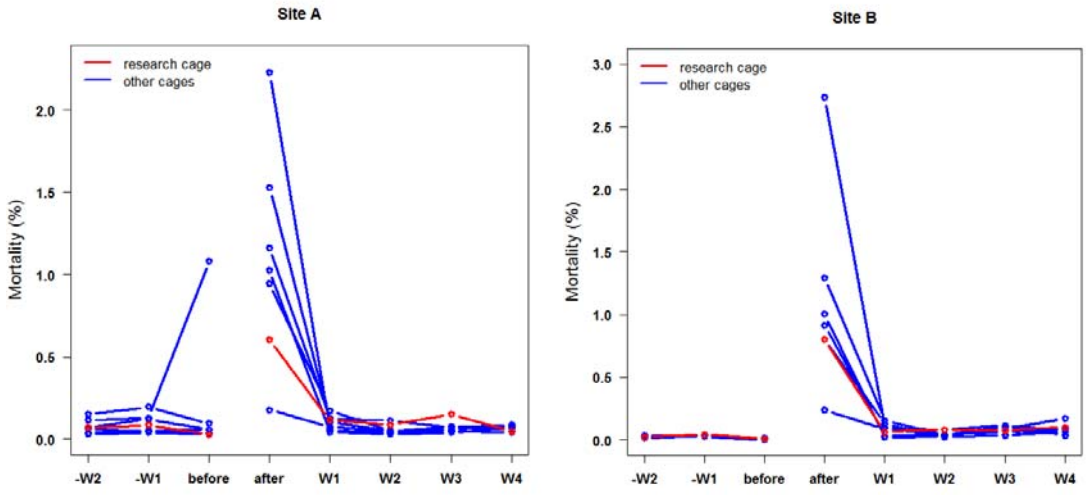


Figure 3: Percentage mortality in selected cages in each of the four research facilities (A, B, C and D) from 2 weeks before to 4 weeks following treatment.



a b

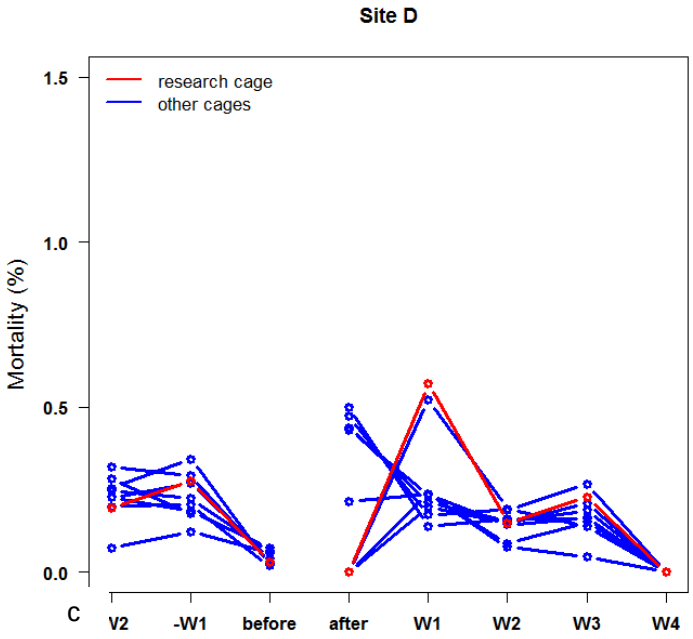


Figure 4: Percentage mortality in all cages on a) site A, b) site B and c) site D for the two weeks before and 4 weeks following treatment. The red line represents selected cages. Detailed welfare investigations were undertaken on fish from this cage.

Feeding – Research facilities

Total feed (kg) provided per cage was recorded for 2 weeks before and up to 4 weeks following treatment. Figure 5 shows kg feed fed per kg fish in the same 4 cages selected for registration of welfare parameters.

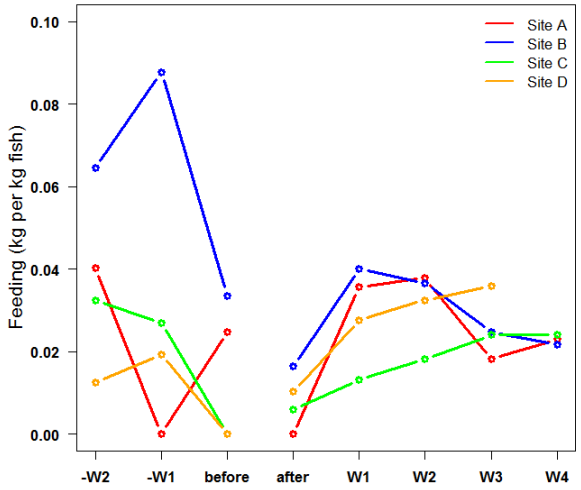


Figure 5: Summarised weekly feed statistics (kg feed per kg fish) for selected cages in each of the four different research facilities.

For research facilities A and B, it was reported that normal feeding did not resume until nearly three weeks post treatment. In site D normal feeding resumed after a couple of days.

Histology

Research facility A
Sampling before Thermal de-licing (TD): Extensive acute lamellar bleeding without proliferation of epithelial tissues was identified in one sample. Proliferation of respiratory epithelial tissues with necrosis and total fusion distally in the lamellae. Unspeciated 'epitheliocysts' were identified.
Sampling after TD: Extensive acute lamellar bleeding without proliferation of epithelial tissues was identified in one sample. Unspeciated 'epitheliocysts' were identified. Bleeding was identified in lamellae with several layers of proliferative respiratory epithelial cells.
Sampling one week after TD: Some proliferation of respiratory epithelium distal on lamellae with clubbing and some bleeding. A few unspeciated 'epitheliocysts' were identified in one sample.
Sampling two weeks after TD: Two of five gills displayed changes with heavy proliferation of respiratory epithelial tissues and bleeding. In the remaining gills small changes such as distal proliferation and clubbing of respiratory epithelial tissues on lamellae were observed. Acute bleeding was identified in one gill.
Sampling three weeks after TD: Bleeding with signs of cellular organization was observed in two gills. The remaining gills displayed small changes considered normal in sea farmed fish.

Research facility B
Sampling before Thermal de-licing (TD): A number of acute bleedings were identified. In the remaining gills only small changes involving proliferation of respiratory epithelial tissues and distal clubbing of lamellae.
Sampling after TD: As before TD.
Sampling one week after TD: Three gills displayed single lamellae with significant proliferation of respiratory epithelial cells.
Sampling two weeks after TD: Three gills displayed single lamellae with significant proliferation of respiratory epithelial cells. In two gills bleedings were identified in proliferative respiratory epithelial cells.
Sampling three weeks after TD: All gills displayed individual lamellae with significant proliferation of respiratory epithelial cells. A few unspeciated 'epitheliocysts' were identified, as were unorganized bleedings.

Research facility C
Note: On this site clinical AGD was diagnosed and treated 4 times in the autumn of 2014 (before de-licing).

Sampling before Thermal de-licing (TD): Bleedings and restructured lamella with central granulomatous tissues were identified. In one gill a degree of proliferation and some fusion of lamellae were observed. In the remaining gills only small changes considered normal in sea farmed fish were seen.
Sampling after TD: Restructured lamellae with central granulomatous tissues were identified. In one gill fusion of opposing lamellae was observed. Amoebae were not identified. In the remaining gills only small changes considered normal in sea farmed fish were seen.
Sampling one week after TD: In the remaining gills only small changes considered normal in sea farmed fish were seen including some clubbing.
Sampling two weeks after TD: In two gills extremely thickened individual lamellae with probable healing processes were observed. One gill showed central bleeding with proliferative respiratory epithelia. In addition small changes considered normal in sea farmed fish (clubbing) were noted.
Sampling three weeks after TD: In three gills a degree of proliferation of respiratory epithelia was observed. Areas of fusion and thickened filaments with central granulomatous tissues were observed.

Research facility D
Sampling before Thermal de-licing (TD): A degree of proliferation of respiratory epithelia distal and along the lamellae was observed in three samples. Acute bleeding distal in lamellae and blood between filaments was observed.
Sampling after TD: Samples taken immediately after TD. Much blood observed between and within lamellae. The bleedings were considered to be acute as they were unorganized and lacked associated tissue proliferation. In one sample a large bleeding was observed. A degree of proliferation of respiratory epithelial cells both distal and along the lamellae was noted.

Sampling one week after TD: Some bleedings showing early signs of organization were observed in lamellae. A degree of proliferation of respiratory epithelia distal on lamellae with some necrosis was observed.
Sampling two weeks after TD: In two gills bleeding was observed both central and distal within lamellae. In one gill a single thickened filament was observed together with fusion of lamellae, bleeding and yellow pigmentation in tissues, indicative of a large bleed. A degree of proliferation of respiratory epithelia distal in lamellae was observed.
Sampling three weeks after TD: Bleeding central to several lamellae and blood between lamellae was observed. A degree of proliferation of respiratory epithelia distal in lamellae was observed.

Water analysis

Results from water analyses show variation in water quality within treatment chambers during treatment of selected cages in sites A, B and C (Table 2). Water samples from site D were not analysed.

On site B 27000 µg/l ammonium (NH_3-N) was measured in the last sample taken from the treatment chamber. The estimated concentration of free ammonia in this sample was 231 ug/l, which is extremely high, and due to a combination of high pH and high temperature. Turbidity in this water sample was also high. This indicates poor water exchange in the treatment chamber during treatment of this cage.

Table 2: Results of selected samples taken from treatment water in the Thermolicer®

Selected cage A			
Parameter	*Control sample1*[1]	*Sample 1*[2]	*Sample 2*[3]
Alkalinity (Mmol/l)	1.842	2.227	3.005
Ammonium (µg N/l)	20	2800	8200
Nitrate og nitrite (µg N/l)	46	44	10
Acidity, pH	7.93	7.33	7.17
Turbidity, FNU	<0.30	3,6	18
Estimated NH3-N, µg N/l	1	51	103
Estimated NH3-N,µg N/l at pH 6.5	0	8	22
Selected cage B			
Parameter	*Control sample1*[1]	*Sample 1*[2]	*Sample 2*[3]
Alkalinity (Mmol/l)	2.146	2.267	2.702
Ammonium (µg N/l)	<5	6600	27000
Nitrate og nitrite (µg N/l)	3	6	4
Acidity, pH	7.92	7.21	7
Turbidity, FNU	<0.30	4.8	26
Estimated NH3-N, µg N/l	0	91	231
Estimated NH3-N,µg N/l at pH 6.5	0	18	73
Selected cage C			
Parameter	*Control sample1*[1]	*Sample 1*[2]	*Sample 2*[3]
Alkalinity (Mmol/l)	2.21	2.33	2.63
Ammonium (µg N/l)	11	2220	4200
Nitrate og nitrite (µg N/l)	84	78	3
Acidity, pH	7.85	7.53	7.35
Turbidity, FNU	<0.30	2	2
Estimated NH3-N, µg N/l	1	63	79
Estimated NH3-N,µg N/l at pH 6.5	0	6	11

[1]*Samples taken in the sea outside treatment chamber*
[2]*Samples taken from treatment chamber early in treatment process*
[3]*Samples taken from treatment chamber late in treatment process*

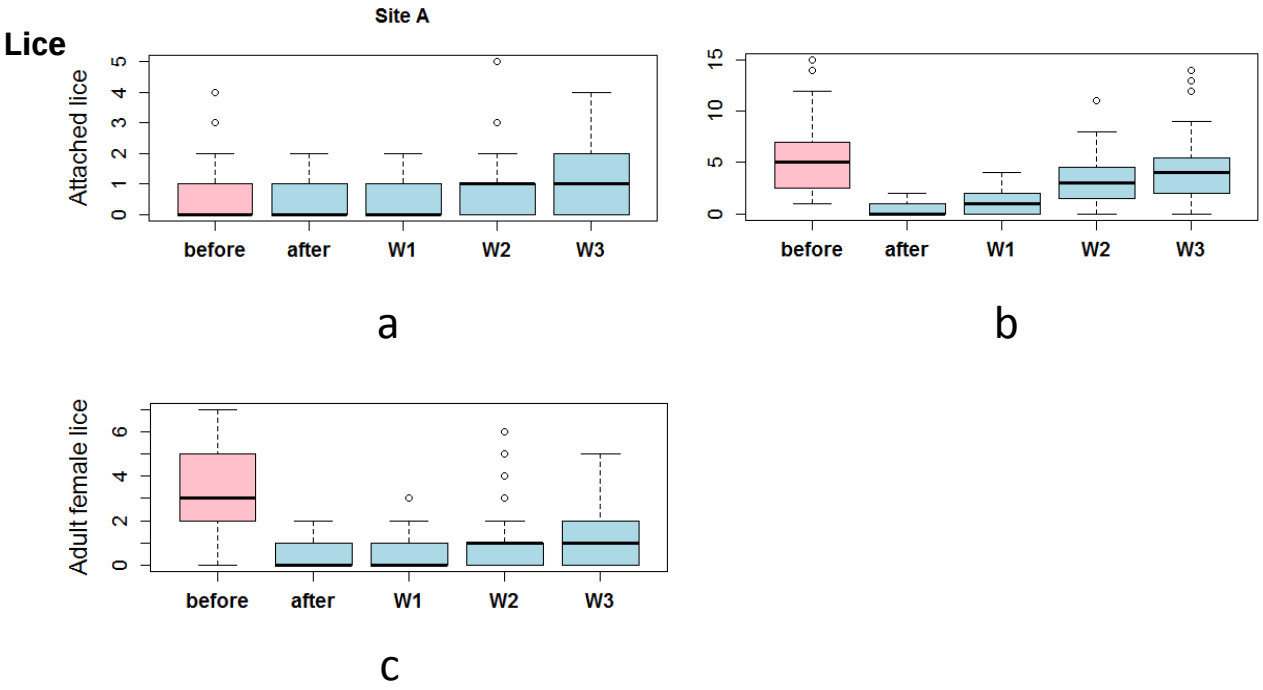


Figure 6: Boxplot of a) attached, b) motile and c) adult lice from selected cages in research facility A before and after treatment and 1, 2 and 3 weeks following treatment. The boxplot shows the distribution of lice from 40 fish per time point with median value (black line) with 25 and 75 percentiles represented by the box. Significantly fewer lice were counted immediately after and 1 week following treatment ($p<0.001$). Significantly fewer adult lice were identified at all time points ($p<0.001$). For attached stages the number of lice counted week three was significantly higher than prior to treatment ($p=0.009$).

From the predictive model, the reduction in motile lice in selected cages in site A was calculated to be 93.7% immediately following and one week after treatment.

All cages in research facility A were treated, and figure 7 shows the collective lice number development 3 weeks after treatment of the whole site.

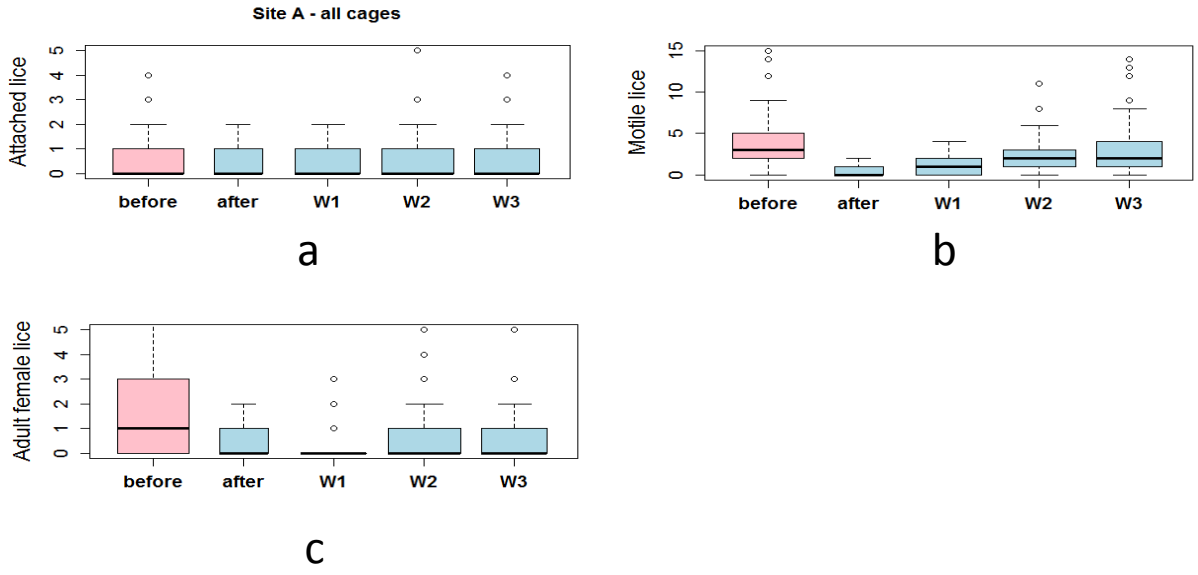


Figure 7: Boxplot of a) attached, b) motile and c) adult lice from selected cages in research facility A before and after treatment and 1, 2 and 3 weeks following treatment. The boxplot shows the distribution of lice from all counted fish in all cages per time point with median value (black line) with 25 and 75 percentiles represented by the box. Significantly fewer motile lice were counted immediately after and 1 and 2 weeks following treatment ($p<0.001$). Significantly fewer adult female lice were identified at all time points ($p<0.001$). Significantly more attached stages were observed 3 weeks post treatment ($p=0.005$).

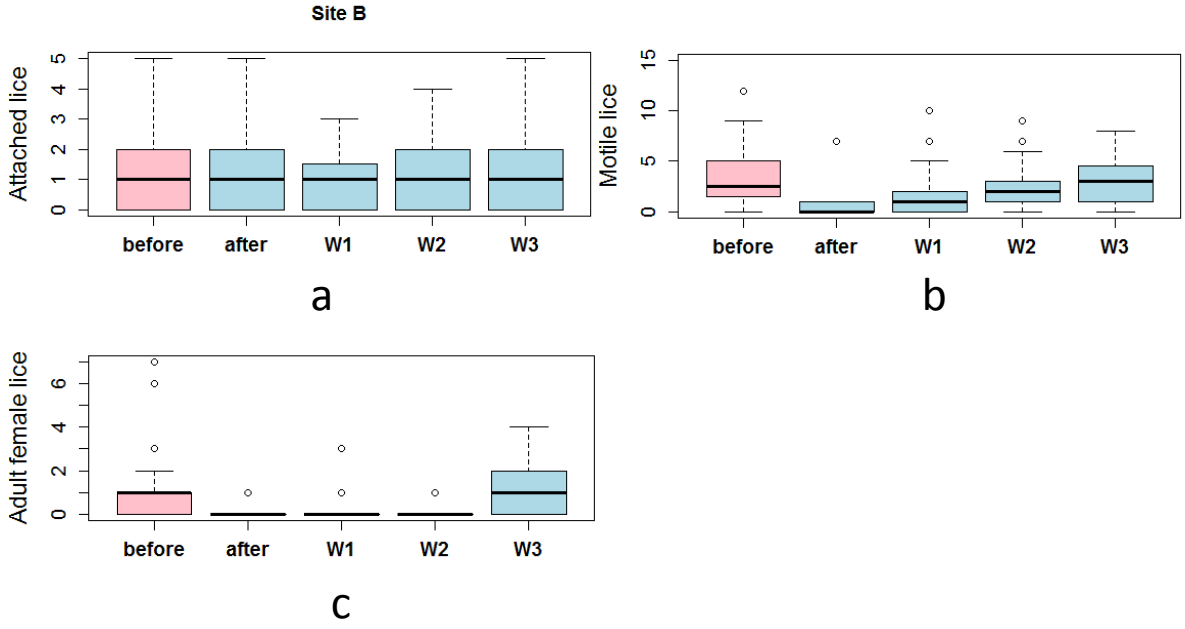


Figure 8: Boxplot of a) attached, b) motile and c) adult lice from selected cages in research facility B before and after treatment and 1, 2 and 3 weeks following treatment. The boxplot shows the distribution of lice from 40 fish per time point with median value (black line) with 25 and 75 percentiles represented by the box. Significantly fewer lice were counted immediately after (p=0.001) and 1 week following treatment (p<0.002). Significantly fewer adult female lice were identified immediately after (p=0.001) and 1 and 2 weeks after treatment (p<0.002).

From the predictive model, the reduction in motile lice in selected cages from site B was calculated to be 87.6% immediately following and 52.6% one week after treatment.

All cages in research facility B were treated, and figure 9 shows the collective lice number development 3 weeks after treatment of the whole site.

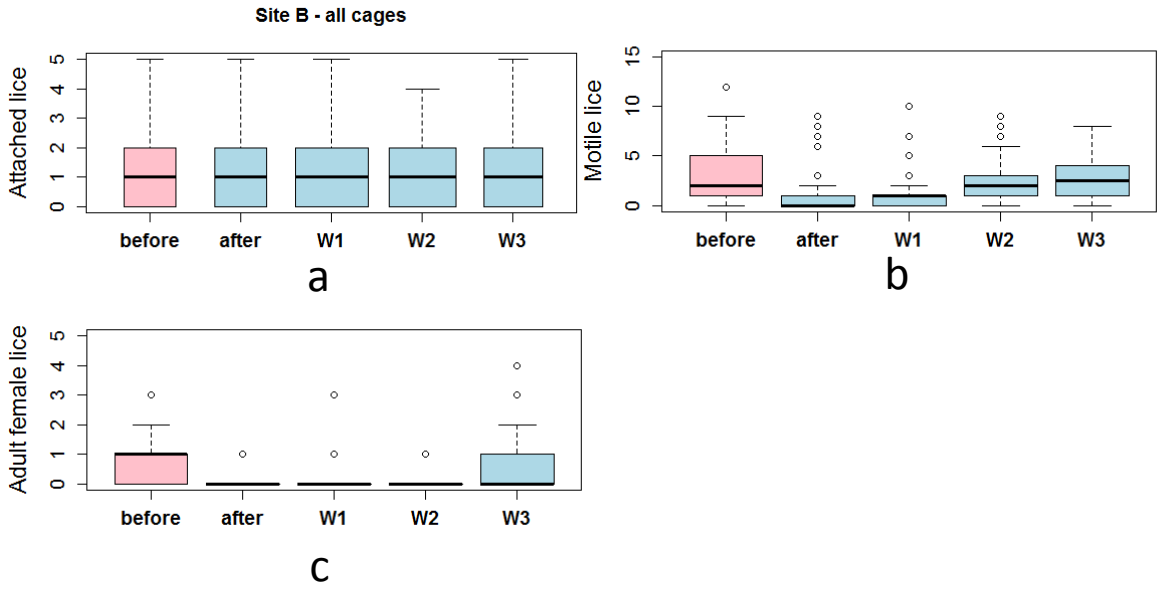


Figure 9: Boxplot of a) attached, b) motile and c) adult lice from selected cages in research facility B before and after treatment and 1, 2 and 3 weeks following treatment. The boxplot shows the distribution of lice from all counted fish in all cages per time point with median value (black line) with 25 and 75 percentiles represented by the box. Significantly fewer lice were counted immediately after and 1 week following treatment (p<0.001). Significantly fewer adult female lice were identified immediately after and 1 and 2 weeks after treatment (p<0.001).

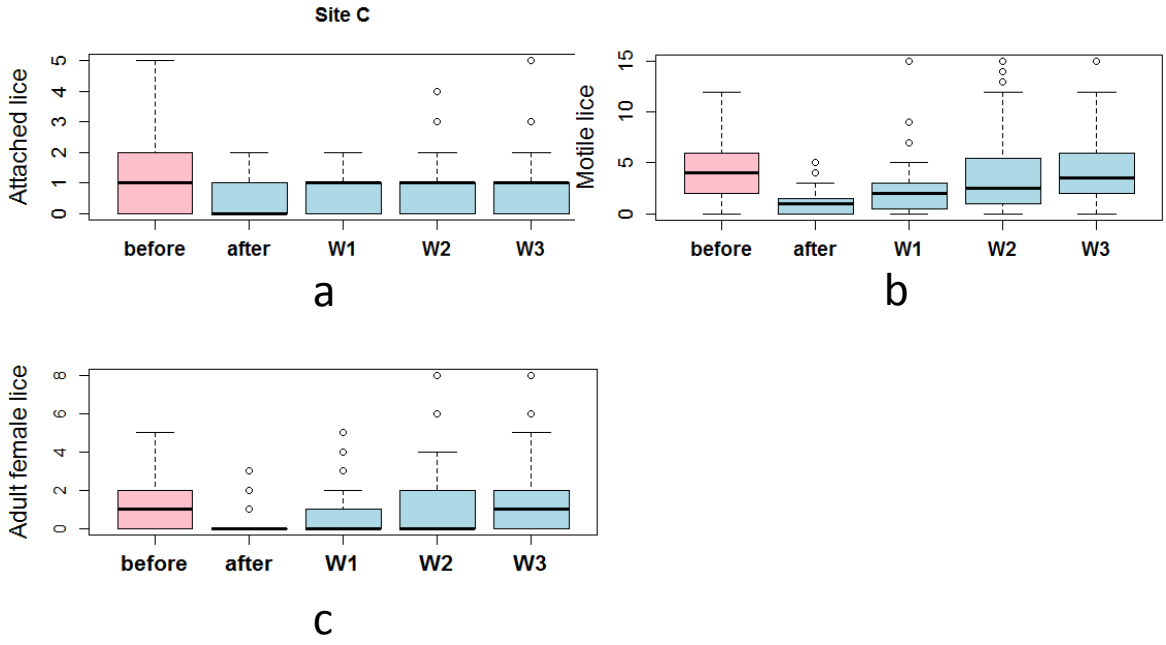


Figure 10: Boxplot of a) attached, b) motile and c) adult lice from selected cages in research facility C before and after treatment and 1, 2 and 3 weeks following treatment. The boxplot shows the distribution of lice from 40 fish per time point with median value (black line) with 25 and 75 percentiles represented by the box. Significantly fewer motile lice were counted immediately after (p=0.001) and 1 week following treatment (p<0.02). Significantly fewer adult female lice were identified immediately after (p=0.001) and 1 and 2 weeks after treatment (p<0.02). Significantly fewer attached lice were identified immediately following (p<0.001) and 1 and 2 weeks following treatment (p=0.02).

From the predictive model, the reduction in motile lice in selected cages from site C was calculated to be 76.7% immediately following and 41.9% one week after treatment.

Only one selected cage was treated in research facility C.

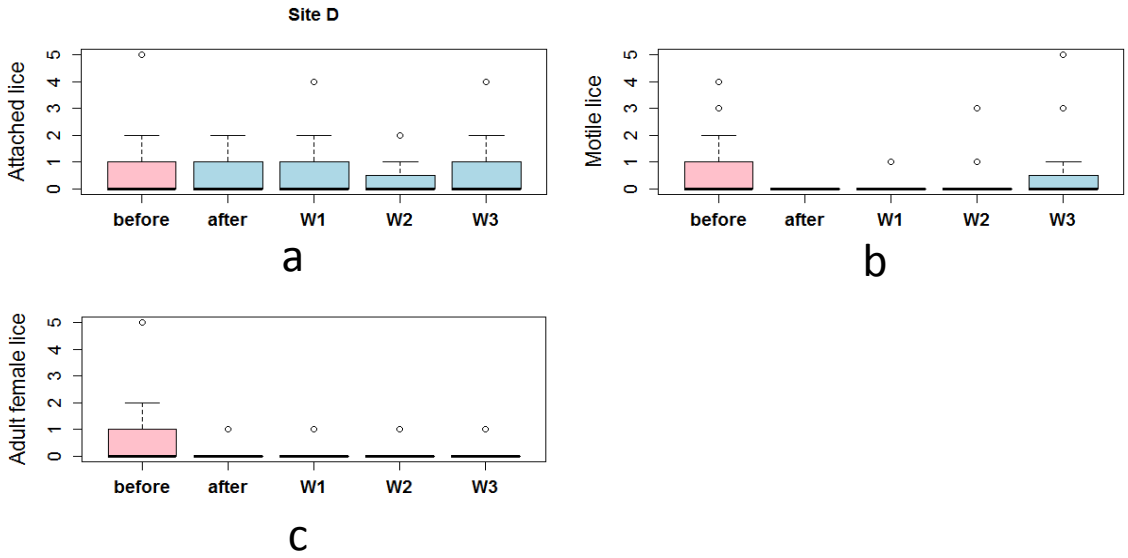


Figure 11: Boxplot of a) attached, b) motile and c) adult lice from selected cages in research facility D before and after treatment and 1, 2 and 3 weeks following treatment. The boxplot shows the distribution of lice from 40 fish per time point with median value (black line) with 25 and 75 percentiles represented by the box. No motile lice were counted immediately after treatment. Further, significantly fewer motile lice after 1 week (p=<0.0001) and 2 weeks following treatment (p<0.02). Significantly fewer adult female lice were identified immediately after (p=0.001) and 1 and 2 weeks after treatment (p<0.005). Significantly fewer adult lice were identified at all time points following treatment (p=0.007).

Model prediction of % reduction of mobile lice numbers in selected cages immediately following treatment in site D was not possible as there were 0 motile lice counted on all fish. One can suppose there was a 100% reduction in motile lice. Percentage reduction of motile lice 1 week after treatment was calculated via predictive modelling to be 87.1%.

While all cages in research facility D were treated, due to the lack of data a boxplot showing lice population development for the whole site is not shown.

For attached lice, only site C showed a significant reduction in lice number between before and after using a site-specific model. On consideration of all sites as a whole (common regression model for all four sites) a significant reduction of attached stages from before and after treatment ($p=0.006$) can be identified. A higher level of infection of attached stages was observed in site B ($p<0.001$) and a lower level in site D ($p<0.001$), compared to site A.

Sea temperature and calculated infection pressure towards research facilities are summarised in figures 12 and 13. Infection pressure from motile stages from neighbouring sites was calculated according to a published infection pressure model (Kristoffersen et al. 2014). Attached larvae which survive thermal treatment have an estimated development time to motility of 10-11 days for sites A and B, 15 days for site C and 17 days for site D.

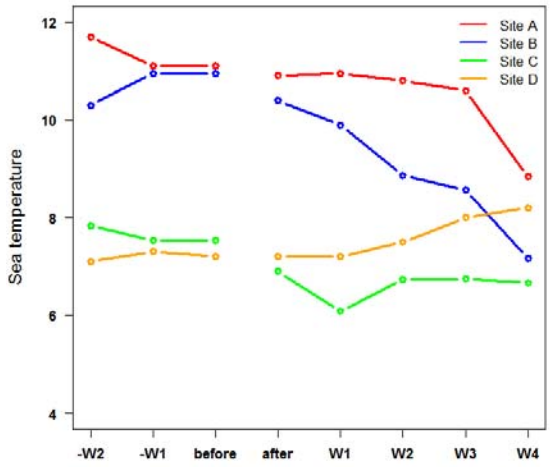


Figure 12: Registered sea temperature for the period 2 weeks before and 4 weeks after treatment in the four research facilities.

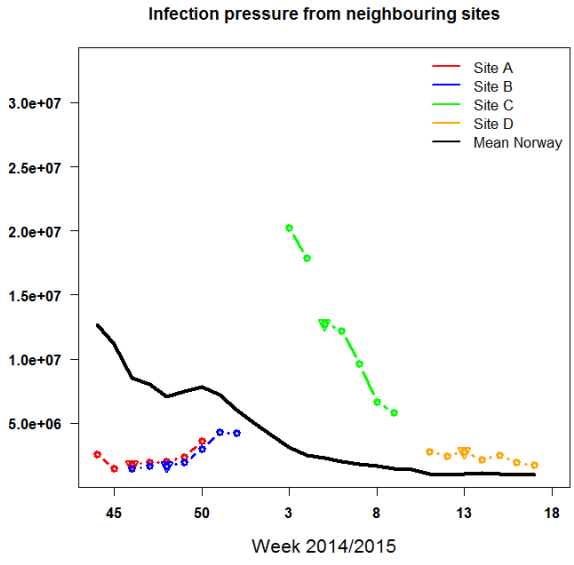


Figure 13: Calculated infection pressure from neighbouring sites towards the different research facilities during the study period. Treatment dates for the various sites: Site A; week 46, site B; week 48, site C; week 5, site D; week 13. The black line represents average infection pressure for the whole of Norway during this period.

Experiences gained from trial sites

Results of lice counting, mortality and feed consumption are not shown for trial sites. In some trial sites, fish with irritated gills and/or diagnosed AGD were treated using Thermolicer®. Extremely high post treatment mortalities were experienced in these sites. Experiences relating to de-licing effect in trial sites were similar to those gained in research facilities.

6. Discussion

Thermolicer® is a new non-medicinal method for de-licing salmonid fish. According to aquaculture legislation § 20 all new methods and technical developments must be proven and documented in regard to fish welfare before they can be taken into general use. The present project has focused on identification of acute injury and/or development of subsequent significant injury towards the skin, eyes, gills and fins over a four week post-treatment period. In addition, histology was performed on selected gills. No samples have been taken in relation to measuring of the stress response in the fish, as it was considered highly likely that the stress response would have been high due to the handling/crowding necessary during this type of treatment. In equivalent new studies, measurement of the stress response should nevertheless be considered as they may be of value for evaluation of fish welfare. Feed consumption records were summarized to provide a general indication of how the fish were affected by the treatment. Mortality records alone are not a good indication of fish welfare. All handling will affect the fish and a less robust or stressed fish will tolerate less handling.

Research facilities A and B were de-liced with machine 1, with no significant alterations made to the machine between treatments. Research facilities C and D were de-liced with machine 2, with considerable changes being made to the machine between treatments. Only one cage was treated in site C, as fish welfare on this site was observed to be unacceptable. Treatment of site D with machine 2, was performed with developments equivalent to those now on commercial sale.

Fish in sites A, B and C were crowded for extended periods while fish in site D were not. Crowding times were unfortunately not documented during the present study. Treatments in sites A, B and C involved salmon, while rainbow trout were treated in site D.

Lice numbers from the research facilities involved show that Thermolicer® treatment reduces the lice burden considerably. The calculated reduction of motile lice varies with site, from between 75-100%. No clear effect was documented on attached lice stages despite identification of statistical trends which indicate a lower number of attached lice immediately following treatment. Lice populations in sites A, B and C, 3 weeks post treatment, were similar to pre-treatment levels. For site D there remained significantly fewer lice 3 weeks post-treatment. Calculation of infection pressure towards the four research facilities show that sites A, B and C were subjected to an infection pressure higher than the average Norwegian infection pressure in the same period. Infection pressure towards sites A and B were in addition considerably higher than that towards site C. All cages in each site were not simultaneously treated. This means that untreated cages may have contributed to internal infection pressure. Further, it is known that calculated developmental times from attached lice to mobile lice are consistent with increasing mobile lice number in these sites. It is likely that both external and internal infection pressures together with development of surviving attached stages following treatment are considerable factors in post treatment lice population development. The degree to which infection pressure and surviving attached stages can explain future lice population development in these sites cannot be established based on the data gathered during the present study. Research facility D, did not experience the same lice population development as the other sites after treatment. Of significance may be that this site is exposed to a lower infection pressure from neighbouring sites than the other experimental sites. In addition this site was treated with a machine in which excess water from the fish pump was filtered, which may have contributed to lower lice population development on this site.

The protocol for welfare documentation included evaluation of gills, snout injury, scale loss, skin bleedings, eye injury, cataract, wounds and fin damage. Focus was placed on acute injuries occurring on the day of treatment, before and after treatment. In the period one to three weeks post treatment many uncontrolled factors may contribute to development of injury. Significant injuries identified during this period are therefore not related directly to the treatment, but are registered as valuable indicators of unintentional effectors on the fish.

Total gill score provides an indication of fish gill-health status and should be considered together with histological changes. This parameter was not expected to indicate acute gill injury following thermal treatment.

Gill pallor was included following introductory observations of varying degree of gill pallor following treatment. Fish from sites B and D displayed significant changes in degree of gill pallor following treatment. These changes are of unknown physiological importance. Histopathological investigation gave no indication of cause or effect.

Significant snout injuries were observed in sites A, B and C with the most serious observed in site C. No significant snout injuries were observed in site D. Injuries to the snout can occur on crowding and fish in sites A, B and C all experienced extended periods of crowding. Fish sampled early in the crowding period from sites A and B displayed significant snout injury. Significant scale loss was documented only in site C. No significant changes were associated with cutaneous bleeding or wounds in any of the trial sites.

No increase in the prevalence of eye injury or cataract was observed immediately following treatment. Eye injury and cataract were observed with time however in site C. This indicates that such injury may only be obvious with time.

Significant fin damage was observed on sites A and C. No significant fin damage was observed on site D.

Histological investigations identified acute bleeding in gills sampled before and after treatment. No detectable changes were identified between samples taken immediately before and after treatment. Gill bleeding was also identified in samples taken two and three weeks after treatment.

Bleeding in gills of euthanized fish is not unusual. The lesions are usually a result of handling or of disease. Blood vessel damage and bleeding are normal findings in gill tissues of fish with epitheliocysts and proliferative gill inflammation which were identified in sites A and B.

Gills with individual extremely thickened or proliferative lamellae with associated and centrally organized /hyaline tissues, probably represent repair processes following bleeding.

Whether Thermolicer® causes injuries which cannot be detected by light microscopy is unknown. Such injuries could conceivably result in increased bleeding on subsequent handling of fish treated with Thermolicer®.

On site C, a degree of lamellar fusing (i.e. the lamellae grow together) was observed. Such changes can be associated with amoebic gill disease (AGD), which had been previously diagnosed on this site. Thickened filaments are observed in fish treated for AGD.

Some proliferation of respiratory epithelia (clubbing) is normal in fish farmed in the sea. These changes are observed both distal and lateral on affected lamellae. The changes identified following thermal de-licing were sparse to moderate with the exception of more extensive AGD associated findings in samples from site C. The findings are therefore within the limits of those expected in sea-farmed fish which have been handled.

Pathological changes which can with a degree of certainty be related to thermal de-licing were not identified. It cannot be completely discounted however that the bleeding identified following treatment with the Thermolicer® is in some way related to thermal de-licing.

Water analyses revealed that sufficient water flow through the Thermolicer® is important. Random samples from the treatment chamber showed variation in turbidity (particle density) and estimated free ammonia. Free ammonia in the last sample from site B was extremely high. This water sample was also extremely turbid. While production and toxicity of ammonia is described by Terjesen and Rosseland, there is little knowledge relating to the importance of short term exposure to the levels identified in the Thermolicer®. There are grounds to believe that the levels involved probably stress the fish. Water quality in the treatment chamber should therefore be monitored closely and water flow/exchange must be sufficient.

Mortality on the trial sites is reported to be within acceptable 'norms' i.e. comparable with other treatments. There are, however, no published mortality figures relating to such treatments, and there are no guidelines for what is in fact an 'acceptable' level of mortality associated with such treatments. Highest observed mortality during the present trial was observed on site C and the lowest on site D. Extremely high mortality was however registered on trial farms in which fish had been diagnosed with AGD and/or gill irritation.

7. Conclusion

Lice counts show that thermal de-licing has an extremely good effect related to reduction of mobile and adult female lice.

There has been continual improvement of the Thermolicer® during the project period. The project results must be considered in light of these improvements. The most significant changes have been related to crowding time and choice of pump. Changes to the pumping system and improvement of the sea water straining chamber improved results. On research facility D the most improved version of the Thermolicer® was used. No significant injuries were identified following treatment of the selected cage on this site. The lowest mortalities (%) were also registered on this site. Feed consumption normalized more quickly on this site compared to other sites. On this basis the effects on fish welfare are considered acceptable.

There are no clear criteria for documentation of new technology in terms of fish welfare. In the present project focus was placed on evaluation of acute injuries, water quality in the treatment chamber together with mortality levels and feed consumption. The results show that improvement of the Thermolicer® resulted in fewer injuries and therefore lower impact on fish welfare. No equivalent documentation relating to other de-licing methods, medicinal or non-medicinal has been identified. The degree of injury considered acceptable in terms of fish welfare is therefore not discussed in the present report.

8. Recommendations

Thermolicer® is a new method which may be used as an alternative to medicinal treatments and in addition to other preventative measures against lice.

Water quality in the treatment chamber must be monitored closely and sufficient water exchange must be ensured. The treatment water must be changed between each treated unit.

A network of Thermolicer® users should be established for transfer of information and further optimisation of the method as it is taken into use by the industry.

Crowding and pumping of fish are challenging in terms of fish welfare. It is recommended that 'gentle' crowding techniques are used. Careful handling of sick fish is also recommended.

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Appendix 1:

Description of scoring criteria for extended welfare registration in fish

AGD-gill score
Based on Taylor *et al*. 2009.
The gill structure on both sides of the fish is scored as a single score according to the criteria described below. The descriptions are based on a poster produced by Skretting.

0 No sign of infection: even red colour, even thickness and no mucus production.
1 One white spot and slight indication of injury or scar development
2 Two to three small, white patches
3 Thick, attached white patches covering up to 20 % of the gills
4 Patches as above covering 50% of the gills
5 Most of the gills (i.e. >50%?) covered by the same type of patch

Total gill score
The principle for scoring is the same as for AGD, without consideration of mucus production. Pale patches are evaluated without considering mucus production.

Gill pallor
This is highly subjective
0 Red, healthy gills
1-2 Lighter areas distally on filaments
3-5 Increasing colour change on the whole gill surface.

Scale loss
0 No scale loss.
1 Loss of individual scales
2 Small areas of scale loss.
3 Large areas of scale loss.

Skin bleeding not including fin bases and fins
0 No bleeding on the body
1 Small bleedings/ colour changes. Often seen on the ventral abdomen
2 Larger area of bleeding often associated with scale loss.
3 Fresh bleeding often associated with significant scale loss, wounds and oedema in the skin.

Wounds
Definition of wounds: A wound is defined as an area with surface or deeper injury in the skin and/or exposure of underlying tissues/musculature
0 No wounds
1 One small wound
2 Several small wounds
3 Large significant wounds

Snout injury
Definition: Wounds on the snout covering the anterior part of the top and/or bottom jaws
0 No wounds
1 Small wound on snout (either jaw).
2 Injury and broken skin on the snout
3 Significant, deep and extensive injury considered serious enough to justify destruction of the fish. Injuries can cover the whole head.

Eye injuries
definition: Eye injuries including bleeding in the eye and clouding of the cornea. Worst case = punctured eye.

0 No injury or bleeding.
1 One small bleeding or slight corneal clouding.
2 Larger bleeding in the eye or obvious clouding of the cornea
3 Larger bleeding and significant corneal clouding.
In some cases the eye is punctured. The fish can be considered blind, and is destroyed.

Cataract
Definition: Clouding of the lens

0 No clouding
1 One small white spot
2 Up to 25% of the lens clouded
3 Up to 50% of the lens clouded
4 Over 75% of the lens clouded

Fin damage
Definition: Bleeding, broken skin with exposed sub-cutaneous tissues in the fins or fin bases and exposed fin-rays.

0 Normal fins for a farmed fish without acute injury
1 One or more shallow breaks. Often with small bleedings
2 One or more deep breaks. Often with small bleedings. Some fin-rays may be exposed.
3 Broken fin base. Fragments of fin may be missing or hanging loose. In cases in which the skin is 'peeled' off the fin these were scored as a 3 (also noted in comments)

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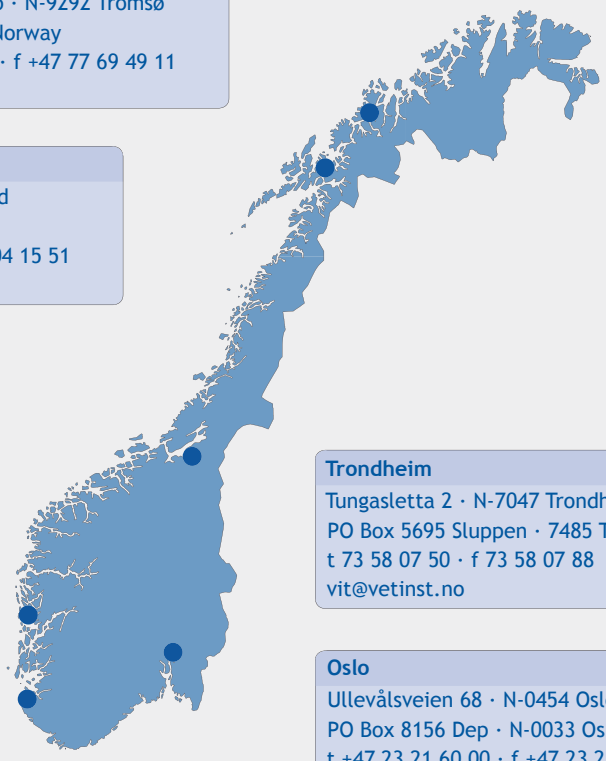

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